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Studies on neoteny in Coleoptera: phylogenomics, classification, and evolutionary interactions

Ph.D. thesis

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I hereby declare that I prepared and wrote the Ph.D. thesis entitled “Studies on neoteny in Coleoptera: phylogenomics, classification, and evolutionary interactions” under the guidance and supervision of prof. Ladislav Bocák and I used literature cited in this study.

Olomouc, 7th March 2024 Mgr. Dominik Kusý

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Abstract

Beetles owe their evolutionary success to metamorphosis. However, their relationships remain controversial, hindering progress in understanding the evolution of different beetle phenotypes. Elateriformia, which includes jewel beetles, pill beetles, fireflies, click beetles, and their relatives, are members of the deepest branch of the "core" Polyphaga (Coleoptera), containing 31 extant families and approximately 43,000 species. These beetles exhibit considerable morphological and ecological diversity. However, their characteristics have led to an unstable classification.

This dissertation investigates the evolution of soft-bodied beetle lineages, focusing on their phylogenetic relationships, temporal dynamics, and the emergence of modified lineages. With eight comprehensive studies, state-of-the-art phylogenomic methods, next-generation sequencing, molecular systematics, and morphology, I aimed to unravel the evolutionary relationships of the studied lineages. The included studies investigated beetle evolution using different data sources such as mitogenomes, nuclear genes, and transcriptomes. I addressed fundamental questions and highlighted the effectiveness of molecular techniques in revealing complex phylogenetic relationships while addressing the possible incongruences and challenges when resolving deep splits.

The primary objective of this study is to clarify the higher phylogeny and phylogenetic placement of enigmatic neotenic soft-bodied lineages within the beetles, especially within Elateriformia. For example, molecular analyses were used to determine the taxonomic affiliations of *Paulusiella*, *Analastesa*, and *Thylodrias*. These analyses challenged conventional classification methods due to the altered morphologies of these species. This highlights the inadequacy of relying solely on morphological characters to accurately infer evolutionary relationships within soft-bodied beetle lineages. Additionally, my research reveals the position of the family Sinopyrophoridae, providing novel insights into the relationships of lineages in elateroid-lampyroid clade and showing that their ancestor was, in fact, a hard-bodied elaterid-like beetle. Moreover, I address the uncertainty surrounding the internal classification of Elateridae, Cantharidae, Lycidae, and Dermestidae by utilizing various molecular datasets.

In addition, my research focuses on the evolutionary basis of life history traits, bioluminescence, and mimicry. Studying the genetic basis and ecological implications of these traits elucidates the adaptive significance of evolutionary innovations and provides a comprehensive picture of beetle diversification through time.

By harnessing the power of molecular techniques and interdisciplinary approaches, I have advanced our understanding of the evolution of soft-bodied beetles and contributed to the broader discourse on biodiversity, adaptation, and evolutionary biology.

Key words: Phylogenomics, evolution, Coleoptera, Elateriformia, Dermestidae, Lycidae, Elateridae, Cantharidae, neoteny, systematics, ontogenetic modifications, molecular dating, bioluminescence, mimicry.

Abstrakt

Brouci vděčí za svůj evoluční úspěch metamorfóze. Nicméně jejich vzájemné vztahy zůstávají kontroverzní, což brzdí pokrok ve výzkumu evoluce jejich rozmanitých fenotypů. Elateriformní brouci, kteří zahrnují skupiny známé jako krasci, světlušky, páteříčci, kovaříci a jejich příbuzné, patřící mezi nejhlubší větve tzv. "core" Polyphaga (Coleoptera), skupinu tvoří 31 recentních čeledí a přibližně 43 000 druhů. Tito brouci vykazují značnou morfologickou a ekologickou diverzitu. Avšak právě jejich ekologická i morfologická rozmanitost vedly k nestabilní klasifikaci.

Tato disertační práce se zabývá evolucí linií měkkotělých brouků s důrazem na jejich fylogenetické vztahy, časovou dynamiku a vznik modifikovaných linií. V osmi komplexních studiích zahrnujících pokročilé fylogenomické metody, sekvenování nové generace, molekulární systematiku a morfologii, jsem se snažil vyřešit evoluční vztahy studovaných skupin. Tyto studie zkoumaly evoluci brouků s využitím různých zdrojů dat, jako jsou mitogenomy, nukleární geny či transkriptomy. Zabýval jsem se základními otázkami příbuznosti a zdůraznil efektivitu molekulárních technik v odhalování složitých fylogenetických vztahů a možných nekonzistencí.

Hlavním cílem této studie je objasnit vyšší fylogenezi a fylogenetické umístění záhadných neotenických linií brouků uvnitř Elateriformia. Molekulární analýzy byly použity k určení taxonomické příslušnosti rodů *Paulusiella*, *Analastesa* a *Thylodrias*. Tyto analýzy vedly k přehodnocení tradičních metod klasifikace kvůli paralelním modifikacím morfologických znaků u těchto druhů. To ukazuje na nedostatečný taxonomický signál morfologických znaků pro přesné určení evolučních vztahů uvnitř linií měkkotělých brouků. Další studie se zabývá pozicí čeledi Sinopyrophoridae. Tato práce poskytuje nové pohledy na vztahy linií v elateroid-lampyroidním kládu a ukazuje, že jejich předek byl ve skutečnosti brouk podobný kovaříkům. Dále jsem se věnoval vyřešením vnitřní klasifikace čeledí Elateridae, Cantharidae, Lycidae a Dermestidae s využitím různých molekulárních datových sad.

Mimo to se mé studie soustředily na evoluci životních strategií, bioluminiscenci a mimikry. A jejich vliv na diverzifikaci a evoluční úspěch studovaných skupin v čase.

Využitím molekulárních technik a interdisciplinárních přístupů jsem snad přispěl k našemu chápání evoluce brouků s „měkkým tělem“ a přispěl k širší diskusi o biodiverzitě, adaptaci a evoluční biologii.

Klíčová slova: Fylogenomika, evoluce, Coleoptera, Elateriformia, Dermestidae, Lycidae, Elateridae, Cantharidae, neotenie, systematika, ontogenetické modifikace, molekularní datování, bioluminescence, mimikry.

Contents

1 Preface

1.1 Holometaboly is the key innovation in the evolution of insects

Holometaboly is the latest major ontogenetic modification in Arthropoda (Engel & Grimaldi, 2005; Jindra 2019, Misof et al. 2014) and it changed the evolutionary history of animals on Earth. Today, holometabolous insects are the largest group of animals in the number of species, they are ubiquitous and play an important role in all terrestrial ecosystems. Fossil records indicate that the first holometabolan insects appeared in the Carbon and gradually rose to dominance in the Perm (Moran 1994; Nel et al. 2013). At least since the Permian extinction event, they dominate the terrestrial biota and they become indispensable parts of the ecosystems when they become important pollinators of angiosperms in the Cretaceous.

The principal trait, the endogenous development of wings (Medved et al. 2015; Almudi et al. 2020; Ross 2022; Prokop et al. 2023), is phenotypically demonstrated by the transition from the last wingless semaphoront, larva, to a pupa already having wing pads. The pupal stage lasts a substantial time of the transition between the last larval instar, still having the nutritional role and the adult with the predominant reproductive role. Hence, the pupa is a specialized larval instar that is inactive and neither takes food nor reproduces (Truman 2019). The separation of the nutritive and reproductive roles enabled phenotypic divergence opening access to energy sources otherwise unavailable. Therefore, the modification of ontogeny is one of the major macroevolutionary factors leading to novelties determining the success of lineages (Martynov et al. 2022). Developmental plasticity, the ability of an individual to modify its development in response to environmental conditions, might facilitate the evolution of such novel traits. Modifications of serial homologs of legs (wings, gills, mouthparts, antennae) are examples of such a process (Moczek et al. 2011; Fisher et al. 2020; Hu et al. 2019). In insects, the substantial phenotypic and ecological transition between larva and adult is hypothesized to be a key innovation leading to the present dominance of Holometabola, (=Endopterygota) in modern biota (Mayhew 2007). There are 11 recent holometabolan orders, combined having over a million recognized species and representing about 60% of the named animal diversity (Engel 2015).

Prevalently, the holometabolan metamorphosis is a fine-tuned ontogenetic process (Jindra 2019). The Holometabola is characterized by a developmental sequence in which the pupal stage services the transitions between larval/adult semaphoronts and their ecological roles. Measured by the dominance in the modern biota, the advantages of the holometaboly are prevalent. Nevertheless,

the pupal stage brings about also disadvantages. Pupa needs time to rebuild the body tissues, is mostly defenseless for a quite long time (only chemical and mechanical protection remains available, e.g., aposematic coloration and setae), exposed to pathogens, and abiotic factors. All organisms pass through ontogenetic phases and metamorphose during their life in some way and there are multiple genes regulating metamorphosis in insects (Truman & Riddiford, 2002; Jindra 2019). As a successive expression of genes can be modified, some stages may be suppressed, or the ontogenetic development prematurely terminated. The modifications of the temporal synchronization of ontogenetic phases are collectively called heterochrony.

The molecular mechanisms of metamorphosis have been intensively studied, but experiments have been limited to a low number of model organisms (Chafino et al. 2019; Minakuchi et al. 2008; Ureña et al. 2014; Ureña et al. 2016; Campli et al. 2024). The beetles remain understudied (Hotaling et al. 2021) and only *Tribolium castaneum* metamorphosis is well described from the gene function perspective (Minakuchi et al. 2009; Richards et al. 2008; Konopova & Jindra 2007, 2008; Belles 2020; Suzuki et al. 2008). The expression of adult characters is blocked in *T. castaneum* larva by the action of juvenile hormone (JH) through its receptor Methoprene-tolerant (Met) and the transcription factor Krüppel homolog 1 (Kr-h1) (Konopova & Jindra 2007, 2008; Belles 2020). The last larval instar is characterized by a temporal decrease of Kr-h1 expression in the early stage and by a connected slight increase of Ecdysone-induced protein 93F (E93) expression (Chafino et al. 2022). An upregulation of Broad-Complex (Br-C) expression reaches a maximum in the prepupal phase and triggers pupation. The Br-C expression and a renewed Kr-h1 expression block E93 in the pre-pupa and postpone adult morphogenesis. In the early phase of the pupa, Br-C and Kr-h1 expression decreases and the strong expression of E93 triggers adult morphogenesis (Chafino et al. 2022). Although neotenics are common in the phenotypically diverse Elateriformia (Lawrence et al. 2011; Kundrata et al. 2014), modifications of their metamorphosis have never been studied and we have only information on *Tribolium* as a model (Konopova & Jindra 2007, 2008; Belles 2020; Suzuki et al. 2008). The expression of various genes during metamorphosis producing neotenous forms has only been described for Strepsiptera and Sternorrhyncha (Chafino et al. 2018; Vea et al. 2019). Currently, it is assumed that neotenic phenotypes reflect the modified hormonal regulation, either absent or differently timed expression of Br-C and E93, but potentially also Kr-h1, HR3, E75 (Chafino et al. 2018; Vea et al. 2019). Additionally, abnormally metamorphosing individuals were recorded and potentially indicate a hormonal disorder. These include the presence of a metathoracic wing in the last instar larva of *Lampyris noctiluca* (in our breeding colony; personal observation), post-imaginal molting of *Lamprigera minor* (Jeng et al. 2021) and a unilaterally winged female of *L. noctiluca* (Maas & Dorn 2003).

Terminology

Holometaboly: the succession of ontogenetic phases i.e., larval instars, inactive pupa, and a sexually morphologically distinct mature adult. Holometabola incl. four big orders wasps, moths, beetles, and true flies.

Heterochrony: temporary shifts in relative onset of developmental stages, prolongation, or contraction of the ontogenetic stages.

Paedomorphosis: the retention of immature traits in sexually mature individuals, reproduction of an individual with a completely partial larval phenotype, heterochrony includes progenesis and neoteny, sometimes termed as paedomorphosis.

Progenesis: short development, early onset of the development of sexual characters; r-strategy. Aphids.

Neoteny: prolongation of the larval stage by deceleration of development of sexual characters, prolonged development, K-strategy. Elateroid beetles, human.

Hypermetamorphosis: some larval instars are functionally and morphologically distinct from each other (false click beetles, blister beetles, Strepsiptera).

(Crowson 1972, Gould, 1977, Bergstrom & Dugatkin, 2011, Burakowski, Muona & Teräväinen 2020, etc.)

1.2 Beetles as a model for studies of neotenic modifications

The enormous biodiversity of insects (>50% of animals are insects, 17.4% of Eukaryotes are beetles) is threatened by environmental changes and the growing human population (Wagner et al. 2020; van Klink et al. 2020). In contrast with their ecological importance, the true diversity of insects, mechanisms affecting their phenotypic and ecological plasticity, as well as their vulnerability to dynamic changes of the environment remain poorly studied (Srivathsan et al. 2019). Due to incomplete knowledge, insects are used less frequently than vertebrates in large-scale studies of evolutionary processes, and in studies aiming to reconstruct past distribution patterns, estimate the impact of climatic fluctuations, and set conservation priorities. Phylogeny provides an indispensable basis for studies dealing with evolutionary questions. A phylogeny-based classification is needed to correctly understand the polarity of phenotypic changes including modifications of ontogenesis that can substantially change evolutionary trajectories.

Even though holometaboly is a key factor in the evolutionary success of insects (Rainford et al. 2014; Jindra 2019), some beetle groups especially many lineages of Elateriformia do not pass through full metamorphosis (the last preimaginal instar is active and feeding, adult traits are absent in imago, which differs in most extreme cases from the last larval instar only in sexual organs and a slightly different structure of cuticle) or their transformation from larva to an adult seems to be 'unfinished', i.e., they show incomplete metamorphosis (McMahon et al. 2016; Chafino et al. 2018). Under incomplete metamorphosis, we include sexually mature individuals that show mixed features of the larva, pupa, and adult. As examples, we can list shortened or vestigial appendages (antennae, legs, mouth parts), vestigial to absent elytra and wings (similar to pupal wing pads), lower meso/metathorax length ratio (the trait characteristic for beetle larvae), weakly sclerotized larviform abdomen, weakly sclerotized and simplified female genitalia, and possibly also incomplete sclerotization of the integument. The neoteny should be strictly separated from the ecologically driven loss of wings and the subsequent modification of some structures that have well-known consequences for female fecundity (Tigreros & Davidowitz 2019). Unlike neotenics, as defined by Gould (1977) and others, the groups affected by the ecologically driven loss of wings are not so deeply modified, usually, their modifications are linked to biological associations that make the loss of wings adaptive. I do not include under incomplete metamorphosis winglessness of both sexes that is putatively caused by ecological adaptations (island and high mountain environments) (Waters 2020; Roff 1990). Soft-bodied Elateriformia, i.e., soldier beetles, most net-winged beetles, and fireflies, etc., are traditionally considered completely metamorphosed, but their aberrant phenotypes can be a result of same or similar modifications of metamorphosis as undisputable neotenic forms but affecting only the last phase of the metamorphosis (Kusy et al. 2019). Females always show deeper modifications than conspecific males. Adult traits are

progressively expressed from the frontal to posterior parts of the body. Males are seldom obviously modified, but if females are larviform, the males are often miniaturized, have shortened elytra, and can be wingless (Takahashi, et al. 2016; Kusy et al. 2019).

Figure 1. Representatives of soft-bodied and neotenic lineages. A) Mating male and female of *Chauliognathus* sp. (Cantharidae), B) Mating male and female of *Malacogaster* sp. (Elateridae, Drilini), C) mating male and female of *Pyrocoelia* sp. (Lampyridae) © Shizuma Yanagisawa, D) mating males and females of *Thylodrias constrictus* (Dermestidae), E) mating male and female of *Stenocladius* sp. (Lampyridae) © Itsuro Kawashima, F) female of *Rhagophthalmus* sp. (Rhagophthalmidae) protecting its eggs © Fang-Shuo Hu, G) freshly molted female adult "larva" of *Platerodrilus* sp. (Lycidae) © Maxs Lee, H) mating male and female of *Phrixothrix* sp. (Phengodidae) © Jim McClarin. Other pictures were taken by the author or Michal Motyka.

Phenotypic traits indicating ontogenetic modifications and their distribution in Coleoptera with a special focus on Elateriformia

Female neoteny in beetles

The extent of juvenile traits preserved till the adult stage is highly variable and sometimes, these forms have not been considered designated as cases of heterochronic development even if analogous modifications are referred to in the literature as text-book examples of neoteny (e.g., Johnston & Gimmel 2020; Dascillidae: *Anorus*). On the contrary, some taxa were incorrectly linked to neotenic females, e.g., *Plastocerus angulosus* (Plastoceridae sensu Crowson 1972, but see Bocak et al. 2018).

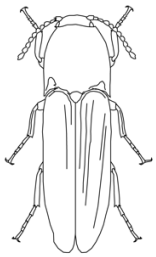
Here, I present an overview of the already described beetle forms that can be designated as incompletely metamorphosed, in other words retaining some juvenile traits. The modifications are gradual. For simplification, they are grouped into five morphological categories defined by the degree of the metamorphosis of the head and thorax, the loss of elytra or brachyptery, the loss of wings, and the presence of an unsclerotized abdomen. In **type 1**, I group the closest relatives of neotenic lineages that are soft-bodied in the adult stage, and simultaneously this category describes the majority of males conspecific with neotenic females. These types can be considered as a transformation series between fully larviform and adult semaphoronts.

Type 1. Soft-bodiedness

Incomplete sclerotization of the adult cuticle (soft-bodiedness, often physogastric)

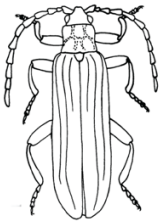
Bocak et al. (2008) proposed that the origin of soft-bodied elateroids might be a result of arrested metamorphosis as neotenic types 2–4. The proposal was based on the absence of the fully sclerotized cuticle of these forms, ***feebly sclerotized,*** sometimes partly physogastric abdomen, the higher number of visible abdominal segments than in the fully sclerotized relatives, the absence of tight coadaptation between lateral elytral margins and the abdomen (absence of a closed sub-elytral

Ancestral clicking



cavity) that decreases the desiccation. These traits were reported by many students of beetle morphology (Ballantyne & Lambkin 2009; Muona 1995; Lawrence et al. 2011; Kundrata & Bocak 2018; etc.). Representatives: members of the following families Cantharidae, Lycidae, Lampyridae, some Elateridae, Omethidae, etc.

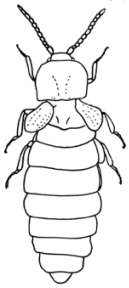
Type 1. Soft-bodiedness



Type 2. Larviform females with adults-like-head, prothorax, and legs; elytra vestigial, wingless

Representatives: *Omalisus*, *Thilmanus* (Elateridae: Omalisinae, female body size similar to males). Documented in *Omalisus* and *Thilmanus*; predicted for the relatives with unknown females: *Phaeopterus*, *Euanoma*, *Pseueuanoma*, *Paradrilus* (Elateridae: Omalisinae).

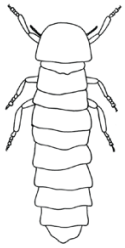
Type 2. Brachypterous



Type 3. Larviform females with adult-like head, prothorax, and legs; elytra absent, wingless

Representatives: *Pyrocoelia*, *Lampyris noctiluca* (some with large-bodied females). There is a loose boundary between types 3 and 4: *L. noctiluca* has no elytra but *L. sardiniae* retains very short vestigial elytra.

Type 3. Apterous



Type 4. Larviform females with adults-like-head and legs, other body parts larviform

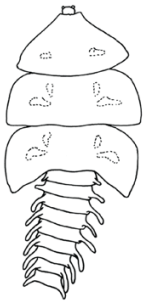
Representatives: *Drilus*, *Malacogaster*, *Selasia*, etc. (Elateridae: Agrypninae: Drilini; female large-bodied); *Oculogryphus chenghoiyanae* (Ototretinae, male and female share similar body size), *Pterotus obscuripennis* (Pterotinae), *Thylodrias constrictus* (Dermestidae, polymorphism – females with vestigial or absent elytra, i.e., type 2 and 3, female moderately large-bodied). *Microphotus dilatatus* (Lampyrinae)

Type 4. Larviform

Type 5. Fully larviform sexually mature females

An undoubtedly neotenic development is represented by fully larviform sexually mature females (Bocak et al. 2008; Makarov & Kazantsev 2022). The groups included in **type 5** have sexually mature females that differ from the last instar larva only by the presence of the genitalia and a different structure of cuticle. For example, Wong (1996) reported that larva-to-larva ecdysis was followed by pigmentation within one day. Conversely, the sexually mature larva turns yellowish after three days.

Type 5. Fully larviform



Representatives: *Stenocladius* (Lampyridae: Ototretinae; *Platerodrilus*, *Lyropaeus*, *Macrolibnetis* (Lycidae: Lyropaeinae, large-bodied females); *Leptolycus* (Lycidae: Lycinae: Leptolycini; similar body size). Predicted for the relatives with unknown females: *Dexoris*, *Lolodorphus*, *Mimolibnetis*, etc. (Lycidae: Dexorinae; females type 4–5), *Ambangia*, *Alyculus*, *Antennolycus*, etc. (Lycidae: Lyropaeinae; males miniaturized), *Atelius*, *Scarelus* (Ateliinae). As females are hardly mentioned unless observed in copula, it is highly probable that further taxa belong to type 1, possibly Ototretinae indet. (Philippines, unpublished), some other Lycidae.

The loss of the pupal stage was expected by Wong (1996). Unfortunately, the transition between larva and adult has not been described for most neotenics. Information is missing either because of the complete absence of knowledge on mature females or due to the absence of research. If pupa is defined by exposed wing pads, then it is surely absent, but if we look for an inactive ontogenetic stage immediately preceding the mature female, and potentially fulfilling the function of a pupa (development of the female genitalia), the situation becomes uncertain. Wong (1996) reported that, unlike larva-to-larva ecdysis, the adult larviform female remains quiescent for three days. Although the larva was closely observed after ecdysis, no further exuvia was shed. Further, the structure of the cuticle is apparently different in the late instar larvae and sexually mature larviform females in *Platerodrilus* (Lycidae) (Makarov & Kazantsev 2022).

Phenotypic diversity of conspecific males

Low interest has been paid to the investigation of the male morphology in lineages with proven neoteny of females (types 2–5). LeConte (1878) described the male of *Micromalthus debilis* as ‘feeble and ill developed’. Crowson (1972) and later students used the concept of 'soft-bodied' beetles. Although not restricted only to, soft-bodiedness is very common in the superfamily Elateroidea, and all males with conspecific neotenic females are soft-bodied. Unlike other soft-bodied elateroids, there have been reported some modifications pointing to parallel evolution of traits identified in neotenic females.

The males of neotenics have sometimes a substantially modified cranium. Unlike close relatives, the head is hypognathous, the cranium is prolonged, the eyes are very small, and the antennae are shifted to the anterior part of the pronotum (Kusy et al. 2018; Kusy et al. 2019). The modified cranium is known in some net-winged beetles (Lycidae): Leptolycini, some Lyropaeini, *Cautires apterus*, and some Calopterini (Kusy et al. 2019; Bocakova & Bocak 1988; Bocak et al. 2013; Motyka, Kusy et al. 2021). In contrast with these, *Platerodrilus* and *Macrolibnetis*, both closely related to *Lyropaeus*, have normally shaped, prognathous heads. A similar modification of the head is known in *Microphotus dilatatus* (Kusy et al. 2019).

Further modifications affect appendages like mouthparts, antennae, and legs. There were reported several cases of the shortened male antennomeres in unrelated lineages. Apparently shorter antennae were illustrated in *Anorus* spp. (Dascilloidea: Dascillidae; Johnson & Gimmel 2020) and *Dexoris chome* (Lycidae: Dexorini; Bocak et al. 2013), and some fireflies (e.g. *Phosphaenus hemipterus*; Novak 2018). Additionally, limited sclerotization and the absence of pigmentation in the terminal antennomere are more common in neotenics than in the related lineages (e.g., net-winged beetles – Leptolycini, some Lyropaeini; *Cautires apterus* in Metriorrhynchini; Bocak et al. 2014; Masek et al. 2014). Nevertheless, the detailed evolution of such modification has never been studied as detailed phylogenies are still absent (Kusy et al. in prep.). Although a linkage to an incomplete metamorphosis can be hypothesized for these modifications, some neotenics have fully developed antennae (some fireflies) and these are sometimes even exceptionally long and robust (net-winged beetles – Ateliini).

The summary of ontogenetic modifications in beetles

In summary, neoteny in beetles is a phenomenon without a clear border at the bottom, i.e., the least modified forms. One may refute the arrested metamorphosis (=neoteny) in the case of some flightless and physogastric fireflies as recently did Ballantyne & Lambkin (2009), but as we move up within the continuum of morphological modifications, we encounter forms that are consensually designated as clear neotenics (levels 3–5).

1.3 Ecological and evolutionary consequences

Research is also being conducted on the macroevolutionary consequences of modified or absent metamorphosis. Neotenics often develop over a longer period than relatives; some are large-bodied and some females invest significant energy in their offspring (McMahon et al. 2016; Waters et al. 2020; Gould 1977; Bocak et al. 2008, Wong 1995) Due to the loss of wings, neotenics are characterized by limited vagility and dependence on stable ecosystems. Such characteristics make them ideal for studying centers of biodiversity with uninterrupted evolution (Bocak et al. 2008; Malohlkava & Bocak 2010, Catalan et al. 2024).

Are progenesis and neoteny in beetles adaptive?

Progeny in beetles is limited only to *Micromalthus debilis* (Micromalthidae) which has a very complex life cycle and stands aside from other beetles in many aspects. *Micromalthus* is the only beetle with individual development from triunguline-like dispersive first larval instar via phenotypically distinct reproductive larvae, parthenogenesis, and vivipary (Beutel & Hörnschemeyer 2002; McMahon & Hayward 2016; Pollock & Normark 2002; Perotti et al. 2016). Additionally, the progeny differs in the ecological consequences. It is characterized as an r-strategy and leads to a shortened life cycle, a small body size, and lower investment in numerous offspring (Gould 1977). No author has yet discussed if the progeny is selectively positive in *Micromalthus*. The family, although evolutionary ancient (Hunt et al. 2007; McKenna et al. 2019) contains only a single rare species.

Neoteny is hypothesized in other modified beetle lineages, and these are concentrated mostly in Elateriformia (Crowson 1972; Gould 1977; Bocak et al. 2008; Jordal et al. 2002). Ecologically, there are three groups of neotenics. The elateriform neotenics have larvae and females freely crawling on the soil surface, in upper soil layers, and in contact with rotten wood (Burakowski 1988; Wong 1996; Masek et al. 2014; Jeng et al. 2021; Rosa et al. 2020). According to the present knowledge, these beetles do not differ in life histories substantially from their close relatives, most are predatory. Among them, drilids (Baalbergen et al. 2014), fireflies (Sato 2019) omalisids (Burakowski 1988, Bocek et al. 2019), phengodids, and rhagophthalmids (Eisner et al. 1998). These groups actively search for the prey in their environment and only drilids regularly hide in shells. These neotenic lineages have uniform life history and during their supposedly long evolution, they never experienced a substantial life history shift. As a notable exception, we can mention some aquatic firefly larvae as they prey on aquatic and their relatives on terrestrial mollusks (Ballantyne & Lambkin 2012). Analogically, the uniform larval life history has been reported for all net-winged beetles, including numerous independently evolved neotenic sublineages (Crowson 1972; Bocak &

Matsuda 2003). Therefore, we can hypothesize, that neotenic development in beetles has probably never been linked with ecology.

Several authors discussed the loss of the pupa as the advantage of neotenic development. The shortcut development could potentially lower the risks connected with deep tissue re-modeling and can avoid the defenseless pupal stage (refs.). The positive impact is limited to the type 5 neoteny only (fully larviform females) and cannot explain the positive selection for neoteny in most neotenic lineages, i.e., types 1–4. However, the resting period in connected to molting is still present in all neotenics including type 5 (Wong 1996).

Table 1. The evolutionary important traits leading to the enormous diversity of beetles as a whole and their presence in neotenic beetles.

Characteristics of beetles as the whole	**Characteristics of neotenic beetle lineages**
the presence of elytra (lower desiccation, protection)	the elytra are absent or vestigial, never closely coadapted with elytral edges, the abdominal never contained to at least partly sealed sub-elytral space, cuticle soft, adults prone to desiccation, absent or rare in arid regions.
ecological innovations (herbivory, high ecological plasticity)	the shifts to neoteny have not been linked to a subsequent change in the trophic strategies; many neotenic lineages are soil or soil surface dwelling, predatory or sucking liquids with high content of microbial life.
diversification rate – continuous diversification, most highly diversified lineages are old (Hunt et al. 2007; McKenna et al. 2019)	diversification rate – most neotenic lineages are species-poor, paedomorphic shifts often lead to the origins of monotypic lineages (Micromalthidae, Dermestidae: Trinodinae: Thylodriini, Lycidae: Metriorrhynchinae: *Cautires apterus*), small clades with a few species (Elateridae: Omalisinae, Scarabaeidae: Pachypodini, Curculionidae: Scolytinae: *Ozopemon*; Telegeusidae: Telegeusinae, Lycidae: Dexorinae, Ateliinae: Ateliini, Lycinae: Leptolycini, etc.). The largest clades have up to 200 species (Elateridae: Drilini, Cebrioninae, some clades in Lampyridae); the most ancient lineages are not the most diversified (Micromalthidae, net-winged beetle and firefly lineages.

high dispersal propensity leads to effective colonization of new areas, cosmopolitics distribution, ability to colonize extreme habitats	very low dispersal propensity of neotenic lineages due to the presence of wingless females (Bray & Bocak 2016), many confined to habitats with a long-term stability
resilience to environmental disturbance – beetles survived three major extinction events; almost all beetle families originated before C/Pg extinction event (Hunt et al. 2007; McKenna et al. 2019)	level of neotenic modification is probably not correlated with time of origin

Returning to the question of the adaptive value of neotenic development, it can be stated that neotenic development does not have a clear positive impact on the long-term survival of the affected lineages. The previous research indicates that **(i)** neotenics never dominate any ecosystem in numbers of individuals (Bocak et al. 2016; Bocek et al. 2018); **(ii)** neotenics are species-poor in comparison with their closest relatives (max. 200 spp. in a single neotenic clade; Bocak et al. 2016; Motyka, Kusy et al. 2021); **(iii)** neotenics have a limited dispersal propensity and small ranges (Bray & Bocak 2016, Masek et al. 2015); **(iv)** the distribution is mostly limited to long-time stable habitats (Bocak et al. 2016; Masek et al. 2014; Bocek et al. 2018; but some lampyrids and drilids, Catalan et al. 2024; Kundrata & Bocak 2019). Nevertheless, some species-poor lineages are ancient (Bocak et al. 2018, Li et al. Cretoph; Kusy et al. in prep.) and their age shows that under some conditions neotenic lineages can evolve for a long time although none of them does attain a similar evolutionary success as their most widespread, common, and diversified relatives. Bocak et al. (2008) proposed for such a situation that the shifts to neoteny are tolerated not selected under specific conditions.

The macroevolutionary role of neoteny in beetles can be also inferred from the investigation of the distribution of neotenics in the phylogenetic trees, i.e., the question if we can recover gradual shifts in the ontogenetic trajectory within a single clade. We can hypothesize that if neoteny is positively selected, the strongly modified terminals should be recovered within the neotenic clades consisting of moderately or slightly modified deep branches. At least for now, there is no evidence of long-term gradual evolution of higher levels of neoteny following the single origin of modified forms. Two levels of neoteny are known in several groups, but these cases are either intraspecific (e.g., *Lampyris noctiluca*, *Thylodrias constrictus*; personal observation, Motyka et al. 2021) or intrageneric (*Lampyris*, *Anorus*; personal observation; Johnston & Gimmel 2020). These neotenics differ only in the presence/absence of vestigial elytra. If the type 2 to 5 is attained all descendants

of a neotenic ancestor are modified at the same level (larviform net-winged beetles - Lyropaeinae, click beetles Drilini reaching the type 4, and Omalisinae type 2).

Further evidence against the profitability of neotenic shifts is the loss of evolvability as the loss of flight is in the neotenic beetles irreversible. Although re-evolution of the flight was proposed for wingless stick insects (Whiting et al. 2003) the consensus on the validity of the hypothesis has not been reached (Stone & French 2003, Trueman et al. 2004, Bang & Bradler 2022; Forni et al. 2022) and there is no indication that the full metamorphosis can re-evolve in beetles. The latter authors suggested that the loss and gain of complex structures or genetic processes are asymmetrical. Therefore, we might analogically speculate that the loss of or incomplete metamorphosis might originate quite more often than has been supposed, but many modified lineages do not survive for long as they are possibly unable to revert to the fine-tuned sequence of steps producing holometabolous adult.

The environment and interactions with other organisms are inherently dynamic and it is a disadvantage if an evolutionary ratchet prevents the lineage from returning to an earlier life strategy enabling a wider spectrum of reactions. Despite the discussed limitations, many origins of neotenic lineages are old (Kusy et al. in prep.) and there was plenty of time for subsequent adaptations of neotenics that can ameliorate the putative disadvantages of neoteny. Here I include an increased investment of females into offspring (invest the resources into fecundity and/or size of eggs), anti-predatory strategies (aposematism, bioluminescence), and miniaturized males (potentially shorter development, low energy investment).

Female large-bodiedness in neotenics

Present knowledge is incomplete and mostly large-bodied females are repeatedly reported (various fireflies, lyropaeine net-winged beetles, drilids, phengodids, and rhagophthalmids). The females comparable in body size to conspecific males are much harder to detect (Bocek et al. 2019, Bocak et al. 2016) and currently, they are only known in Dascillidae (some *Anorus*), Jurasaidae, some net-winged beetles (Lycidae: Leptolycinae), some fireflies, and omalisids (Rosa et al. 2020; Johnson & Gimmel 20xx). For other groups, same-size females are hypothesized (most Lyropaeinae, Iberobaeniidae, Lycidae: Dexorinae, Ateliinae, etc.). The detailed phylogeny of neotenics and their closest relatives is only available for net-winged beetles (Bocak et al. 2008, Masek et al. 2018; Kusy et al. 2019). These studies indicate that neoteny sets repeatedly lead to the evolution of large-bodied females. The duration of larval growth and the body mass at the point of pupation are highly variable in the neotenic lineages (personal observation of *Lampyris* in a breeding colony). Although females are usually larger than males in beetles, the neotenic lineages tend to have very large females, up to ten times larger than conspecific males (Wong 1996, Masek et al. 2014; Jeng et al. 2021) and other

groups retained similar body sizes of both sexes (Elateridae: Omalisinae, Jurasaidae, Dascillidae: *Anorus*).

Female body size is tightly related to the amount of energy that an individual female can invest in the offspring and thus could represent an important fitness component (Roff 2002; Hayashi & Suzuki 2003). However, in insects, correlation has never been found between body and egg sizes (Church et al. 2019). Hayashi and Suzuki (2003) found that if a species did not produce spermatophores, the females had marked degeneration of wings (and usually very large bodies), suggesting that these females had a larger nutrient reserve than the males and did not receive many nutrients from spermatophores. We can sum up, that although the adaptive value is hardly disputable, the large neotenic female body size is not the rule in neotenic beetles and according to available analyses evolved with some delay in the already neotenic groups (Bocak et al. 2008).

Aposematism

The effectiveness of escape reactions depends in insects on the support of the cuticle for muscles (Bolmin et al. 2019). But the regular result of the prematurely terminated metamorphosis is moderate to pronounced soft-bodiedness that excludes effective running or flight. Alternative defensive strategies become critical for beetles that cannot escape if a predator attacks. One of the common strategies is chemical protection and effective signaling of the unprofitability of prey to predators (Motyka et al. 2021; Eisner et al. 1978).

Miniaturization of males

Small-bodied males are known in Omethidae, Jurasaidae, Elateridae: Omalisinae, Drilini), net-winged beetles (Lyropaeinae, Leptolycini, Antennolycini, Alyculini, some neotenic Calopterini). Many of these groups were only recently described (Rosa et al. 2020; Masek et al. 2014; Takahashi et al. 2016, Bocakova 2006) and their females have been identified only for Jurasaidae, Omalisinae (*Thilmanus*; Bocek et al. 2018), and Leptolycini (*Leptolycus*; Ferreira & Ivie 2022), all of them of similar body sizes as their males, although much smaller and feeble than their relatives.

The extremely small males are most common in net-winged beetles. The body size of some males is less than 2 mm, they are soft-bodied, brachelytrous, and eventually wingless, in contrast with their close relatives (Kazantsev 1999, Takahashi et al. 2016; Miller 1996; Ferreira et al. 2023). Surely, these species are hardly able to effectively disperse over long distances, and especially under unfavorable environmental conditions. Such characterization is also supported by their preference for the lowest canopy strata, not higher than 50 cm from the soil surface (personal observation). As their conspecific females are flightless, it has been assumed that natural selection does not penalize such modifications.

1.4 Phylogenomics as a powerful tool to reveal morphological homoplasy

It is widely accepted that genetic and morphological divergence are loosely linked, and that radical phenotypic differences between close relatives can be caused by a relatively simple modification of genetic information that controls metamorphosis (McMahon et al. 2016; Vea et al. 2019). Therefore, the phylogeny can be correctly revealed only with an independent phylogenetic signal. Similar to some parasites, most neotenic taxa were correctly placed in the classification only after molecular evidence was obtained (Kusy et al. 2018; Kusy et al. 2021).

There are over 43,000 spp. of Elateriformia in 4–6 superfamilies and 31 families (Bocak et al. 2014; McKenna et al. 2019; Kusy et al. in prep.). After an era of unstable morphology-based classifications and amendments based on short DNA fragments (Hunt et al. 2007; Lawrence et al. 2011; Bocakova et al. 2007, Kundrata et al. 2014), phylogenomics elucidated some contentious relationships in elateroids (Kusy et al. 2018, 2019), and provided a phylogenetic backbone for the entire order (McKenna et al. 2019). Nevertheless, many questions remain, as only one-third of elateriform families were included in the largest genomic study of beetles (McKenna et al. 2019). Controversial placement of Rhinorhipoidea, either as the deepest polyphagan grade or the deepest split in Elateriformia, affects the reconstruction of the ancestral morphology and biology of the earliest Polyphaga (McKenna et al. 2019; Hunt et al. 2007; Kusy et al. 2018, 2019). Dascilloidea were under-represented and their position was poorly supported. Furthermore, the monophyly of Byrrhoidea remained contentious, even after phylogenomic analyses (McKenna et al. 2019, Cai et al. 2022). The most recent analyses have cast doubt on the relationship between Buprestoidea and Byrrhoidea/Dryopoidea (Zhang et al. 2018), and by extension keep open the origin of a large jewel beetle radiation which is possibly connected with angiosperms. Similarly, little is known about the shifts between terrestrial and aquatic habitats in Dryopoidea/Byrrhoiodea (Kundrata et al. 2017). Relationships within Elateriformia have been discussed based on morphological and molecular data (Bocakova et al. 2007, Sagegami-Oba et al. 2007, Bocak et al. 2014; McKenna et al. 2019; Lawrence et al. 2011; Kundrata et al. 2014). However, due to disparate morphology, the homology of characters is difficult to assess, and whether phenotypic similarity is a result of common ancestry, or parallel evolutionary processes is often unclear (Lawrence et al. 2011; Kusy et al. 2019; Kusy et al. 2021). Although phylogenomic data are limited, recent advances in sequencing and analytical methods have provided important new information for comparative evolutionary studies and rigorous testing of hypotheses is now possible using various types of data (Boudinot et al. 2023; Simon et al. 2018; Kusy et al. in prep.; Steenwyk & King 2024).

The phylogenetic position of modified lineages can be resolved with an independent phylogenetic signal mined from an unaffected ontogenetic stage even if both sexes have retained neotenic traits. The morphological analyses of the larval dataset resolved robustly the position of *Thylodrias* in Trinodinae (Kiselyova & McHugh 2006), despite deep morphological differentiation of their adults, in that case, both males and females. In some cases, preferences for a limited number of characters, and suppression of contradictory evidence led to repeated transfers between unrelated groups. For example, Micromalthidae was temporarily placed even in Polyphaga (Cantharoidea, Lymexyloidea; Beutel & Hörnschemeyer 2002) although the shared wing venation and similar mouthparts were pointing to Archostemata (Crowson 1981). Strepsiptera were placed either in beetles, as a sister to beetles, or as sister to flies or as sister to entire holometabola (Niehuis et al. 2012). Only the phylogenomic analyses definitively resolved the conundrum of their relationships (Niehuis et al. 2012; Misof et al. 2014).

Till molecular data were analyzed, the systematic positions of most neotenic elateroids have been the subject of controversy. They have been given inappropriately high ranks or were incorrectly placed in unrelated groups. Crowson (1972) defined Cantharoidea in which he merged all elateriform soft-bodied and neotenic groups. This concept has been held by all morphology-based phylogenetics (Branham & Wenzel 2001, 2003, Lawrence et al. 2011). Only quite recently, the concept was rejected by DNA data (Bocakova et al. 2007; Hunt et al. 2007; Bocak et al. 2014; Kundrata et al. 2014; McKenna et al. 2015, 2019; Zhang 2018). The rejection of the close relationships of all neotenics leads to the conclusion that many characters that were originally considered to be synapomorphies to be homoplasies.

The mtDNA rRNA-based studies indicated some relationships but usually with low support and limited stability of the tree backbone as has been pointed out by Muona & Teräväinen (2020). As a result, the rank and position of several neotenics still have not been accepted by some students of the group (Muona & Teräväinen 2020, Kovalev et al. xxxx) even if recovered by transcriptomic analyses (Kusy et al. 2019, 2020; McKenna et al. 2019). The families Omalisidae, Drilidae, and Plastoceridae were downranked and included in click beetles (Elateridae), and Telegeusidae were merged with Omethidae and transferred to the relationships with Artematopodidae (Bocakova et al. 2007). The position of *Cydistus* was only solved with molecular data and the genus was placed in the Phengodidae (Kundrata et al. 2019). Molecular data also contributed to the discovery and definition of two new families with presumable neotenic females – Iberobaeniidae (Bocak et al. 2016) and Jurasaidae (Rosa et al. 2020).

Phylogenomics helped solve the relationships of currently recognized families with a common occurrence of female neoteny. Kusy et al. (2020) analyzed the phylogenomic data and

proposed the elaterid-lampyroid clade uniting the monophyletic Elateridae, newly defined Sinopyrophoridae, and all bioluminescent soft-bodied elateroid families, i.e., Phengodidae, Rhagophthalmidae, and Lampyridae. These relationships were recently rejected by Hume et al. (2021) who prefer paraphyletic or polyphyletic click beetles. Incongruence between various datasets is common and substantial changes were proposed also in the phylogenomic analyses of net-winged beetles in deep contrast with earlier morphology-based and mtDNA and rRNA based phylogenies (Bocak et al. 2008, Kusy et al. 2019).

Reconstructing the Tree of Life remains a central goal in biology. Despite the obvious benefits of the large-scale datasets, new challenges appear (Yeates et al. 2016). Investigations based on phylogenomics, which use hundreds to thousands of loci for phylogenetic inquiry, have provided a clearer picture of life's history, but certain branches remain problematic (Steenwyk & King 2024). Therefore, the use of large-scale omics-datasets will not end the presence of conflicting and often incongruent hypotheses but will enable us to evaluate the sources of the inconsistencies and in many cases provide a more complex picture of evolution (Steenwyk & King 2024).

2 Aim of the thesis and included studies

My Ph.D. thesis aims to provide an understanding of the evolution of beetles and Elateriformia through the robust phylogeny, timing, and the reconstruction of the origins of modified (soft-bodied) lineages. I have considered a wide range of information sources, and the studies presented not only resolve some crucial questions about the evolution of beetles and soft-bodied lineages in general, but also demonstrate the power of molecular methods and the need for rigorous evaluation of inferred phylogenies. I wanted to extend the sampling to focus on neotenic lineages to provide critical information about their closest relatives. Therefore, I wanted to estimate the age of heterochronic lineages and their closest relatives, regardless of their morphological divergence. Research in this direction has been minimal, and our current understanding of the diversification rates of the group in question is severely hampered by the lack of such data, which was partially changed by my work.

In total, my dissertation includes eight studies dealing with state-of-the-art phylogenomic methods, divergence time estimation, fossil placement, convergent/parallel changes of ontogeny and their evolutionary and ecological consequences. I also wanted to explore the evolution of life history traits such as mimicry and bioluminescence which might be tightly linked with soft-bodiedness and neotenic modifications.

The first study (Part I) focuses specifically on the Elateridae family and the placement of enigmatic lineages of modified soft-bodied elateroids, namely *Paulusiella* and *Analastesa*. Both are only known from males, with modified morphology and are unable to click. Here I used mitogenomes and nuclear genes to test their placement in Cebrionini and Elaterinae incertae sedis. *Paulusiella* was recovered as a sister lineage to the subfamily Hemiopinae, and *Analastesa* was found to be one of the serially splitting branches in Cardiophorinae. It has been shown that click beetles affected by ontogenetic changes in many cases converge to similar forms. Therefore, their phylogenetic position cannot be reliably inferred by analyzing morphological characters alone and must be validated by molecular data. This highlights the need for cautious interpretation of morphology in other soft-bodied groups, including fossil taxa described mostly from amber deposits.

In the second study (Part II), the new family Sinopyrophoridae is recovered and detailed phylogenomic analyses of the elateroid-lampyroid clade are performed. In this study, I first begin to explore the sources of conflicting topologies for some of the lineages of Elateriformia that cloud our understanding of the evolution of bioluminescence and neoteny. I also examine in detail the

sources of conflicting signals in the data and propose likely solutions. Furthermore, this study shows that the common ancestor of the soft-bodied Lampyridae, Rhagophthalmidae, and Phengodidae was in fact a hard-bodied elaterid like click beetle. And highlight the inability of morphological characters to resolve deep splits in groups with lineages that shifted to soft-bodiness and neoteny from ancestral hard-bodied forms. This study also discusses the general trends and timing of the evolution of bioluminescence in beetles.

The third study (Part III) deals with phylogeny and divergence time estimation of the family Dermestidae. Boundaries between subfamilies were recovered and their origin was dated between the Middle Jurassic and the Upper Cretaceous. Mitogenomics was used to reconstruct their phylogeny and evolution of life history strategies. This family represents another clade with neoteny origins outside of Elateriformia. The position of *Thylodrias contractus* with brachypterous females and flightless males is resolved here. The evolution of Dermestidae represents a new example of ecological shifts already observed in the evolution of beetles: mycetophagy as a feeding habit predisposing a shift to saprophagy. Also discussed are the effect of flightlessness and host specificity as drivers of diversification; a shift from free-living in crevices to commensalism in the nests of eusocial Hymenoptera; parallel shifts from general saprophagy to keratin feeding; and the increased diversification rate of beetles associated with angiosperms. As part of this study, I have also succeeded in establishing a laboratory breeding colony of *Thylodrias*, which will serve as a distant comparison to neotenic lineages of Elateroidea in further studies of neotenic development.

The fourth study (Part IV) focuses on resolving the phylogeny of the family Cantharidae (Elateroidea) using different types of data and analytical approaches. This group of beetles represents another beetle lineage affected by soft-bodiness. Using a holistic approach and different types of data, relationships between subfamilies and the timing of diversification of lineages is resolved. In addition, characters used in previous morphology-based studies are re-evaluated and the classification and placement of Cretaceous Cantharidae are questioned.

The fifth study (part V) is a large-scale study using Lycidae (Metriorrhynchini), soft-bodied, appropriately colored beetles as a model to propose an effective way how to accelerate research and our understanding of unknown hyperdiverse lineages of tropical insects. Samples were collected from ~700 localities on three continents. The species-rich dataset included ~6500 terminals, and ~1850 putative species delimited at a 5% uncorrected pairwise threshold, of which ~1000 may be unknown to science. The study combines a phylogenomic approach using transcriptomes and genomes to build a phylogenetic backbone that will later be used together with traditional Sanger amplicon sequencing. The phylogenetic position of the *Cautires apterus* male with a modified ontogeny is also discussed. This study was only possible because of the many

collaborators who provided the material and most importantly the lifelong efforts of my supervisor. The fact that my supervisor and I were able to collect all the necessary lineages for transcriptome sequencing during a single expedition to New Guinea, which mitigates the problem of large Lycidae genomes, also contributed to the success of this study.

The sixth study (part VI) is a detailed taxonomic work directly related to the previous study **(part V)**. Using a combination of top-down and bottom-up approaches, detailed taxonomic work is conducted for the porrostomine lineage using mtDNA and morphology. The 352 analyzed species were assigned to genera and 8 new genera are described in honor of the local people. Repeated origins of several external morphological characters previously used to delimit genera were identified. Therefore, concordant evidence from the densely sampled mitochondrial phylogenies and male genitalia is preferred. The analyses reveal high phylogenetic diversity and species richness in New Guinea, much lower phylogenetic diversity of the Australian continental fauna, and the limited permeability of Wallacea resulting in a single porrostomine genus in Asia. The study also points to the general acceptance of paraphyletic and polyphyletic taxa in the current classification.

The seventh study (Part VII) deals with the phylogeny of the tribe Lycini (Coleoptera: Lycidae) and shows how sexually dimorphic characters and common aposematic patterns in soft-bodied, aposematically colored, and chemically protected beetles can often mislead morphology-based classification. In this study, a mito-ribosomal dataset was assembled representing ~100 species from across the range. Results show that each specific aposematic pattern occurs in a limited range and that similar body shape and coloration evolved in unrelated sympatric lineages. High intraspecific polymorphism is presumably a result of adaptation of different populations to local mimetic assemblages. Therefore, the delimitation of many phenotypically diverse species should be investigated using molecular data.

The eight study (VIII) is a detailed phylogenomic work showing an independent, delayed origin of bioluminescence in the Elateridae. *Campyloxenus pyrothorax* reveals a separate, recent origin of bioluminescence in elateroids no older than ~ 53 mya. The study focuses on neglected and difficult-to-sample Southern Hemisphere lineages of putative Gondwanan origin. The sampling of many studies is biased toward developed countries and the South Hemisphere endemics are a valuable source of information for true worldwide phylogenies. The possible precipitation of *C. pyrothorax* in assemblages of unique aposematic rings of soft-bodied elateroids is also discussed. This discovery highlights the fourth or fifth origin of bioluminescence in Elateroidea, alongside the lampyroid clade, click beetles Pyrophorini, *Alampoides* and *Coctilelater* in Anaissini (Agrypninae), and *Balgus schnusei* (Thylacosterninae). While phylogenetic findings illuminate the phylogenetic

aspects, the complete story awaits further field observations and in-depth genomic analyses of genetic and biochemical pathways used by bioluminescent elateroids.

3 Conclusion and future directions

My Ph.D. thesis and a set of presented studies relate to the uniting of a single goal: I wanted to resolve the phylogenetic relationships of diverse beetle lineages, especially soft-bodied groups of Elateriformia in particular. To effectively study the genetic and molecular mechanisms underlying diverse phenotypic traits, their role in evolution, and how they shape the origin of diversity. I hope that my research has contributed to the understanding of these phenomena in the soft-bodied lineages with modified ontogenetic development.

The progress in phylogenomic methods substantially changes our understanding of the evolution of ontogenetic modifications. Recent studies definitively rejected the concept of monophyletic soft-bodied elateroids (McKenna et al. 2019, Kusy et al. 2018; Kusy et al. 2020, Kusy et al. in prep.). The concept of the superfamily Cantharoidea or the cantharoid clade and the merging of ontogenetically modified lineages in a single clade have been held until recently if morphology was used as a sole source of phylogenetic information (Lawrence et al. 2011). Other groups of neotenics were also correctly placed in the tree of life (Kusy et al. 2019; Rosa et al. 2019). Importantly, the recent molecular studies also solved many questions on internal relationships in the large families that contain multiple neotenic lineages, i.e., Lampyridae, Lycidae, Dermestidae and Elateridae (Martin et al. 2019; Kusy et al. 2019; Motyka, Kusy et al. 2021; Motyka et al. 2022; Kusy et al. 2018, 2019). The recent studies robustly proved that the origins of heterochronic development are more common than earlier thought and now over 30 origins are hypothesized in Elateriformia (Kusy et al. in prep.). However, substantially increased numbers of newly discovered neotenics also indicate that we are far from complete knowledge and that we should intensify research in regions with ancient ecosystems that potentially preserve the non-flying neotenics (Bocak et al. 2016, Rosa et al. 2019, etc.)

In the upcoming months, I would like to finish my work dealing with the phylogeny of the entire Elateriformia. The study includes extensive sampling of almost all families and subfamilies, with a particular focus on sampling as many neotenic lineages as possible. A total of 200 samples were analyzed, with 125 newly sequenced. The study uses genomic and transcriptomic sequencing, advanced phylogenomic analyses, and divergence time estimations to comprehensively understand the relationships between higher clades, as well as the timing and patterns of neotenic lineage origins in general. Working on this study, I would say that I have reached the limit of what is currently achievable in employing phylogenomic methodology and large omics datasets to answer questions about the evolution of any group of organisms.

Recovery of true phylogeny and disentangling causes of possible incongruence in the phylogeny is essential to effectively investigate the genetic and molecular mechanisms underlying diverse phenotypic traits in biology e.g. evolution of neotenic lineages and their development. It is not surprising that a lot of work remains ahead, especially at the genetic and molecular level. The genetic control of metamorphosis is a hot topic but remains limited to model organisms. However, the nature of the changes that lead to neoteny, and progeny, is not understood. Therefore, it is critical to develop detailed studies of the modification of gene expression and genome structure in neotenic lineages together with gene modification experiments, preferably using several unrelated models, to investigate whether similar heterochronic modifications are controlled by the same or different changes in the genetic machinery. Such studies must be designed with well-understood phylogenetic relationships in mind. **Because nothing in biology makes sense except in the light of evolution.**

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6 List of publications included in the Ph.D. thesis

Part I

Kusy, D., Motyka, M., & Bocak, L. (2023). Ontogenetic modifications produce similar phenotypes in distantly related click beetles (Coleoptera: Elateridae). *Insect Systematics and Diversity*, 7(4), 7.

Part II

Kusy, D., He, J. W., Bybee, S. M., Motyka, M., Bi, W. X., Podsiadlowski, L., Li, X. Y. & Bocak, L. (2021). Phylogenomic relationships of bioluminescent elateroids define the 'lampyroid'clade with clicking Sinopyrophoridae as its earliest member. *Systematic Entomology*, 46(1), 111-123.

Part III (Kusy, D., Motyka, M., Háva, J. contributed equaly)

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Part IV (Kusy, D., Motyka, M., contributed equaly)

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PART I

Studies on neoteny in Coleoptera: phylogenomics, classification, and evolutionary interactions

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Ontogenetic modifications produce similar phenotypes in distantly related click beetles (Coleoptera: Elateridae).

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Research

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Morphology and Ontology

Ontogenetic modifications produce similar phenotypes in distantly related click beetles (Coleoptera: Elateridae)

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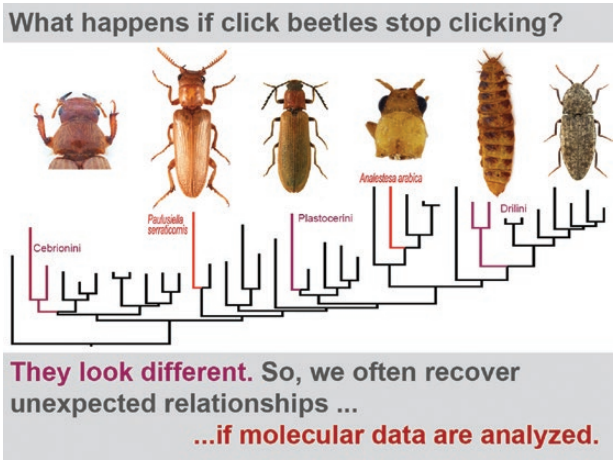
We analyze the relationships of the click beetles (Elateridae) *Paulusiella* Löbl, 2007, and *Analestesa* Leach, 1824 (=*Cebriognathus* Chobaut, 1899). Both are incapable of jumping, with soft-bodied habitus caused by the incomplete sclerotization of the cuticle during the metamorphosis and unknown females. Their phylogenetic positions have been an uncertain issue. We use mitochondrial genomes and nuclear genes to test their current placement in Cebrionini (=Cebriognathini) and Elaterinae *incertae sedis*, respectively. We recover *Paulusiella* as a sister to *Hemiops* Laporte, 1838 (Hemiopinae) and *Analestesa* as one of the serially splitting branches in Cardiophorinae, both with robust support. Paulusiellini **trib. nov.** is proposed for *Paulusiella* in Hemiopinae due to high morphological disparity. *Analestesa* is transferred to Cardiophorinae, and Cebriognathini Paulus, 1981, an earlier synonym of Elaterinae: Cebrionini, is a synonym of Cardiophorinae Candèze, 1859. The click beetles affected by ontogenetic modifications converge to similar forms. As a result, their phylogenetic position cannot be reliably inferred by morphological analyses and needs to be validated by molecular data. *Paulusiella* and *Analestesa* represent two additional cases of the shift to incomplete sclerotization in elaterids raising the total number to 6. The present transfers of extant taxa between subfamilies call for a cautious interpretation of morphology in other soft-bodied groups, including the taxa described from amber deposits.

Key words: classification, molecular phylogeny, morphology, evolution, ontogeny

Graphical Abstract



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Introduction

Recent research produces growing evidence of the common parallel evolution of similar morphological structures (San Jose et al. 2018, Xu et al. 2021). The closely related taxa can also substantially differ in morphology if some pass through modified metamorphosis (Crowson 1972, Gould 1977). Molecular analyses handle data unrelated to morphological modifications and can identify discrepancies between phenotypic similarity and relationships, i.e., morphological characters potentially resulting from parallel modifications of ancestral characters. Only phylogeny-based classification can serve other fields, such as modern evolutionary biology and functional genomics (Hennig 1950, Carroll 2008). The availability of large DNA datasets and new analytical methods have been first applied at high classification levels and solved such conundrums as the position of insects in Pancrustacea (Oakley et al. 2013), the evolution of social behavior (Inward et al. 2007, Warner et al. 2019, Wipfler et al. 2019), and the systematic position of Strepsiptera and Siphonaptera (Wiegman et al. 2009, Misof et al. 2014). Now, the research projects focus on lower-rank taxa that, although not so generally known public and scientific community, are crucial for our understanding of the evolution of enormous diversity of insects (e.g., Martin et al. 2019, Keegan et al. 2021). Here, we study click beetles (Elateridae) that are known for their jumping behavior (Ribak and Weihs 2011). The clicking elateroids are morphologically similar, yet it is a question if non-clicking elateroids are really as related as their morphology suggests (Lawrence et al. 2011). Therefore, we analyze the relationships of *Paulusiella* Löbl, 2007 and *Analestesa* Leach, 1824 (=*Cebriognathus* Chobaut, 1899) that have been recently placed in the Cebrionini—a group that was considered a unique high-rank beetle lineage already by Latreille (1804), downranked to the family (Lacordaire 1857), the subfamily (Lameere 1900, Lawrence et al. 2021) and to the tribe rank in modern classifications (Kundrata et al. 2014, Douglas et al. 2021).

For almost 200 years, all clicking beetles were considered close relatives as their common names suggest—click-beetles (Elateridae), false click beetles (Eucnemidae), rare click beetles (Eucnemidae), and false metallic wood-boring beetles (Throscidae) (Latreille 1804, 1825, Lameere 1900, Crowson 1955, 1981, Lawrence and Newton 1982, Muona 1995). The non-clicking elaterids and elateroids were initially merged with some groups of Cleroidea (now Cucujiformia) in Malacodermata. Later, the more narrowly defined Cantharoidea contained only groups currently placed in Elateriformia (Crowson 1955, 1981, Lawrence and Newton 1982). Morphology-based classifications regularly separated the clicking beetles from their non-clicking relatives, placing them in different groups of families (Kolbe 1908) and divisions (Jeannel and Paulian 1944). Recently, the morphological analyses suggested that non-clicking elateroids (all except *Cebrio* and relatives) hypothetically share the non-clicking and soft-bodied most recent common ancestor (Lawrence et al. 2011).

Molecular phylogenetic studies profoundly affected the family-level classification of Elateroidea. Omalisids, drilids (false firefly beetles), and plastocerids were separate families considered the relatives of fireflies, soldier- and net-winged beetles (Crowson 1972, Branham and Wenzel 2003, Lawrence et al. 2011). Only recently, they were included in Elateridae (Bocak et al. 2018, Kusy et al. 2019). Cebrionids (Elaterinae) traditionally contained other non-clicking, at least partly soft-bodied elaterids. Although cebrionids were earlier treated as a family (Crowson 1955), they are so slightly modified (Arnett 1949, Rattu 2016) that they were firmly included in Elateridae since applying Hennig's phylogenetic systematics (Crowson 1981, Bouchard et al. 2011). Still, their monophyly and generic classification have never benefited from a molecular study, and the relationships of constituent genera have been corroborated exclusively by the morphology. The recent authors dealing with *Analestesa* and *Paulusiella* placed them in Cebrionini or Cebrioninae (Paulus 1981, Löbl 2007, Sánchez-Ruiz and Löbl 2007, Costa et al. 2010, Mortazavi et al. 2011, Platia and Ghahari 2016, Lawrence et al. 2000—see https://www.delta-intkey.com/elateria/www/elatce.htm, accessed on 16 May 2023). Only Ivie and Barclay (2011) placed *Paulusiella* as a taxon with an uncertain position in Elateridae. Concerning the known effect of modified metamorphosis on the phenotype of elateroids (Crowson 1972, Gould 1977, Kusy et al. 2018, 2019, 2021b), reexamining the phylogenetic position of further non-clicking elaterids with molecular data is needed not only to update the classification but to test the monophyly of Cebrionini and to understand the evolution of ontogenetic modifications better.

Metamorphosis is a crucial innovation supposedly connected with the enormous diversity of insects (Nicholson et al. 2014). The transition between larva, pupa, and adult is a complex, fine-tuned cascade of steps (Jindra et al. 2015, Jindra 2019) that can be prematurely terminated. If the metamorphosis stops prematurely, some larval and pupal characters can persist in the adult semaphoronts (Crowson 1972, Gould 1977, Bocak and Brlik 2008, McMahon and Hayward 2016, Bocek et al. 2018). Rarely, the external imaginal characters are not expressed, and larva-like females are sexually mature. It means that the larvae and adult females are phenotypically similar, but mature females have developed ovaria and an open sexual duct (Wong 1996, Cicero 2008, Masek et al. 2014, 2015, Makarov and Kazantsev 2022).

Some elaterids, like drilids, omalisids, cebrionids, and so on, have phenotypically divergent adults that are weakly sclerotized and do not click, unlike their relatives. Further, we can encounter shortened or vestigial elytra, often connected with a loss of wings. The abdomen can be larviform (drilids), or at least the ventrites are loose (e.g., Omalisinae, Dendrometrinae: Plastocerini; Crowson 1972, Bocak et al. 2018, Kusy et al. 2018). The incomplete metamorphosis results in the loss of phylogenetically younger traits in agreement with Baer's recapitulation law (Løvtrup 1978). The modified soft-bodied forms have been confusing to systematists till an independent source of phylogenetic information became available with the sequencing of DNA. Due to absent apomorphic traits, some affected lineages have been assigned inappropriate high ranks (see above). Alternatively, the unrelated groups were merged into a single taxon (Lawrence et al. 2011, Kazantsev 2013). Using only morphological characters for phylogenetic reconstructions, the morphologists defined the earlier superfamily Cantharoidea and the cantharoid clade in Elateroidea. These hypothesized relationships are in deep conflict with findings recovered by all molecular studies. We can see it if we compare the topologies proposed by Crowson (1972), Lawrence (1988), Muona (1995), and Lawrence et al. (2011) with multigene molecular studies by McKenna et al. (2015, 2019) and all earlier published molecular phylogenies by other authors.

Several obstacles have delayed studies on modified elaterids. Most neotenic groups are poorly represented in world museum collections compared to their close relatives. Obtaining individuals properly fixed for molecular analyses has been difficult, as information on their occurrence is limited. Additionally, their females often remain unknown, and we cannot study the sexual dimorphism in such cases. We only estimate from the morphology of males that females are affected by paedomorphic syndrome. The possibility that females are wingless can also be indicated by the absence of females in collections in contrast with numerous males. Known wingless females remain in the soil or disperse across the surface (surface crawling observed in Drilini only). A female

sometimes only exposes the abdomen during copulation (observed in *Cebrio*, https://www.youtube.com/watch?v=MlEz6jHCgLo&ab_channel=RobertoLascaro, accessed on 3 November 2022; Bocak et al. 2013, Bocek et al. 2018). Although observations are limited, and we do not have direct evidence in all cases, it is possible that other neotenic females are also endogenous and therefore have not been collected. Some larvae and females (omalisids, Iberobaeniidae) were collected from soil samples, but nobody observed them crawling on the soil surface (Bocak et al. 2016, Bocek et al. 2018).

Our mitogenomic study reinvestigates the morphology-based relationships of the soft-bodied *Paulusiella* and *Analestesa* currently placed in Elateridae *incertae sedis* and Cebrionini (Elaterinae), respectively. Our null hypothesis predicts that if some organisms are morphologically similar, they are phylogenetically related (i.e., placed in the same higher taxon, e.g., Cebrionini). If the null hypothesis is rejected by an independent source of evidence, e.g., molecular data, we need to consider an alternative explanation that similar phenotypes might evolve in distantly related species due to similar ontogenetic modifications (Gould 1977, Kusy et al. 2019). Then, it is valuable to revisit morphological evidence for the earlier proposed concept of cebrionids. We will look for the morphological traits that could potentially support the molecular relationships and evaluate if the characters supporting the null hypothesis also occur in other morphologically divergent lineages putatively affected by similar ontogenetic modifications.

The changes in the Linnean classification of soft-bodied elateroids have been reluctantly accepted by some researchers studying phylogeny with morphological methods, even if the newly proposed relationships were supported by the thorough analyses of thousands of orthologs (e.g., the refusal of the ranks of Drilini, Cebrionini, Omalisinae, and Sinopyrophoridae without any new data and analyses by Kovalev et al. 2019, Ruchin and Egorov 2019, and Lawrence et al. 2021). Unfortunately, the phylogeny-based classification is often counterintuitive. The taxonomic history of elateroid groups started with the clear clicking versus non-clicking dichotomy (Latreille 1804, Crowson 1955, 1981, Lawrence and Newton 1982). This user-friendly classification was abandoned in the late 20th century. Recently, it reached the stage when morphologically distinct Cantharoidea and the soft-bodied "families" lost their ranks and were included in Elateroidea and Elateridae, respectively (Bocakova et al. 2007, Kundrata and Bocak 2011, McKenna et al. 2015, 2019, Bocak et al. 2018, Kusy et al. 2019, 2021b, Douglas et al. 2021). Still, phylogeny-based classification is preferred nowadays. The study of relationships of additional extant soft-bodied elaterids can help better understand morphological evolution. Hopefully, it will also attract attention to further taxa not yet available for molecular analyses. The ambiguous relationships of extant soft-bodied forms should also be considered in works on fossils for which only morphological and often incomplete data are available.

Methods

Compilation of the Dataset

The mitogenomic dataset was assembled from newly sequenced mitogenomes of *Cebrio* sp., *Cebriorhipis* sp., *Analestesa arabica* (Paulus, 1981), *Paulusiella serraticornis* (Paulus, 1972), *Quasimus* sp., and *Hemiops* sp. The voucher numbers and complete locality data are reported in Table 1. Further mitogenomic data were taken from the dataset published by Kusy et al. (2021a).

Laboratory Procedures, Data Handling, and Morphological Investigation

Total DNA was isolated from alcohol-preserved or dry-mounted samples. Voucher specimens were used for morphological investigation. They were dissected after short relaxation in 50% ethanol. The structures were treated in hot 10% KOH for a short time. The photographs were taken by Canon M6 Mark II camera attached to Olympus SZX16 binocular microscope. Stacks were assembled using Helicon Focus software and processed in Photoshop 6.0. Vouchers are deposited in the collections of the collectors and of Biodiversity & Molecular Evolution at CATRIN, Olomouc.

DNA was extracted using Qiagen MagAttract HMW DNA extraction kit, and eluted in AE buffer. Short insert size library constructions (~320 bp) and subsequent paired-end (2 × 150 bp) sequencing of the samples were done by Novogene, Inc., Beijing, using Illumina NovaSeq 6000. Raw Illumina reads were quality checked with FastQC and filtered with fastp 0.21.0 (Chen et al. 2018) using -q 28 -u 50 -n 15 -l 50 settings. Filtered reads were used for final mitogenome assemblies. The mitogenomes were built de novo using the NOVOPlasty v.2.7.2 pipeline (Dierckxsens et al. 2017). NOVOPlasty was run with the default settings except the kmer value when we used a multi-kmer strategy with the following kmer sizes of 25, 39, 45, and 51. We used as seed the single fragment of *Oxynopterus* sp. cox1 gene available in GenBank (HQ333982). In the case of *Quasimus* sp., the mitochondrial fragments were mined from unpublished transcriptomic data that were mapped on the mitochondrial genome of *Cardiophorus signatus*, and manually curated in Geneious v.7.1.9. The newly assembled mitochondrial genomes were annotated using the MITOS2 webserver (Bernt et al. 2013) with the invertebrate genetic code and RefSeq 63 metazoa reference. The annotation, circularization, and start + stop codons corrections of protein-coding genes (PCSGs) were performed manually in Geneious v.7.1.9. The *SSU* and *LSU* rRNA nuclear genes were extracted by mapping reads to the closest taxon (*Hemiops* for *Paulusiella*; *Cebrio* for *Cebriorhipis*, and *Cardiotarsus* for *Cebriognathus*). The sequences of newly produced mitochondrial genomes were deposited into the Mendeley database DOI:10.17632/73dmw4czm3.1.

Phylogenetic Analyses

The 6 mitochondrial genomes of click beetles were merged for the purpose of phylogenetic analyses with 30 earlier published mitochondrial genomes (36 ingroup taxa and 1 outgroup, Kusy et al. 2021a). The dataset contained terminals belonging to 10 subfamilies of Elateridae. The nucleotide sequences of protein-coding genes (PCG) were aligned using TransAlign (Bininda-Emonds 2005). In addition, nucleotide sequences of rRNA genes and translated amino acid sequences of PCGs were aligned with Mafft v.7.407 using the L-INS-i algorithm (Katoh and Standley, 2013). The aligned data were concatenated with FASconCAT-G v.1.04 (Kück and Longo 2014). We compiled the following datasets: (A) 13 PCG mtDNA and 2 rRNA mtDNA genes partitioned by gene or unpartitioned; (B) 13 mitochondrial PCGs and by gene or unpartitioned; (C) 13 mitochondrial PCGs masked by degen software (Steenwyk et al. 2020) partitioned by a gene or unpartitioned; (D) amino acid level analysis of 13 mitochondrial PCGs. The degree of missing data and overall pairwise completeness scores across all datasets was inspected using AliStat v.1.7. (Thomas et al. 2020; Supplementary Fig. S1). Additionally, to explore the relationships of focal taxa with a densely sampled dataset, we assembled the data set (*rrnL*, *cox1* mtDNA, and *SSU*, *LSU* nuclear rRNA) consisting of focal taxa and Elateridae lineages from Kundrata et al. (2014). Furthermore, we assembled the dataset consisting only of nuclear genes. All genes were aligned as stated earlier.

Phylogenetic inferences were performed under maximum likelihood (ML) optimization using IQ-TREE v.2.1.2 (Minh et al. 2020),

Table 1. The list of newly sequenced samples. For the list of publicly available samples, see Supplementary Table S1

Taxon	Voucher	Geographic origin
Paulusiella serraticornis	G21004	Iran, Kerman prov., Gebal Barez mts., 1345 m, 26 km N of Jiroft, wadi, 28°54′14″N 57°40′32″E, 27. May 2018, Vit Kuban leg., coll. V. Kuban.
Analestesa arabica	G21003	Kingdom of Saudi Arabia, Hieth, 2. May 1975, 40 km S of Riyadh, 24°18′9″, 46°42′27″, W. Büttiker leg., coll. L. Bocak.
Cebrio sp.	G19010	Italy, Sardinia, 2 km W of Irgutosu, 62 m, 39°31′31″N, 8°28′20″E, D. Ahrens & S. Fabrizzi leg., coll. L. Bocak.
Cebriorhipis sp.	G22001	Indonesia, Bali, Tamblingan Lake, 1000–1300 m, 8°15′33″S, 115°6′14″E. 2.–17. Feb 2004, S. Jakl leg., coll. D. Kusy.
Quasimus sp.	R19010	Japan, Kunimidake, 35°27′3 ″N, 136°21′37″E, 14. May 2015, T. Sota leg., coll. L. Bocak.
Hemiops sp.	G19002	Malaysia, Perak, km 24 Rd Tapah-Ringlet, 230 m, 4°18′39″N, 101°19′52″E, 19. Apr. 2013, L. Dembicky leg., coll. L. Dembicky.

and Bayesian inference (BI) using PhyloBayes MPI v.1.8 (Lartillot et al. 2013). Before ML tree searches, best-fitting model selection for each partition was performed with ModelFinder (Chernomor et al. 2016, Kalyaanamoorthy et al. 2017) using the -MFP. All datasets were tested against a complete list of models. We used the edge-linked partitioned model for tree reconstructions (-spp option), allowing each partition to have its own rate.

Ultrafast bootstrap values (Hoang et al. 2018) were calculated for each tree using -bb 5000 option. In the PhyloBayes analysis, unpartitioned datasets A and D were analyzed under the site-heterogeneous mixture CAT + GTR + Γ4 model for all searches. Two independent Markov chain Monte Carlo (MCMC) were run for each dataset. We checked for the convergence in the tree space with bpcomp program and generated output of the largest (maxdiff) and mean (meandiff) discrepancy observed across all bipartitions and generated a majority-rule consensus tree using a burn-in of 30% and sub-sampling every 10th tree. Additionally, we used the program tracecomp to check for convergence of the continuous parameters of the model.

We employed several tests to investigate alternative phylogenetic relationships, including the approximately unbiased AU test (Shimodaira 2002), the p-SH (*P*-value of the Shimodaira–Hasegawa test) (Shimodaira and Hasegawa 1999), the KH test (1-sided Kishino–Hasegawa test) (Kishino and Hasegawa 1989), the p-WKH (*P*-value of weighted KH test), the p-WSH (*P*-value of weighted SH test), c-ELW (Expected Likelihood Weight) (Strimmer and Rambaut 2002), and bp-RELL (bootstrap proportion using RELL method) (Kishino et al. 1990). To test if the data support former placement of *A. arabica*, and *P. serraticornis* within Elaterinae (Cebrionini), we analyzed the results of the maximum likelihood (ML) and Bayesian inference (BI) tree searches and 3 alternative topologies suggested by earlier classifications: (A) the clade containing all focal taxa—null hypothesis based on earlier classification and the acceptance of the rule that similar forms are related. (B) *Analestesa arabica* placed to a sister relationship to *Cebrio* and *Cebriorhipis*, and (C) *P. serraticornis* as sister to *Cebrio* and *Cebriorhipis*. IQ-TREE v.2.1.2 (Minh et al. 2020) was used to perform all tests, with per-site log-likelihoods calculated using the --test-weight --test-au --sitelh parameters and 10,000 replications.

Results

Molecular Phylogeny

The Bayesian and maximum likelihood mitogenomic analyses indicate high support for the polyphyly of Cebrionini in the traditional sense (Fig. 1B,C; Supplementary Figs. S2–S11). Only *Cebrio* sp. and *Cebriorhipis* sp. are members of Elaterinae (BS 99%, PP 0.97). *Paulusiella serraticornis* was regularly a sister to *Hemiops* sp. (BS 100%, PP 1.00). Still, the clade was variably a sister to the remaining elaterid subfamilies or a sister to the non-Elaterinae subfamilies (Fig. 1B,C, Supplementary Figs. S2–S11). *Analestena arabica* was firmly placed within Cardiophorinae (BS 100%, PP 1.00) as the second split following *Globothorax femoralis*. The position of Negastriinae as a putative sister of Cardiophorinae was recovered only by some analyses (Supplementary Figs. S5, S6, S8). Alternatively, the genus was found as a sister to the Agrypninae (Fig. 1B), or the Cardiophorinae + Agrypninae clade (Fig. 1C). The analysis of the mt/nDNA gene fragments dataset confirmed the same positions of focal taxa as in mitogenomic analyses (Fig. 1A; Supplementary Figs. S12–S14). No tree search analysis suggested alternative positions for the focal taxa. Additionally, all topological tests strongly rejected placement of *Paulusiella* or *Analestesa* in Cebrionini (Elaterinae), or topology with Cebrionini containing both aforementioned taxa (null hypothesis, close relationships to *Cebriorhipis* and *Cebrio*; Table 2). We separately considered both genera in Cebrionini, and alternatively, either of them as a sister to *Cebrio* and *Cebriorhipis* (Table 2). We did not test the relationships between subfamilies and the monophyly of Elateridae as the dataset does not provide enough support for the deepest relationships. The 8 click-beetle superfamilies were included in the analysis to set the monophyly of Cebrionini in the earlier sense.

Morphology

Paulusiella serraticornis (Paulus, 1972)

Escalerina serraticornis Paulus, 1972: 38 (in Karumiidae; now Dascillidae: Karumiinae).

(Fig. 2A–P)

Redescription

Male. Body 5–7 mm long, slender, slightly flattened, light brown colored, vestiture of upper surfaces with erect, long bristles (Fig. 2A–C).

Head slightly declined, transverse, including eyes greater than prothoracic width, gradually narrowed posteriorly. Antennal sockets small. Frons and vertex flat; lateral carinae behind antennal pits, without ocelli (Fig. 2F,G). Eyes protuberant, rounded, finely facetted. Antennal insertions widely separated, covered by protuberant edge from above (Fig. 2B). Anterior edge of clypeus straight (Fig. 2C), gular sutures narrowly separated, cervical sclerites well-sclerotized (Fig. 2F). Antennae reaching elytral humeri, antennomere 1 robust, long, antennomere 2 small, longer than wide, antennomeres 4–10 flabellate, terminal antennomere flat (Fig. 2D). Labrum concealed

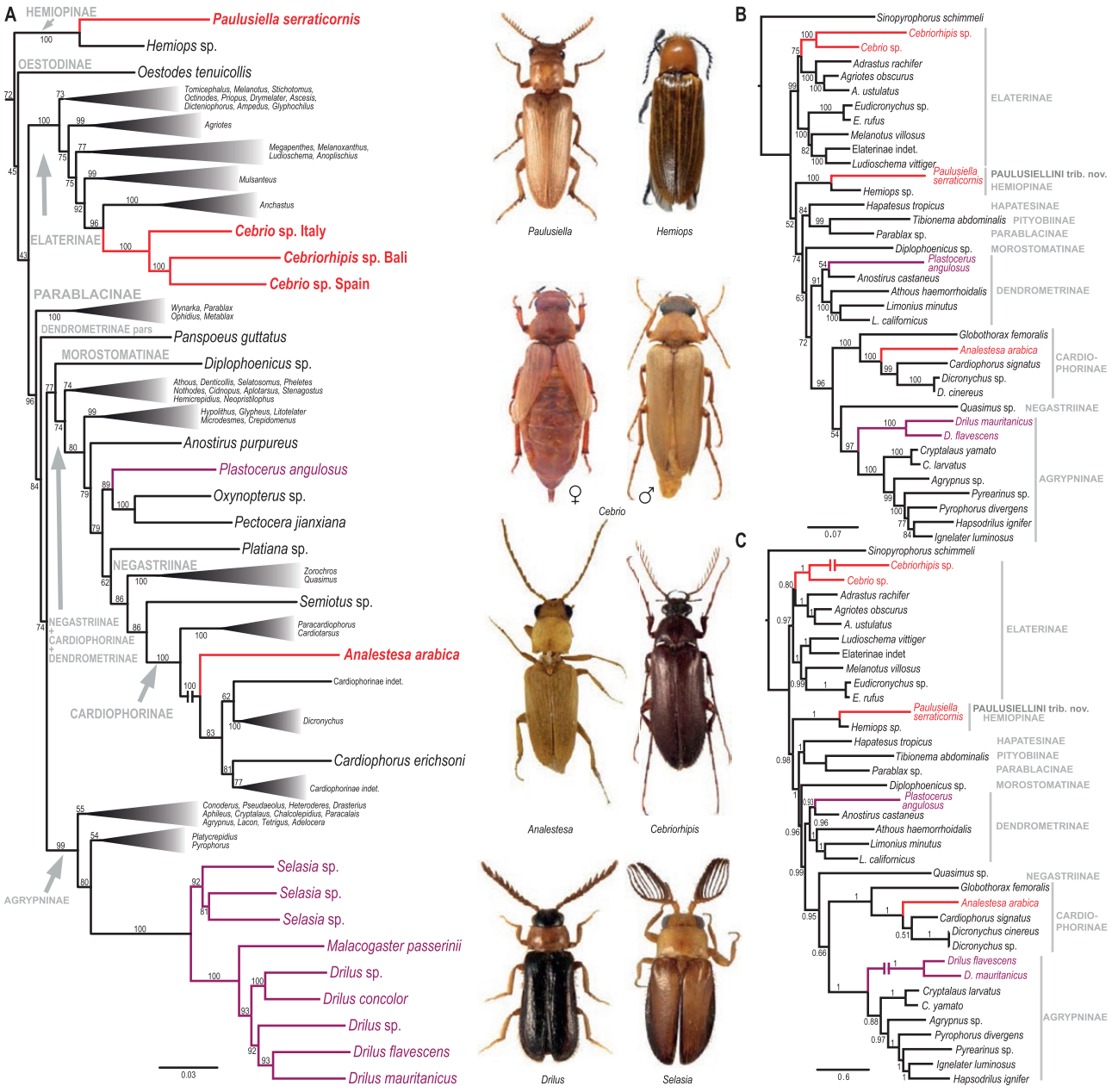

Fig. 1. The mitogenomic relationships of soft-bodied and/or non-clicking genera *Paulusiella, Cebrio, Scaptolenus, Analestesa, Plastocerus, Drilus,* and their clicking relatives. The numbers at branches designate ultrafast bootstrap values and posterior probabilities. (A) The maximum likelihood analysis of the mt/nDNA dataset with single partition, non-focal clades are collapsed, Lissominae and Pityobiinae omitted. (B) The maximum likelihood analysis of thirteen protein-coding mitochondrial genes at the nucleotide level with coding masked by the degen software and partitioned by genes. (C) The Bayesian analysis using PhyloBayes at the nucleotide level. The trees with full-length branches and the results of additional analyses are shown in Supplementary Figs. S2–S14. *Paulisiella, Cebrio,* and *Cebriorrhipis* (red or grey colored)—the taxa earlier placed in Cebrionini; *Plastocerus, Selasia, Drilus,* and *Malacogaster* (magenta or grey colored)—non-clicking elaterids earlier placed in families Drilidae and Plastoceridae.

beneath clypeus; mandibles robust, curved, with dorso- and ventro-lateral edges (Fig. 2E). Mandibular apex unidentate, incisor edge simple, without mola (Fig. 2F,G), maxilla with setose mala; maxillary palpi slender, 4-segmented, apical palpomere cylindrical; labium tiny, labial palpi 3-segmented, cylindrical (Fig. 2F).

Prothorax transverse (Fig. 2B), pronotum without carinae, maximum width 1.23 times its length, widest in anterior third, only slightly narrower at base, sides sinuate (Fig. 2B,J). Prothorax basally narrower than elytral bases; lateral pronotal carina visible posteriorly. Posterior angles of pronotum strongly acute (Fig. 2K). Posterior edge of pronotum sinuate. Prosternum about as long as prosternal process; process long, slender, its edge curved from lateral view (Fig. 2C,K). Apex of prosternal process does not reach mesosternal pit (Fig. 2L); promesothoracic clicking mechanism non-functional; procoxal cavities open, narrowly separated (Fig. 2C). Elytra cover whole abdomen, tapering to apex, widest at humeri, without apparent costae or rows of punctures (Fig. 2I). Elytra free apically (Fig. 2A), Elytral pleuron very short. Scutellum well developed; abruptly elevated; anteriorly straight, posteriorly broadly rounded and surpassing elytral surface, anterior edge of mesoventrite rounded (Fig. 2H), meso- and meta coxal cavities narrowly separated. Metaventrite long, discrimen complete, posterior transverse suture

Table 2. Results of the alternative tree topologies likelihood testing

Topology	logL	deltaL	bp-RELL	p-KH	p-SH	p-WKH	p-WSH	c-ELW	p-AU
ML	**–79,060.25**	**0.00**	**0.823**	**0.826**	**1.000**	**0.826**	**0.994**	**0.819**	**0.820**
PB	**–79,069.89**	**9.64**	**0.177**	**0.174**	**0.570**	**0.174**	**0.370**	**0.181**	**0.181**
(Aa(Ce,Cr))	–79,486.96	426.70	0.000	0.000	0.000	0.000	0.000	0.000	0.000
(Ps(Ce,Cr))	–79,109.91	49.66	0.000	0.000	**0.098**	0.000	0.000	0.000	0.000
(Ps(Aa(Ce,Cr)))	–79,532.71	472.46	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Aa, *Analestesa arabica*; Ce, *Cebrio* sp.; Cr, *Cebriorhipis* sp.; Ps, *Paulusiella serraticornis;* deltaL, logL difference from the maximal logL in the set; bp-RELL, bootstrap proportion using RELL method; p-KH, *P*-value of 1-sided Kishino–Hasegawa; p-SH, *P*-value of Shimodaira–Hasegawa test; p-WKH, *P*-value of weighted KH test; p-WSH, *P*-value of weighted SH test; c-ELW, Expected Likelihood Weight; p-AU, *P*-value of approximately unbiased (AU) test. Bold text represents the accepted test.

apparent, posterior margin deeply emarginate between coxae. Hind wing present.

Legs slightly compressed, with long, gradually widened trochanters, femoral attachments oblique; femora twice wider than tibiae (Fig. 2C), tibiae with simple outer edge and 2 long apical spines; tarsomeres slender, 5 segmented, without ventral pads, claws paired, long, slender, and simple.

Abdomen with 6 visible abdominal ventrites, ventrite 1 divided by metacoxae, ventrite 2 without process; penultimate tergite deeply emarginate, ultimate tergite very small, narrow (Fig. 2Q). Male genitalia trilobate; symmetrical. Phallus stout, short, basally merged with parameres (Fig. 2N–P).

Female unknown.

Biology, distribution, and species diversity

All species have been reported from semidesert ecosystems of southwestern Asia. Unfortunately, the collectors did not describe the ecosystems in more detail. The highest diversity is known in Iran. Males are commonly collected at the light, i.e., they are actively flying at night. It means that the males are crepuscular or nocturnal. The larvae and females are unknown, and are presumably endogenous. The dispersal stage of the mite *Trochometridium kermanicum* Mortazavi & Hajiqanbar, 2011 was found on *Paulusiella* sp. in Iran (Mortazavi et al. 2011).

The genus *Paulusiella* contains 6 species: *P. serraticornis* (Paulus, 1972) (Iran); *P. richteri* (Mandl, 1974) (Iran); *P. fossulatipennis* (Mandl, 1974) (Pakistan); *P. pallida* (Mandl, 1974) (Iran); *P. holzschuhi* (Mandl, 1979) (Iran); *P. sweihana* (Geisthardt, 2009) (United Arab Emirates).

Analestesa arabica (Paulus, 1981)

Cebriognathus arabicus Paulus, 1981: 261.

(Fig. 3A–P)

Redescription

Male. Body 6 mm long, slender, slightly flattened, light brown colored, vestiture dense and short (Fig. 3A).

Head prognathous, slightly transverse, including width across eyes equals prothoracic width; gradually narrowed posteriorly; dorsally flat, without ocelli (Fig. 3B–D). Eyes slightly protuberant, rounded, finely facetted. Antennal insertions widely separated, covered by protuberant edge from above (Fig. 3B,C,E); clypeus concave (Fig. 3C), regular sutures narrowly separated, and cervical sclerites sclerotized (Fig. 3D). Antennae reaching midlength of elytra, the diameter of antennomere 1 1.8 times basal diameter of antennomere 2, antennomere 1 longest of all antennomeres, antennomere 2 shorter than antennomere 2, apically narrower than antennomere 1, antennomeres 4–11 filiform, terminal antennomere shorter than preceding one (Fig. 3I). Labrum concealed beneath clypeus; mandibles robust, abruptly curved (Fig. 3E). Mandibular apex and incisor edge simple, maxilla with setose mala; maxillary palpi slender, 4-segmented, apical palpomere parallel-sided; labium tiny, with 3 palpomeres (Fig. 3D).

Prothorax transverse, pronotum without carinae, maximum width 1.18 times length, widest in middle, sides convex (Fig. 3B). Prothorax basally narrower than elytral bases; lateral pronotal edge absent (Fig. 3F–H). Posterior angles of pronotum short, acute. Posterior edge of pronotum sinuate. Prosternum anterior portion slightly longer than prosternal process, process slender, its edge curved from lateral view (Fig. 3F,G); procoxal cavities open, narrowly separated (Fig. 3D); promesothoracic clicking mechanism non-functional. Elytra cover abdomen, tapering to apex, widest at humeri, with inconspicuous costae (Fig. 3A). Elytra close to each other in basal part, each elytron free apically, separately rounded. Scutellar shield well-developed; widest anteriorly (Fig. 3A) Hind wings well developed (see Paulus 1981, Fig. 2E,F for wing venation and folding scheme).

Legs slightly compressed, with long trochanters (Fig. 3D), tibiae with simple outer edge, bearing setae, and 2 apical, long spines; tarsomeres slender, 5 segmented, without ventral pads, claws paired, long, slender, and simple.

Abdomen with 6 free abdominal sternites, ventrite 1 entire, with inter-coxal process; penultimate tergite simple, ultimate tergite large (Fig. 3P). Phallus slender, short (Fig. 3K,M).

Female unknown.

Cebrio igelmimen Rattu et François, 2021

(Fig. 4A)

Remarks

Cebrio females are rare in collections, but two females of this species were described in detail by Rattu (2016) and Rattu and François (2021). Figure 4A shows the female *C. igelmimen* (photo provided by R. Rattu).

The *Cebrio* females differ from conspecific males in several traits: substantially larger body, smaller eyes, shorter antennae. Legs, especially tarsi, are substantially shorter than in males (compare Figs. 4A and 4L), often shortened elytra, physogastrous abdomen, and weak cuticle sclerotization (see Fig. 1).

Cebriorhipis sp.

(Fig. 4B–M)

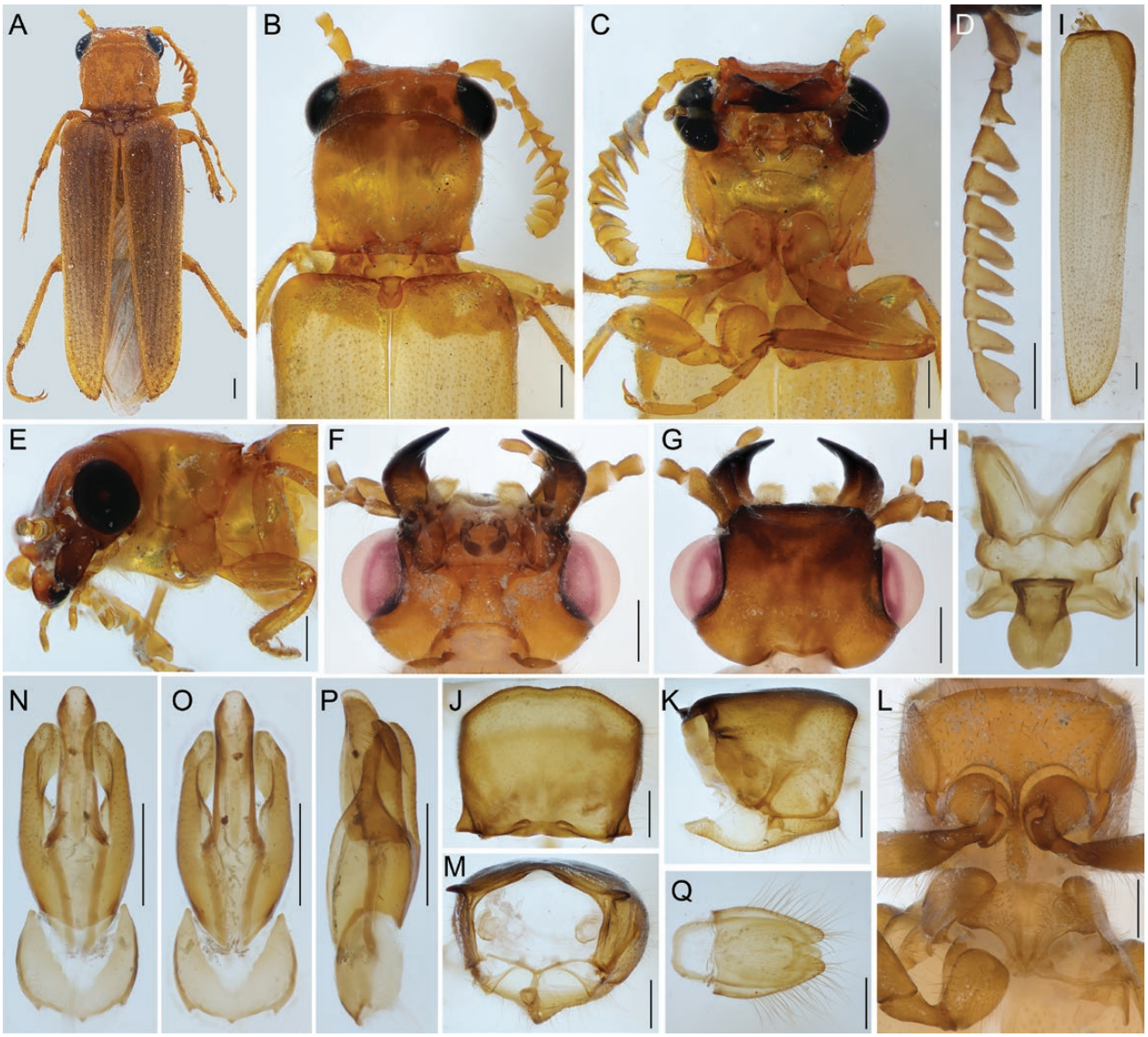

Fig. 2. *Paulusiella* sp. from Iran. A—general appearance, dorsal view; B, C—frontal part of the body, dorsally and ventrally; D—antenna; E—head and pronotum in lateral view; F, G—head, ventral and dorsal view; H—mesonotum; I—elytron; J–L—pronotum, dorsally, laterally, posterior view, M prosternal process, and mesosternal pit; N–P male genitalia, ventrally, dorsally, laterally; Q—terminal abdominal segments. Scales 0.5 mm.

Description

Male. Body 12–14 mm long, robust, slightly flattened, brown colored, vestiture dense, long short (Fig. 4B–E).

Head prognathous to slightly declined, transverse, width across eyes narrower than prothoracic width, gradually narrowed posteriorly (Fig. 4B). Eyes slightly protuberant, rounded, ocelli absent. Antennal insertions widely separated, gular sutures narrowly separated; cervical sclerites sclerotized (Fig. 4B). Antennae slender, shortly flabellate, reaching almost mid of elytra, antennomere 1 robust, long, antennomeres 2 and 3 short, antennomeres 4–11 with short lamella, terminal antennomere similar length as preceding one. Mandibles robust, abruptly curved (Fig. 4E). Mandibular apex and incisor edge simple, maxilla with short setose mala; maxillary palpi slender, 4-segmented, apical palpomere parallel-sided; labium tiny, with 3 palpomeres (Fig. 4B).

Prothorax transverse, pronotum without carinae, maximum width 1.5 times length, widest at posterior angles, sides convex (Fig. 4C,D). Prothorax basally narrower than elytral bases; lateral pronotal edge conspicuous in whole length (Fig. 4C). Posterior angles of pronotum long, acute. Prosternal process 4 times longer than anterior portion of prosternum (Fig. 5D); procoxal cavities open, narrowly separated; prothoracic process does not reach mesothoracic pit (Fig. 4D). Elytra cover abdomen, tapering to apex, widest at humeri, with inconspicuous costae (Fig. 4E). Elytral suture almost complete. Scutellum well developed; pointed posteriorly (Fig. 4K). Hind wings well developed.

Legs slightly compressed, with tarsi longer than femora and tibiae; slender pro- and mesotrochanters and widened metathoracic trochanters (Fig. 4L), tibiae with simple outer edge bearing setae, and 2 apical, long spines; tarsomeres slender, 5 segmented, without ventral pads, claws paired, long, slender, and simple (Fig. 4L).

Abdomen with 7 free abdominal sternites, ventrite 1 entire, without inter-coxal process; penultimate tergite simple (Fig. 4J). Male genitalia trilobate; symmetrical. Phallus robust, basally fused with parameres (Fig. 4F–H). Females of *Cebriorhipis* are unknown.

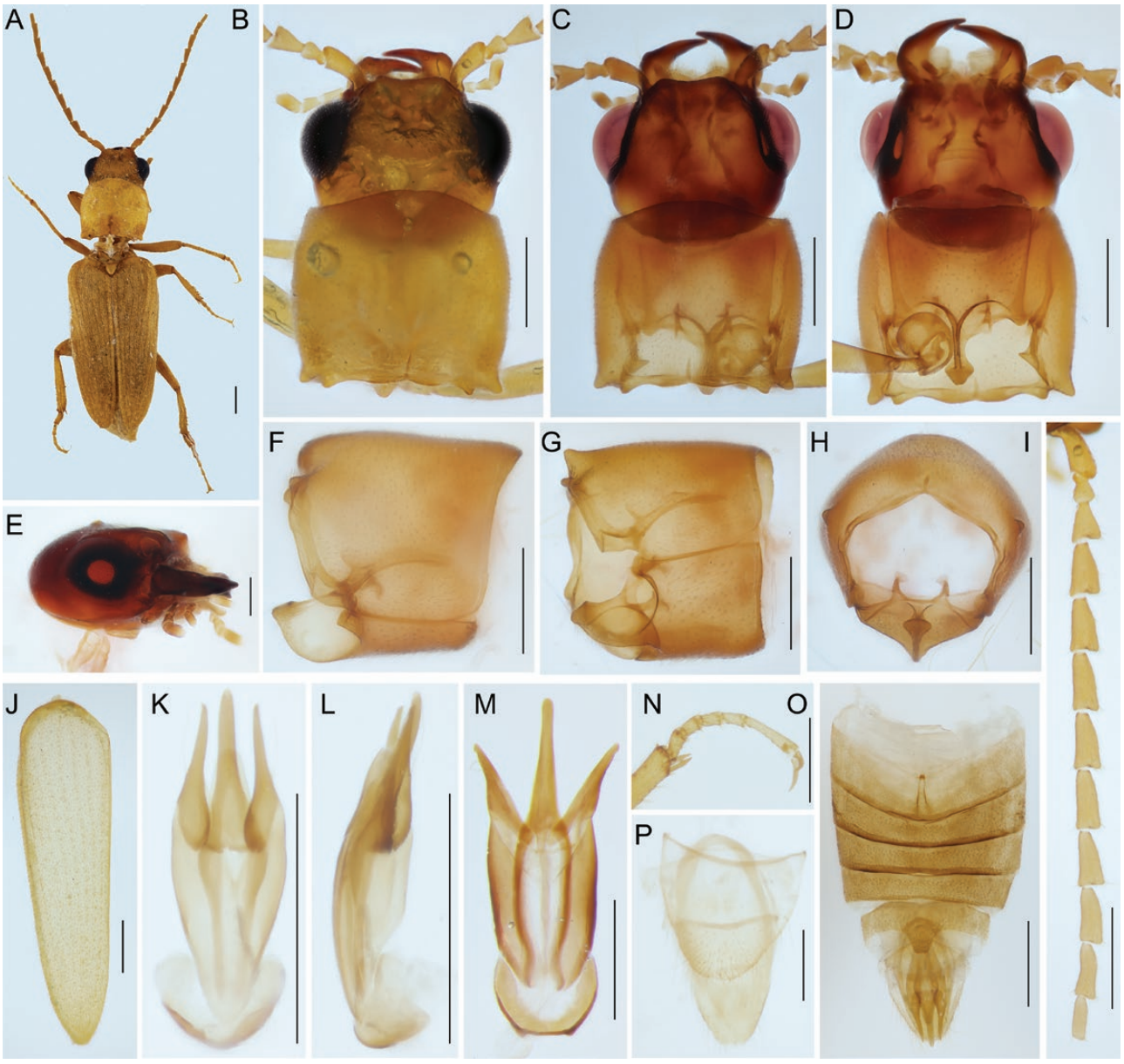

Fig. 3. *Analestesa arabica* (Paulus, 1981) from Saudi Arabia (except for Fig. 3I). A—general appearance, dorsal view; B–D—frontal part of the body, dorsally (B, C) and ventrally); E—head in lateral view; F–H—pronotum, lateral, dorsolateral, and posterior view; I—antenna; J—elytron; K, M—male genitalia, ventrally, dorsally, laterally; I—Male genitalia of *Dicronychus cinereus* (Herbst, 1784); N—hind tarsus and apical part of tibia; O—abdomen, ventral view; P—terminal abdominal segments. Scales 0.5 mm.

Taxonomy

Subfamily Hemiopinae Fleutiaux, 1941

Hemiopinae Fleutiaux, 1941: 31.

Type genus

Hemiops Laporte, 1838.

Tribe Paulusiellini Kusy, Motyka & Bocak, new tribe

urn:lsid:zoobank.org:act:9C68A2F0-4ED8-44F4-9F68-FCD9C3B3184C

Type Genus

Paulusiella Löbl, 2007 (monotypic).

=*Paulusiella* Mandl, 1974 (invalid name).

Diagnosis

The erection of the new tribe is based on molecular relationships that rejected the relationships of *Paulusiella* and *Cebrio* (the Cebrionini concept *sensu* Paulus 1982, Lawrence et al. 2000, Sánchez-Ruiz and Lobl 2007, Platia and Ghahari 2016) and the significant morphological disparity of hemiopine genera and *Paulusiella*. We recovered *Paulusiella* as a sister to *Hemiops* Laporte, 1838 (Elateridae: Hemiopinae; Fig. 1). The tribe is monogeneric, and the description of *P. serraticornis* is given above. The morphology is illustrated in Fig. 2 and summarized in Table 3.

Justification of the erection of the tribe Paulusiellini

The morphology does not provide sufficient guidance for the placement of the genus in a natural classification. The absence of apparent diagnostic characters is documented by the initial invalid description

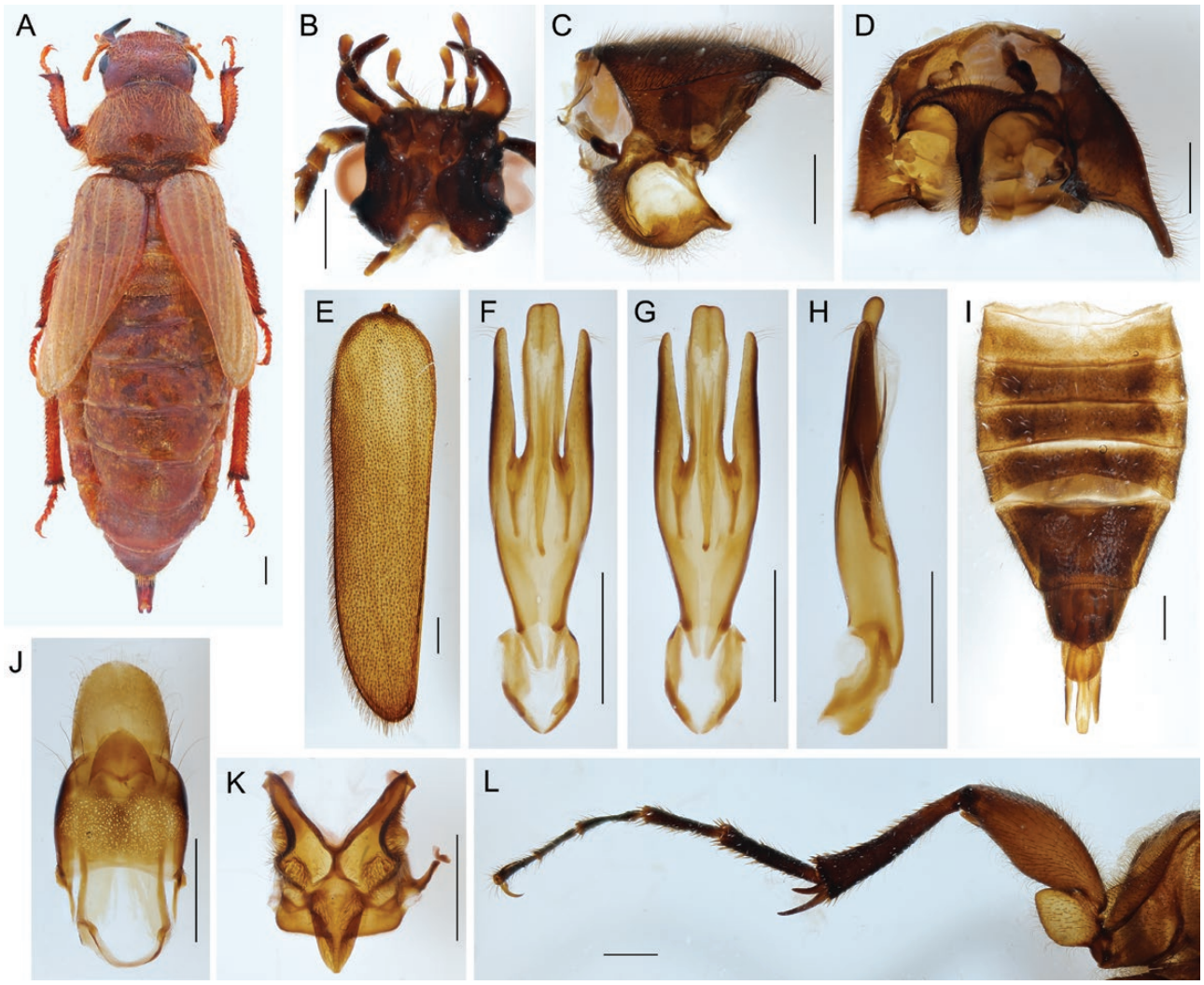

Fig. 4. A—*Cebrio (Tibesia) igelmimen* Rattu et François, 2021 from Morocco, female, general appearance. *Cebriorhipis* sp. from Bali. B—head, ventral view; C, D—pronotum, lateral and ventral view; F—elytron; G–I male genitalia, ventral, dorsal, and lateral view; J—abdomen, ventral view; K—terminal abdominal segments; L—mesoscutellum; M—metathoracic leg. Scales 1.0 mm. Figure 4A was published by Rattu and François (2021) and is here reprinted with permission of the authors who retain the copyright of this photo.

of *Paulusiella* in Dascilloidea (Mandl 1974, 1979), the inclusion of an originally species in the genus *Escalerina* (*E. serraticornis* Paulus, 1972; Dascilloidea: Dascillidae), and the recent description of a *Paulusiella* species in *Selasia* (*Selasia sweihana* Geisthardt, 2009; Elateridae: Agrypninae: Drilini; Geisthardt 2009, Ivie and Barclay 2011). The type-genus *Paulusiella* was validly erected in Cebrioninae: Cebrionini (now Elaterinae: Cebrionini) by Löbl (2007) and later transferred to Elateridae *incertae sedis* (Ivie and Barclay 2011).

Here, *Paulusiella* was recovered with robust support (BS 100%) as a sister to *Hemiops* Laporte, 1838 by all analyses (Figs. 1A–C, 5A–P, Supplementary Figs. S2–S14). Hemiopinae is a small elaterid subfamily with only 3 morphologically similar genera—*Hemiops* Laporte, 1838 (Fig. 5A–P; East and Southeast Asia), *Legna* Walker, 1858 (Sri Lanka), *Parhemiops* Candèze, 1878 (Nepal) and morphologically somewhat distant *Exoeolus* Broun, 1893(New Zealand) that was excluded from Cardiophorinae and provisionally placed in Hemiopinae by Douglas (2011). Comparing *Paulusiella* and *Hemiops*, we can see similar abruptly elevated scutellum that is anteriorly straight in *Paulusiella* but bilobate in *Hemiops* (Figs. 2H and 5M) and similar morphology of trochanters (Figs. 2D and 5B,K; Paulus 1972, Fig. 3). In both taxa, scutellum posteriorly surpasses elytral surface, but projected posterior part of the scutellum is commonly encountered in elateroids. The antennae are different, but the relative length of the 3 basal antennomeres is similar (Figs. 2D and 5D). There are several structures that are present in *Hemiops* and absent in *Paulusiella*: sharp lateral prothoracic edge (Figs. 2K and 5F), apparent elytral longitudinal costae (Figs. 2I and 5A), complex posterior shape of the prothorax (Figs. 2J,M and 5N,O). The taxa also differ in the relative length of the prosternal process, prosternum, shape of elytra, abdominal terminal segments, and the shape of phallus (Figs. 2A–Q and 5A–P). *Exoeolus* is morphologically distinct and does not share any diagnostic characters with *Paulusiella*. We do not discuss the position of this genus as we do not have access to click beetles from New Zealand.

We prefer to assign the tribe rank to the *Paulusiella*-based taxon due to the morphological disparity of the aforementioned genera. The morphologically distinct forms are regularly given some family-group rank to demonstrate in the Linnean classification their hypothesized sister relationships. We cannot propose any reliable diagnostic character that would morphologically define the clade

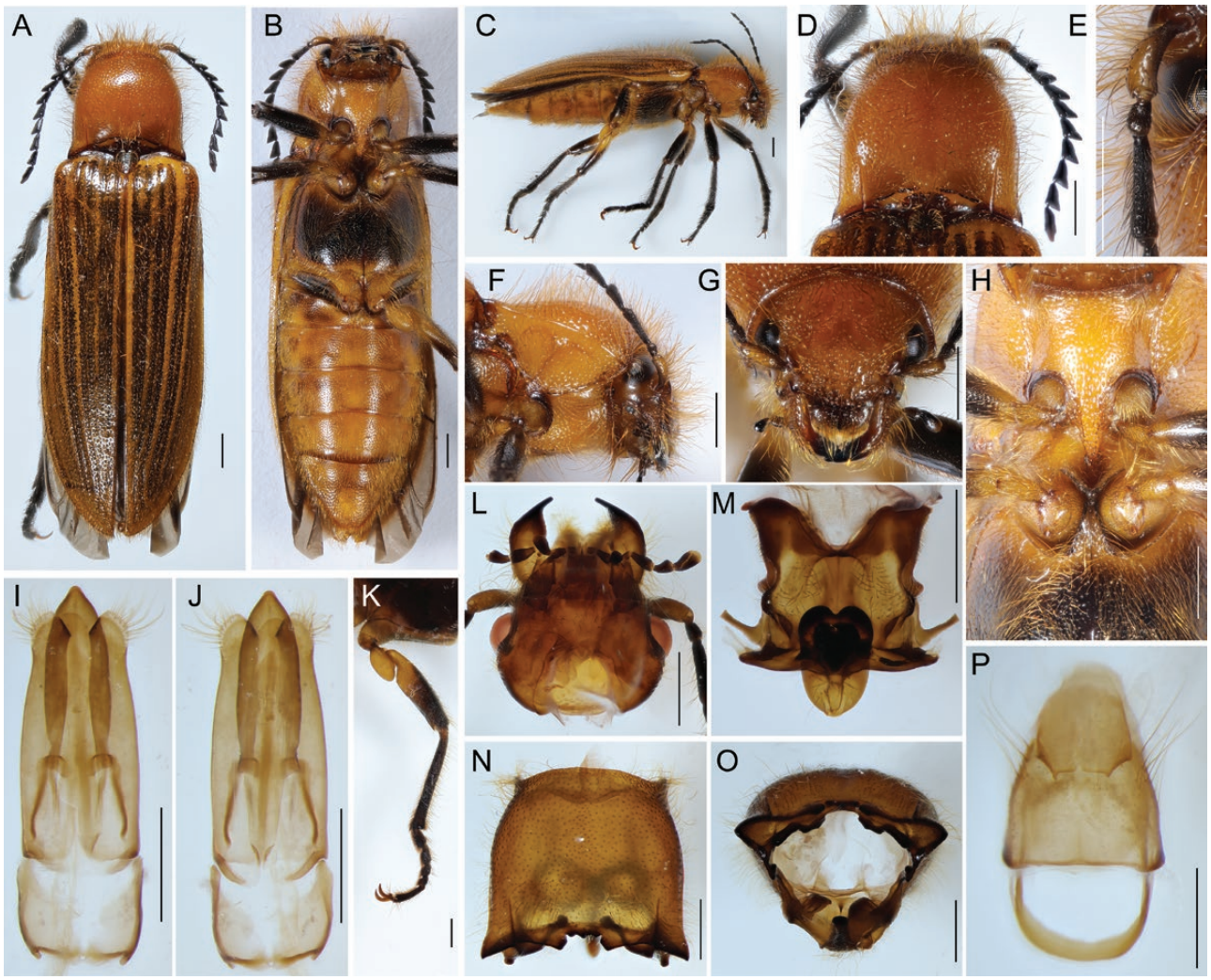

Fig. 5. *Hemiops* sp. from Laos. A–C general appearance, dorsal, ventral, and lateral aspects; D—pronotum; E—basal antennomeres; F—pronotum and head, ventrolateral view; G—head, dorsally; H—prosternal process and mesosternal pit; I, J—male genitalia; K—metathoracic leg; L—head ventrally; M—mesonotum; N, O ° pronotum, dorsal, and posterior view; P—terminal abdominal segments. Scales 1.0 mm.

of all hemiopine genera and *Paulusiella*. Their distinctiveness was probably the reason why *Paulusiella* was earlier placed in different superfamilies and families or was kept in an uncertain position in Elateridae by earlier students of Elateriformia.

Subfamily Cardiophorinae Candèze, 1859

Cardiophorites Candèze, 1859: 4.
Cardiophori: LeConte 1861: 166.
Cardiophorinae: Burakowski et al. 1985: 227.

Type genus

Cardiophorus Eschscholtz, 1829.
=Aphrici LeConte, 1861: 173.
Type genus: *Aphricus* LeConte, 1853.
=Aptopina Jakobson, 1913: 760.
Type genus: *Aptopus* Eschscholtz, 1829.
=Esthesopinae Fleutiaux, 1919: 76.
Type genus: *Esthesopus* Eschscholtz, 1829.
=Nyctorini Semenov-Tian-Shanskij et Pjatakova, 1936: 102.
Type genus: *Nyctor* Semenov-Tian-Shanskij et Pjatakova, 1936 [=*Cardiophorus* Eschscholtz, 1829 *sensu* Cate (2007) but not Douglas (2017)].
=Cebriognathinae Paulus, 1981: 264 (recently Elaterinae: Cebrionini or as a synonym of Cebrioninae), **a new synonym of Cardiophorinae.**
Type genus: *Cebriognathus* Chobaut, 1899 (=*Analestesa* Leach, 1824).

Remarks

The molecular analysis robustly recovered *A. arabica* in close relationships with *Globothorax* Fleutiaux, 1891 (=*Teslasena* Fleutiaux, 1892), *Cardiophorus* Eschscholtz, 1829, and *Dicronychus* Brullé, 1832 (Fig. 1A–C, Supplementary Figs. S2–S11). This position is also supported by the structure of male genitalia (Fig. 3K–M). Although Paulus (1981) erected Cebriognathinae within that time accepted Cebrionidae, he mentioned the similarity of the male genitalia of Cebriognathus and Cardiophorinae erected Cebriognathinae within that time accepted Cebrionidae, he mentioned the similarity of the male genitalia of *Cebriognathus* and Cardiophorinae(confirmed here) and discussed the possibility that *Cebriognathus* is a modified click beetle. Bouchard et al. (2011) listed Cebriognathinae as a synonym of Cebrioninae, and the GBIF database (https://www.gbif.org/species/4428817 accessed on 7 November 2022) lists *Analestesa* as a junior synonym of *Cebrio*. Neither of the referenced sources

Table 3. The overview of morphological characters in the elaterid lineages: clicking *Hemiops* and soft-bodied non-clicking taxa

	Male							Female
Character Taxon	Antennae	Apical antennomeres	Prosternum/ prosternal process	Lateral pronotal edge	Elytral rows or costae/ suture; shape	Abdominal segments	Mesocox. Process (ventr. 1)	Differences when compared with conspecific male
Hemiops (Hemiopinae)	Short	10<11	Long/ long, clicking	Present	Present/ full; widest in middle	Connate	Present	Fully sclerotized, flying, similar to male
Cebrio (Elaterinae)	Short	10<11	Short/long, non-clicking	Present	Absent/ divergent, widest basally	Free	Absent	Some females with short elytra and wingless; physogastric abdomen, short antennae, and legs (Fig. 4A).
Paulusiella (Hemiopinae)	Short	10<11	Short/ short, non-clicking	Vestige posteriorly	Absent/ divergent, widest basally	Free	Absent	Unknown
Analestesa (Cardiophorinae)	Long	10>11	Long/ short, non-clicking	Absent	Absent/ absent; widest basally	Free	Present	Unknown
Plastocerus (Dendrometrinae)	Long	10<11	Long/ short, non-clicking	Present	Present/ full; parallel-sided	Free	Short	Obtuse posterior pronotal angles, shorter antennae than male
Drilus (Agrypninae)	Short	10<11	Short/ short, non-clicking	Present	Absent/ full suture; widest posteriorly	Free	Absent	Head and appendages adult-like, rest of body larviform
Omalisus (Omalisinae)	Long	10<11	Short/ short, non-clicking	Present	Present; full suture, parallel-sided	Free	Absent	Pupa-like: short legs and antennae, vestigial elytra, similar length of meso- and metathorax

gives the reasons for their statements. Still, *Analestesa* is a valid name within the Cardiophorinae.

Analestesa is much less sclerotized than most cardiophorine click beetles and has no clicking mechanism (Fig. 3A,D). It differs from most Cardiophorinae by a quadrate mesoscutellar shield and the small and short prosternal process that does not reach the mesothoracic pit. The only cardiophorine species with weakly sclerotized cuticle has hitherto been *Nyctor expallidus* Semenov-Tian-Shanskij et Pjatakova, 1936 (=*Cardiophorus expallidus*, *sensu* Cate 2007). Its males have possibly slender antennae, while the antennae of females are shortened and robust. Although Douglas (2017) described both illustrated specimens as males, the intraspecific variability in the morphology of antennae is improbable (Douglas 2017; Figs. 60, 61).

There are several characters defining Cardiophorinae + Negastriinae and the monophyly of Cardiophorinae (Douglas 2017) that could also be observed in *Analestesa*: the shape of the parameres (Fig. 3K–M) and the straight lateral edge of the prosternum. Another diagnostic character was defined in female genitalia, but females are unknown for *Analestesa*. The modified external morphology does not provide evidence for the placement of *Analestesa* in Cardiophorinae, and only the similar male genitalia support the recovered DNA-based topology. The recovered relationships (*Globothorax*(*Analestesa*(*Cardiophorus*, *Dicronychus*))) suggest that the earlier proposed morphology-based topology (*Dicronychus*(*Cardiophorus*, *Globothorax*)) needs further investigation (Douglas 2017).

Subfamily Elaterinae Leach, 1815

Elaterides Leach, 1815: 85.

Type genus: *Elater* Linnaeus, 1758.

Cebrionini Latreille, 1802

Cebrionates Latreille, 1802: 97.

Type genus: *Cebrio* Olivier, 1790.

Remarks

Females of *Cebrio* have variably developed elytra and large bodies. The known females are flightless (Fig. 5A; Rattu 2016, Rattu and François, 2021, Martinez-Luque et al. 2022) and differ from conspecific males by smaller eyes and differently shaped cranium. Appendages of females are shorter: very short antennae, with low differentiation between the antennomeres, short legs, with longer femora and tibiae than tarsi, shortened elytra, and vestigial wings. *Cebrio gigas* (F., 1787) has a physogastric female, but its elytra form complete elytral suture, although they do not completely cover the abdomen (https://inpn.mnhn.fr/espece/cd_nom/240515, accessed on 3 November 2022).

Revised composition of Cebrionini

Cebrionina Latreille, 1802: *Cebrio* Olivier, 1790; *Cebriorhipis* Chevrolat, 1875, *Musopsis* Chevrolat, 1875, *Scaptolenus* LeConte, 1853; *Selonodon* Latreille, 1834; *Stenocebrio* Solervicens, 1988. The analysis of the mitochondrial and nuclear DNA dataset revealed that *Cebriorhipis* sp. is nested within *Cebrio*, making it paraphyletic.

Aplastina Stibick, 1979: *Aplastus* LeConte, 1859; *Euthysanius* LeConte, 1853; *Octinodes* Candèze 1863; (=*Plastocerus sensu* LeConte, 1853); *Cylindroderus* Latreille, 1834 (=*Cylindroderoides* Schwarz, 1907); *Dodecacius* Schwarz, 1902 (Arnett 1949, Johnson 2002, 2017, Sánchez-Ruiz and Löbl 2007, Johnson and Chaboo 2015).

Stibick (1979) listed Pleonomini Semenov et Pjatakova, 1936 as a coordinate taxon of Aplastini in Aplastinae. The group was listed as Pleonominae by Cate (2007) and as the tribe Pleonomini in Dendrometrinae by Bouchard et al. (2011). *Pleonomus* Ménétriés, 1849 has both sexes with fully developed elytra but apparent sexual dimorphism (Reitter 1900) resembling the modifications observed in other soft-bodied elaterids.

Discussion

Origins of Soft-bodied Forms

Some 15 years ago, Elateridae was a morphologically homogenous beetle family with few slightly modified, weakly sclerotized taxa

concentrated in Cebrionini (Elaterinae; Sánchez-Ruiz and Löbl 2007, Bouchard et al. 2011, Lawrence et al. 2011). Until now, cebrionids have served as a collective taxon for at least partly soft-bodied, sometimes non-clicking elaterids with flightless or unknown females (Arnett 1949, Johnson 2002, Rattu 2016, Rattu and François 2021, etc.). The strict link between morphological similarity (i.e., shared character states by all taxa now placed in Cebrionini) and relationships recovered by phylogenetic analyses (the monophyly of Cebrionini) is considered a null hypothesis. We reject it based on the analysis of molecular data ($P = 0.000$; Table 2).

This is not the first case of the conflict between morphology- and DNA-based relationships. Since 2007, several DNA-based studies have targeted Elateriformia, including soft-bodied elateroids. They have advocated that Drilidae, Omalisidae, and Plastoceridae, earlier placed in Cantharoidea, are modified click beetles and telegeusids and omethids were regularly related to Artematopodidae instead of Cantharidae, Lampyridae, and Lycidae (Bocakova et al. 2007, Kundrata and Bocak 2011, Bocak et al. 2014, 2016, 2018, Kundrata et al. 2014, McKenna et al. 2015, 2019, Kusy et al. 2018, 2021b, Zhang et al. 2018, Douglas et al. 2021). The role of ontogenesis in the evolution of these groups was already discussed by Crowson (1972) and Gould (1977) and later by many different authors (the review by McMahon and Hayward 2016). Although some characters might be attributed to the selection for endogenous life of females, such an approach does not explain why are also modified females that are not endogenous (*Drilus* spp., *Platerodrilus* spp., and various lampyrid, phengodid and rhagophthalmid genera) and why the males are similarly modified (e.g., compare the male of net-winged beetle *Dexoris chome* Bocak et al., 2013 and the female of click beetle *Omalisus* spp.).

Earlier phylogenetic hypotheses had a clear evolutionary connotation: the shift leading to flightless, soft-bodiedness, the retention of some larva- or pupa-like characters in adults, and even larviform females of some taxa, was understood as a rare phenomenon. Therefore, almost all elateroid lineages with weak sclerotization, including some taxa with neotenic females, shared a hypothesized most recent common ancestor (the Cantharoidea and cantharoid clade concepts; Crowson 1955, 1972, Branham and Wenzel 2003, Lawrence et al. 2011). The rejection of the cantharoid clade (Bocakova et al. 2007, Sagegami-Oba et al. 2007, etc.) and the robust placement of some "cantharoid" groups in Elateridae (Kusy et al. 2019) suggested a different view: the process of metamorphosis is less stable than previously thought, and numerous elaterid groups independently lost the clicking mechanism, are soft-bodied (i.e., cantharoid-like), their known females are physogastric, have short appendages, and short, vestigial or absent elytra (Fig. 4A, Table 3).

Here, we studied the position of 2 taxa, *Paulusiella* and *Analestesa* (=*Cebriognathus* or *Cebrio*), earlier placed in Elateridae *incertae sedis* or Elaterinae: Cebrionini) (Lawrence et al. 2000, Sánchez-Ruiz and Löbl 2007, Costa et al. 2010, Ivie and Barclay 2011). Both genera are known only from males, and their females are putatively flightless. These are small-bodied, non-clicking, weakly sclerotized beetles, with shortened and posteriorly slender elytra, without a full-length elytral suture and coadaptation between the lateral elytral margins of the abdomen (Figs. 2 and 3). Due to these traits, they are superficially similar to some Cebrionini, but the DNA analysis placed them in very distant positions (Fig. 1A,B, Supplementary Figs. S2–S11). *Paulusiella serraticornis* was recovered as a sister to Hemiopinae and *A. arabica* as one of the serial splits in Cardiophorinae. These positions are robustly supported by molecular data (see Table 2 for the tests of alternative relationships). Conversely, their relationships are hardly supported by morphology (see Results). Still, the comparative morphology neither clearly supports their relationships to *Cebrio* (Figs. 2–4). Cebrionini, i.e., *Cebrio*, *Cebriorhipis*, and *Scaptolenus*, contain medium-sized beetles with a characteristically robust body, short prosternum, fully developed sharp lateral pronotal margin, and 7 segments of the abdomen (Fig. 5; Table 3). Yet, all have elaterid-like pronotum with acutely projected posterior angles. *Selonodon* is an elaterid-like cebrionid (Galley 1999), and *Stenocebrio* is similar in general appearance to *Paulusiella* or *Analestesa* (Solervicens 1988), but the representative of this genus was not available for the study.

Although the divergent morphology is sometimes referred to only as flightlessness and soft-bodiedness, the morphological modifications affect almost all body parts, and similar modifications are known in unrelated lineages (e.g., Paulus 1972, Johnston and Gimmel 2020, Table 3). Besides shortened to vestigial elytra and wings, we often notice the shortened antennae and legs; the loss or substantial simplification of complex structures, e.g., the pronotum (keels, lateral edge, shortened prosternum, shortened prosternal process, the loss of acutely projected posterior angles, unfunctional click mechanism), a lower ratio between the length of meta and mesothorax (the female of *Omalisus*; Bocak and Brlik 2008), free abdominal segments, the loss of inter-coxal keel in visible abdominal segment 1, loss of costae of rows of punctures in elytra (Figs. 2–4; Table 3).

Fully sclerotized elaterids have thick-walled thoracic segments, strong muscles, a mesoventral pit, long prosternum, and the elytra held closed by the mesoscutellar catch. Additionally, the prothorax and mesothorax are coadapted, and pivots and flanges enable precisely defined click action (Ewans 1972). This complex mechanism is lost in all soft-bodied groups. The affected groups do not necessarily have all traits modified (e.g., compare the antennal length of *Analestesa* and *Paulusiella*; Figs. 2D and 3I), but the presence of these characters is often recorded in the males of elateroid taxa for which we have modified, flightless females (Crowson 1972, Cicero 1988, Bocakova et al. 2007, Bocek et al. 2018, Kundrata and Bocak 2019, Kusy et al. 2019, Rosa et al. 2020).

The phylogenetic distribution of non-flying and soft-bodied groups is biased to Elateroidea or Elateriformia, respectively (Gould 1977, McMahon and Hayward 2016). Within click beetles, modified forms are known in Elaterinae: Cebrionini, Omalisinae, Dendrometrinae: Plastocerini, and Agrypninae: Drilini (Bocakova et al. 2007, Kundrata and Bocak 2011, Bocak et al. 2018, Bocek et al. 2018, Kusy et al. 2019). The relationships of *Analestesa* and *Paulusiella* are hypothesized as further two independent origins of these modifications: *Paulusiella* is a sister to Hemiopinae, and *Analestesa* is one of the numerous genera of Cardiophorinae (Fig. 1A–C; Supplementary Figs. S2–S14). Similar phenotypes are known in the lampyroid families. Telegeusinae (Omethidae), which are closely related to Artematopodidae, and Jurasaidae, related to Eucnemidae and Cerophytidae, are further examples of the independent origin of soft-bodied forms (Bocakova et al. 2007, Bocak et al. 2014, Zhang et al. 2018, McKenna et al. 2019, Rosa et al. 2020). An additional case of lost sclerotization was hypothesized in Ptilodactylidae, which now contains one morphologically modified species, earlier the type of Podabrocephalidae (Kundrata et al. 2019). Analogical modifications were also reported in one species of *Anorus* (Dascillidae; Johnston and Gimmel 2020) and other dascillids (Karumiinae; Paulus 1972). In agreement with earlier studies, we suppose that the morphological modifications result from earlier termination of metamorphosis that is more pronounced in females but also has some effect on males (Gould 1977, Bocak et al. 2008, McMahon and Hayward 2016, etc.).

Modified Morphology and Systematics

The modification caused by incomplete metamorphosis (i.e., the retention of some larval and pupal characters and the loss of some derived traits) leads to two kinds of taxonomic misplacements. These beetles are often merged into a single clade as the modifications have a similar effect on unrelated lineages (see above). Hence, the morphological analyses using the parsimony criterion prefer these shared characters before fewer, if any, contradicting characters shared with close, yet unmodified, relatives. As a consequence, earlier authors defined taxa as Cantharoidea and the cantharoid clade (Crowson 1972, Lawrence et al. 2011), the subfamily Cebrioninae or the tribe Cebrionini (Arnett 1949, Bouchard et al. 2011), the clade of neotenic lineages in net-winged beetles (Kazantsev 2013), or suggested *Podabrocephalus* Pic, 1913 (Byrrhoidea) as a sister of the cantharoid clade (Lawrence et al. 2011). Additionally, some taxa were initially placed in distantly related but similarly modified groups. For example, Paulus (1972) described *P. serraticornis* as *Escalerina serraticornis* in Dascillidae, and Geisthardt (2009) described *P. sweihana* in *Selasia* (Agrypninae: Drilini; Ivie and Barclay 2011).

The unrealistically deep rooting of modified forms is a second kind of misplacement. The loss of derived character states leads to the inference of a deeper position of incompletely metamorphosed groups than those of their fully sclerotized relatives. Consequently, the inappropriately high rank is given to modified lineages. This effect led to the earlier discussion on the ancient origin of neotenics (Crowson 1972), the proposed sister-relationships of *Selonodon* Latreille (Cebrionini) and other elaterids (Lawrence et al. 2011) or descriptions of Pleonominae (=Pleonomini, Dendrometrinae) and Nyctorini (=Cardiophorinae). The absence of female characters in the analyzed matrices (unknown or sometimes completely larviform females) lowers the number of informative characters coded for neotenic taxa. Thus, missing data negatively affect the stability of phylogenies.

The above-described pitfalls cannot be solved by any methodological modification of the morphology-based phylogenetic analyses. Earlier analyses handled many taxa and characters and were correctly conducted by experienced comparative morphologists (Branham and Wenzel 2003, Lawrence et al. 2011). For these groups, we urgently need information unaffected by the pedogenetic syndrome. Now, we can access information-rich genetic data and reinvestigate the traditionally accepted relationships. The growing evidence suggests common shifts from the clicking, well-sclerotized elaterids to weakly sclerotized forms with highly modified females (Fig. 1A–C; Supplementary Figs. S2–S14). We can look for similar evolutionary pathways in other groups and test the new hypotheses with even more extensive data in the future. The striking conflict between morphology- and DNA-based relationships of extant lineages also calls for cautious analyses of soft-bodied forms preserved in amber deposits, as their relationships cannot be validated with molecular data.

Supplementary Data

Supplementary data are available at *Insect Systematics and Diversity* online.

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Author Contributions

Dominik Kusy (Conceptualization [Equal], Data curation [Equal], Formal analysis [Equal], Funding acquisition [Equal], Investigation [Equal], Methodology [Equal], Validation [Equal], Writing – original draft [Equal], Writing – review & editing [Equal]), Michal Motyka (Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources [Equal], Validation, Visualization [Equal], Writing – original draft, Writing – review & editing), and Ladislav Bocak (Conceptualization [Equal], Formal analysis [Equal], Funding acquisition [Equal], Investigation [Equal], Methodology [Equal], Project administration [Equal], Resources [Equal], Supervision [Equal], Validation [Equal], Visualization [Equal], Writing – original draft [Equal], Writing – review & editing [Equal])

Specimen Collection Statement

The authors attest that all legal and regulatory requirements, including export and import collection permits, have been followed for the collection of specimens from source populations at any international, national, regional, or other geographic level for all relevant field specimens collected as part of this study.

Data Availability

The analyzed sequences are available in the Mendeley depository. Kusy, Dominik; Motyka, Michal; Bocak, Ladislav (2023), "Data for „Ontogenetic modifications produce similar phenotypes in distantly related click beetles (Coleoptera: Elateridae)", DOI:10.17632/73dmw4czm3.1.

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PART II

Studies on neoteny in Coleoptera: phylogenomics, classification, and evolutionary interactions

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Phylogenomic relationships of bioluminescent elateroids define the ‘lampyroid’clade with clicking Sinopyrophoridae as its earliest member.

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Phylogenomic relationships of bioluminescent elateroids define the 'lampyroid' clade with clicking Sinopyrophoridae as its earliest member

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Abstract. Bioluminescence has been hypothesized as aposematic signalling, intersexual communication and a predatory strategy, but origins and relationships among bioluminescent beetles have been contentious. We reconstruct the phylogeny of the bioluminescent elateroid beetles (i.e. Elateridae, Lampyridae, Phengodidae and Rhagophthalmidae), analysing genomic data of *Sinopyrophorus* Bi & Li, and in light of our phylogenetic results, we erect Sinopyrophoridae Bi & Li, **stat.n**. as a clicking elaterid-like sister group of the soft-bodied bioluminescent elateroid beetles, that is, Lampyridae, Phengodidae and Rhagophthalmidae. We suggest a single origin of bioluminescence for these four families, designated as the 'lampyroid clade', and examine the origins of bioluminescence in the terminal lineages of click beetles (Elateridae). The soft-bodied bioluminescent lineages originated from the fully sclerotized elateroids as a derived clade with clicking *Sinopyrophorus* and Elateridae as their serial sister groups. This relationship indicates that the bioluminescent soft-bodied elateroids are modified click beetles. We assume that bioluminescence was not present in the most recent common ancestor of Elateridae and the lampyroid clade and it evolved among this group with some delay, at the latest in the mid-Cretaceous period, presumably in eastern Laurasia. The delimitation and internal structure of the elaterid-lampyroid clade provides a phylogenetic framework for further studies on the genomic variation underlying the evolution of bioluminescence.

Introduction

Bioluminescence has been intensively studied by numerous researchers (Costa, 1975; Branham & Wenzel, 2003; Nakatsu *et al.*, 2006; Fallon *et al.*, 2018). The production of light occurs sporadically in insects (Watkins *et al.*, 2018; Martin *et al.*, 2019); nevertheless, beetle bioluminescence is well known to both researchers and the general public. Most luminous beetles belong to Elateroidea and we know of ~2000 firefly species (Lampyridae, Fig. 1F–J), ~300 glow-worms or railroad-worm beetles (Phengodidae, Rhagophthalmidae), as well as >100 bioluminescent click beetle species (Elateridae; Fig. 1A–D), especially in the Neotropical region (Costa, 1975, 1984). With the recent discovery of the first Palearctic bioluminescent clicking beetle, the number of bioluminescent beetle lineages has increased (He *et al.*, 2019; Bi *et al.*, 2019; Fig. 1D–E).

The phylogenetics of Elateroidea have been studied with morphology since the late 1980s, where the bioluminescent

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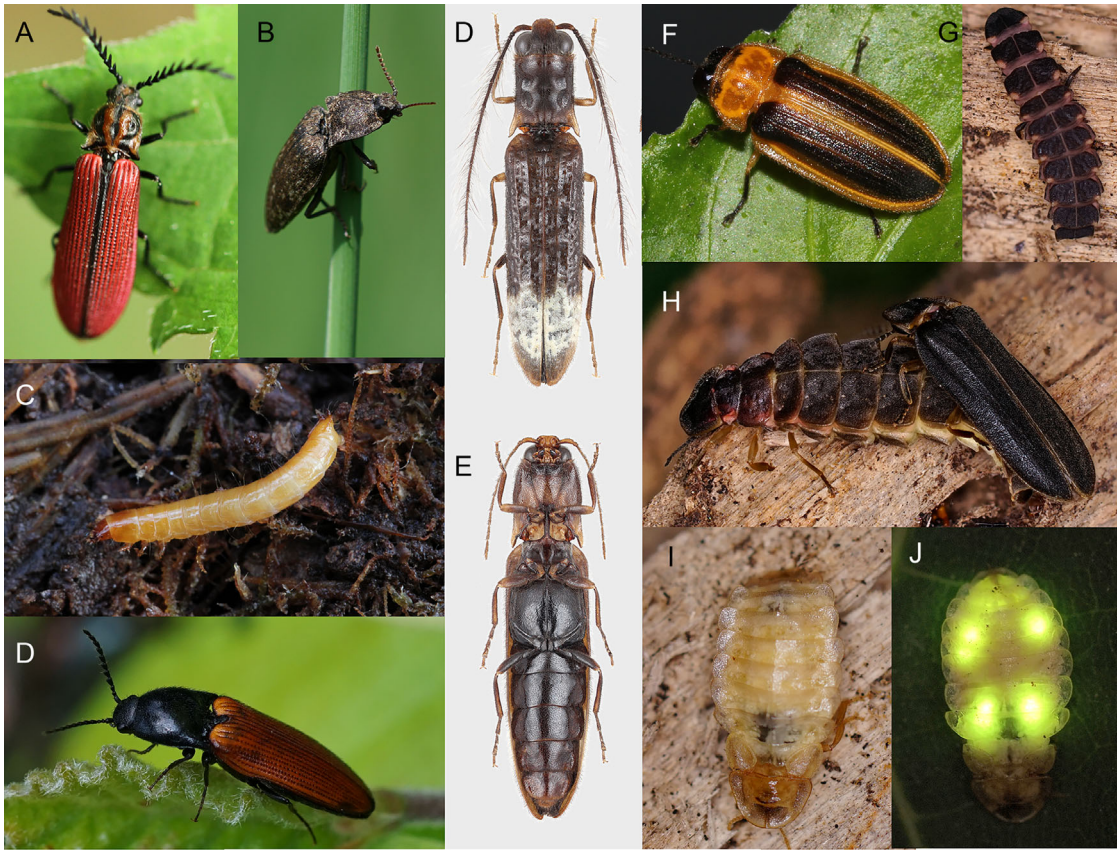

Fig 1. Morphological diversity of the elaterid-lampyroid clade. Elateridae: (A) *Denticollis* sp.; (B) *Agrypnus murinus* (L.); (C) click beetle larva; (D) *Ampedus* sp. The lampyroid clade. Sinopyrophoridae: (D, E) *Sinopyrophorus schimmeli* Bi & Li; Lampyridae: (F) *Asymmetricata circumdata* (Motschulsky); (G) *Lampyris noctiluca* (L.), larva; (H) ditto, in copula; (I, J) *Lamprohiza splendidula* (L.), female. Photographs by M. Motyka (B, D, G–J), L. Bocak (A,C) and Z.-W. Dong (F); (D, E) from Bi *et al.*, 2019. [Colour figure can be viewed at wileyonlinelibrary.com].

soft-bodied 'cantharoids' and fully sclerotized click beetles were merged in a single superfamily, Elateroidea (Lawrence, 1988; Branham & Wenzel, 2003; Lawrence *et al.*, 2011). Later studies using DNA data supported showed that the soft-bodied elateroids were polyphyletic (Bocakova *et al.*, 2007). Some relationships among these groups remained inconclusive when topologies were inferred from a few widely used molecular markers and the controversies persisted even when protein-coding nuclear fragments were employed (McKenna *et al.*, 2015), or when taxon sampling was substantially increased (Kundrata *et al.*, 2014; Bocak *et al.*, 2016; Linard *et al.*, 2018) (Fig S1A–C). A clade of bioluminescent elateroids, that is, Elateridae, Lampyridae, Phengodidae and Rhagophthalmidae, was first identified by the analyses of 13 mitochondrial genes (Timmermans *et al.*, 2010) and confirmed by further analyses of these mitogenomes but with more extensive taxon sampling (Amaral *et al.*, 2016; Bocak *et al.*, 2016). Nevertheless, the analyses were relatively limited by the volume of data and resulted in ambiguous support for critical clades and contradictory results (Kundrata *et al.*, 2014; Linard *et al.*, 2018). Recent progress has been made possible by high throughput sequencing, transcriptomes and whole-genome analyses. Zhang *et al.* (2018a) used 99 nuclear markers and Kusy *et al.* (2018a) analysed ~4000 orthologous genes. Both studies provided evidence for a clade that included five elateroid families with at least some bioluminescent taxa. However, these two studies either used a low number of orthologs or restricted taxon sampling. A phylogenomic analysis for all of Coleoptera provided a timeframe for the evolution of the order based on multiple calibration points, confirming the elateroid backbone but contained only six elateroid terminals (McKenna *et al.*, 2019) (Fig S1D,E).

Recently, the subfamily Sinopyrophorinae Bi & Li was proposed in Elateridae for the bioluminescent *Sinopyrophorus schimmeli* Bi & Li. Bi *et al.* (2019) included homologous fragments of *S. schimmeli* in a phylogenetic reconstruction from earlier published click beetle rRNA and mtDNA genes (Bocakova *et al.*, 2007; Timmermans *et al.*, 2010, 2016; Kundrata *et al.*, 2014; Amaral *et al.*, 2016) and used glow-worms as outgroups. They recovered *Sinopyrophorus* with the elaterid subfamilies Hemiopinae and Oestodinae, but with limited statistical support for many relationships among elaterid subfamilies.

Herein, several thousand orthologs of *S. schimmeli* are used to investigate the evolution of the clade of bioluminescent

elateroid families. Large genomic datasets are proving empirically necessary if the relationships among old lineages can be recovered with some level of confidence (Misof *et al.*, 2014; Kusy *et al.*, 2018a; McKenna *et al.*, 2019). Based on these results, we discuss (i) the origins of bioluminescence in Elateroidea, (ii) loss of the clicking mechanism, and (iii) loss of a fully sclerotized body in most bioluminescent elateroids. This phylogeny will enable further evaluation of the similarity of the luciferase genes and track their evolution (Fallon *et al.*, 2018).

Methods

Genomic data

The genomic DNA of a single male adult of *S. schimmeli* from Yunnan Province (Collecting data: Husa village, 1770 m a.s.l., Longchuan County, 15–25 Jun 2017, coll. Wenxuan Bi) was shotgun-sequenced on the Illumina HiSeq4000 by Novogene Co., Ltd. (Tianjing, China) for 150 bp paired-end reads and ∼30 Gbp of total data (Bi *et al.*, 2019). Raw paired-end reads were filtered using fastp v.0.20.0 (Chen *et al.*, 2018) and low-quality reads were removed. The draft genome was assembled using SPAdes v.3.13.1 (Bankevich *et al.*, 2012), with k-mer sizes of 21, 33, 55, 77 and 99. Contigs were used to train 'Augustus' (Stanke & Waack, 2003) for species-specific gene models with BUSCO v.3 (Waterhouse *et al.*, 2018). Predicted models were used for ab initio gene predictions and protein-coding sequences were used for analyses. Basic statistics of genome assembly were evaluated with QUAST v.5 (Mikheenko *et al.*, 2018). We performed k-mer counts on the filtered data in Jellyfish 2.2.10 using 17 and 21-mer sizes (Marcais & Kingsford, 2011). Based on the distribution of k-mer occurrences, we estimated the genome size using GenomeScope (Vurture *et al.*, 2017).

We compiled the phylogenomic dataset using *Sinopyrophorus* and 42 publicly available transcriptomes or genomes (Poelchau *et al.*, 2014; Sanders & Hall, 2015; Amaral *et al.*, 2017, 2019; Wang *et al.*, 2017; Fallon *et al.*, 2018; Ye *et al.*, 2018; Kusy *et al.*, 2018b, 2019; McKenna *et al.*, 2019) (Table S1). The single-copy ortholog set was collated by searching the OrthoDB v.9.1 database (Zdobnov *et al.*, 2016) (Tables S2, S3). We carried out Orthograph v.0.6.3 searches (Petersen *et al.*, 2017) on assembled transcriptomes and protein-coding gene sets. Terminal stop codons were removed and internal stop codons at the translational and nucleotide levels were masked using the Perl script summarize_orthograph_results.pl (Petersen *et al.*, 2017). The amino acid sequences were aligned using Mafft v.7.407 with the L-INS-i algorithm (Katoh & Standley, 2013). Resulting alignments from each ortholog group were checked for the presence of outliers using the script checker_complete.1.3.1.2.pl and earlier reported methods (Misof *et al.*, 2014; Peters *et al.*, 2017). Outlier sequences were removed from alignments. Then, the non-elateriform taxa were removed. The multiple sequence alignments of nucleotides were generated using Pal2Nal (Suyama *et al.*, 2006) and Aliscore v.2.2 (Misof & Misof, 2009; Kück *et al.*, 2010) was used to identify ambiguous and randomly similar aligned sections. Aliscore was invoked with a custom –r 10^{27} option, with a scoring approach for gap-filled amino acid sites (option -e). After that, we used Alinuc.pl (Misof *et al.*, 2014) to create a list of corresponding codons to be removed from nucleotide alignments. Identified random or ambiguous similarities were masked using ALICUT v.2.3 (Kück *et al.*, 2010). In each gene alignment, the short randomly aligned fragments were replaced with gaps and sequences with ≥80% missing data were removed using Python scripts (Zhang *et al.*, 2020). We used MARE v.0.1.2-rc (Misof *et al.*, 2013) to calculate the information content of each gene partition. Partitions with zero information content were removed.

For the remaining 4199 genes, we used AMAS (Borowiec, 2016) to calculate statistics (alignment length, GC content, number of missing taxa, number of parsimony informative sites) and individual gene alignments were retained for coalescent analyses. The concatenated datasets were generated using FasConCat-G v.1.4 (Kück & Longo, 2014). From the 4199 genes, we generated the datasets A-4199-AA and B-4199-NT (designation: name-number of taxa-amino acid or nucleotide level, Table S4). To reduce the effect of saturation (Breinholt & Kawahara, 2013), we created dataset C-4199-NT12, with third-codon positions excluded. To increase the data decisiveness, we constructed additional supermatrices using only partitions with all 43 species present: datasets D-968-AA and E-968-NT. The degree of missing data and overall completeness scores across all datasets was inspected using AliStat v.1.7 (https://github.com/thomaskf/AliStat).

Compositional heterogeneity tests

To explore the effect of compositional heterogeneity, we inspected the dataset A-4199-AA with BaCoCa v.1.105 (Kück & Struck, 2014). We considered compositional heterogeneity among species in a given partition to be high when the overall RCFV value was ≥0.1 (Fernandez *et al.*, 2016; Vasilikopoulos *et al.*, 2019). Heterogeneous partitions were excluded from the dataset A-4199-AA to generate dataset F-2195-AA. We used Maximum Symmetry Test (Naser-Khdour *et al.*, 2019) to exclude the deviating genes from the dataset B-4199-NT (*P*-value cutoff <0.05) and the dataset G-958-NT contains only partitions that passed the test. The software SymTest v.2.0.49 (https://github.com/ottmi/symtest) was used to calculate the deviation from stationarity, reversibility and homogeneity (Jermiin *et al.*, 2008) (SRH). Heatmaps were generated for all datasets to visualize the pairwise deviations from SRH conditions. To eliminate synonymous signal (Kawahara *et al.*, 2011), we used Degen v.1.4 (Zwick *et al.*, 2012) (http://www.phylotools.com/ptdegendocumentation.htm). All sites with synonymous substitutions were replaced by the corresponding ambiguity codes. The synonymous signal was removed from dataset B-4199-NT and H-4199-DEGEN-NT was generated.

Phylogenetic analyses

Phylogenetic reconstructions were performed using maximum likelihood (ML) criterion with IQ-TREE v.2.0-rc2 (Minh *et al.*, 2020). Model selection for each gene was performed with ModelFinder (Chernomor *et al.*, 2016; Kalyaanamoorthy *et al.*, 2017) using the -MFP option. For amino acid supermatrices, the substitution models LG, DCMUT, JTT, JTTDCMUT, DAYHOFF, WAG, and free rate models LG4X and LG4M were tested and all combinations of rate heterogeneity among sites were allowed (options: -mrate E,I,G,I+G,R -gmedian -merit AICc). We used the edge-linked partitioned model for tree reconstructions (-spp option) allowing each gene to have its own rate. The optimized partition schemes and best-fitting model were inferred for the datasets A-4199-AA, B-4199-NT, D-968-AA, and E-968-NT using -m MFP+MERGE − merit AICc -gmedian options and considering the same substitution models as above. The fast-relaxed clustering algorithm was used (Lanfear *et al.*, 2017). The top 10% of partitions schemes −rclusterf 10 and maximum 10 000 partitions pairs −rcluster-max 10 000 were considered for all datasets except for D-968-AA and E-968-NT, where −rclusterf 10 −rcluster-max 5000 were used. Ultrafast bootstrap (Hoang *et al.*, 2018) and SH-like approximate likelihood ratio test (SH-aLRT) were calculated using options -bb 3000 and -alrt 10 000.

To account for variation among gene trees owing to incomplete lineage sorting and to account for potential gene tree heterogeneity and discordance (Degnan & Rosenberg, 2006; Edwards, 2009; Mirarab *et al.*, 2016), the datasets A-4199-AA, B-4199-NT and C-4199-NT12 were analysed using the coalescent-based species-tree method. For every single-gene partition, we calculated an ML gene tree, with 1000 ultrafast bootstrap replicates (-bb option) and using the same substitution models as earlier. For coalescent species tree estimation, the Accurate Species Tree Algorithm was used [ASTRAL-III v.5.6.3 (Zhang *et al.*, 2018b)]. ASTRAL accuracy is reduced when poorly resolved gene trees are included (Barrow *et al.*, 2018). Therefore, we calculated average ultrafast bootstrap and branch length for every gene tree using an R script (https://github.com/marekborowiec/good_genes/tree_props.R) and reduced gene trees datasets were created. To account for very poorly resolved branches on gene trees, branches with ultra-fast bootstrap ≤10 were collapsed using Newick utilities v.1.6 (Junier & Zdobnov, 2010) in every ASTRAL analysis. Local posterior probabilities (Erfan & Mirarab, 2016) and quartet frequencies of the internal branches in every species tree were calculated using the parameter '-t = 2'. Furthermore, we used DiscoVista v.1.0 (Erfan *et al.*, 2018) to visualise gene-tree quartet frequencies of three topologies around focal internal branches of the inferred ASTRAL nucleotide species tree in the datasets A-4199-AA, B-4199-NT, and C-4199-NT12. Here, the following regularly recovered monophyletic groups were considered: nine elateriformian outgroups, Throscidae, Lycidae, Cantharidae, Elateridae (except Elaterinae), Elaterinae, *Sinopyrophorus*, Lampyridae, Phengodidae, Rhagophthalmidae.

Analyses of alternative relationships

We used Four-cluster Likelihood mapping (Strimmer & von Haeseler, 1997; Misof *et al.*, 2013) (FcLM) analysis to investigate alternative topologies in the datasets A-4199-AA, B-4199-NT and C-4199-NT12. The analysis determines if incongruent or confounding signals are present, which may be obscured in a multispecies phylogenetic tree. The tree-likeness graph for the three possible quartet topologies shows the support for each topology. Additionally, we tested positions of Elaterinae as (i) a sister to clade of *Sinopyrophorus*, Lampyridae, Phengodidae, Rhagophthalmidae (paraphyletic Elateridae) and (ii) a sister to other Elateridae (monophyletic Elateridae) by evaluating which gene partitions of the datasets A-4199-AA and B-4199-NT favour the alternatives. We calculated log-likelihood scores and differences of each pL score for each gene partition using option -wpl in IQ-TREE (Minh *et al.*, 2020).

66-gene dataset

We assembled a dataset from earlier published data (Kusy *et al.*, 2018a,b, 2019; Zhang *et al.*, 2018a) and newly produced homologs (Table S5). Sequences of 84 elateroids and outgroups were used as an input to Orthograph, 66 beetle orthologs were extracted, and aligned at an amino acid level using mafft v.7.394 with the L-INS-i algorithm (Katoh & Standley, 2013). Multiple sequence alignments of nucleotides were generated using Pal2Nal (Suyama *et al.*, 2006) to produce datasets J-66-NT and K-66-AA. We then manually checked all alignments for the presence of outliers and alignment errors. The matrices for both amino acid and corresponding nucleotide alignments were generated using FasConCat-G v.1 (Kück & Longo, 2014). The software SymTest v.2.0.49 (https://github.com/ottmi/symtest) was used to calculate the overall SRH deviation. The fully partitioned reconstructions were performed using the ML criterion with IQ-TREE. Full methods and the complete list of analyses are provided in File S1.

Results

Newly generated shotgun DNA reads of *S. schimmeli* produced a genome assembly with ∼190x read coverage (Fig S34A). Despite fragmentation, the assembly contained a sufficient amount of information for ortholog extraction and downstream phylogenomic analyses (Table S3). The genome completeness and assembly statistics are summarized in Figs S33A, B, S34A. The genome size of *S. schimmeli* was estimated to 171.8 and 190.7 million base pairs (Mbp) (Fig S34B, C) using k-mer sizes 17 and 21, respectively.

Definition and relationships of lineages

Two sets of topologies were produced which differ in the density of sampling and the number of orthologs. The first

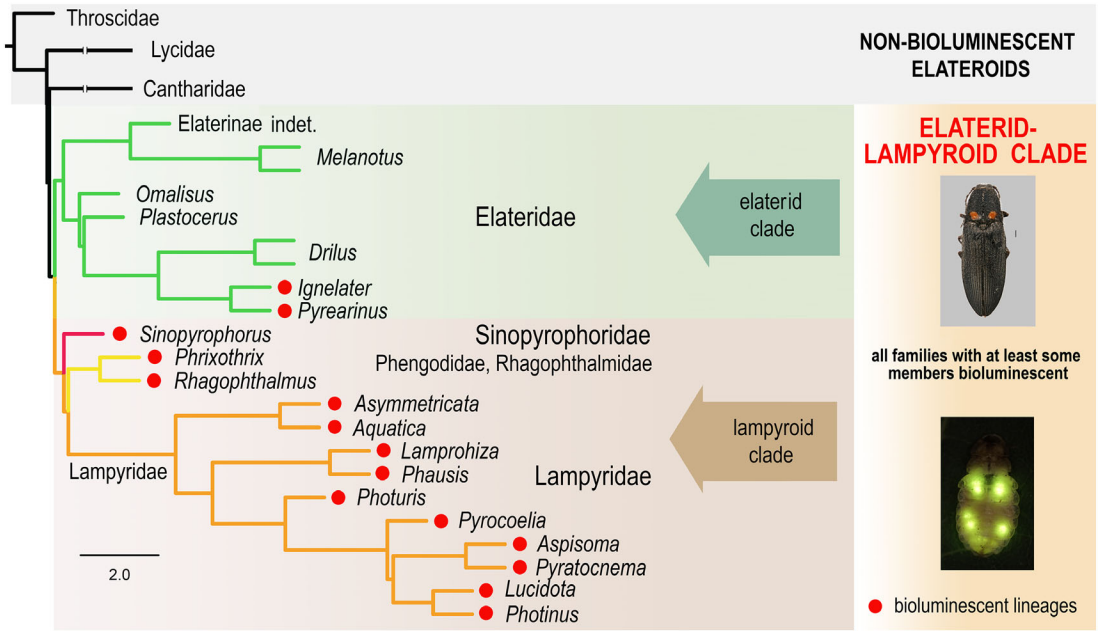

Fig 2. Phylogenetic relationships. The topology recovered by the ASTRAL coalescent phylogenetic method applied to the full set of single-gene trees inferred at nucleotide level from the dataset B-4199-NT; only the elaterid-lampyroid clade shown, see the full trees in Figs S17, S18. [Colour figure can be viewed at wileyonlinelibrary.com].

set was based on 4199 orthologs and 43 taxa, nine of them as outgroups; the topologies were inferred under the ML criterion and the coalescent method by the analyses of amino acids and nucleotides (Fig. 2). The second set was based on the 66-orthologs, 83 elateroids and the topologies were inferred by ML analyses (Figs 3A, B, S2–S22). All analyses recovered a clade containing exclusively the families with at least some taxa that produce light, that is, Elateridae (~2% of taxa bioluminescent, in three subfamilies), Sinopyrophoridae (*Sinopyrophorus*, 1 sp., bioluminescent in an adult stage, larvae unknown), Phengodidae, Rhagophthalmidae and Lampyridae (all bioluminescent at least in the larval stage, except Phengodidae: Cydistinae for which larvae and females are unknown and males are non-luminescent). Hereafter, these widely accepted families and *Sinopyrophorus* are designated as the 'elaterid-lampyroid clade' (Figs 2–4).

Sinopyrophorus was found outside of Elateridae and as a sister to Lampyridae, Phengodidae and Rhagophthalmidae. The topology was stable regardless of the dataset and inference method employed (Figs 2–3, S2–S22). Its position was also supported by the FcLM analysis (Figs 5A, B, S25, Table S6) and alternative topologies were rejected by an approximately unbiased test (AU test, Table S7). All phylogenomic analyses indicate that phenotypically elaterid-like *Sinopyrophorus* (originally Elateridae: Sinopyrophorinae, Fig. 6) and the here redefined Elateridae do not share a common exclusive ancestor and form a serial paraphylum towards the branch of Lampyridae, Rhagophthalmidae and Phengodidae. The clade of *Sinopyrophorus* and the soft-bodied bioluminescent families is here designated as the 'lampyroid clade'.

In contrast to the relatively well-supported lampyroid clade, we found confounding signal for some relationships among elaterid lineages (Figs 2, 3, 5B, D). Most analyses of genomic datasets suggested the paraphyly of Elateridae with Elaterinae rooted as a sister to the lampyroid clade and other elaterids as a sister to both of them (Figs S2–33). Such a result was inferred from all ML analyses and the coalescent analysis of the amino acid data, but not by the coalescent method applied to the nucleotide dataset (Fig. 2). The distribution of the signal for alternative topologies prefers the monophyly of Elateridae. The FcLM analysis of the nucleotide dataset B-4199-NT returned 58.7% for the monophyly of Elateridae versus 39.6% for their paraphyly (Figs 5B, S25). Similarly, the DiscoVista relative frequency analysis of the B-4199-NT dataset (node 9; Fig. 5D) preferred the monophyly of Elateridae, but paraphyly is supported by the datasets A-4199-AA and C-4199-NT12 (Figs S23, 24). The 66-gene amino acid dataset suggests Elateridae without *Sinopyrophorus* as a serial paraphylum of three elaterid groups to the lampyroid clade (Fig. 3A), but the same dataset produced a monophyletic Elateridae at the nucleotide level (Fig. 3B).

With regard to the positions of the clicking and non-clicking forms in the elaterid-lampyroid clade, our analyses always recovered the clicking forms as the earliest splits. Bioluminescent taxa are always recovered in a derived position within the elaterid-lampyroid clade, regardless of monophyly or paraphyly of click beetles inferred from various analyses (Figs 2–3), that is, the most recent common ancestor of the elaterid-lampyroid clade was clicking and non-bioluminescent. All topologies and additional information on data are shown in Figs S2–S34.

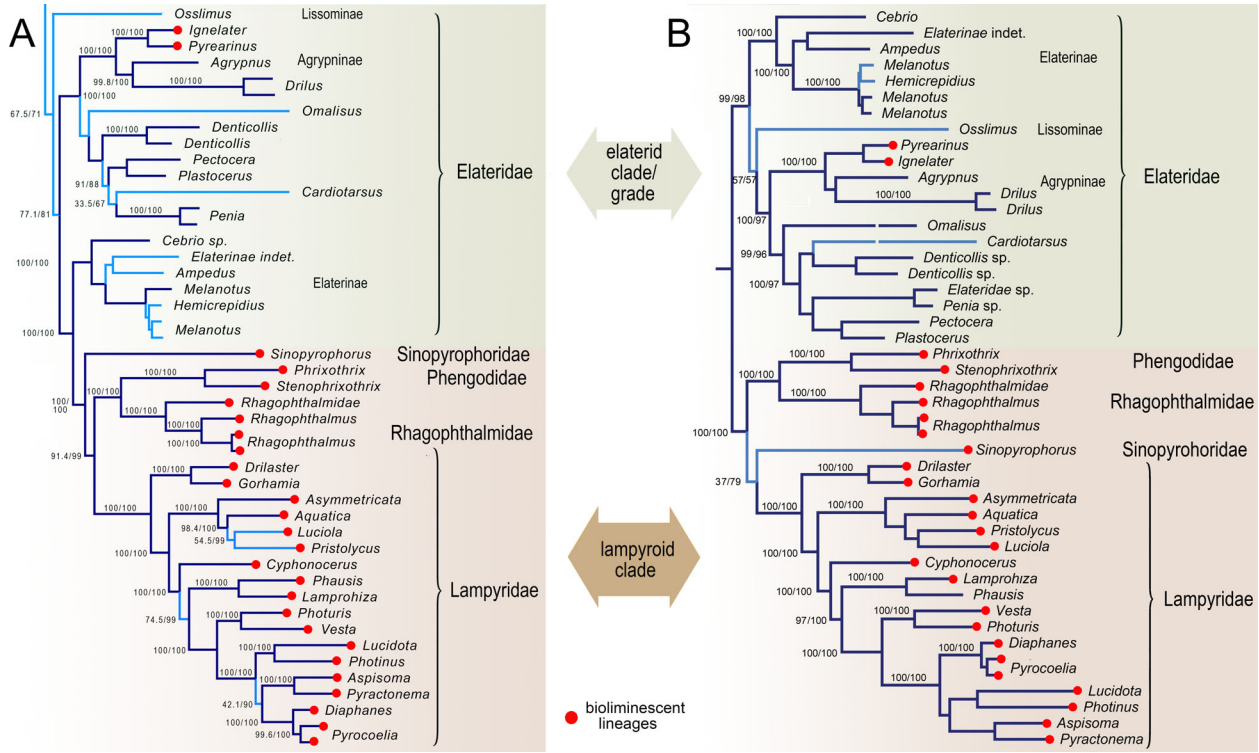

Fig 3. (A) The topology recovered by the maximum likelihood analysis of the 66-gene dataset J-66-AA at the amino acid level. (B) The topology recovered by the maximum likelihood analysis of the 66-gene dataset J-66-NT at the nucleotide level. The dark blue branches are significantly supported (both SH-aLRT >80% and UFboot >95%). [Colour figure can be viewed at wileyonlinelibrary.com].

Taxonomy

Sinopyrophoridae Bi & Li, 2019, new status

Sinopyrophorinae Bi & Li, 2019 in Bi *et al.*, 2019: 83.

Type genus: *Sinopyrophorus* Bi & Li, 2019 in Bi *et al.*, 2019: 89.

= *Sinopyrophorus* He *et al.*, 2019: 565, unavailable name due to the absence of a description in the study where the name was proposed (International Committee for Zoological Nomenclature, 1999).

Based on the recovered relationships, *Sinopyrophorus* (earlier Elateridae: Sinopyrophorinae) cannot be a member of Elateridae. Despite its morphological similarity and a shared clicking mechanism, its phylogenetic position requires it to be classified as a separate taxon of the same rank as true click beetles, that is, family. The proposed rank fulfils the requirement of the reciprocal monophyly of all taxa and, simultaneously, keeps traditionally recognized families valid.

Discussion

Phylogenomic relationships

The uncertain homology of characters in phenotypically disparate elateroids, ambiguities in length variable alignments and low information content in the Sanger data have produced contradicting phylogenies (Bocakova *et al.*, 2007; Sagegami-Oba *et al.*, 2007; Lawrence *et al.*, 2011; Kundrata *et al.*, 2014; McKenna *et al.*, 2015). Here, we present analyses based on more than 4000 orthologs that support three principal relationships that have not been well supported in earlier studies:

1 The monophyly of the elaterid-lampyroid clade (Figs 2–4; Timmermans *et al.*, 2010; Amaral *et al.*, 2016; Bocak *et al.*, 2016; Kusy *et al.*, 2018b; McKenna *et al.*, 2019) is preferred over alternative hypotheses (Bocakova *et al.*, 2007; Sagegami-Oba *et al.*, 2007; Kundrata *et al.*, 2014; McKenna *et al.*, 2015; Zhang *et al.*, 2018a).
2 We prefer the monophyly of redefined click beetles, that is, including Elaterinae and Lissominae without *Sinopyrophorus*, as suggested by the B-4199-NT dataset and the coalescent method, the J-66-NT dataset and ML methods, and as additionally supported by the FcLM analyses, DiscoVista analyses and AU test (Figs 2, 3B, 5B, D, S20, S22B, S23–S26; Table S7) over a paraphyletic Elateridae obtained from all ML analyses of the genomic datasets, from the coalescent method analyses of C-4199-NT12/A-4199-AA datasets, and the ML analysis of the I-66-AA dataset (Figs 3A, S2–S19, S21, S22A). The paraphyletic or polyphyletic Elateridae were recovered in earlier estimates where datasets representing all Coleoptera were analysed (McKenna

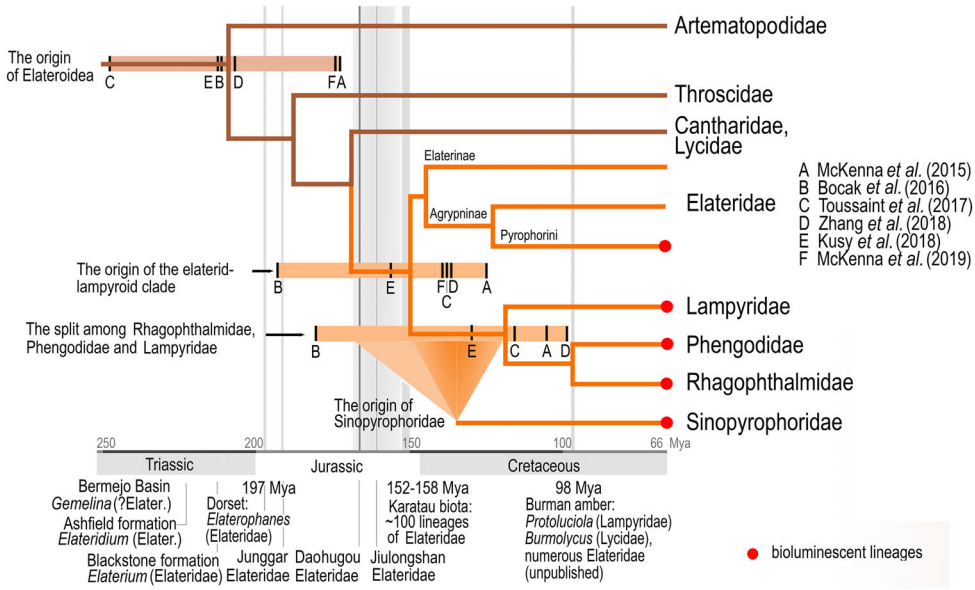

Fig 4. The summary cladogram of Elateroidea with bars showing the distribution of estimated origin of Elateroidea, the earliest split within the bioluminescent clade, and the splits within the [Lampyridae (Rhagophthalmidae, Phengodidae)] clade. The origin of Sinopyrophoridae is supposed after the origin of the bioluminescent clade and before the earliest split between Lampyridae and Rhagophthalmidae + Phengodidae. [Colour figure can be viewed at wileyonlinelibrary.com].

et al., 2015, 2019; Zhang *et al.*, 2018a). In this case, only a slight majority of genes recover the monophyly of click beetles over their paraphyly (Figs 5B, S26). Generally, the amino acid and nucleotide first + second codon position sequences are highly conservative when the set of orthologs for whole Coleoptera is assembled and possibly, due to incomplete lineage sorting, the analyses of these datasets do not support the monophyly of click beetles is analysed using the maximum likelihood approach (Figs 3A, S2–16). The ASTRAL coalescent phylogenetic method applied to the full set of gene trees inferred the monophyly of Elateridae from the dataset B-4199-NT at the nucleotide level (Fig. 2). Additionally, the FcLM and DiscoVista relative frequency analyses of the dataset B-4199-NT support, albeit weakly, the monophyly of Elateridae (Figs 5B, D, S24, 25). Similarly, the 66-gene dataset is based on highly conservative genes and Elateridae are split into three serial groups of the lampyroid clade if amino acids are analysed (Figs 3A, S17), but the family is monophyletic when the tree is inferred with the maximum likelihood analysis at the nucleotide level (Figs 3B, S18). The earlier 99-gene (among them the here employed 66 orthologs) analysis of the whole Coleoptera by Zhang *et al.* (2018a) suggested the polyphyly of click beetles when Lissominae was the sister to net-winged beetles and the rest of Elateridae formed two serial sister groups to the lampyroid families. We are aware that further analyses of a much larger dataset will be needed to test the monophyly of Elateridae but, based on the current results, we prefer to accept the monophyly of Elateridae. Although the genome-scale data offer a powerful tool for phylogenetics, the relationships of elaterid subfamilies remain poorly supported and need rigorous testing with a dense sampling of taxa and a higher number of orthologs specifically designed for this question.

3 Sinopyrophoridae is decisively placed as a sister to Lampyridae, Phengodidae and Rhagophthalmidae (Figs 2, 3A, S2–S25), or as a sister to Lampyridae (Fig. 3B) rather than a member of the click beetles (Bi *et al.*, 2019). Therefore, we propose to accept *Sinopyrophorus* as a member of the 'lampyroid clade', that is, the ancient lineage closely related to fireflies and glow-worms (Figs 2, 3, S2–S26). The elaterid-like morphology of *Sinopyrophorus* supports the idea that the characteristic well-sclerotized body form and clicking escape mechanism were abandoned multiple times during the evolution of Elateroidea. Here, a single shift to incomplete sclerotization in the common ancestor of soft-bodied fireflies and glow-worms was recovered by all analyses (Figs 2, 3). Such a process was earlier inferred for drilids and omalisids now included in Elateridae (Kundrata *et al.*, 2014; Kusy *et al.*, 2018a). The clicking mechanism is unique, relatively complex and we can assume that it evolved once if the probabilities of the origin and the loss of the clicking mechanism are substantially different (Trueman *et al.*, 2004).

Formal classification

The elevation of Sinopyrophoridae as a separate family is our preferred, but not a single possible solution of the formal classification. Our decision is conservative in the sense that it keeps the family rank for all earlier designated reciprocally monophyletic lineages, that is, click beetles, fireflies, glow-worms and

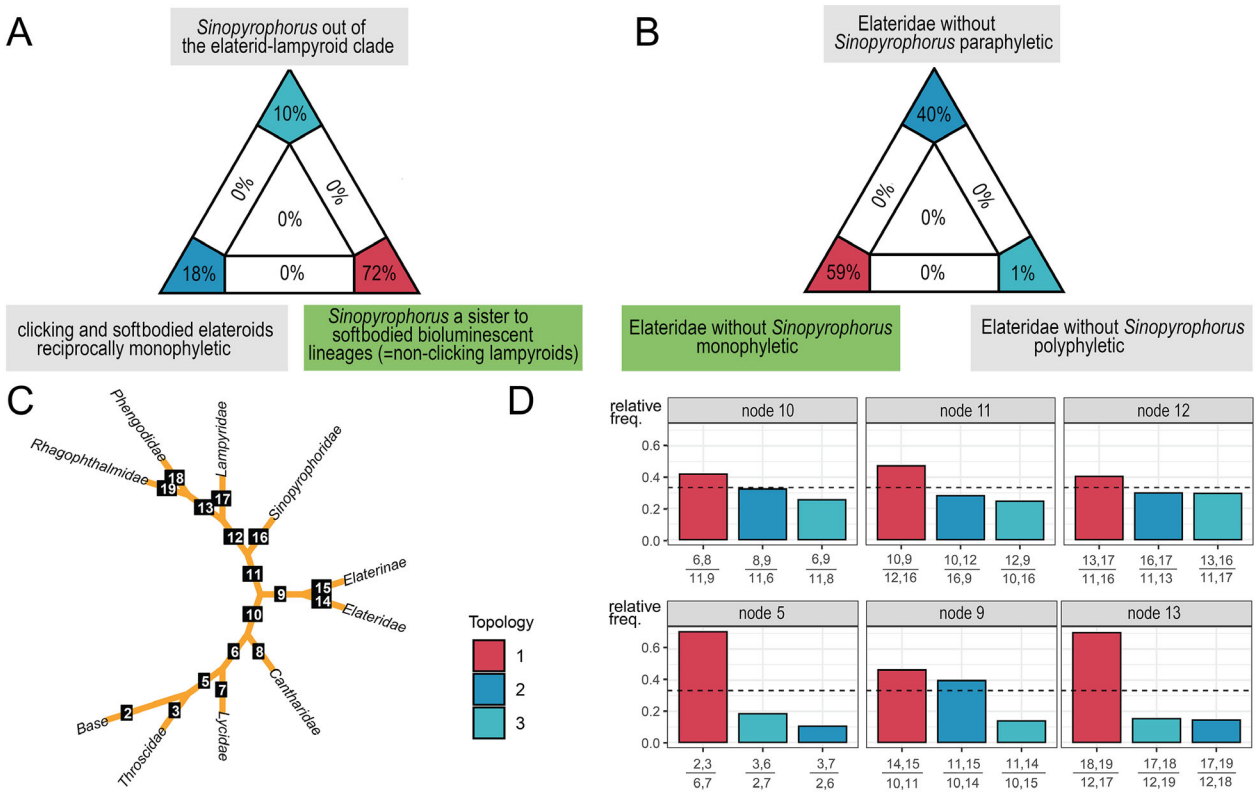

Fig 5. Four cluster Likelihood Mapping tests of the selected phylogenetic hypothesis applied at the nucleotide level of the dataset B-4199-NT. (A) The test of the position of *Sinopyrophorus*. (B) The test of the monophyly of Elateridae. (C) Evaluated topology. (D) DiscoVista relative frequency analyses of the dataset B-4199-NT. [Colour figure can be viewed at wileyonlinelibrary.com].

railroad-worm beetles. There are several alternative solutions. We could merge Sinopyrophoridae, Phengodidae, Rhagophthalmidae and Lampyridae under a single family, that is, the here recovered lampyroid clade will be called Lampyridae Latreille, and contain five subfamilies. Lampyridae in a new wide sense would contain several morphologically distinct lineages with differing natural history and the whole internal classification of fireflies would be down-ranked. The formal requirement for the monophyly of all named taxa would also be fulfilled by the redefinition of Elateridae sensu lato that would contain eight traditional families Elateridae, Drilidae, Omalisidae, Plastoceridae, Lampyridae, Phengodidae, Rhagophthalmidae and Sinopyrophorinae. (Kusy *et al.*, 2018a,b; Bi *et al.*, 2019). Considering also the logical extreme, we could accept whole Elateroidea as a single family (*sans* the artemotopodid clade) as recently mentioned by Muona & Tarävainen (2020). The clade would be defined by the clicking mechanism that was secondarily lost in half of its members. Nevertheless, for convenience, we prefer to keep the family status for all traditional, widely accepted families whenever possible. Click beetles and fireflies are known to non-specialists and they are intuitively distinguished also by the general public, for example, naturalist involved in the Firefly Citizen Science Project by the Natural History Museum of Utah among others. Therefore, the family rank is appropriate for their separation and the expansion of fireflies to a heterogeneous assemblage of biologically and morphologically disparate forms would not be practical.

Origins of the bioluminescence

Our analyses indicate a unique origin of bioluminescence in the common ancestor of the clade Sinopyrophoridae, Rhagophthalmidae, Phengodidae and Lampyridae clade and further independent origin of bioluminescence within click beetles as defined here, that is, without *Sinopyrophorus*. The relatively distant position of bioluminescent taxa in the formal classification was confirmed by the earliest molecular phylogenies (Bocakova *et al.*, 2007; Sagegami-Oba *et al.*, 2007) and has been inferred due to the genetic structure around the luciferase genes from the genomes of *Photinus*, *Aquatica* and *Ignelater* (Fallon *et al.*, 2018). The position of photic organs is variable in adult click beetles: prothoracic spots are reported in *Balgus*, *Campyloxenus*, and Pyrophorini: Nyctophyxina, the prothoracic and abdominal organs in Pyrophorini: Hapsodrilina and most Pyrophorina, and only the abdominal photic organs in the pyrophorine genus *Hifo* (Costa, 1975, 1984). Unlike these, only abdominal photic organs are shared by all adult bioluminescent forms in the lampyroid clade (Branham & Wenzel, 2003; Bi *et al.*, 2019).

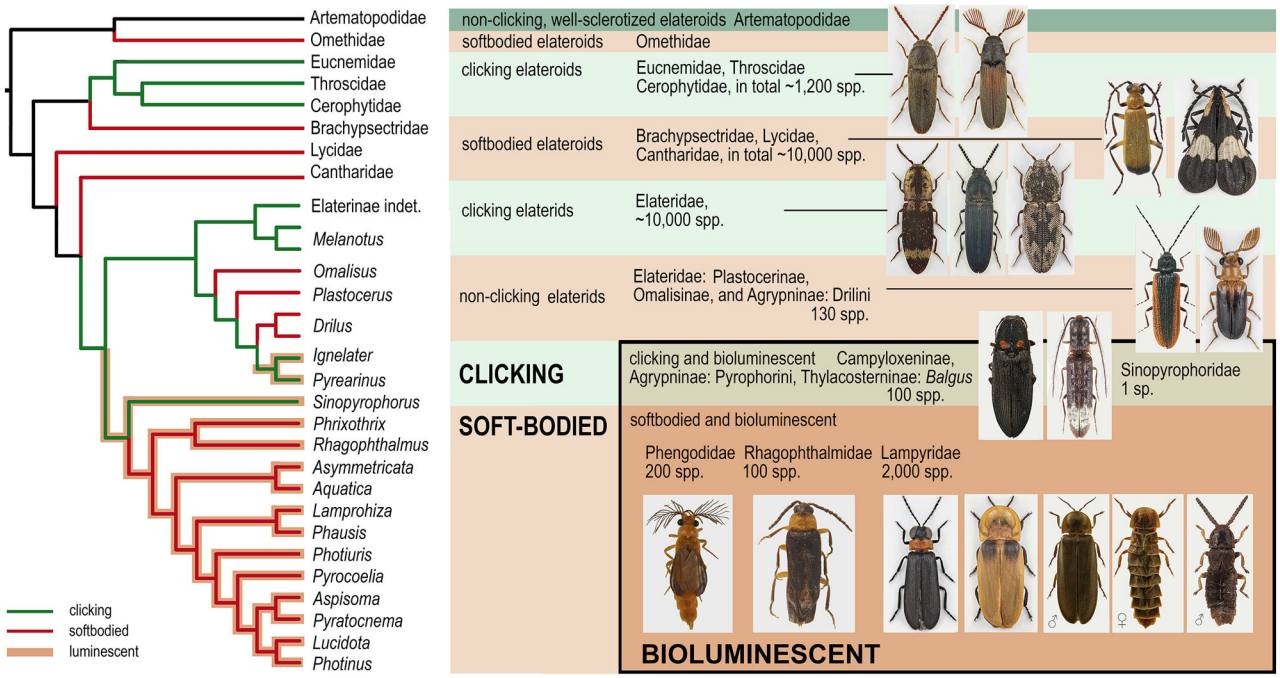

Fig 6. The summary of Elateroidea relationships with the distribution of clicking, soft-bodied, and bioluminescent forms. Photographs J. Klváček and authors. [Colour figure can be viewed at wileyonlinelibrary.com].

About 2300 species of the lampyroid clade inherited bioluminescence from their most recent common ancestor and we must suppose that if this ancestor was a fully sclerotized elaterid-like beetle, the origin of bioluminescence preceded the shift to being soft-bodied (Fig. 6). Among bioluminescent groups, only lampyrids are species-rich today (~2000 spp.) and the second largest bioluminescent clade are glow-worms (>200 spp.). Both are common in the Neotropical region along with bioluminescent elaterids (>100 spp.) (Costa, 1975, 1984). The effectiveness of bioluminescence as an aposematic signal depends on the number of taxa and individuals sharing the signal. As flashing can serve as an isolating mechanism (Branham & Wenzel, 2003), the intensive interactions among bioluminescent elateroids might have played a role in the high diversity of extant fireflies, glow-worms and luminous click beetles in the Neotropical region (Ellis & Oakley, 2016).

Since South America is a major epicentre for bioluminescent species, the region has been hypothesized as the ancestral region for the diversification of fireflies (Amaral *et al.*, 2016). The sister group position of the elaterid-like East Asian Sinopyrophoridae (Figs 2, 3), the predominantly Laurasian distribution of two of the deepest subfamilies of fireflies, Ototretinae and Luciolinae (Kundrata *et al.*, 2014; Martin *et al.*, 2017, 2019; Zhang *et al.*, 2018a) (Fig. 3), Southeast Asian Rhagophthalmidae, and the presence of Phengodidae in Burmese amber (unpublished data) suggest as an alternative hypothesis that the early diversification of the bioluminescent lineages took place in eastern Laurasia and was followed by subsequent intensive radiations of fireflies in the Neotropical region.

Bioluminescence is scattered among some terminal lineages of click beetles, each of them being fully sclerotized and with a clicking mechanism. Further investigation of the origins of bioluminescent elaterids will need denser sampling of all bioluminescent genera, including as many species as possible. Nevertheless, we support the earlier proposed hypothesis that elaterid photic organs evolved several times (Bocakova *et al.*, 2007; Sagegami-Oba *et al.*, 2007; Fallon *et al.*, 2018). The known examples of elaterid bioluminescence are placed in three different subfamilies (Costa, 1975, 1984), and a non-luminescent most recent common ancestor and sister taxa have already been recovered for *Balgus* (Thylacosterninae) and Pyrophorini (Agrypninae) (Bocakova *et al.*, 2007; Kundrata *et al.*, 2014).

How old is elateroid bioluminescence?

We do not attempt a formal dating analysis as our data partly overlap with the datasets used earlier for the analyses of all beetles with multiple fossil calibration points (McKenna *et al.*, 2015, 2019; Bocak *et al.*, 2016; Toussaint *et al.*, 2017; Kusy *et al.*, 2018a; Zhang *et al.*, 2018a). Most calibration points would not be available if we attempt an analysis restricted only to elateroids. Previous studies and the elateroid fossil records already serve as a guide to estimating of the origins of both bioluminescence and Sinopyrophoridae. The recovered topologies place *Sinopyrophorus* between the first split of the elaterid-lampyroid clade and the deepest split between soft-bodied bioluminescent fireflies, railroad and glow-worms (Figs 2, 3). Most published analyses dated the earliest split

within the elaterid-lampyroid clade to the lower Cretaceous periods, ∼135 Ma (Toussaint *et al.*, 2017; Zhang *et al.*, 2018a; McKenna *et al.*, 2019) (Ma; Fig. 4). More shallow estimations of 115–125 Ma (McKenna *et al.*, 2015; Fallon *et al.*, 2018), as well as a deeper one at 165 Ma (Kusy *et al.*, 2018a,b) are also hypothesized. The estimations for the earliest split between the fireflies and glow-worm clade are similarly inconclusive, at ∼98 Ma (Zhang *et al.*, 2018a), ∼122 Ma (Martin *et al.*, 2019), and ∼140 Ma (Kusy *et al.*, 2018a,b), but the presence of a lucioline firefly in Burman amber supports older dates (Kazantsev, 2015). If we consider median estimations, the elaterid-lampyroid clade would have originated in the lower Cretaceous and the subsequent split of the Lampyridae, Rhagophthalmidae and Phengodidae clade in the mid-Cretaceous. As a result, the ancestor of Sinopyrophoridae had to have split from their closest relative ∼120 Ma (Fig. 4). That period must also be considered as a moment when bioluminescence evolved in Elateroidea for the first time.

Dating analyses using different taxon sampling, analysed markers, applied models and calibrations often come to different age estimates (Fig. 4). Some studies recovered the early origins of the bioluminescent elateroids (Bocak *et al.*, 2016; Kusy *et al.*, 2018a) and we suggest these should be inspected as a possibility. The shallower estimates (McKenna *et al.*, 2015, 2019; Toussaint *et al.*, 2017; Fallon *et al.*, 2018; Zhang *et al.*, 2018a) set the origin of the elaterid-lampyroid clade of which click beetles is a principal branch to 115–140 Ma in contrast with the upper Jurassic 152–158 Ma old Karatau deposits contain a rich click beetle fauna (Doludenko *et al.*, 1990). Additionally, *Elaterophanes* (Whalley, 1985) was assigned to Elateridae, and even if it is not a true click beetle but an ancestral non-artematopodid elateroid lineage, its age is older than some estimates of the origin of Elateroidea (Fig. 4). Similarly, false click beetles, Eucnemidae, were reported from the lower Jurassic Xiwan deposits (Lin, 1986) and multiple species are known also from the upper Jurassic–lower Cretaceous period (Chang *et al.*, 2011). These fossils also support quite an early origin of the Elateroidea. Consequently, an earlier origin of bioluminescence is also possible.

Conclusion

The phylogenetic studies dealing with Elateroidea produce conflicting results (Bocakova *et al.*, 2007; Sagegami-Oba *et al.*, 2007; Lawrence *et al.*, 2011) and similarly, the placement of *Sinopyrophorus* as a terminal clade within Elateridae is ambiguous (Bi *et al.*, 2019; He *et al.*, 2019). Phylogenomic data presented here provide strong evidence that Sinopyrophoridae, **stat.n.**, is the sister group of fireflies, railroad and glow-worm beetles, despite its morphological similarity to the extant click beetles (Fig. 6). Our finding suggests that lampyrids and their closest relatives are in fact modified click beetles. The definition of the Sinopyrophoridae+Phengodidae+Rhagophthalmidae+Lampyridae clade supports an early origin of bioluminescence in their common clicking ancestor, likely ∼120 Ma, in the lower Cretaceous, the delayed shift to being soft-bodied and further radiation and signal diversification in the lineages which already used some form of luminescence.

Author contributions

DK analysed genomic data, carried out sequence alignments and phylogenetic analyses, XYL and JWH produced genomic data, MM and JWH participated in analyses, all co-authors contributed to the draft of the manuscript; WXB collected the specimen, DK, LB, LP, and SB conceived and designed the study, XYL and LB coordinated the study, LB, DK, SB, XYL and JWH drafted the manuscript. All authors commented the drafts and gave final approval for publication.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Classification of Elateroidea: an overview of hypothesized phylogenies.

Figure S1. An overview of Elateroidea topologies recovered by earlier studies.

Figure S2–S22. Topologies recovered by individual analyses.

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Data availability statement

The data that support the findings of this study are openly available in GenBank, accession number PRJNA64573 and the datasets are openly available in the Mendeley Data repository at https://www.doi.org/10.17632/g4xkckycph.1.

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PART III

Studies on neoteny in Coleoptera: phylogenomics, classification, and evolutionary interactions

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Mitogenomic data elucidate the phylogeny and evolution of life strategies in Dermestidae (Coleoptera).

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Mitogenomic data elucidate the phylogeny and evolution of life strategies in Dermestidae (Coleoptera)

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Abstract. Dermestidae (Bostrichoidea) exploit diverse food sources including fungal mycelia, but notably they as saprophagous, feeding on decomposing and dried flesh and keratin of animals and plants. Some of them live in spider webs, vertebrate and social insect nests, while others cause damage in human dwellings. Here, we use mitogenomics to reconstruct their phylogeny and evolution of life history strategies. We recovered serial splits of Orphilinae, Thorictinae + Dermestinae, Attageninae, Trinodinae and Megatominae, and we dated the origins of all subfamilies between the Middle Jurassic and Upper Cretaceous. Extant genera started their diversification in the Middle Cretaceous, except for *Dermestes* that originated in the Eocene. Mycetophagy, the likely feeding style of the common ancestor with Endecatomidae, was retained only by Orphilinae. Since the Late Jurassic, most dermestids have been saprophagous with the preference for desiccated tissue. We infer a scenario of feeding preferences from mycetophagy moving to saprophagy, always depending on food with low water content, followed by the shift from cryptic life in crevices and wood, to commensalism with social Hymenoptera, and ultimately feeding on angiosperm pollen as adults. The dependence on spider larders evolved already in the Early Cretaceous, but lineages with this specialized strategy remained species-poor. We date the origin of exploitation of vertebrate carcasses to the Eocene when modern mammalian fauna became dominant. The diversification of Megatominae (62% of known dermestids) and *Attagenus* Latreille (17%) coincides with the radiation of angiosperms.

Introduction

Dermestidae (skin, larder, hide, leather, carpet, and khapra beetles) are among a few coleopterous families widely known to the public due to their occasional presence in homes where they pose a hygiene problem, damage wool fabrics and furs, and infest stored products (Battisti et al., 2011; Hussain et al., 2019). Some species are well known to curators of natural history collections, forensic entomologists, industrial storage space keepers and disinsection specialists (Anton et al., 2011; Querner, 2015). Flower visiting species are commonly noticed in nature as they often occur *en masse*. In contrast to anthophagous groups, some species escape attention due to their cryptic life in rotten wood, under bark, tree hollows, rock crevices and nests of eusocial insects (Figure 1a–o; Zhantiev, 2009).

Unlike many beetle families, the biology of dermestid larvae is well known due to their economic importance and propensity for breeding under laboratory conditions. Almost all species feed on materials with very low water content and this trait is shared with other bostrichoid families possibly due to a unique

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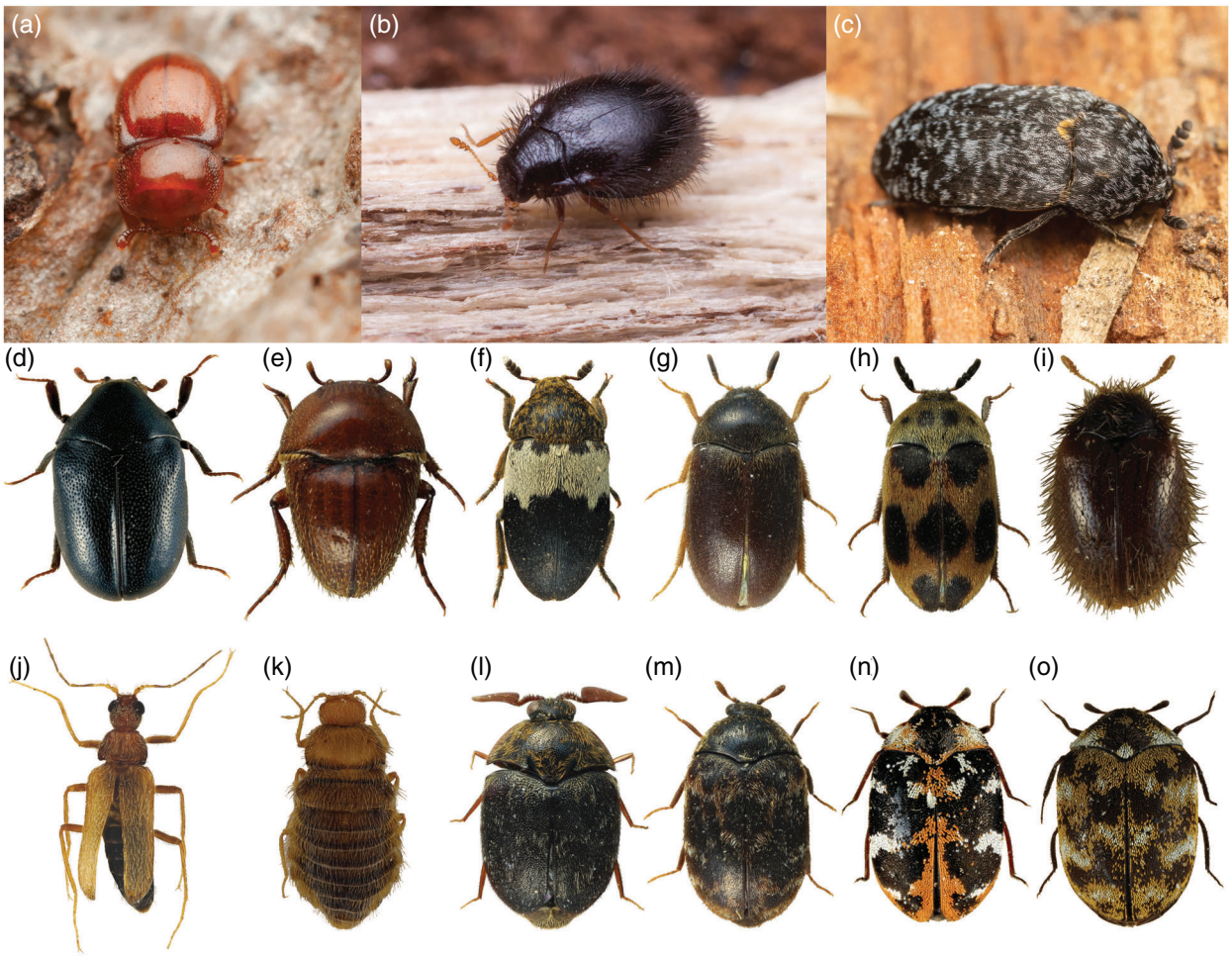


Fig. 1. The morphological diversity of Dermestidae (a) *Thorictus canariensis* Wollaston; (b) *Trinodes hirtus* (F.); (c) *Dermestes murinus* L.; (d) *Orphilus niger* (Rossi), Orphilinae; (e) *Thorictus castaneus* Germar, Thorictinae; (f) *Dermestes elegans* Gebler, Dermestinae; (g) *Attagenus unicolor* (Brahm); (h) *Attagenus suspiciosus* Solsky, both Attageninae; (i) *Trinodes hirtus* (F.); (j) *Thylodrias contractus* (Motschulsky), male; (k) ditto, female; all Trinodinae; (l) *Thaumaglossa rufocapillata* Redtenbacher; (m) *Trogoderma versicolor* (Creutze); (n) *Anthrenus scrophulariae* (L.); (o) *Attagenus verbasci* (L.), all Megatominae. Photos a–c by P. Krásenský, d–o by M. Motyka

cryptonephridial system (Saini, 1964). Despite this shared trait, life histories vary greatly in this relatively small family of 1721 described species and 61 genera (Table 1; Háva, 2015, 2021). Many dermestids predominantly feed on desiccated insect bodies. *Dermestes* L. feed on animal carcasses and some Megatominae: Anthrenini and Attageninae are among only a very small number of insect lineages able to feed on keratin. Thorictinae and a few Megatominae are commensals in ant nests, trinodines feed on the remnants of insect bodies in spider webs, while Orphilinae are mycetophagous. Adults live together with larvae in the case of Thorictinae, Trinodinae and Dermestinae, while Orphilinae, Megatominae and Attageninae have anthophagous adults. The most recent compilation of bionomical data, including many original observations, provides life history information for 90 species (Zhantiev, 2009), and additional studies have reported biological observations on individual taxa (Háva et al., 2021; Kadej et al., 2013; Lenoir et al., 2013).

Our understanding of the evolution of Dermestidae is hampered by conflicting phylogenetic hypotheses (Table S1). Recent analyses considered ecological and morphological data (Kiselyova & Mchugh, 2006; Lawrence & Slipinski, 2005; Zhantiev, 2000, 2009). Several studies have been based on dense sampling and a high number of morphological characters of larvae and adults. However, each character system indicated slightly different relationships and the latest classification contains some ambiguously placed taxa. Depending on the analysis, the Orphilinae has been placed either as sister to other dermestids, as sister to Megatominae + Trinodinae, or altogether more distantly related to the other Dermestidae (Kiselyova & Mchugh, 2006; Lawrence & Slipinski, 2005; Lawrence et al., 2011; Zhantiev, 2009). The subfamily rank for Thorictinae as the next serial sister after Orphilinae and their placement in Dermestinae are alternatively supported by the analyses of larval and adult characters (Kiselyova & Mchugh, 2006; Lawrence & Slipinski, 2005). Attageninae,

Table 1. An overview of the classification and diversity of Dermestidae (diversity data summarized from Háva, 2021); aextinct.

Subfamily tribe	# of genera/species
Orphilinae	2/14
Orphilini	1/7
Ranolini	1/7
Thorictinae	3/190
Thorictini	3/185
Thaumaphrastini	1/5
Dermestinae	6/95
Dermestini	3/91
Marioutini	2/3
[a]Paradermestini	1/1
Attageninae	14/295
Apphianini	1/1
Attagenini	10/290
Egidyellini	1/2
[a]Cretodermestini	1/1
[a]Eckfeldattagenini	1/1
Trinodinae	7/67
Thylodriini	4/7
Trinodini	4/55
Trinoparvini	1/4
[a]Cretonodini	1/1
Megatominae	32/1064
Anthrenini	2/278
Megatomini	30/786

[a]extinct.

recently elevated to subfamily rank, were inferred either as sister of Dermestinae or as a more deeply rooted clade (Kiselyova & Mchugh, 2006; Lawrence & Slipinski, 2005). The dermestid classification currently recognizes six dermestid subfamilies (Orphilinae, Thorictinae, Dermestinae, Attageninae, Trinodinae and Megatominae) whose relationships was recovered by weighted parsimony analysis of larval characters (Kiselyova & Mchugh, 2006).

No densely sampled DNA dataset has been assembled to specifically study the phylogeny of Dermestidae. Existing phylogenetic studies of Coleoptera at the whole order-level contained a limited number of dermestid taxa, but these analyses did not specifically address intrafamily relationships. Bocak et al.'s (2014) tree of some 8000 taxa of Coleoptera suggested an early branching of Orphilinae and serial splits of Dermestinae, Trinodinae, Attageninae and Megatominae. Multimarker and phylogenomic studies included only three taxa and supported Orphilinae as sister of Dermestinae + Megatominae (McKenna et al., 2015, 2019) or Trinodinae as sister to Dermestinae + Megatominae (Zhang et al., 2018). Recently, a mitogenomic analysis by Zeng et al. (2021) using 11 samples, including six species of *Dermestes*, proved inconclusive. All estimations of divergence times using molecular data for Coleoptera have provided several estimations for Dermestidae. All analyses supported an ancient split from other Bostrichoidea. The youngest age estimation placed the separation of Dermestidae and the Ptinidae/Bostrichidae clade at 180 Ma and the earliest dermestid crown age at 155 Ma (McKenna et al., 2015). A reanalysis of the dataset pushed these dates slightly deeper to 192 and 172 Ma, respectively (Toussaint et al., 2016). Other studies provided even older estimates of the Bostrichidae/Dermestidae split at 205 Ma (Bocak et al., 2016) or 220 Ma (McKenna et al., 2019).

Information on fossil Dermestidae is invaluable for understanding their evolution. The oldest fossilized specimens assigned to Dermestidae were reported from Late Triassic and Early Jurassic deposits (Dunstan, 1923). These taxa are preserved as unattached elytra and thus are difficult to interpret (Háva & Prokop, 2004). Therefore, no subfamily or tribe rank has been proposed for them. The oldest well-preserved fossil of a dermestid was reported from the Middle Jurassic Jiulongshan Formation and the specimen was placed in Dermestinae (Deng et al., 2017a). Kirejtshuk et al. (2009) described Lower Cretaceous Cretonodini (Dermestinae, Table 1). Furthermore, undisputable dermestid beetles were reported from Burmese amber and their descriptions have substantially increased the volume of our knowledge. The tribe Cretodermestini was proposed based on these specimens and placed in Attageninae (Deng et al., 2017b), while other inclusions were assigned to extinct *Cretoattagenus* Deng et al. and the extant genera *Attagenus* Latreille and *Megatoma* Herbst (Attageninae and Megatominae; Cai et al., 2017; Deng et al., 2017b; Háva, 2020; Háva & Damgaard, 2017). The Tertiary fauna is highly similar to extant dermestids, and individuals trapped in Dominican and Baltic amber have been placed in 13 extant genera (Háva & Prokop, 2004; Háva & Wappler, 2014; Table S2).

The importance of dermestids for human activities makes the lineage worth of detailed study, and we present phylogenetic analyses of the first densely sampled molecular dataset of Dermestidae. We present a robust mitogenomic, dated phylogenetic tree that will serve as a framework for the investigation of the evolution of life strategies. We specifically test hypotheses such as when this bostrichiform lineage underwent life history changes from the presumed ancestral mycetophagy, origins of feeding on dry tissue of dead vertebrates and insects, and whether their diversification can be linked to the rapid evolution of flowering plants, which provide nutrition for many lineages in the adult stage.

Material and methods

Laboratory procedures

All specimens were collected in 96% ethanol and genomic DNA was extracted using the DNeasy tissue kit (Qiagen N.V.) from thoracic muscles. The 3′ portion of the mitochondrial *cox1* gene (∼1000 bp) was amplified, purified and sequenced using ABI technology following the procedures described by Bocek et al. (2018).

Mitogenomes were produced by shotgun sequencing of total DNA on the Illumina MiSeq platform using a 2 × 300 bp paired-end kit and generating ∼200 Mbp per sample. Raw reads were filtered using FASTP v.0.20.0 (Chen et al., 2018) with the parameters -q 5 -u 50 -l 50 -n 15, and the quality was visualized with fastqc (bioinformatics.babraham.ac.uk/projects/fastqc). In

the first step, we de novo assembled mitogenomes using the NOVOPLASTY v.2.7.2 pipeline (Dierckxsens et al., 2017) under the default settings and using the full-length *cox1* gene as a seed for the initial search. If NOVOPLASTY did not assemble a circular genome, we mapped reads directly to the closest complete mitochondrion. All mitogenomes were annotated using the online gene prediction tool MITOS 2 (Bernt et al., 2013) and manually refined in GENEIOUS v.7.1.9 (identification of the start and stop codons by eye). The GC content of mitochondrial genes and the mean GC (guanine-cytosine) content per taxon were analysed using AMAS (Borowiec, 2016) and visualized in R (R core team, 2020). All newly produced sequences were submitted to GenBank under accession numbers listed in Table S3. The *cox1–3′* mtDNA fragments are listed in Table S4.

Sequence handling and phylogenetic analyses

The newly generated data were combined with previously published sequences representing all subfamilies of Dermestidae and the closest families as outgroups. The published mitogenomic and *cox1–3′* data were obtained from GenBank (www.ncbi.nlm.nih.gov/ genbank), and the *cox1–5′* data (barcode dataset) from The Barcode of Life Data Systems (v3 .boldsystems.org, both databases accessed 1 February 2021, Tables S3, S4). For simplicity, we applied 2% DNA divergence as a threshold for species delimitation using CD-hit-est (Fu et al., 2012). The length invariable protein-coding mtDNA genes were aligned using TRANSALIGN (Bininda-Emonds, 2005) and the rRNA genes using MAFFT v.7.2 under default parameters (Katoh & Standley, 2013). Our phylogenetic approach combines two successive steps of inferring topologies.

The GC content of mitochondrial genes and the mean GC content per taxon were analysed using AMAS (Borowiec, 2016) and visualized in R (R core team, 2020). The proportion of missing data and overall completeness scores (Ca) across all datasets were inspected using ALISTAT v.1.7 (https://github.com/thomaskf/AliStat). Moreover, we generated heatmaps of pairwise completeness scores for all analysed datasets. The software SYMTEST v.2.0.49 (https://github.com/ottmi/symtest) was used to calculate the overall deviation from stationarity, reversibility and homogeneity (SRH; Jermiin et al., 2008) at the amino acid level (AA) and nucleotide level (NT). Heatmaps were generated for all datasets to visualize the pairwise deviations from SRH conditions.

First, we analysed the mitogenomic dataset consisting of 13 protein-coding and two rRNA genes under maximum likelihood (ML) using IQTREE v.2.1.1 (Minh et al., 2020). The matrices were analysed at the nucleotide and amino acid levels, with R/Y site masking, ClipKIT sites masking (Steenwyk et al., 2020) or using various partitioning schemes (Table S6). MODELFINDER (Kalyaanamoorthy et al., 2017) was used to identify substitution models for each matrix. Support of inferred splits was tested using UFboot (Minh et al., 2020), sh-ALRT test (Guindon et al., 2010), both with 5000 replicates, and a Bayes factor (Anisimova et al., 2011). The topology recovered by mitogenomic analyses was used for the subsequent constrained analysis of the *cox1–3′* dataset. We ran constrained and unconstrained tree searches using the same parameters as listed above to test topological differences.

To overcome deviations from SRH conditions, we used Bayesian inference in the PhyloBayes software implementing a site-heterogeneous mixture model. Two datasets, NT and first + second codon positions, were analysed under the CAT + GTR + Γ4 model. Additionally, convergence was checked with tracecomp for continuous parameters of the model. The majority-rule consensus tree using a burn-in of 30% and sub-sampling every tenth tree.

We conducted topology tests available in the IQTREE package with NT and AA datasets produced by transAlign. Four hypotheses were considered by computing log-likelihood with the -zb 50 000 -zw -au parameters. We tested the topology recovered by the analysis of the NT dataset, the AA dataset (Trinodinae as a serial split), the larva-based morphological dataset (Thoractinae as a serial split; Kiselyova & Mchugh, 2006) and the adult-based morphological dataset (the Attageninae, Thoractinae and Dermestinae clades; Lawrence & Slipinski, 2005). The tests included bp-RELL – bootstrap proportion using the RELL method; p-KH – one-sided Kishino–Hasegawa test; p-SH – Shimodaira–Hasegawa test; p-WKH – weighted KH test; p-WSH – weighted SH test; c-ELW – expected likelihood weight; p-AU – approximately unbiased (AU) test.

As the methodology of phylogenetic inference has further developed in the last two decades, we reanalysed morphological datasets published by Lawrence and Slipinski (2005) and Kiselyova and Mchugh (2006) under the ML criterion using IQTREE v.2.1.1 and -st MORPH argument.

Voucher specimens of newly collected individuals are deposited in the collections of the Laboratory of Biodiversity and Molecular Evolution, CATRIN-CRH, Olomouc, and the Natural History Museum, London. J. Háva identified sequenced specimens using morphological characters.

Divergence dating

The *cox1–3′* dataset was used for dating the preferred mitogenome topology with BEAST v.1.8.1 (Drummond et al., 2012). As the fossil calibration points we chose the amber inclusion of *Cretonodes antounazari* Kirejtshuk & Azar from Lebanese amber (Cretaceous, 130.0–125.45 Ma) and Cretaceous Attageninae preserved as inclusions in Burmese amber (Cretaceous, 99.7–94.3 Ma; Cai et al., 2017; Deng et al., 2017b; Háva, 2020; Háva & Damgaard, 2017). Our conservative approach calibrated the split of Trinodini and Thylodriini and the split of Attageninae to offset: 130; log(mean): 0.01; log(SD): 2.2 and offset: 99; log(mean): 0.01; log(SD): 2.2, respectively. A potential calibration point based on a doubtful record of *Dermestes larvalis* Cockerel was omitted (Háva & Prokop, 2004, see Table S2 for details). The BEAST runs were conducted on an existing topology under an HKY + I + G model of sequence evolution, data partitioning according to genes and codon positions, and a birth–death speciation prior. The analyses were run for 10^8 generations with a sampling

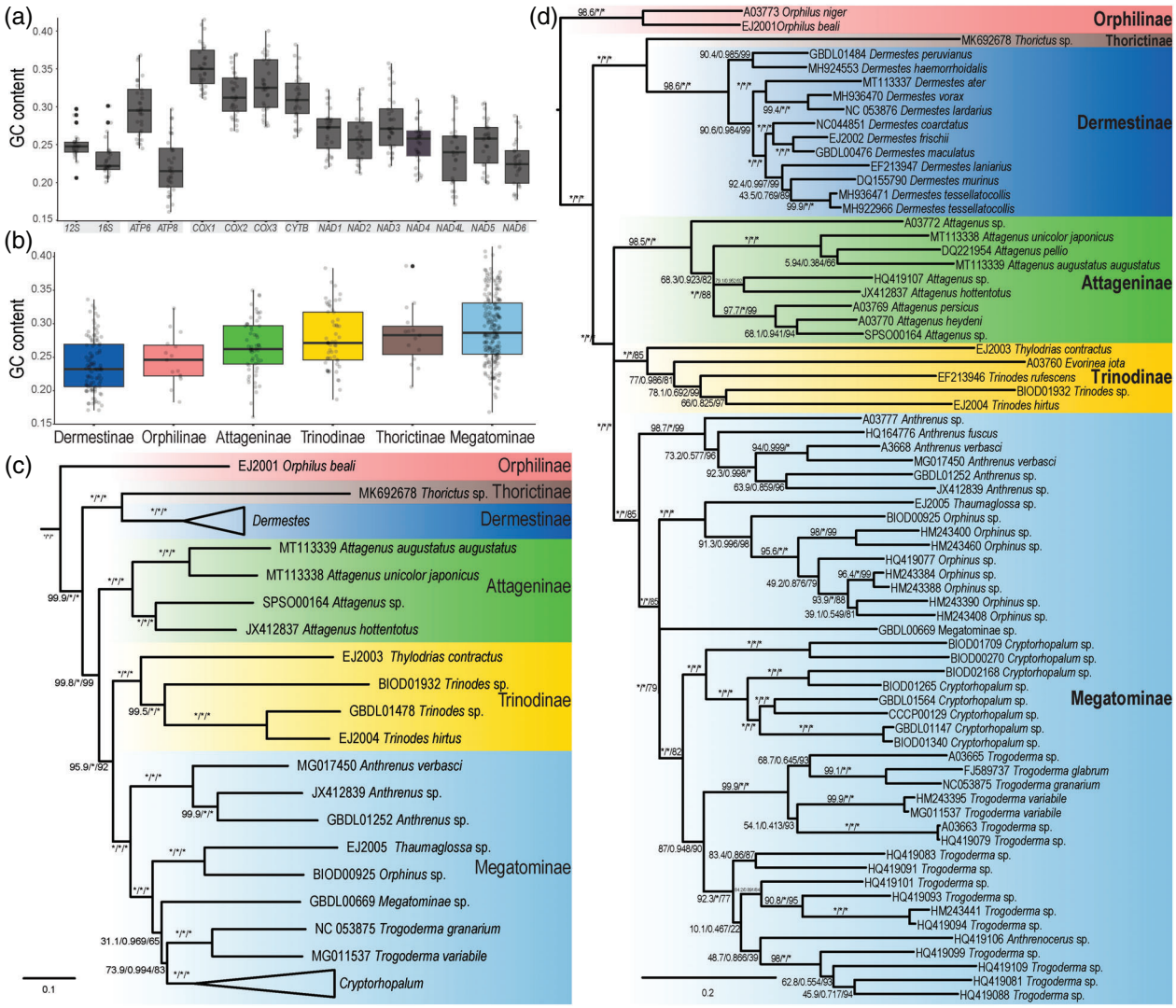


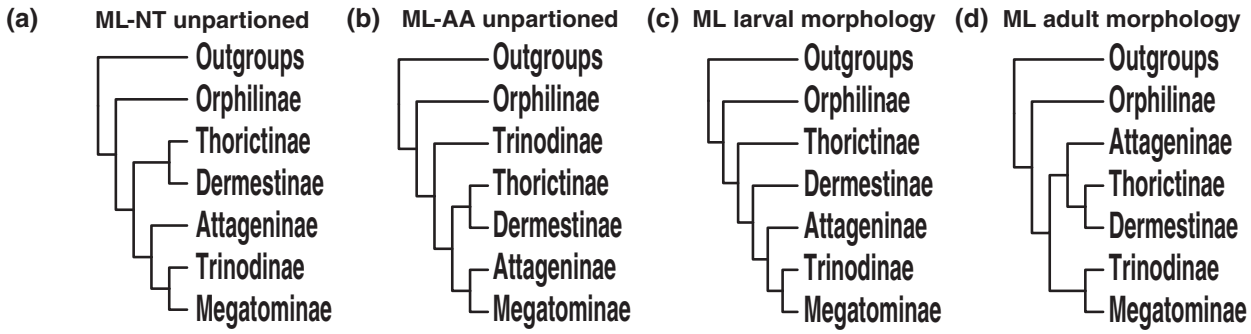
Fig. 2. (a) The GC content for mitochondrial genes, with genes clustered by functional mitochondrial complexes; (b) GC content for the subfamilies of Dermestidae; (c) phylomitogenomic relationships recovered by the maximum likelihood (ML) analysis of the unpartitioned mitogenomic dataset. The numbers above branches show SH-aLRT, aBayes and UFboot values; for the full tree see Figure S2D; (d) ML constrained analysis of the *cox1–3′* dataset, support values as in Figure 3c. * indicates 100% bootstrap support

frequency of 10,000 generations. The plateau phase and lineage through time plot were analysed in TRACER v.1.6 (beast.bio.ed.ac.uk/Tracer). A maximum credibility tree was generated with TREEANNOTATOR (Drummond et al., 2012), discarding 25% of the trees as a burn-in after checking the effective sample size.

Results

Mitogenomes of Dermestidae consisted of the canonical set of genes coding for two rRNAs, 22 tRNAs and 13 PCGs, in addition to the control region. The gene order was conserved corresponding to the presumed ancestral mitogenome of insects (Clary & Wolstenholme, 1985). The combined length of the 13 PCGs ranged from 11,355 bp (*Trinodes* sp.) to 11,430 bp (*Attagenus augustatus* Ballion). The CG content varied across subfamilies and genes; it reached higher values in *cox1*, *cox2* and *cox3*, whereas the lowest values found were *atp8* and *rrnL* (Figure 2a, b). The results of the SYMTEST analyses showed high heterogeneity in the nuclear dataset (Figure S1A, B).

To analyse relationships among dermestid subfamilies and tribes, protein coding and rRNA genes were concatenated from 70 dermestid mitogenomes, 37 of which included the complete set of genes or had at most one gene missing. Furthermore, we assembled a *cox1–5′* mtDNA datasets with 965 terminals, representing 175 operational taxonomic unit (OTU) at ≤2% uncorrected pairwise distance, and a *cox1–3′* mtDNA dataset with 258 terminals and 76 OTUs. The samples belong to all six recognized subfamilies and 8 of 14 extant tribes. The six missing tribes represent 22 known species in total (Tables S1–S3).



	NT	AA	NT	AA	NT	AA	NT	AA
ΔLikelihood	0	43.677	121.48	0	115.91	127.9	74.793	48.622
bp-RELL	**0.879**	**0.0393**	4.00E-05	**0.921**	0	0	0.00034	0.00672
p-KH	**0.876**	**0.05**	8.00E-05	**0.954**	0	0.0001	0.00072	0.0164
p-SH	**1**	**0.0781**	0.0007	**1**	0.00154	0.00012	0.0161	0.0772
p-WKH	**0.876**	**0.05**	8.00E-05	**0.95**	0	2.00E-05	0.00072	0.0164
p-WSH	**0.998**	**0.16**	0.00024	**0.995**	0	0.00014	0.00226	0.0485
c-ELW	**0.878**	**0.0395**	3.71E-05	**0.92**	7.35E-26	3.77E-11	0.000391	0.00697
p-AU	**0.884**	**0.0761**	7.25E-05	**0.967**	4.74E-07	0.00045	0.00102	0.0196

Fig. 3. The AU tests of competing hypotheses of subfamily relationships. Accepted topologies in bold. (a) Mitogenomic dataset, nucleotide level (NT); (b) the same dataset, amino acid level AA); (c) relationships recovered from larval morphology (Kiselyova & Mchugh, 2006); (d) relationships recovered from adult morphology (Lawrence & Slipinski, 2005). The outgroup taxa recovered within ingroup were omitted from the topologies c and d. bp-RELL, bootstrap proportion using RELL method; p-KH, one-sided Kishino–Hasegawa test; p-SH, Shimodaira–Hasegawa test; p-WKH, weighted KH test; p-WSH, weighted SH test; c-ELW, expected likelihood weight; p-AU, approximately unbiased (AU) test

Our analyses recovered successive splits of Orphilinae, Thorictinae + Dermestinae, Attageninae, Trinodinae and Megatominae (Figures 2c, d, S2, Table S6). All subfamilies, tribes and genera were monophyletic, except for the paraphyletic *Trogoderma* Dejean. The resulting trees were well resolved and well supported when analysed at the nucleotide level, while a similar topology with lower support was recovered at the amino acid level using Bayesian inference (Figure S2E). The analyses of the AA and first + second codon position datasets indicated a basal split of Trinodinae as sister to the four large subfamilies versus a sister relationship with Megatominae in the nucleotide analysis (Figures 3B, S2F–H). The ML tests rejected the alternative morphology-based topologies (Figure 3c,d). The FcLM analysis of mitogenomic dataset at the NT level preferred the (Attageninae [Trinodinae, Megatominae]) topology recovered by the analyses at nucleotide level (Figure 2c).

To broaden the taxonomic sampling, we also analysed the *cox1–3′* mtDNA data with a higher number of terminals and *Evorinea* that was unavailable for the mitogenomic analysis (Figures 2d, S2). The constrained topology produced by the analysis of the *cox1–3′* fragment is shown in Figures 2d and S3A, B. The unconstrained topology based on the barcode fragment (*cox1–5′*) did not recover numerous monophyletic groups that were otherwise well supported in other analyses (Figure S3C, D). Furthermore, we reanalysed the morphological datasets. The ML topology based on larval characters is highly similar to those produced by the originally weighted parsimony analysis. Conversely, the topology from reanalysed adult traits was incongruent with the original parsimony analysis reported by Lawrence and Slipinski (2005) (Figure S4A, B). The tests of fit of nucleotide and amino acid data rejected both morphology-based topologies (Figure 3c,d). Specifically, the tests rejected an independent deep position of Thorictinae indicated by larval traits (Figure 3c, Kiselyova & Mchugh, 2006) and the clade (Attageninae [Thorictinae, Dermestinae]) suggested by adult traits (Lawrence & Slipinski, 2005).

We dated the origin of the Dermestidae crown group to the Middle Triassic (223.9 Ma; Figure 4a, the full tree available in Mendeley), while the splits among extant subfamilies was placed into a period of ~50 Ma from 178.2 to 122.7 Ma. The diversification within the major genera began already in the Middle Cretaceous (*Attagenus* 105.0 Ma; *Trinodes* Dejean 109.0 Ma; *Anthrenus* Geoffroy 98.2 Ma; *Cryptorhopalum* Guérin-Méneville 98.6 Ma; *Trogoderma* 103.9 Ma; *Orphinus* Motschulsky 75.4 Ma). *Dermestes* is the only genus with a later crown group diversification (54.3 Ma). The diversification rate slowed down in the last 25 Ma, given the taxa included in the combined mitogenome and *cox1–3′* data set (Figure 4b).

Discussion

Phylogeny and classification

The present mitogenomic trees resolve the debated relationships within Dermestidae and provide a robust framework for the formal classification (Figures 2c,d and 4a). Unlike whole mitogenomes, *cox1* topologies are informative only for shallow relationships (Figures 4c, S3A–D). Using ingroup fossil calibration, we recovered the split between Dermestidae and Bostrichidae in the Middle Triassic at 223.9 Ma. This is broadly

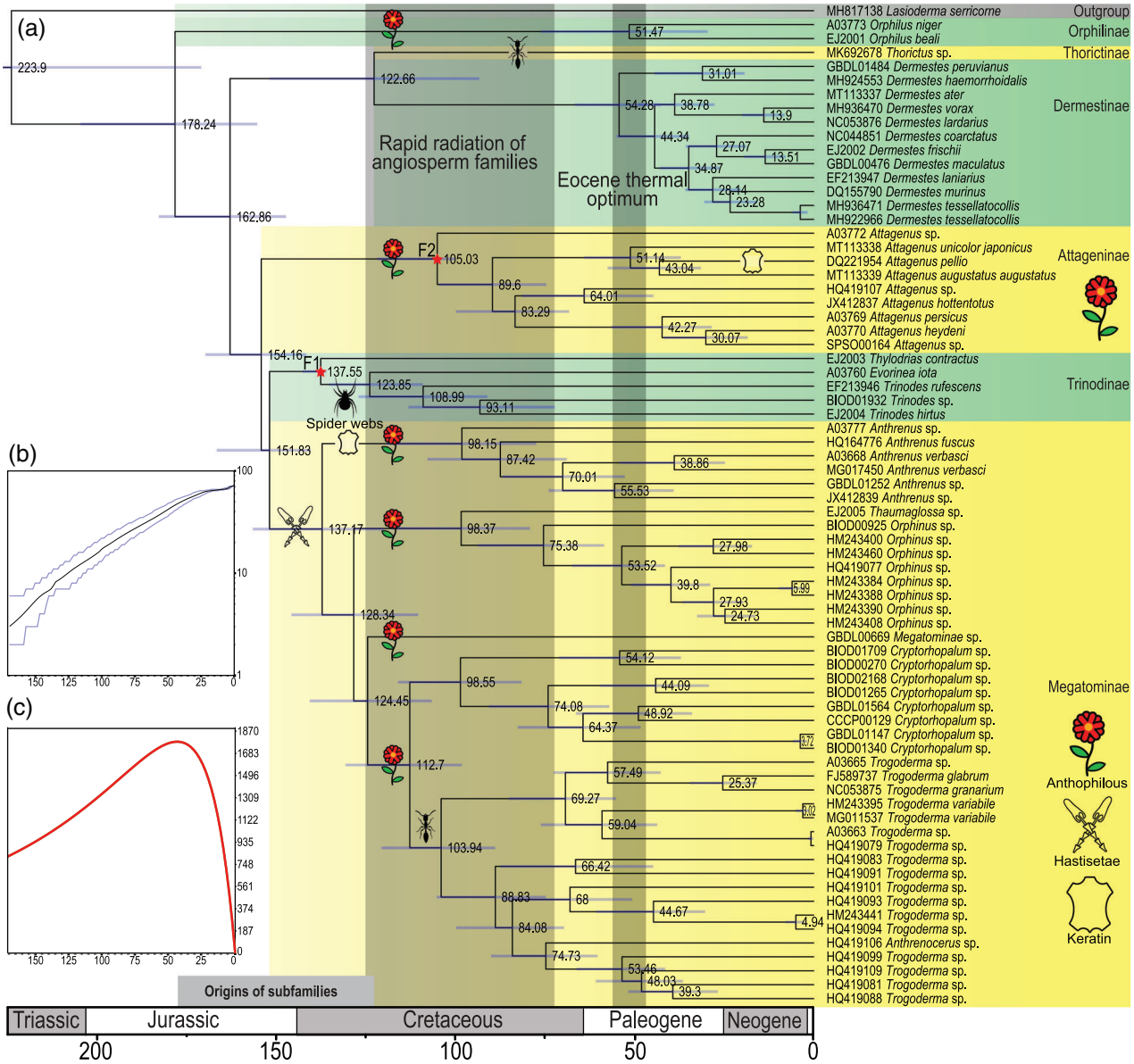


Fig. 4. (a) Dated phylogenetic tree of Dermestidae inferred from the Bayesian analysis of the *cox1–3′* dataset using maximum likelihood and the fixed topology from the mitogenomic analysis; calibration points F1 and F2. (b) The accumulation of the lineages through time. (c) Phylogenetic informativeness and qualitative utility of the *cox1–3′* gene. The ant icon was provided by phylopic.org (CC0)

consistent with the estimates of ~240 Ma for the split of Dermestidae and other Bostrichiformia based on nuclear genomes, a different calibration set and a single terminal per family by McKenna et al. (2019). The diversification of major lineages followed rapidly after the origin of Dermestidae and all extant lineages at subfamily rank had been in existence by the end of the Jurassic, except for Thorictinae (Figure 4a). No doubts remain about the inclusion of Orphilinae in a monophyletic Dermestidae. Its proximity was poorly supported by larval characters (Kiselyova & McHugh, 2006), and variable positions within Dermestidae were recovered by Lawrence and Slipinski (2005) and Lawrence et al. (2011). Unlike morphological analyses, the sister relationships of Orphilinae and the rest of Dermestidae are well supported by mitogenomic data, and this result is congruent with the previous study of the whole Coleoptera based on an rRNA and mtDNA data (Bocak et al., 2014).

In our analyses, Thorictinae and Dermestinae are consistently recovered as sister groups (Figures 2c,d, 4a), as has been inferred from adult characters by Lawrence and Slipinski (2005) and is supported by topological ML tests (Figure 3a). Thorictinae is a species-rich group with a highly modified general appearance (Figure 1a,e) that led earlier authors to establish a separate family, Thoricidae (Crowson, 1981). This was supported in particular by larval characters that indicated the divergent

position of *Thorictus* Germar (Kiselyova & Mchugh, 2006). Thorictinae can now be considered as the evolutionary youngest dermestid lineage at subfamily rank and its origin came some 15 Ma after the earliest splits within Trinodinae and Megatominae (Figure 4a). We recovered Dermestinae + Thorictinae as a clade and considering their delayed origin (122.7 Ma), the latter might be considered a tribe of Dermestinae as proposed previously (Beal, 2003; Zhantiev, 2000, 2009). In our analysis, these subfamilies are represented only by *Thorictes* and *Dermestes*, while the finding of the Middle Jurassic *Paradermestes* Deng et al. keeps open the possibility that even some genera within Dermestinae might have diversified before the origin of the ecologically and morphologically divergent Thorictini. Knowledge about the relationships and the late origin might be considered if the rank of Thorictinae was to be revised in the future.

Attageninae is the next serial lineage and deserves the subfamily rank as proposed by Lawrence and Slipinski (2005) when these beetles were excluded from Megatominae (Zhantiev, 2000, 2009). Unlike Lawrence and Slipinski (2005), we recovered Attageninae as a lineage distant from Dermestinae + Thorictinae (Figure 3a,d). Their early origin is supported by the presence of *Attagenus* and *Cretoattagenus* Deng et al. in Burmese amber (Deng et al., 2017b; Háva, 2020).

The clade of Trinodinae and Megatominae split in the Upper Jurassic (151 Ma; Figure 4a) and the crown group diversification in either lineage was inferred to have originated in the Lower Cretaceous (137.0 Ma). The estimated ages fully support the high rank assigned to them, as their origins are contemporary with the diversification of the other subfamilies of Dermestidae. Within Trinodinae, Thylodriini is sister to Trinodini and the split is dated as Early Cretaceous (137.5 Ma). Such age and morphological distinctiveness might resurrect the subfamily rank given earlier to this lineage (Beal, 2003; Zhantiev, 2000). Unexpectedly for such a morphologically uniform lineage, we found that *Evorinea* Beal and *Trinodes* diverged very early (Early Cretaceous, 123.9 Ma) at a time when other more distinct trinodines lived (Kirejtschuk et al., 2009). Similarly, early origins of major subgroups are indicated in Megatominae. Anthrenini split 137.2 Ma and all genera included in the analysis were established by the Middle Cretaceous. Two of them, *Anthrenus* and *Megatoma*, have already been reported from Burmese amber (Cai et al., 2017; Deng et al., 2017b). The formal classification of Dermestidae was based on a consensus, but some relationships have not been unanimously supported in earlier analyses (Kiselyova & Mchugh, 2006; Lawrence & Slipinski, 2005; McKenna et al., 2019; Zhang et al., 2018). Our results do not call for formal changes to the taxonomy as the currently defined subfamilies are reciprocally monophyletic in our topologies.

Life strategies

We primarily base our estimation of the evolution of life strategies on the mitogenomic phylogeny (Figure 2c). As biased sampling has a strong impact on the quality of ancestral state reconstruction (Kalkauskas et al., 2021), rather than attempting a formal analysis, we summarized relationships and discussed directions of life history evolution. We consider the previously published topology that suggested the position of Endecatomidae as sister to all other Bostrichiformia (McKenna et al., 2019), the morphology-based genus-level relationships in Dermestinae and Trinodinae (Lawrence & Slipinski, 2005), and the current *cox1–3′* mtDNA phylogeny (Figure 2d).

Endecatomidae are mycetophagous and Ptinidae + Bostrichidae are predominantly xylophagous and mycetophagous, although some exhibit similar life strategies as dermestids (e.g., saprophagy and myrmecophily; Philipps, 2000). The Orphilinae, as the lineage splitting at the deepest node, is the only mycetophagous dermestid group (Zhantiev, 2001). They excavate galleries in rotten wood, and mycelia are a major component of their diet. Although *Orphilus* larvae can occasionally feed on dead insect bodies, they cannot finish their development on them (Zhantiev, 2001). Based on our topology (Figure 2c) and the established phylogeny of Bostrichiformia (McKenna et al., 2019, but see Lawrence et al., 2011), the feeding on fungi is shared with Endecatomidae as their closest deeply rooted relative. Concerning the similar structure of fungal and insect cell walls (chitin, glucans, mannans and glycoproteins; Bowman & Free, 2006), the earliest bostrichoid lineages are predisposed for the shift to dead insect bodies as a major component of their diet.

Three serially splitting clades of more derived dermestids, that is, Thorictinae + Dermestinae, Attageninae and Trinodinae, are predominantly xerophilous necrophages with a preference for dessicated insect bodies and only some internal groups shifted to the digestion of keratin and dried vertebrate tissues in an advanced stage of decomposition (*Attagenus*; Zhantiev, 2009). The deepest of these clades, Dermestinae + Thorictinae, contains myrmecophilous *Thorictus* and free-living necrophagous *Dermestes*. The Early Cretaceous separation of *Thorictus* and *Dermestes* at 122.7 Ma coincides with the origin of Formicidae (125 Ma; Branstetter et al., 2017) and keeps open the possibility of an ancient *Thorictus* shift to life in ant nests (Figure 4a). The shift of Thorictini to obligatory commensalism does not require any dietary adaptation, but in ant nests *Thorictus* faced a potentially antagonistic host (Lenoir et al., 2013). The necessity to interact with a specific ant species and flightlessness are potential mechanisms that drive their diversification and lead to observed high species richness (>10% of Dermestidae; Bray & Bocak, 2016; Brucker & Bordenstein, 2012; Ikeda et al., 2012). As other Dermestinae and Thorictinae genera were unavailable for our analyses, we can discuss their biology only with the morphology-based phylogeny (Lawrence & Slipinski, 2005). Species of *Rhopalosilpha* (tribe Marioutini) live facultatively with ants, while the related *Mariouta* is found under stones but according to present observations without an apparent connection to ants. *Derbyana* Lawrence & Slipinski (Dermestini) shares some morphological traits with myrmecophilous species and might be also an ant commensal (Lawrence & Slipinski, 2005; Zhantiev, 2009). These dermestids live cryptically, hiding in crevices and ant nests, and feed on dead insect bodies (Lenoir et al., 2013). The only available phylogenetic analysis suggests that these genera with similar life history (Thorictini,

Thaumaphrastini, Marioutini and *Derbyana*) form a paraphylum that also includes *Dermestes* (Lawrence & Slipinski, 2005).

In agreement with the supposed morphology-based position, our analysis shows that *Dermestes* diversified since the Early Eocene (54.3 Ma, Figure 4a). *Dermestes* includes commensals in bumblebee nests (subgenus *Montandonia* Jacquet), nidicolous species (*Dermestes* **s.str.**) and species feeding on decaying carcasses of vertebrates, molluscs and insects, always in contact with soil, that is, a substrate with a higher water content (*Dermestinus* Zhantiev; Zhantiev, 2009). Feeding on vertebrate carcasses is a derived trait and we date this to the Upper Eocene (~34 Ma) based on the split between the *D. (Dermestinus) frischii* and *D. (Dermestinus) laniarius* clades (Figure 4). The current subgeneric classification of *Dermestes* was proposed with life history in mind (Zhantiev, 2009), but mitogenomic analyses indicate that nidicolous *Dermestes* **s.str.** are paraphyletic and the carcass feeding *Dermestinus* are nested within them (Figures 3c,d, 4a). *Montandonia* was represented only in the *cox1–5′* mtDNA dataset and was recovered within the *Dermestes* (**s.str.**) *bicolor/lardarius* clade (Figure S6) and *Montandonia* possibly does not deserve the subgeneric status assigned to it based on the divergent life history. Similarly, we did not confirm reciprocal monophyly of *Dermestes* **s.str.** and *Dermestinus* and the possibility that some species of *Dermestinus* independently shifted to a diet based on vertebrate tissues in the advanced stage of decomposition should be investigated with more taxa and markers. We found that feeding on carcasses coincides with the Eocene mammalian diversification and dominance in ecosystems (Upham et al., 2019).

The attagenine species mostly feed on dead insect bodies, but some *Attagenus* can feed on keratin (Zhantiev, 2009). The adults are anthophagous and the exploitation of flowers might increase their fitness in arid habitats as we assume in Megatominae (see later; 290 spp., 16.8% of dermestids; Háva, 2021). Trinodinae feed on dry insect bodies similar to Thorictinae, Attageninae and most genera of Dermestinae (Zhantiev, 2009). Little is known about the natural feeding requirements of Thylodriini, but *Thylodrias contractus* Motschulsky is an occasionally serious pest in insect collections and simultaneously can complete development on dried fish under laboratory conditions. Other trinodines live in spider webs and feed on dry remnants of prey. The dated topology indicates that the dependence on spiders is very old (Figure 3a), although the lineage remains species-poor (67 spp, <4% of Dermestidae diversity).

Megatominae is the taxonomically most diverse dermestid subfamily with 1064 species (61.7% of all Dermestidae; Háva, 2021). Their larvae do not differ in their life history from most relatives and depend on dead insect bodies, while some can digest keratin (*Anthrenus*) or become commensals of social insects (*Trogoderma*, 37 spp.) similar to some Dermestinae and Thorictinae (Figure 4a). Unlike their relatives, adults of Megatominae frequently feed on pollen as an additional food resource and potentially also take some nectar as source of water that is crucial for adult survival in arid areas. The association with flowers possibly enabled adult activity and opened an adaptive zone in semi-desert areas where desiccated insects and other animal tissues are widely accessible and where Megatominae possibly do not encounter high competition from other saprophagous organisms. The preponderance of Dermestidae in arid areas stands in contrast with their relative rarity in the moist tropics where tissue quickly decomposes. Another evolutionary innovation of Megatominae is the presence of hastisetae, which are detachable setae located on the thoracic and abdominal segments of the larvae that entangle and potentially kill invertebrate predators (Ruzzier et al., 2020). These defence mechanisms, together with the switch to a new feeding source after the origin of the angiosperms, may have contributed to the exceptional diversification of megatomines in arid areas. The dominance of angiosperms significantly changed ecosystems and triggered the evolution of many beetle groups (Hunt et al., 2007; McKenna et al., 2015, 2019) and may have indirectly also promoted the diversification of dermestids.

We can summarize the evolution of feeding strategies in Dermestidae as a sequence from mycetophagy shared by Orphilinae with bostrichiform ancestors to widespread feeding on dead insect bodies. Only after the diversification and ecological dominance of birds and mammals, some restricted groups of Dermestidae shifted to decomposing carcasses with a higher water content (*Dermestes* feeding on bodies 20–50 days after death in the butyric fermentation stage) and keratin available when the mammal and bird bodies are dried up (*Attagenus* feeding on body remnants in the dry decay stage, 50–365 days after death). Most extant dermestid lineages resemble their Jurassic and Cretaceous relatives and several existing genera, tribes and subfamilies (Orphilinae, Trinodinae, Marioutini in Dermestinae) are species-poor despite their great lineage age. The most diversified groups, Attageninae and Megatominae, differ from their relatives in the exploitation of flowers as a source of energy for adults.

Incomplete metamorphosis and sexual dimorphism

Dermestids are usually strongly sclerotized beetles, but they include a few lineages with incompletely metamorphosed females and soft-bodied males (McMahon & Hayward, 2016). These include *T. contractus* as the most modified species in Thylodriini (Figure 1j,k), which belong to a deeply rooted lineage together with soft-bodied *Trichodryas* and two genera, *Trichelodes* Lawrence & Slipinski and *Hexanodes* Blair, with a more compact and sclerotized body (Háva, 2021; Lawrence & Slipinski, 2005). Male modifications include weakly sclerotized body, shortened elytra, aptery or brachyptery (intrapopulation variability) and body miniaturization (Figure 1j). *Thylodrias* females are much larger than males, they have larviform meso- and metathorax and abdomen, shortened appendages, and are completely apterous, unlike their males and related species (Figure 1k). Another example of a modified dermestid is *Egidyella prophetea* Reitter whose males are similarly modified, but females remain unknown and are potentially wingless (Zhantiev, 2009). The morphological traits found in modified dermestids are analogous to those in some neotenic elateroids (Bocek et al., 2018; Kusy et al., 2019; McMahon & Hayward, 2016). These modified elateroids have been identified as ancient lineages, just like the

peculiar Thylodriini that originated already in the Cretaceous (Figure 3a) but remain species-poor (*Thylodrias* is monotypic, and the other genera comprise six species combined).

Conclusion

Dermestidae are of Triassic origin but remain relatively species-poor compared to other beetle families of similar age. Although dermestids share with other bostrichiform beetles feeding on substrates with low water content, they have evolved various life history modifications. Orphilinae are the only mycetophagous lineage and were already present in the Middle Jurassic. Our analysis placed the common ancestor of all other dermestids to the Upper Jurassic. They diversified subsequently to become saprophagous since the Upper Jurassic, and they dominantly feed on desiccated insect bodies on the soil surface, in crevices, depressions between dunes, in nests of vertebrates and social insects, or inside galleries in wood (Zhantiev, 2009). A similar shift from life in open situations to nests of other animals has been reported also in the related Ptinidae (Bell & Philips, 2008). Adults of Thorictinae, Trinodinae and Dermestinae do not visit flowers. In contrast with these relatively species-poor subfamilies (252 species), Orphilinae, Megatominae and Attageninae have anthophagous adults and represent 79.8% of dermestid alpha-diversity. We hypothesize that protection of larvae by hastisetae and anthophagy in adults are plausible triggers of diversification of Megatominae (Figure 4a). The evolution of Dermestidae thus may include new examples of already observed ecological shifts in the evolution of beetles: mycetophagy as a feeding habit that predisposes a shift to saprophagy; flightlessness and host specificity as a driver of diversification; a shift from free living in crevices to commensalism in the nests of eusocial Hymenoptera; parallel shifts from general saprophagy to keratin feeding; and the enhanced diversification rate of beetles associated with the angiosperms.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Overview of Dermestidae classification.

Table S2. Overview of the Dermestidae distribution and fossil records with the justification of calibrations.

Table S3. Mitogenomes included in the phylogenic analysis.

Table S4. List of cox1-3′ mtDNA fragments included in the phylogenic analysis.

Table S5. List of cox1-5′ mtDNA (barcode) included in the phylogenic analysis.

Table S6. Overview of tree topologies.

Figure S1. Symtest of nucleotide (NT) and amino acid (AA) datasets. Heat maps calculated with SymTest showing p-values for the pairwise Bowker's tests in produced datasets: (A) 15 mitochondrial genes, NT level, (B) amino acids of 13 mitochondrial PCGs, AA level.

Figure S2. The analyses of the mitochondrial datasets: (A) IQTREE analysis of the whole dataset at nucleotide level (NT) (2rRNA + 13 PCGs), partitioned by genes; (B) PhyloBayes analysis of the whole NT dataset (2rRNA + 13 PCGs); (C) IQTREE analysis of the whole NT dataset (2rRNA + 13 PCGs), masked by CLIPkit and partitioned by genes; (D) IQTREE analysis of the whole NT dataset (2rRNA + 13 PCGs) unpartitioned; (E) PhyloBayes analysis of the dataset without 3rd codon position (13 PCGs); (F) IQTREE analysis of the dataset without 3rd codon position (13 PCGs), partitioned by genes; (G) IQTREE analysis of the dataset without 3rd codon position; (13 PCGs), unpartitioned; (H) IQTREE analysis of the amino acid (AA) dataset (13 PCGs), partitioned by genes. Numbers above branches designate alrt, aBayes, UFBoot values.

Figure S3. The analyses of the mitochondrial fragment datasets: (A) IQTREE analysis of the full cox1-3′ dataset; (B) IQTREE analysis of the reduced cox1-3′ dataset; (C) IQTREE analysis of the full cox1-5′ dataset; (D) IQTREE analysis of the reduced cox1-5′ dataset. Numbers above branches designate alrt values.

Figure S4. IQTREE analyses of earlier published morphological datasets. (A) dataset of adult characters containing 24 taxa and 104 characters (Lawrence & Slipinski, 2005); (B) dataset of larval characters containing 41 taxa and 79 characters (Kiselyova & McHugh 2006). Numbers above branches designate alrt, aBayes, UFBoot values.

Figure S5. Four cluster likelihood mapping tests for alternative topologies (A) NT and (B) AA dataset.

Figure S6. IQTREE analyses of the reduced cox1-3′ dataset focused on Montandonia subgenus.

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Conflict of Interest

The authors declare no conflict of interest.

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Data availability statement

The additional supporting data and all the analysed supermatrices are deposited in the Mendeley Data repository doi: https://doi.org/10.17632/jfkdznm29m.1.

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PART IV

Studies on neoteny in Coleoptera: phylogenomics, classification, and evolutionary interactions

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Untangling the evolution of soldier beetles (Coleoptera: Cantharidae) and the evaluation of the morphological phylogenetic signal in a soft-bodied elateroid lineage.

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Untangling the evolution of soldier beetles (Coleoptera: Cantharidae) and the evaluation of the morphological phylogenetic signal in a soft-bodied elateroid lineage

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Abstract

This study addresses the long-standing uncertainty about the internal classification of soldier beetles (Elateroidea: Cantharidae). Four datasets were compiled and analysed: 66 genes for 14 terminals, 15 mtDNA genes for 79 terminals, one mtDNA and two rRNA genes for 217 terminals, and barcodes for 576 terminals. Based on congruent topologies, Chauliognathinae is proposed as a sister to the remaining Cantharidae, followed by the redefined Malthininae (including Tytthonyxini), the paraphyletic "dysmorphocerine" lineages (Dysmorphocerinae *sensu stricto* and Heteromastiginae subfam. nov.), and Silinae + Cantharinae as a terminal clade. The present phylogeny supersedes earlier morphology and short-fragment molecular hypotheses that have not converged on a consensus. Few morphological characters corroborate the DNA-based relationships (see the adults and larval keys). However, morphology-based hypotheses have relied on a few informative characters, and no evidence strongly rejects the preferred molecular topology. The interpretation of morphological characters and uncertain polarity resulting from the high phenotypic disparity of Elateroidea are discussed in detail. The dated phylogeny hypothesizes the earliest split within the Cantharidae in the Berriasian stage (Early Cretaceous, ~141 Myr) and the diversification of most extant subfamilies and tribes already in the Late Cretaceous. The most diverse subfamily, Cantharinae, represents a delayed radiation that started during the Eocene climatic optimum, 55.5 Myr. The late origin of Cantharinae questions the classification of Cretaceous Cantharidae as members of Cantharinae. Instead, the results suggest their deeper rooting after separating from dysmorphocerine lineages and before the node between Cantharinae and Silinae.

Introduction

Comprising more than 5500 species, soldier beetles (Cantharidae) are the second-largest family of the Elateroidea (Delkeskamp, 1977; Brancucci, 1980; Bocak et al., 2014). Being widespread and common in the temperate zone of the Northern Hemisphere (Kazantsev and Brancucci, 2007; Ramsdale, 2010) and widely known to the public, the cantharids attracted the interest of entomologists, who have studied them since the mid-nineteenth century. The work of the founders of cantharid systematics, e.g. J. Bourgeois, G. C. Champion, H. S. Gorham, J. L. LeConte and H. Kiesenwetter, was continued by the French entomologist M. Pic in the 1920s and 1930s (Pic, 1927, 1934, etc.). After

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World War II, an additional >2000 species were proposed, and the most productive student of the family was W. Wittmer, who published >100 alpha-taxonomic studies dealing with Cantharidae (e.g. Wittmer, 1969, 1974). Further taxa were described by Brancucci (1980, 1982), Green (1966), Švihla (2011), and others.

Cantharid taxonomy was recently advanced with new material, especially from East Asia and the tropics. The currently active authors include Biffi (2015), Constantin (2020), Geiser (2017), Hsiao et al. (2016), Kazantsev (2005), Okushima (2005), Takahashi (2018) and Yang (Yang et al., 2018). Cantharidae are also relatively well represented in the fossil record, and three extant subfamilies have already been reported from lower and mid Cretaceous amber (Kirejtshuk and Azar, 2013; Fanti, 2017; Hsiao and Huang, 2018; Peris and Fanti, 2018; Ellenberger and Fanti, 2019; Yang et al., 2021; Fanti and Müller, 2022; Zhao et al., 2022). The Eocene species partly belong to extant genera, and about 100 species have been described (Kazantsev, 2013; Fanti and Kupryjanowicz, 2018; Kazantsev and Perkovsky, 2019, etc.).

Although quite closely related to click beetles (Elateridae) and false click beetles (Eucnemidae), Cantharidae appear phenotypically distant because, unlike elaterids and eucnemids, they are weakly sclerotized. Cantharids are small to medium-sized (2–20 mm), elongate and dorsoventrally depressed. Family membership can be assigned intuitively, but the formal diagnosis requires less apparent morphological characters, specifically a membranous labrum, reduced wing venation, characteristic complex trilobate male genitalia and the presence of paired glandular pores on the abdominal tergites (Fig. 1; Brancucci, 1980; Ramsdale, 2010; Hsiao et al., 2021). Additionally, all species have a freely movable posteriorly constricted head, a transverse prosternum, and open prothoracic coxal cavities. The majority of species have the pronotum and elytra without any robust carinae or costae, the elytra are flexible and leathery, and the abdominal sclerites are never connate and are feebly sclerotized (Brancucci, 1980; Ramsdale, 2010; Hsiao et al., 2021). Finally, the adults and larvae are non-luminescent. These characters are also found in other soft-bodied elateroids, such as Lampyridae, Phengodidae and Lycidae (fireflies, phengodids and net-winged beetles, respectively), but never in this combination. The larvae are campodeiform with dense hydrophobic vestiture and have a pair of glandular pores on the thoracic and abdominal terga (Fig. 1n,p,q). They are mainly predatory but occasionally feed on plant material. As they have a feebly sclerotized integument, they live in humid environments, such as the upper soil layers, under rocks, in rotten wood and bracket fungi, and inside bromeliads or rotten fruits (Biffi and Casari, 2017; Biffi and Rosa, 2019). Larvae can be seen crawling on the soil surface or even snow. Adults emerge in spring and summer and sit on leaves or visit flowers, where they feed on nectar, pollen and small arthropod prey (Fig. 1d). Cantharidae contain noxious compounds that make them unpalatable (Meinwald et al., 1968; Eisner et al., 1981; Brown et al., 1988; Durvaux et al., 2007). Some species are aposematically coloured (Fig. 1c,d,o; Machado et al., 2004), and they are commonly involved in Müllerian mimetic complexes dominated by net-winged beetles (Motyka et al., 2021).

The family gave the name to the superfamily Cantharoidea Imhoff, 1856, an assemblage of soft-bodied beetle families proposed earlier after the dissolution of Malacodermata that additionally contained some weakly sclerotized groups of the distantly related superfamily Cleroidea (Crowson, 1972). Lawrence (1988) merged cantharoids and elateroids in the widely defined Elateroidea, but based on the morphology, he and other authors defended the monophyly of the cantharoids lineages (Lawrence et al., 1995, 2011; Branham and Wenzel, 2003). The Cantharoidea superfamily was contradicted by molecular analyses of rRNA and mtDNA markers (Bocakova et al., 2007; Sagegami-Oba et al., 2007), but the position of Cantharidae relative to the other elateroid families remained unclear, even in subsequent analyses of increasingly larger datasets (Bocak et al., 2014; Kundrata et al., 2014). Similarly, mitogenomics and multiple nuclear markers did not solve the backbone of Elateroidea with sufficient confidence (McKenna et al., 2015; Bocak et al., 2016; Timmermans et al., 2016; Linard et al., 2018). The relative position of Cantharidae, Lycidae, Elateridae and Lampyridae remains problematic even with the use of phylogenomics data (Kusy et al., 2018a, b; 2021; Zhang et al., 2018; McKenna et al., 2019; etc.). Yet, the monophyly of Cantharidae is widely agreed upon and recovered in almost all recent analyses, especially after the Chauliognathidae *sensu* Miskimen (1961a) were included in Cantharidae, and Omethinae earlier placed in Cantharidae were excluded by Crowson (1972), which are now considered a part of the distantly related Omethidae (Bocakova et al., 2007). Yet, only Lawrence et al. (2011) (Fig. 1c) suggested *Matheteus* (Omethinae), but not other Omethidae, as the sister to Cantharidae, and they inferred *Podabrocephalus* (Byrrhoidea, Ptilodactylidae) in Elateroidea as a sister to *Matheteus* + Cantharidae.

Early authors only superficially addressed the supergeneric classification of Cantharidae. Initially, four subfamilies or seven tribes were delimited (Bøving and Craighead, 1931; Delkeskamp, 1939). Later, the major lineages were grouped into the subfamilies Cantharinae and Chauliognathinae (Delkeskamp, 1977). After Omethinae were excluded, five major lineages remained in Cantharidae (Table 1; Crowson, 1972), and they were assigned into the subfamilies Cantharinae,

Fig. 1. Cantharidae in nature. Photos by authors.

Dysmorphocerinae, Malthininae, Silinae and Chauliognathinae by Brancucci (1980), who additionally presented a detailed comparative morphology and the first cladistic analysis of subfamily relationships. The relationships between three subfamilies were defined by Lawrence et al. (2011). The latest morphology-based analyses were presented by Hsiao et al. (2021) and Zhao et al. (2022) (Figs 2c,d), which also included fossils from Burmese amber. These studies favour the basal node of Cantharinae from all other subfamilies and a more derived position of Chauliognathinae. However, the proposed morphology-based topologies have not converged on a consensus (Fig. 2a–d), and studies using molecular data also revealed different subfamily relationships and suffered from incomplete taxon sampling (Fig. 2e–g).

Table 1
Overview of the historical and current classification of Cantharidae (Cantharidae)

Bøving and Craighead (1931)	Delkeskamp (1939)	Delkeskamp (1977)	Brancucci (1980) with extinct tribes added	Genera/ spp.*	Distribution
	Omethini (excl.)				
Chauliognathinae	Chauliognathini	Chauliognathinae	Chauliognathinae	18/600	Cosmopolitan
	Ichthyurini	Chauliognathini	Chauliognathini	11/300	NEO, NEA,AUS
Cantharinae	Cantharini	Ichthyurini	Ichthyurini	6/300	Cosmopolitan
		Cantharinae	Cantharinae	64/1300	Cosmopolitan
			Cantharini	54/1100	Cosmopolitan
	Podabrini		Podabrini	5/200	PAL, OR, NEA
			Cacomorphocerini†	5/40	Eocene amber
	Silini		Silinae	48/2100	Cosmopolitan
			Silini	48/2000	Cosmopolitan
			Tytthonyxini	1/50	NEA, NEO
Malthininae	Malthinini		Malthininae	16/1000	PAL, OR, NEA, AFR, NEO
			Malthinini	5/290	Cosmopolitan
			Malchinini	1/6	PAL
Malthodinae			Malthodini	5/627	Cosmopolitan
			Dysmorphocerinae	15/250	AFR, NEO, AUS
			Mimoplatycini†	1/3	Eocene amber
Total				161/5250	

AFR, Afrotropical; AUS, Australian; NEA, Nearctic; NEO, Neotropical; OR, Oriental; PAL, Palearctic.

*The numbers of genera as in Appendix, i.e. reflecting the changes in the classification; the *incertae sedis* genera are only included in the higher taxon; the number of species in extant taxa are as reported by Delkeskamp (1977) with some modifications.

†Extinct taxon.

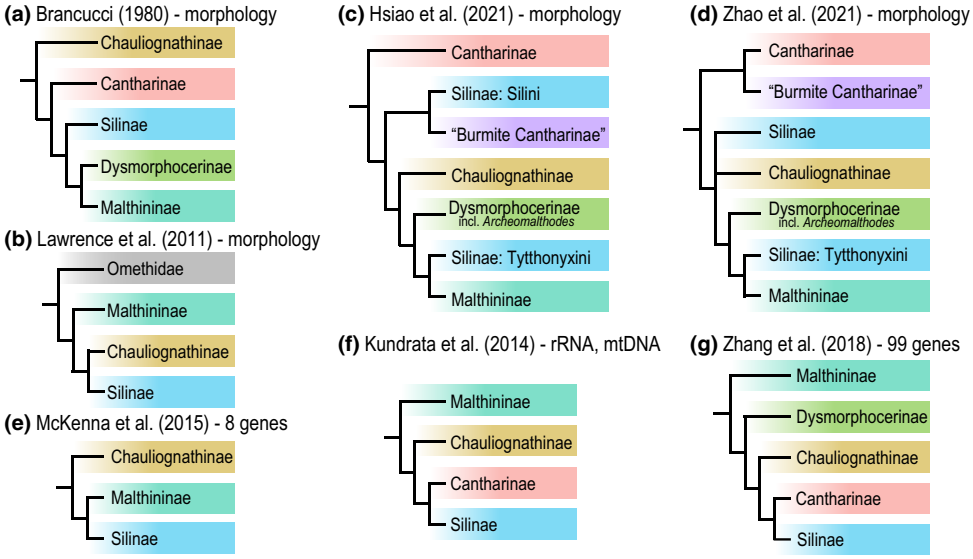

Fig. 2. Phylogenetic relationships among cantharoid subfamilies presented in previous studies. References to topologies a–g are shown in the figure.

Morphological analyses unanimously indicated the monophyly of soft-bodied elateroids (Lawrence, 1988; Lawrence et al., 1995, 2011; Branham and Wenzel, 2003) in deep contrast with all molecular studies, including the latest phylogenomic analyses (Bocakova et al., 2007; Sagegami-Oba et al., 2007; McKenna et al., 2015, 2019; Zhang et al., 2018; Kusy et al., 2018b, 2021). We assume that the sequenced molecular markers and phenotypes are unlinked. Therefore, this study aims to produce a robust phylogeny-based classification and evaluate the phylogenetic history of morphological characters. Although Cantharidae is not as severely modified owing to ontogenetic reprogramming as the net-winged or some click beetles (Kusy et al., 2019, 2021), the possible homoplasy of morphological characters should be considered within the framework of the whole Elateroidea.

In addition, morphological analyses can also contribute to the reliable placement of extinct taxa.

Materials and methods

Material

The newly sequenced specimens were collected mainly by the study's authors during various expeditions from 2001 to 2020. Part of the material was provided by our colleagues (see the Acknowledgements and the List of Material). All specimens for whole genome sequencing, the analysis of mitogenomes, mtDNA, and rRNA fragments were fixed in 96% ethanol in the field. Material for mitogenomic analyses was obtained via standardized collection in various traps (Bian et al., 2022) and preserved in 96% alcohol after extraction from traps using 70% alcohol for short-term fixation of tissues. Voucher specimens of newly analysed individuals are deposited in the collections of the Laboratory of Biodiversity and Molecular Evolution, CATRIN-CRH, Olomouc and the Natural History Museum, London (NHMUK). M. Geiser, G. Biffi and F. Fanti identified sequenced specimens.

Laboratory procedures

Genomic DNA was extracted from thoracic muscles using the DNeasy tissue kit (Qiagen N.V., Hilden, Germany). The total genomic DNA of *Plectonotum laterale* Pic, 1906 (Dysmorphocerinae) from Brazil and Silinae gen. sp. from the Dominican Republic was shotgun sequenced with the Illumina Novaseq 6000 platform (Illumina Inc., San Diego, CA, USA) for 2 × 150 bp paired-end reads. The sequencing service was provided by Novogene Co. Ltd (Beijing, China).

Mitogenomes were produced by the shotgun sequencing of total DNA on the Illumina MiSeq platform using a 2 × 300 bp paired-end kit and generating ~200 Mbp (base pairs) per sample. Raw reads from both sequencing methods were filtered using fastp v.0.20.0 (Chen et al., 2018) with the parameters -q 5 -u 50 -l 50 -n 15, and the quality was visualized with fastQC (bioinformatics.babraham.ac.uk/projects/fastqc/). In the first step, we *de novo* assembled mitogenomes using the NOVOPlasty v.2.7.2 pipeline (Dierckxsens et al., 2017) under the default settings and using the full-length *cox1* gene as a seed for the initial search. All mitogenomes were annotated using the online gene prediction tool MITOZ (Meng et al., 2019) and subsequent refinement of start and stop codons with the mitocorrect tool (Creedy, 2022).

For a subset of taxa, the complete 18S rRNA (~1850 bp), the D2 loop of 28S rRNA (~800 bp) and the mitochondrial *cox1*-5′ (~800 bp) mtDNA fragment were amplified, purified and sequenced using ABI technology following the procedures described by Bocek et al. (2018).

Datasets

The new genomic data were obtained from three samples, *Plectonotum laterale*, Silinae gen. sp. and *Malthodes* sp. Further genomic data were extracted from publicly available genomes (two samples: *Rhagonycha* sp., and *Chauliognathus* sp.). These were compiled in a single dataset with orthologues used earlier for the analysis of beetle phylogeny (an additional nine samples; Zhang et al., 2018). Non-orthologues were not included in the compiled matrix. The dataset is further designated as CAN66 (Table S1).

The mitogenomic dataset was newly assembled from data produced by biodiversity studies and some published mitogenomic data downloaded from GenBank (www.ncbi.nlm.nih.gov/genbank). The samples were collected from various localities included in the NHM Biodiversity Initiative, the Leverhulme Trust Project and the SITE100 projects at the NHMUK (Bian et al., 2022). The dataset contained 79 ingroup taxa and two outgroups. The dataset contained 13 protein-coding and two ribosomal mitochondrial genes. The dataset analyses used all or only the protein-coding genes (described in the phylogenetic analysis section). The dataset is designated as CANMITO (Table S2).

The dataset of two rRNA and one mtDNA fragments contains: (1) 92 samples designated by voucher numbers starting "UPOL VM" and produced by our team specifically for this project to ensure proportionate representation of family-group taxa; (2) samples from our earlier Elateroidea studies (designated with voucher numbers starting "UPOL" and "UPOL RK" respectively); and (3) samples produced by NHMUK coauthors within their ecological studies—other samples). No similar dataset has been analysed earlier in any study dealing with soldier beetle phylogeny. The dataset is designated as CAN3 (Table S3).

The barcode dataset, i.e. *cox1*-3' mtDNA fragments, was compiled from publicly available data; only CANMITO samples with full *cox1* gene were included as a topology guiding taxa, and no newly sequenced barcodes were generated. The dataset is further designated as CANBAR (Table S4). The terminals represent 576 operational taxonomic units defined by 2% uncorrected pairwise divergence. The analysis of such data cannot provide any reliable information on deep relationships at the subfamily or tribe levels. Still, the phylogenetic tree can guide taxonomic research dealing with species relationships and generic limits. The 2% threshold is an arbitrary value commonly used to identify units representing putative species. Although intraspecific genetic variability can be higher in some widespread species, there are also known examples of pairs of morphologically well-defined species with an uncorrected pairwise divergence under 2% (Baselga et al., 2013a, b; Li et al., 2015). The number of terminals corresponds to ~10% of the family's known diversity (downloaded from www.boldsystems.org; search word Cantharidae; accessed September 2022).

We did not compile a supermatrix combining CAN66, CANMITO, CAN3 and CANBAR, as these datasets are not overlapping. Instead, we used the following divide and conquer strategy. We used two gene-rich (CAN66 and CANMITO) datasets to recover the backbone of the tree (66 orthologues, 14 ingroup terminals, all subfamilies and all tribes except Podabrini and Malchinini). As the analysis of the CAN66 is key for identifying the earliest nodes, we merged cantharid data with nine outgroups from four elateroid families (Table S1). Further, we used complete mitogenomes (dataset CANMITO) to recover relationships among subfamilies and tribes with more taxa to test the monophyly of tribes and subfamilies (Table 2). The outgroup was limited in the mitogenomic analysis owing to computational limitations.

The analysis of Sanger data (CAN 3) focused on the highest possible coverage of phylogenetic diversity at the subfamily, tribe and genus level and geographic origins. We used sequences of the closest relative, net-winged beetles (*Cautires* sp. and Metriorrhynchina gen. sp.) for outgroup comparison (dataset CAN3; 217 ingroup terminals, Table S3). The other potential outgroups, such as fireflies and relatives, substantially differ from Cantharidae in the length of rRNA loops and partly also in the GC (guanine–cytosine) content (Bocak et al., 2014; Timmermans et al., 2016). The alignment of highly length-variable sequences may lead to incorrect homology assignments and often results in rogue positions of some outgroup taxa (Bocak et al., 2014). Additionally, the compositional heterogeneity further complicates the phylogenetic analyses. It leads to unexpected artefacts such as the polyphyly of Elateridae and the resulting rogue positions of some elateroid groups in other distantly related families (Timmermans et al., 2016). The barcode dataset (CANBAR) was analysed using ML with constrained topology to show already sequenced diversity and not used to discuss suprageneric relationships (see above).

Table 2
The structure of analysed datasets. The columns are arranged by decreasing information per terminal and increasing number of terminals in the dataset

Datasets	CAN66 nuclear protein coding	CANMITO mitogenomes	CAN3 rRNA and mtDNA	CANBAR barcodes
Length	51,000 NT;17,000 AA	14,000 NT; 3,800 AA*	4,500 NT	650 NT
	Number of analysed taxa			
Outgroups	9/4 families	2 (Lycidae)	2 (Lycidae)	2 (Lycidae)
Chauliognathinae	2	7	41	50
Malthininae	2	20	55	168
Heteromastiginae	1	–	3	2
Dysmorphocerinae	1	1	1	2
Silinae	2	18	53	92
Cantharinae	6	33	62	259
Total	23	81	217	576

*All mtDNA genes/protein-coding mtDNA genes only.

The information on datasets is summarized in Table 2 and the supermatrices are deposited in the Mendeley Data repository, doi: 10.17632/m8hbh5dwrx.1.

Alignment of sequences and phylogenetic analyses

All length-invariable protein-coding genes were aligned using transalign (Bininda-Emonds, 2005) and the rRNA genes using MAFFT v.7.2 under default parameters (Katoh and Standley, 2013). The proportion of missing data and overall completeness scores across the CAN66 and CANMITO datasets were inspected using AliStat v.1.7 (Wong et al., 2020; Fig. S1). Moreover, we used SymTest v.2.0.49 with Bowker's test to calculate the deviation from stationarity, reversibility and homogeneity (SRH; Jermiin et al., 2008; Fig. S2). Our phylogenetic approach combines gradual successive steps of inferring topologies from data-rich matrices to data-poor datasets. Using the aligned datasets, we conducted 32 different analyses listed in Table 3 (Figs S3–S13).

In a first step, we analysed the CAN66 datasets under the parsimony criterion (MP) using TNT v.1.6 (Goloboff et al., 2021; Goloboff and Morales, 2023) and MPBoot v1.1.0 (Hoang et al., 2017). TNT analyses were conducted using concatenated nucleotide sequences and amino acids using the following settings: the New Technology search method (Sectorial Search, Ratchet, Drift and Tree Fusing); nstates NOGAPS; and xmult = hits 10 level 10 noupdate nocss replic 10 ratchet 10 fuse 10 drift 10 hold 100 noautoconst verbose keepall and branch support calculated with 1000 bootstrap replicates (for a complete list of used commands and analysis settings see Data S1–S3). Additional MP analysis were run using MPBoot software with the default settings (only -multihits and -numpars 500 modified); the consensus tree was calculated from 1000 replications. Further, we conducted maximum likelihood (ML) tree searches using IQ-TREE v.2.2.0 (Minh et al., 2020) for various sets of the partitioning schemes (Table S3). ModelFinder (Kalyaanamoorthy et al., 2017) implemented in IQ-TREE was used to identify substitution models for each matrix and the level of the analysis (a single model for unpartitioned analyses and specific models for each partition, respectively). The model search was conducted using the -m MFP option using extended model selection followed by tree inference or -m MFPMERGE merging partitions to increase model fit. The best model was chosen according to the Bayesian information criterion. The support values of inferred nodes were tested using UFboot (Minh et al., 2020), sh-ALRT test (Guindon et al., 2010), both with 5000 replicates, and a Bayes factor (Anisimova et al., 2011), respectively.

Next, we conducted the MP and ML analysis (with identical settings as mentioned above in the CAN66 section) of the mitogenomic dataset consisting of 13 protein-coding and two rRNA genes and, alternatively, only 13 protein-coding mitochondrial genes. The matrix CANMITO represents the data with the highest ratio sequence/taxa sampling, and therefore, it was analysed at the nucleotide and amino acid levels, with the third codon position removed, with R/Y site masking or Degen sites masking (Steenwyk et al., 2020; Fig. 4k, Fig. S4), or using various partitioning schemes (by genes, by codon positions and/or with merge option; Fig. 4c,e,g,i,k; Table 3). The GC content of mitochondrial genes and the mean GC content per taxon/subfamily were analysed using AMAS (Borowiec, 2016) and visualized in R (R Core Team, 2021). Further, to overcome deviations from SRH conditions, we used Bayesian inference in the PhyloBayes v.1.8 (BI; Lartillot et al., 2013), implementing a site-heterogeneous mixture model for the analysis of the CANMITO dataset. The dataset was analysed under the CAT + GTR + Γ4 model (Fig. S5). The convergence was checked with tracecomp for continuous parameters of the model. The majority-rule consensus tree was constructed using a burn-in of 30% and subsampling every tenth tree.

The congruent topology recovered from CAN66 and CANMITO under ML, BI and MP (Figs 3, 4a, 5) analyses was used for the subsequent constrained analyses of the CAN3 and CANBAR datasets. We ran backbone constrained and unconstrained tree searches using the same parameters as above to identify topological differences. Both CAN3 trees (guided vs. unguided) were compared using the cophylo library (phytools; Revell, 2012) with argument rotate = TRUE (Figs S8–S11).

The barcode dataset (CANBAR) was analysed only with the ML approach at the nucleotide level and with the constrained subfamily-level topology (as in Figs 3, 4a and 5). The unconstrained analysis was not conducted as the short fragment (only ~650 bp) of the mitochondrial DNA data cannot reliably recover the deep nodes.

Divergence dating

The CAN3 dataset was used for dating the preferred topology with BEAST v.1.8.1 (Drummond et al., 2012). As the fossil calibration placements are challenging owing to the problematic taxonomy of the fossil taxa, we chose to conservatively calibrate the first node in the clade of Cantharidae, Lycidae, Elateridae and lampyroid families to 161.18 Ma (uniform distribution, lower 144.0 and upper 173.0; Zhang et al., 2018). The amber inclusions of Cretaceous Cantharidae (Cenomanian, Burmese amber, 99.7 Myr; Spanish amber, 94.3 Myr) have been placed into different subfamilies and, therefore, could not be used to calibrate the tree. The BEAST analysis was conducted under an HKY + I + G model of sequence evolution, data partitioning according to genes (*cox1*-5′ set to 0.0115 rates), and birth–death

Table 3
The overview of the conducted analyses

Dataset	Method	AA-NT/PCG-RNA	Partition scheme	Figure	Topology
CAN66	ML	AA/PCG	Unpartitioned	3a, S3A	As Figs 3, 4
CAN66	ML	AA/PCG	Gene	S3B	As Figs 3, 4
CAN66	ML	AA/PCG	Gene, merged	S3C	As Figs 3, 4
CAN66	MP/TNT	AA/PCG	Unpartitioned	4b	As Figs 3, 4
CAN66	MP/MPB	AA/PCG	Unpartitioned	S6A	As Figs 3, 4
CAN66	ML	NT/PCG	Unpartitioned	S3D	(Chauliogn, *Plectonotum*)
CAN66	ML	NT/PCG	Gene	S3E	(Chauliogn, *Plectonotum*)
CAN66	ML	NT/PCG	Gene, merged	S3F	(Chauliogn, *Plectonotum*)
CAN66	MP/TNT	NT/PCG	Unpartitioned	4d	(Sil, Can), (Malth (Heterom (Chauliogn (Dysmorph, *Trixagus*))))
CAN66	MP/MPB	NT/PCG	Unpartitioned	S6B	(Sil, Can), (Malth (Heterom (Chauliogn (Dysmorph, *Trixagus*))))
CANMITO	ML	AA/PCG	Unpartitioned	S4A	Paraphyletic Silinae
CANMITO	ML	AA/PCG	Gene	S4B	Paraphyletic Silinae
CANMITO	ML	AA/PCG	Gene, merged	S4C	Paraphyletic Silinae
CANMITO	MP/TNT	AA/PCG	Unpartitioned	4h	Paraphyletic Silinae
CANMITO	ML	NT/All	Unpartitioned	3c, S4D	As Figs 3, 4
CANMITO	ML	NT/All	Gene	S4E	Paraphyletic Silinae
CANMITO	ML	NT/All	Gene, merged	S4F	Paraphyletic Silinae
CANMITO	MP/TNT	NT/All	Unpartitioned	4a,f	Paraphyletic Silinae
CANMITO	MP/MPB	NT/All	Unpartitioned	S7A	Paraphyletic Silinae
CANMITO	ML	NT/PCG	Gene, codon	S4G	As Figs 3, 4
CANMITO	ML	NT/PCG	Gene, codon, merged	S4H	As Figs 3, 4
CANMITO	ML	NT/PCG	Unpart., 3rdcodon excl.	S4I	As Figs 3, 4
CANMITO	ML	NT/PCG	Gene, 3rd excl., merged	S4J	As Figs 3, 4
CANMITO	ML	NT/PCG	Unpartitioned, Degen	S4K	As Figs 3, 4
CANMITO	ML	NT/PCG	Gene, Degen	S4L	As Figs 3, 4
CANMITO	ML	NT/PCG	Gene, Degen, merged	S4M	As Figs 3, 4
CANMITO	BI	NT/All	Unpartitioned	S5	As Figs 3, 4
CANMITO	MP/TNT	NT/PGS	Degen	4J	Paraphyletic Silinae
CANMITO	MP/MPB	NT/PGS	Degen	S7C	Paraphyletic Silinae
CAN3	ML	NT/All	Gene	S9	(Chauliogn (Canth, Silin)), (Dysmorph, Malth.)
CAN3	ML	NT/All	Gene, partly constrained	S8	As Figs 3, 4
CAN3	MP/MPB	NT/All	Unpartitioned	S11A	(Heterom (other Canth.)); (Malth, (*Plecton*, Chauliogn.))
CAN3	MP/TNT	NT/All	Unpartitioned	S11B	(Heterom (other Canth.)); (Malth, (*Plecton*, Chauliogn.))
CANBAR	ML	NT/PCG	Single gene, constr.	S12	As Figs 3, 4

Datasets: CAN66, 66 orthologues; CANMITO, complete mitogenomes; CAN3, 18S rRNA, 28S rRNA, and *cox1* mtDNA fragments; CANBAR, barcodes. Methods: ML, maximum likelihood; MP, parsimony; BI, Bayesian inference; TNT, TNT software, v.1.6, (Goloboff and Morales, 2023); MPB, MPBoot v1.1.0 (Hoang et al., 2017). Levels and subsets: NT, nucleotides; AA, amino acids; PGC, protein-coding genes.

speciation prior. The analyses were run for 10^8 generations with a sampling frequency of 10 000 generations. The plateau phase and lineage through time plot were analysed in Tracer v.1.6 (beast.bio.ed.ac.uk/Tracer). A maximum credibility tree was generated with Treeannotator (Drummond et al., 2012), discarding 25% of the trees as a burn-in after checking the effective sample size (Fig. S13).

Results

Molecular phylogenies

We analysed altogether four DNA datasets rich in markers (66 protein-coding orthologues in the CAN66 dataset and 15 genes if complete mitogenomes were analysed or 13 genes if only protein-coding mitochondrial genes were included in the analyses). The more densely sampled datasets contained 217 terminals in the CAN3 (rRNA and mtDNA) dataset (Figs 3, 4) and 576 arbitrarily delimited terminals in the CANBAR dataset (barcode; Table 2). The datasets were analysed to recover the phylogeny of Cantharidae and test the congruence between morphology- and DNA-based relationships. The approaches included different levels of the analyses (amino acids, nucleotides), partition schemes (unpartitioned, partitioned by gene, partitioned by gene and codon position—PCG only, merged or unmerged partitions with the same model) or different coding (third position excluded—PGS only, Degen coding). The list of analyses is shown in Table 3, with reference to the illustration where the

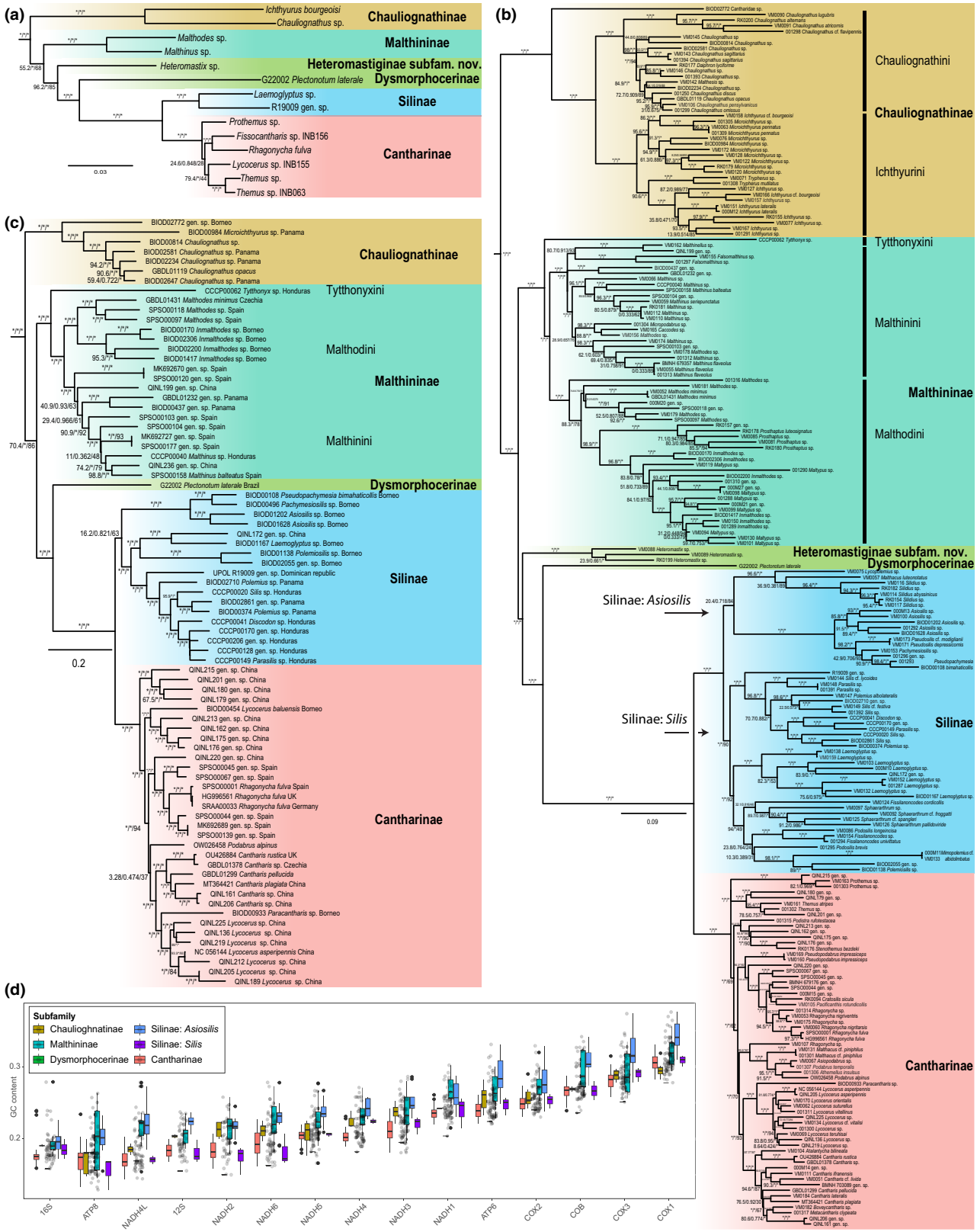

Fig. 3. The recovered topologies: (a) the maximum likelihood (ML) analysis of the CAN66 dataset at the amino acid level; (b) the ML analysis of the CAN3 dataset; and (c) the ML analysis of the CANMITO dataset. * Significant statistical support (depending on the applied method—aLRT support values >95%/ aBayes >98%/UFbootstrap >95%).

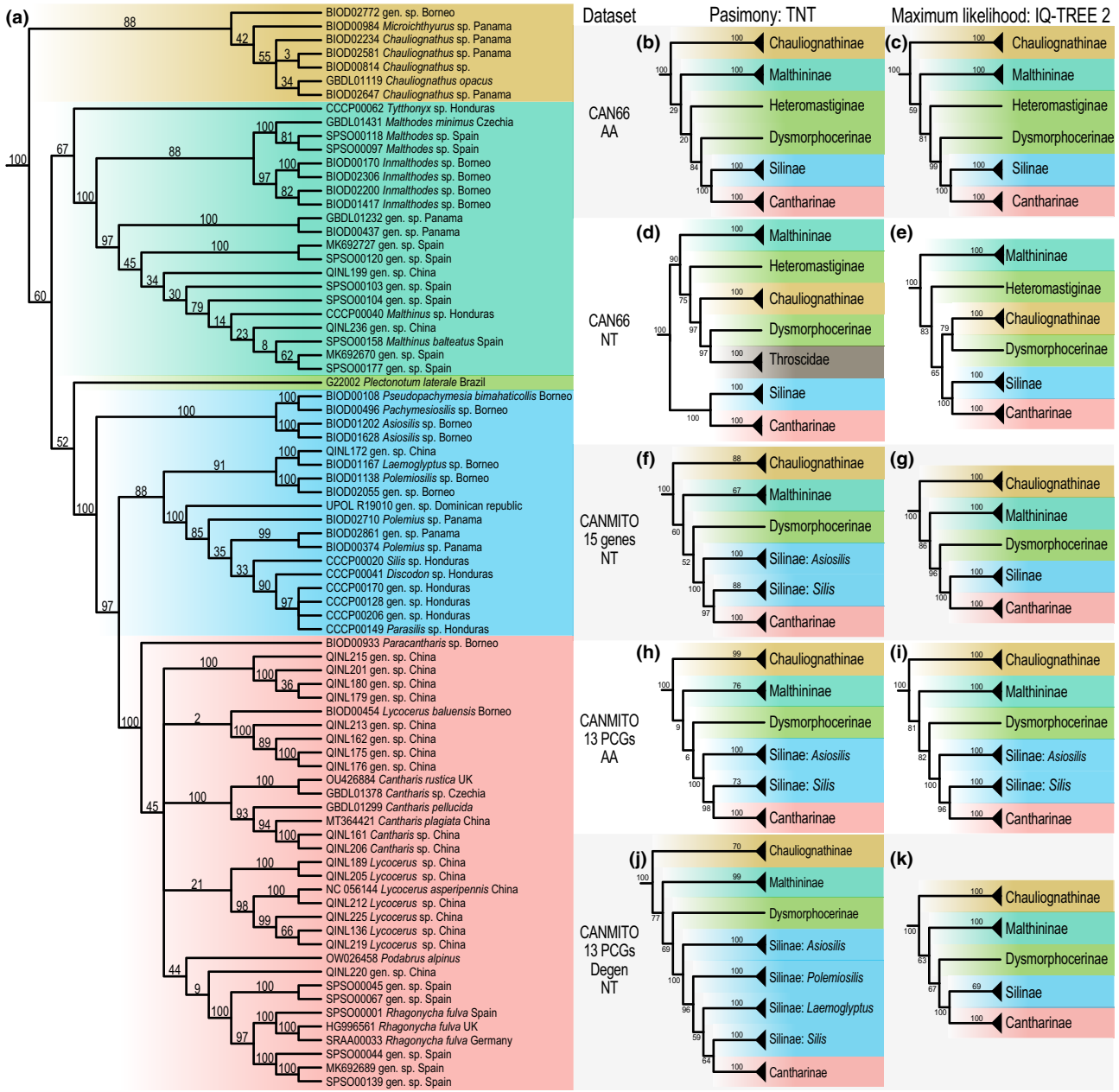

Fig. 4. (a) The tree of Cantharidae recovered by the parsimony analysis of the mitogenome dataset (13 protein-coding and two ribosomal mitochondrial genes, designated as CANMITO in the main text); (b–k) comparison of the results of parsimony analyses conducted with the TNT software (left column) and maximum likelihood analyses conducted with IQ-Tree v.2 software (right column). The rows contain the results of repective analyses of various datasets: (b, c) 66 protein-coding nuclear genes at amino acid level (CAN66 in the main text); (d, e) the same dataset at the nucleotide level; (f, g) all mitochondrial genes at the nucleotide level (CANMITO in the main text); (h, i) 13 mitochondrial protein-coding genes at the amino acid level (CANMITO); (j, k) the same dataset as above but masked with the degen software, and analysed and nucleotide level. Figure (f) shows the same topology as (a), but collapsed. Abbreviations: AA, amino acid level; NT, nucleotide level. Designation of datasets as above.

tree is shown and a short description of the recovered topology.

The monophyly of Cantharidae was well supported (Figs 3, 4a), and all analyses (maximum likelihood, Bayesian inference and parsimony) agreed on the position of Chauliognathinae as the sister group to the remaining Cantharidae, except for the nucleotide level MP (TNT and MPBoot) analysis of the CAN66 dataset that also misplaced *Trixagus* (Throscidae) within the ingroup (Table 3; Figs 3a–c, 4, Fig. S3–13). The deepest node was followed by the Malthininae clade (CAN66; *Tytthonyx* unavailable) or Malthininae +

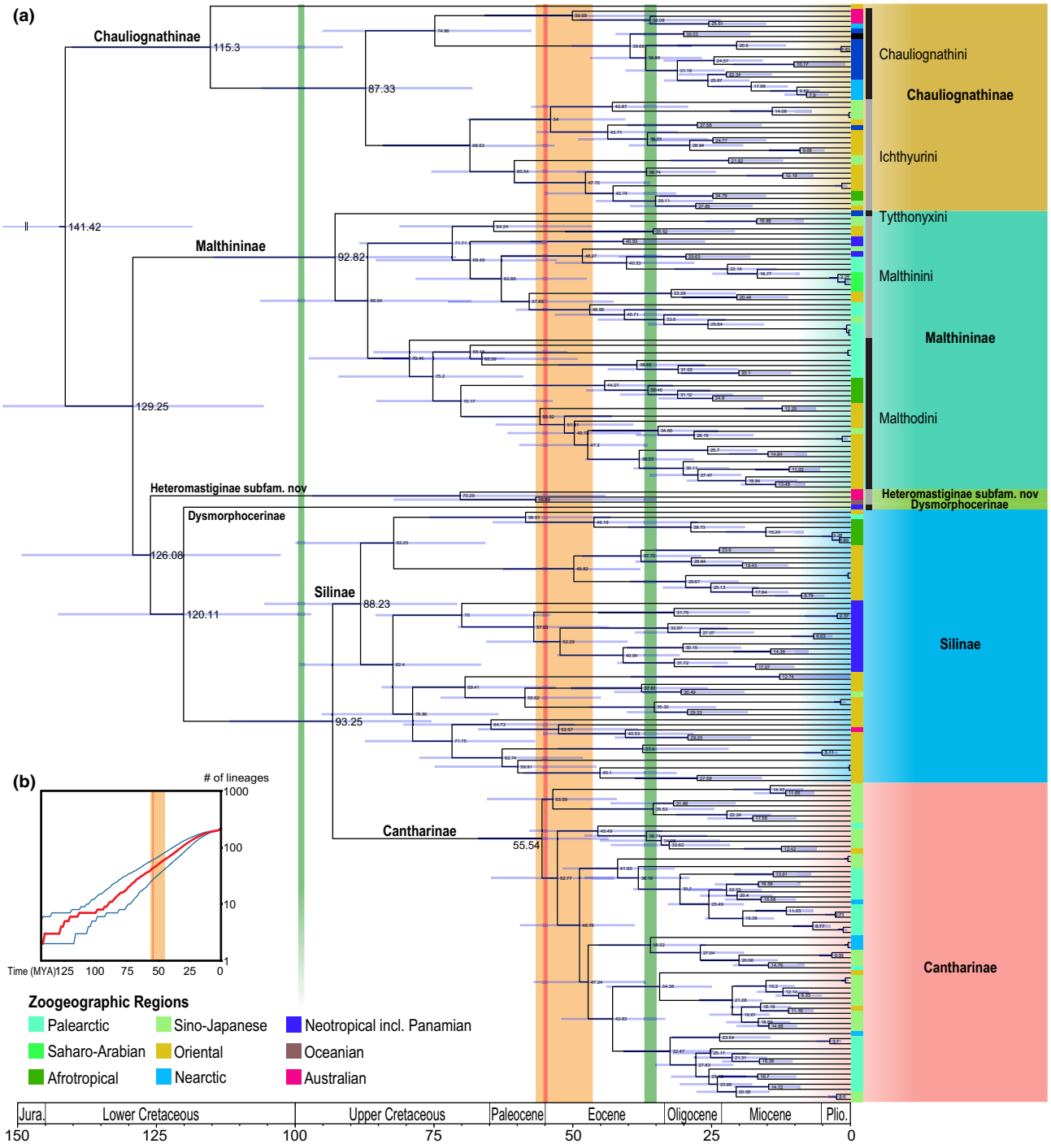

Fig. 5. (a) The dated tree of Cantharidae; (b) the lineage-through-time plot. The biogeographic origin of terminals is indicated by different colours. The left green vertical line designates the occurrence of Burmese amber fossils; the right green line designates the occurrence of European Eocene amber; the red vertical line designates the global Palaeocene/Eocene temperature maximum; the orange area designates the period of the warm and humid climate.

Tytthonyx (CANMITO, CAN3), and two serial branches representing the dysmorphocerine taxa *Heteromastix* spp. and *Plectonotum laterale* (CAN66, CAN3), or only by *Plectonotum laterale* (CANMITO, *Heteromastix* unavailable). All analyses recovered a monophylum consisting of Silinae and Cantharinae, but about a third of the analyses did not support the monophyly of Silinae (see Table 3 for the list of

analyses and results). Branch support was very high for all nodes except for the node of the non-Chauliognathinae clade in the CANMITO (70/1/86 for ML, BI, and parsimony) and CAN66 analyses (56/1/72) (Fig. 3b). If both Dysmorphocerinae taxa are included in the analysis, we robustly recovered their paraphyly (97/1/91 and 100/1/100, respectively, in the CAN66 analysis). We never recovered the clade *Heteromastix* + *Plectonotum*. The analysis of the CANBAR dataset showed a highly supported clade containing *Plectonotum laterale* and *Afronycha caffra* (91.8/0.996/100), suggesting that *Afronycha* should be retained in Dysmorphocerinae. The data of Afrotropical Dysmorphocerinae taxa were not available for the compilation of other datasets. Tytthonyxini was sister to Malthininae in the trees recovered from the CANMITO and CAN3 datasets (absent in CAN66); in both analyses, the position of *Tytthonyx* nested in Malthininae is well supported (100/1/100).

Molecular dating placed the origin of Cantharidae at 156.3 Myr and the earliest node between Chauliognathinae and the other lineages at 141.4 Myr (Fig. 5). The Chauliognathinae, Dysmorphocerinae and Heteromastiginae subfam. nov. were established already in the early Upper Cretaceous. Tytthonyxini/Malthinini and Cantharinae/Silinae nodes were dated at ~93 Myr. The Cantharinae and Silinae differed in their diversification history. The deep branches of the Silinae node were already present in the Upper Cretaceous, and we recovered more than 10 Silinae lineages with their origin before the Tertiary/Paleogene extinction. Unlike them, Cantharinae started their diversification with a considerable delay, and the first node within the subfamily is estimated to be close to the Palaeocene/Eocene boundary at only 55.6 Myr (Fig. 5).

Morphological characters shared by subfamilies

The relationships of subfamilies vary among the used datasets and approaches (Fig. 2). There is no exact congruence between earlier morphologically based trees or a limited congruence between DNA-based trees. The results of our molecular hypothesis confirmed only some earlier proposed relationships.

We investigated which characters support alternative topologies (Figs 2–4) recovered by molecular and morphological analyses. Only eight characters, i.e. those used in earlier published morphological datasets, were shared by two or more taxa belonging to different subfamilies. Other characters were present in a single family (either in all or in only some members of a subfamily), were invariable in all soldier beetles or were present in a single taxon included in the analysis. See Data S1–S3 for a detailed evaluation and the keys for adult and larval morphological characters.

The morphological support for the tree's backbone recovered by molecular analyses was variable. The monophyly of non-Chauliognathinae Cantharidae is supported by the contiguous mesothoracic preepisterna, four-segmented larval maxillary palpi, elongated larval galea and a median tooth of the larval nasale. The monophyly of Dysmorphocerinae, Heteromastiginae subfam. nov., Silinae and Cantharinae is suggested by the presence of a cu-a vein or at least its incomplete remnants. The sister position of Tytthonyxini and Malthinini (Figs 2c,d, 3, 4) is indicated by the completely open radial cell and reduced gonostyli. We did not find any adult morphological evidence for the DNA-based relationships of Silinae and Cantharinae (Figs 1d,f, 3, 4). The larvae of Silinae and Cantharinae share the long maxillary palpomere 2 and very short palpomere 3. The labial palpomere 1 is much longer than palpomere 2, unlike the subequal palpomeres of other groups.

The nodes of the previous morphology-based topologies were mostly supported by a single or two characters. The monophyly of Malthininae + Dysmorphocerinae (Fig. 2a) was supported by strongly ventrally developed parameres, and male abdominal segments 9 and 10 completely retracted into segment 8. The alternative topology with Tytthonyxini inserted within the previous clade (Fig. 2c,d) is defined by globular and pointed terminal palpomeres. The support for the position of Dysmorphocerinae within such a clade is ambiguous, as we noticed the variable shapes of their palpi. The DNA-based clade Tytthonyxini + Silinae is supported by a deep fissure in the centre of the posterior edge of ventrite 8. The enlarged bursa copulatrix indicates the relationships of non-Cantharinae subfamilies (see Data S1–S3 for details). Some characters are not exclusively present in the defined clades and have low consistency. The median wing area with three separate veins supports the non-Cantharinae clade, but *Tytthonyx*, a member of this clade, has the media further reduced, and three veins were also found in some outgroups (e.g. Lycidae: *Scarelus*). The relationships of Dysmorphocerinae, Malthininae and Silinae (Fig. 2a) are supported by an uninvaginable internal sac that is also known in other elateroid families. The cu-a vein is absent in Chauliognathinae and Malthininae. The prolonged gula was presented as a further possible indication of their relationships. However, we found that it is variable and can be confluent or parallel, distinct or indistinct, very elongated or absent (*Ichthyurus*).

Systematics

Cantharidae Imhoff, 1856
Type genus. *Cantharis* Linnaeus, 1758.

The monophyly of Cantharidae is supported by the membranous labrum (but independently evolved in Eucnemidae and Lampyridae: *Pyractonema*) and the presence of lateral glandular pores in abdominal tergites.

Chauliognathinae LeConte, 1861

Type genus. *Chauliognathus* Hentz, 1830.

The monophyly of Chauliognathinae is supported by four morphological characters of adults: the knife-shaped terminal maxillary palpomeres, the emarginate scutellum (shared with net-winged beetles), asymmetrical genitalia and terminal abdominal segments owing to the position of males during copulation (Fig. 1a), and the distant position of mesothoracic preepisterna. The larvae have three-segmented maxillary palpi, minute galea and nasale without a median tooth (see Biffi and Casari, 2017 for details). There are other characteristic adult features, such as the unidentate inner margin of the mandibles, the apparent frontoclypeal suture and the reduced or absent tibial spurs, but they are not exclusively present in Chauliognathinae (see Data S1–S3 for details).

Composition. Chauliognathini LeConte, 1861; Ichthyurini Champion, 1915.

Malthininae Kiesenwetter, 1852

Type genus. *Malthinus* Latreille, 1806.

Malthininae species are small-bodied (<10 mm, often <5 mm) with the radially symmetrical apical maxillary palpomeres, their apex is pointed, the anterior branch of the tentorium is filamentous and the elytra are shorter than the abdomen (non-exclusive character, see Ichthyurini). Consequently, the folded wings are partly exposed. The phallobase is extensive, sclerotized and ventrally expanded, and the styli and coxites are fused (Brancucci, 1980; Hsiao et al., 2021). The larvae have a strongly laterally projected nasale, with an elevated median tooth, antennomere 2 with large subapical sensorium, mandibles without a fringe of setae beneath the mesal channel and abdominal segment 9 without glandular pores (Biffi et al., 2022).

Composition. Malchinini Brancucci, 1980; Malthinini Kiesenwetter, 1852; Malthodini Bøving et Craighead, 1931; Tytthonyxini Arnett, 1963; †Mimoplatycini Kazantsev, 2013 (Eocene).

Remark. Tytthonyxini share with other Malthininae the mandibles with retinacle, globular apical labial and maxillary palpomeres, an open radial cell, the basally reduced Cu vein, setose C vein, short elytra and reduced gonostyli. Brancucci (1980) noted the morphological differences between Tytthonyxini and Silini but placed the tribe provisionally in relationships with Silinae (*incertae sedis* in Silinae). Hsiao et al. (2021; Fig. 2c) recovered Tytthonyxini as a sister to Malthininae but did not formally modify the classification, so the tribe remained a part of Silinae. Here, considering molecular topologies and morphology (Figs 3, 4, Data S1–S3), we formally transfer Tytthonyxini to Malthininae.

Heteromastiginae Motyka, Biffi et Bocak, subfam. nov.

urn:lsid:zoobank.org:act:B8E380C7-C716-4BB5-9FAC-2E9DC0EDDB9F

Type genus. *Heteromastix* Boheman, 1858.

Diagnosis. The definition of this family is based on our phylogenetic analyses (Figs 3, 4). We have not identified any shared character in adults. Heteromastiginae is a heterogeneous assemblage of genera. The adults can be characterized by the terminal maxillary palpomere ovate to securiform, with apex rounded or acute, antennae filiform, simple or variously modified, with glabrous spots, swellings and torsions. The elytra are long, completely covering the abdomen, smooth, rough, slightly to deeply punctate, or with rows of large foveae. Legs with tibial spurs and claws are variously shaped, simple, cleft or with basal tooth. Abdomen with eight tergites and seven ventrites, abdomen and male genitalia never asymmetrical. The larvae of Heteromastiginae (*Heteromastix*, *Asilis*, and *Neoontelus*) have antennomere 2 with a large subterminal sensorium (shared with Malthininae and Neotropical Dysmorphocerinae) and large rounded sensilla flanking each thoracic and abdominal glandular pore (Biffi et al., 2022).

Composition. Heteromastiginae subfam. nov. comprises four genera distributed in Australia, New Zealand and New Guinea. All were formerly classified as Dysmorphocerinae (see Appendix).

Dysmorphocerinae Brancucci, 1980, new sense

Type genus. *Dysmorphocerus* Solier, 1849.

Diagnosis. Dysmorphocerinae has female genitalia with an extensive paraproctus, simple mandibles without any teeth (shared with Cantharinae), and globular to securiform apical palpomeres (Brancucci, 1980). The larvae are morphologically variable. *Plectonotum* has antennomere 2 with a large subterminal sensorium, maxillary palpomeres 1–3 are subequal in length, and labial palpomere 1 is almost as long as antennomere 2. Alternatively, *Afronycha* has antennomere 2 with a terminal sensorium, maxillary palpomeres 2 is very long, antennomere 3 short and palpomere 2 is much longer than antennomere 2 (Biffi et al., 2022).

Composition. After *Heteromastix* Boheman, 1858 and related genera are excluded owing to their independent origin recovered by molecular analyses (Figs 3–5). Dysmorphocerinae Brancucci, 1980, sensu novo comprises eight genera distributed in the Neotropical and Afrotropical regions (see Appendix). The Afrotropical genera are left in this subfamily, as *Afronycha* seems close to *Plectonotum* (Fig. S12).

Silinae Mulsant, 1862

Type genus. *Silis* Charpentier, 1825

Remark. Silinae have male abdominal segments 9 and 10 completely retracted into the preceding segment, the reduced phallobase, sclerotized, often fused parameres, sometimes forming a ventral plate. The phallus is membranous, female genitalia have a sclerotized vagina, the abdomen bears large glandular pores and similar pores are present on the pronotum (Brancucci, 1980). The brief larval descriptions of only three Palaearctic taxa are insufficient for generalization, but the known species share mandibles with a minute basal tooth.

Composition. Silini Mulsant, 1862.

Remark. *Malthinocantharis* Pic, 1914 is transferred from Chauliognathini to Silinae based on the morphological examination of the syntypes in the Muséum national d'Histoire naturelle (Paris) by G. Biffi.

Cantharinae Imhoff, 1856

Type genus. *Cantharis* Linnaeus, 1758.

Remark. Cantharinae is difficult to define with clear and reliable morphological characters. Hsiao et al. (2021) defined the subfamily with a tube-like diverticulum arising from the apex of the vagina. However, some Cantharinae have modified female genitalia, and this character is not present.

Composition. The subfamily contains three tribes: Cantharini Imhoff, 1856, Podabrini Gistel, 1856 and †Cacomorphocerini Fanti et Kupryjanowicz, 2018 (Eocene). Brancucci (1980) pointed to the uncertain morphological separation of the former two tribes. The present analyses do not indicate two reciprocally monophyletic tribes (Fig. 3c). *Podabrus* (type genus of Podabrini) was recovered among five to seven serially splitting branches before the terminal clade containing *Cantharis* (type genus; Figs 3b,c, 4). The revision of the subfamily's internal classification is beyond the scope of this study, but unless up to seven tribes can be morphologically defined, Podabrini cannot be retained as a formal taxon owing to its potential paraphyletic character and should be dropped from the formal classification.

Detailed information on the classification of Cantharidae can be found in the Supporting Information.

Discussion

The molecular phylogeny of Cantharidae has only been recovered in the context of broader studies dealing with Elateriformia or Coleoptera (Kundrata et al., 2014; McKenna et al., 2015, 2019; Zhang et al., 2018; Kusy et al., 2021, etc.) and has never been discussed as these studies suffered from sparse sampling. Here, we focus specifically on Cantharidae and consider the analyses of four datasets (Figs 3, 4, Figs S3–S9) that greatly surpass the previous studies in taxonomic coverage and data volume. We use published morphological data to investigate the congruence between various data sources. Three morphology-based phylogenetic analyses have specifically dealt with subfamily-level relationships (Brancucci, 1980; Hsiao et al., 2021; Zhao et al., 2022), and three subfamilies were included in the whole-order morphological analysis (Lawrence et al., 2011; Fig. 2a–g).

The monophyly of Cantharidae is well supported (Figs 3, 4; Brancucci, 1980; McKenna et al., 2015; Zhang et al., 2018; Hsiao et al., 2021; Zhao et al., 2022, etc.) and has been recovered by all analyses except for Branham and Wenzel (2003) and our analyses under some settings (the misplacement of Throscidae in Cantharidae; see Table 3, Table S5). A monophyletic Cantharidae has regularly obtained medium to high support (Figs 3, 4). The latest morphological analyses supported the monophyly of Cantharidae by nine and eight characters, respectively (Hsiao et al., 2021; Zhao et al., 2022) compared with the two characters proposed earlier (Brancucci, 1980). However, the interpretation of synapomorphies mapped by the latest studies is contentious owing to limited outgroups. If more non-cantharid taxa were included in the dataset, most hypothesized synapomorphies of Cantharidae would be homoplasies or plesiomorphies (see Supporting Information). These earlier proposed synapomorphies included the distance of antennal insertions, the long pedicel, the basal mandibular width nearly the same width as the antennal scape, simple pronotum, cylindrical femora and tibiae (coded as two separate characters by Hsiao et al., 2021 and Zhao et al., 2022), the plate-like, long ovipositor, and the base of gonocoxite situated mesad of the paraprocts (see Data S1–S3 for detailed analysis). The simplified wing venation proposed by Brancucci (1980) is not clearly separating the Cantharidae from other elateroid families, as similar simplification was also found in other elateroids. Consequently, only two morphological characters support the monophyly of the family, namely, the membranous labrum and the presence of lateral glandular pores in abdominal tergites.

Morphology-based relationships among subfamilies

Unlike the monophyly of subfamilies, earlier analyses recovered markedly different backbone relationships (Fig. 2a–g). Brancucci (1980) identified only two informative characters for subfamily-level relationships: an uninvaginable internal sac (Dysmorphocerinae *s. l.* + Malthininae + Silinae) and modified parameres (Dysmorphocerinae + Malthininae). The third character that suggested the monophyly of non-chauliognathine cantharids was coded as present in

some Chauliognathinae. Brancucci (1980) did not code any outgroups and rooted the tree with Chauliognathinae as a sister to other cantharids in agreement with Miskimen (1961a). Then, Silinae and Cantharinae were serial nodes before the Dysmorphocerinae + Malthininae clade. Even after Brancucci's very detailed morphological analysis, no synapomorphy supported the monophyly of the widely accepted concept of Cantharinae.

Substantially different morphology-based topologies were produced by the recent analyses of 16 extant and five Mesozoic taxa cantharids by Hsiao et al. (2021) and 13 extant and 11 extinct taxa by Zhao et al. (2022). The authors scored in total 75 characters, partly following Brancucci (1980) and Lawrence et al. (2011) and adding new characters in respective analyses. Zhao et al. (2022) excluded four characters from Hsiao's dataset and added 14 new ones. However, 26 and 24 characters, respectively, were invariable in the ingroup, and four and six, respectively, doubled other characters (e.g. the separately coded shape of maxillary and labial palpi, the presence of a furrow in respective apical palpomeres, the shape of tibiae and femora, prolonged antennal lamellae and the terminal antennomere). Therefore, these characters only increased their weight. A further possible pitfall was the treatment of linked characters. For example, the acquisition of a specific position of males and females during copulation of chauliognathines affects not only genitalia but also terminal abdominal sclerites (Fig. 1a; asymmetrical phallus, phallobase and sternite 8 were coded identically). Unlike the earlier analyses (Fig. 2a,c), the hypothesis preferred by Hsiao et al. (2021) and Zhao et al. (2022) designates the Cantharinae as the earliest node of Cantharidae, followed by Silinae (a part of the polytomy in Zhao et al., 2022), Chauliognathinae, Dysmorphocerinae + Malthininae. Hsiao et al. (2021) suggested a tubelike diverticulum arising from the apex of the vagina as a synapomorphy of Cantharinae, yet Brancucci (1980) did not find it in some cantharines. The shortage of characters supporting subfamily-level relationships remained acute. Each deep node was supported by a single or two characters, eventually by a low-consistency and retention character with doubtful polarization (e.g. the clade Chauliognathinae + Dysmorphocerinae + Silinae is supported only by toothed mandibles that would be differently polarized if outgroups were not restricted to liquid-feeders; see Data S1–S3).

There are two aspects of the uncertainty of morphological analyses. The first question is which subfamily marks the earliest node within Cantharidae. Only Lawrence et al. (2011) tried to analyse Cantharidae in the context of the whole Elateroidea. Yet their topology (Fig. 2c) proposed the monophyletic "cantharoid" clade merging the soft-bodied elateriform beetles, in contrast to all molecular studies (see above). Additionally, Lawrence et al. (2011) suggested Podabrocephalidae (now Byrrhoidea: Ptilodactylidae, McKenna et al., 2015) as a member of Elateroidea and the sister of the "cantharoid" clade. We suggest that the soft-bodiedness of distantly related elateroids has such a profound effect on the morphological analysis that it is nearly impossible to avoid grouping all soft-bodied lineages in a single clade.

Brancucci (1980) did not code any outgroup, and Hsiao et al. (2021) and Zhao et al. (2022) coded only three outgroup terminals (one firefly and two very derived net-winged beetles, all with simplified mandibles owing to liquid feeding). Owing to the scarcity of informative characters, the outgroup choice resulted in the recovery of Silinae and Cantharinae (both having mostly untoothed mandibles) as deeply rooted branches (Fig. 2c; Hsiao et al., 2021; see Data S1–S3). When the shape of the incisor was excluded from the analysis, Cantharinae remained a sister to all other Cantharidae, and Silinae formed a polytomy (Fig. 2d; Zhao et al., 2022).

Even if we ignore the possible effect of root misplacement and compare all proposed phylogenies as unrooted, we encounter highly inconsistent topologies. It is difficult to identify the reason for such high uncertainty. Yet the shortage of clearly defined synapomorphies and parallel evolution of similar characters in soft-bodied forms was earlier proposed as a possible source of topological uncertainty (Bocakova et al., 2007; Bocak et al., 2018; Kusy et al., 2019). The reappearance of some characters is also known in soldier beetles, e.g. the presence of conspicuous elytral costae in *Oontelus* (Dysmorphocerinae) and some other cantharid genera (only Cantharinae). The ancestral elateroid phenotype seen in Artematopodidae, Eucnemidae, Throscidae, Elateridae and most Byrrhoidea is highly modified in soft-bodied elateroids, including Cantharidae (Bocakova et al., 2007, McKenna et al., 2019; Kusy et al., 2021, etc.). Possibly owing to a low level of sclerotization, some complex characters are lost, and morphology-based phylogenies have to rely on a few characters. Owing to these difficulties, the analyses of extant soft-bodied elateroids have not recovered a consensus widely accepted by the students of the group.

DNA-based relationships

Given the uncertainty of morphology-based hypotheses, we had expected support for some of them from DNA analyses. Yet DNA-based relationships are incongruent (Fig. 2) except for the three-subfamily topology proposed by McKenna et al. (2015). The phylogenetic signal emerging from Sanger data suggested Malthininae as a sister to the remaining

subfamilies (Bocakova et al., 2007; Bocak et al., 2014; Kundrata et al., 2014; Linard et al., 2018; Zhang et al., 2018). McKenna et al. (2015) proposed deeply rooted Chauliognathinae, but Cantharinae and Dysmorphocerinae were absent (Fig. 2e). The terminal clade Silinae + Cantharinae is the only node shared by most molecular analyses.

The present molecular analyses predominantly recovered the topology that morphology and Sanger data-based phylogenies have not. Most analyses recovered the arrangement (Chauliognathinae (Malthininae (Dysmorphocerinae (Heteromastiginae (Silinae + Cantharinae))))) (Table 3). We inferred an aberrant position of Chauliognathinae only in the ML analyses of the CAN66 dataset at the nucleotide level (Chauliognathinae as a sister to *Plectonotum*) and in the MP analyses of the same dataset and setting (the latter possibly affected also by the misplacement of Throscidae; Fig. S6). If the 66-gene dataset was analysed under different settings, Chauliognathinae were recovered as a sister to other Cantharidae (Fig. 3a; Kusy et al., 2018, 2021). The monophyly of Silinae is another uncertainty identified by our analyses (Table 2). The monophyly of Silinae is supported by most analyses (Table S5) and morphology (Brancucci, 1980; Hsiao et al., 2021; Zhao et al., 2022). The possible source of topological instability is an extremely different GC content in the subclade I (*Silis*) and subclade II (*Asiosilis*) of Silinae (Fig. 3d). Using all evidence, we prefer the relationships shown in Figures 3, 4a, and 5: (Chauliognathinae (Malthininae (Heteromastiginae (Dysmorphocerinae (Silinae, Cantharinae))))).

Zoogeography

The oldest cantharid fossils were reported from Gondwanan Lebanese amber and Laurasian Spanish amber (125–130 and 110 Myr; Kirejtshuk and Azar, 2013; Peris and Fanti, 2018). Further fossil taxa are known from Burmese amber. Hsiao et al. (2021) followed Poinar (2019) and considered the Burmese amber fauna as Gondwanan. Geological evidence places the terrain as a part of Laurasia long before amber was deposited (Metcalfe, 2011; Westerweel et al., 2019; Dew et al., 2021), and therefore, the fossil record cannot decisively point to an area where Cantharidae originated.

Miskimen (1961b) suggested that Chauliognathinae, a sister to other Cantharinae, originated in the Mesozoic in East Asia, spinning off an ancient colonization of Gondwana (Chauliognathini). Using data from environmental sequencing by the NHMUK Biodiversity Initiative, we found an unknown Oriental cantharid as a sister group to predominantly Gondwanan Chauliognathini and mostly Old World Ichthyurini (Figs 3b, 4, 5). Unfortunately, no voucher specimen is available, and further research will be needed. Similar relationships between the East Asian and Australian fauna were also identified in other soft-bodied elateroids (Motyka et al., 2017; Masek et al., 2018). The geographic distinctiveness of these groups has been preserved since the Upper Cretaceous (87.3 Myr; Fig. 5).

The subsequent three serial nodes of the Cantharidae phylogeny (Figs 3–5) contain deep Gondwanan lineages, i.e. Tytthonyxini as a sister of the other tribes of Malthininae and the subfamilies Heteromastiginae and Dysmorphocerinae. The node of Tytthonyxini is estimated in the Late Cretaceous (92.8 Myr), i.e. contemporaneous with the node of Gondwanan Chauliognathinae (87.3 Myr). The other two groups are older and presumably contemporaneous with node 120–126 Myr (Fig. 5). The Heteromastiginae and Dysmorphocerinae have an allopatric distribution. The Dysmorphocerinae *sensu stricto* are Neotropical (most genera) and Afrotropical (*Afronycha* and *Compsonycha*), and Heteromastiginae subfam. nov. are Australian.

Within these Gondwanan lineages are nested two lineages as terminal clades: Malthininae *sensu stricto* and Silinae + Cantharinae. The traditionally delimited Malthininae (except Tytthonyxini) has the centre of diversity in the Oriental and Palearctic region and does not occur in Australia (Delkeskamp, 1977), although they are now distributed in Nearctic, Oriental, Neotropical and Afrotropical regions. Cantharinae and Silinae separated from Gondwanan dysmorphocerine lineages in the mid-Cretaceous (120.1 Myr, Fig. 4), and their extant members are widely distributed (Delkeskamp, 1977). Cantharinae predominantly occurs in the Palearctic region, and the Nearctic species resulted from at least three independent dispersal events (Fig. 5). Cantharinae does not occur in Australia (Delkeskamp, 1977). Silinae is predominantly a Palearctic and Oriental group but occurs in Australia, unlike Cantharinae. However, the Australian Silinae belong to a single genus, *Sphaerarthrum*, which might have colonized Australia from the north. The analysed Neotropical Silinae are concentrated in a single terminal clade that split from Laurasian relatives in the Upper Cretaceous (82.4 Myr, Fig. 5). This means that groups of Cantharinae and Silinae known in southern continents are much younger than Gondwanan Chauliognathini, Tytthonyxini, Heteromastiginae and Dysmorphocerinae (Fig. 5).

We can sum up that distribution patterns were established already in the Upper Cretaceous and that only the terminal hyperdiverse clade of Silinae and Cantharinae managed to colonize other continents shortly before or after the K/Pg extinction event. The Gondwanan groups (Chauliognathinae: Chauliognathini, Malthininae: Tytthonyxini, Dysmorphocerinae, and Heteromastiginae) are relatively small (combined, they represent ~12% of soldier beetle

diversity). The highly diversified Malthininae (except for Tytthonyxini), Silinae and Cantharinae diversified from the beginning in Laurasia. Our study is not primarily focused on zoogeography, and denser sampling is needed for a formal ancestral area reconstruction to give deeper insight into the dispersal history.

Origin and diversification of Cantharidae

The exact position of Cantharidae in Elateroidea gradually emerged from the latest analyses. There is a general agreement that Cantharidae and Lycidae represent serial nodes, but it is somewhat uncertain which branch split first. An initial separation of Lycidae was suggested by McKenna et al. (2015, 2019), Bocak et al. (2016) and Kusy et al. (2018a, b, 2019), but an earlier origin of Cantharidae was proposed by Zhang et al. (2018). This topology was used by Hsiao et al. (2021) as the basis for dating. As the topology profoundly affects dating analyses, it is better to compare the age of the earliest node within Cantharidae crown group than the age of separation from other elateroid stem node.

We dated the first node in the cantharid clade to the Berriasian stage of the Early Cretaceous (141.4 Myr; 95% confidence interval 118.5–166.2 Myr). Most earlier studies proposed the origin within our 95% confidence interval limits (Hunt et al., 2007—120 Myr; McKenna et al., 2015—105 Myr; Kusy et al., 2018a—152 Myr; Toussaint et al., 2017—127.0 Myr; Bocak et al., 2016—162 Myr; Zhang et al., 2018—121.3 Myr). The oldest soldier beetle fossil record from Barremian to Upper Hauterivian Lebanese amber (unidentified species; 125–130 my old, Kirejtshuk and Azar, 2013) is only 11–16 Myr younger than our estimate and older than some shallower estimations by the earlier authors. Therefore, if the present estimation is modified, the origin of Cantharidae cannot be significantly younger.

The oldest formally described fossil soldier beetle, *Molliberus albae* Peris and Fanti, 2018 (Cantharinae: Cantharini), was reported from an Albian Spanish amber outcrop (~110 Myr; Peris and Fanti, 2018). Other Mesozoic Cantharidae, all dated to Cenomanian (93–99 Myr), have been placed to Dysmorphocerinae (*Archaeomalthodes*; Hsiao et al., 2017; *Asiopodabrus* was labelled by error as *Archaeomalthodes* in their fig. 6) or Cantharinae (Burmese amber fauna—11 genera, ~50 spp., and Agdzhakend amber, a monotypic genus; Kazantsev and Perkovsky, 2019; Fanti and Müller, 2022). Hsiao et al. (2021) and Zhao et al. (2022) analysed the morphology of five and 11 Mesozoic cantharids and proposed *Archaeomalthodes* as a sister to *Oontelus* in Dysmorphocerinae consisting of *Heteromastix*, *Oontelus* and *Archaeomalthodes*. Yet the monophyly of this clade was supported by a single character—an enlarged paraproctus that is coded "?" for *Archaeomalthodes*. The relationship to extant *Oontelus* is based on deeply punctate elytra, but similar elytra are also coded for other Cenomanian Cantharidae (Yang et al., 2021; Zhao et al., 2022), and the rows of deep punctures are also known in various elateroids (Bocek et al., 2018; Kusy et al., 2020). The position of *Archaeomalthodes* is kept only by the pointed apical palpomeres (coded as two independent characters) and the highly plastic shape of mandibles; see Data S1–S3). Therefore, its position remains open for reevaluation. Further, Hsiao et al. (2021) recovered a monophylum, designated as "Burmite Cantharinae" and placed it as a sister to Silinae (Fig. 2c). Silini and "Burmite Cantharinae" share only lateral, eversible glands in the abdominal intersegmental membranes, but this is unlikely to be a shared ancestral character as similar eversible glands can be observed in various cantharids if killed in alcohol. This character was excluded from the analysis by Zhao et al. (2022), and most Cenomanian Cantharidae were recovered as a terminal clade in Cantharinae following the deep nodes of *Cantharis* and *Asiopodabrus*.

Hsiao et al. (2021) abandoned their morphology-based phylogeny (Fig. 2c; Hsiao et al., 2021, cladogram depicted in Fig. 1) when they estimated the age of cantharid subfamilies. Instead, they mapped the position of extinct taxa on a very different topology proposed by Zhang et al. (2018) (Fig. 2g; Hsiao et al., 2021, fig. 9). They declared that their time-dated cladogram is based on time estimations by Zhang et al. (2018) and McKenna et al. (2019), but the latter study contained only a single soldier beetle terminal. Additionally, Hsiao et al. (2021) moved the node between Cantharinae + Silinae from 52.1 Myr, estimated by Zhang et al. (2018), to ~110 Myr in their study. As such a claim is unjustified, all conclusions on the relationships of Mesozoic Cantharinae need revision. The position of ~99 Myr old Burmese cantharids as a terminal clade in Cantharinae is in deep conflict with the inferred node between *Asiopodabrus* and *Cantharis* at ~47 Myr.

Our dating analysis points to a possibility that Mesozoic Cantharidae may represent a deeper branch originating after the serial branching of Malthininae, Heteromastiginae and Dysmorphocerinae (diversification completed ~120 Myr) and the node between Silinae and Cantharinae (93.2 Myr; Fig. 5). Such a position may explain the earlier uncertainty of their placement either in Cantharinae or as a sister to Silinae (Hsiao et al., 2021; Zhao et al., 2022). Additionally, the poor support for the monophyly of the Cenomanian genera leaves the possibility that they represent remnants of an earlier, much larger mid-Cretaceous radiation (129–93 Myr; Fig. 5) and might not be monophyletic. The shortage of well-defined morphological characters supporting the relationships between families (Data S1–S3), serious

conflicts in recovered relationships (Fig. 2a–d) and the absence of general agreement on morphology-based relationships in the community of specialists make speculations about the exact position of Mesozoic Cantharidae premature. However, with high confidence, we can reject their placement within Cantharinae.

Conclusion

Very often, neither molecular nor morphological analyses recover a single well-supported phylogeny. The molecular topologies reflect the dataset composition, completeness, applied models, analysis parameters and in the case of large and complex datasets also stochastic processes such as a starting seed (Bocak et al., 2014; Young and Gillung, 2020). The hypotheses based on a single analysis are problematic, and we must consider all evidence (Vasilikopoulos et al., 2021; Boudinot et al., 2022). Further, we must evaluate independent information sources (Eernisse and Kluge, 1993; Kusy et al., 2022). In this study, we re-evaluated morphological characters concerning their linkage, plasticity and hypothesized polarity and compared the morphology- and DNA-based hypotheses.

Our DNA-based relationships were recovered from non-overlapping datasets. They consensually suggest the early node of Chauliognathinae, deeply rooted Malthininae, serial nodes of Dysmorphocerinae and Heteromastiginae, and the crown clade Silinae + Cantharinae. In contrast with robust support from molecular analyses, a very ambiguous phylogenetic signal has been found from the morphology. The relationships between subfamilies and tribes have been based on a few characters, and if only soft-bodied liquid-feeding relatives are coded as outgroups, the polarity of several characters is contentious. Our study clearly shows that conflicting hypotheses must be tested with independent data sources. In this study, we analysed molecular data and compared the results with morphology-based hypotheses (Brancucci, 1980; Branham and Wenzel, 2003; Lawrence et al., 2011; Hsiao et al., 2021; Zhao et al., 2022). We expect that further studies will be based on denser sampling and can also use a higher number of orthologues (e.g. data produced by the sequencing of ultraconserved regions, transcriptomes or whole genomes). The uncertain interpretation of the morphological phylogenetic signal of extant taxa seriously questions the reliability of the phylogenetic placement of many extinct groups, including Burmese amber fossils.

The unexpected DNA-based hypotheses are often only reluctantly accepted, and the names designating traditional taxa continue to be used because of inertia (e.g. the earlier cantharoid family Drilidae; Lawrence et al., 2011; McKenna et al., 2015, 2019; Hsiao et al., 2017; Kovalev et al., 2019, etc.). Additionally, various re-analyses continually produce conflicting topologies (e.g. Hsiao et al., 2021; Zhao et al., 2022). Here, we tried to apply various methods, and we analysed all information to propose a better-supported phylogeny of Cantharidae and to date the origins of their main lineages. The hypotheses are here to be falsified (Figs 3, 4). We showed how uncertain morphology-based phylogenies are and that we apparently still do not have strong morphological evidence for the high-level classification of Cantharidae. Therefore, we do not erect any family-group taxa for Mesozoic Cantharidae that will surely be better studied in the future. Such an important, widely distributed species-rich group as Cantharidae surely deserves further research. Therefore, we also present the barcode-based tree as a source for species- or genus-level taxonomy (Fig. S12).

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Author contributions

Conceptualization, L.B., D.K., M.M., A.P.V., M.G., G.B., E.J., S.V.K.; molecular analyses, M.M., D.K.,

E.J.; writing, original draft preparation, L.B., M.M., D.K.; comparative morphology, L.B., G.B., S.V.K., M.G.; Eocene fossil record interpretation, S.V.K.; writing, reviewing, and editing, all co-authors; visualization, L.B., M.M., D.K., E.J.; funding acquisition, L.B., M.M., D.K., S.V.K. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

None declared.

Data availability statement

The analysed sequences are available in the Mendeley depository. Motyka, Michal; Kusy, Dominik; Geiser, Michael; Biffi, Gabriel; Kazantsev, Sergey; Bilkova, Renata; Jahodarova, Eva; Vogler, Alfried; Bocak, Ladislav (2023), Data for: "Untangling the evolution of soldier beetles (Coleoptera: Cantharidae) and the evaluation of morphological phylogenetic signal in a soft-bodied elateroid lineage", Mendeley Data, doi: 10.17632/m8hbh5dwrx.1.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Fig. S13. The result of the dating analysis using BEAST software and constrained topology of the CAN3 dataset.

Appendix 1.

Key to subfamilies and tribes of Cantharidae adults

1. Three basal maxillary palpomeres different in length; preepisterna of mesothorax joined medially. Tibial spurs large and well visible. Male sternite IX and aedeagus generally symmetrical (if asymmetrical, other characters different)—**4**.

   Three basal maxillary palpomeres subequal in length; preepisterna of mesothorax separate or weakly joined medially. Tibial spurs usually absent, if present, they are very small, barely visible and restricted to fore tibia. Male sternite IX and aedeagus always asymmetrical—**subfamily Chauliognathinae—2**.
2. Elytra long, completely concealing the hind wings and abdomen or exposing up to three distal tergites (except for very few Australian and Neotropical species with extremely reduced elytra); fronto-clypeal suture well visible, strongly arched; head longer than wide, genae elongate between eyes and base of mandibles; occipital region with a shallow median longitudinal furrow; tibial spurs absent; abdominal pores not prominent. Neotropical, Nearctic, Australasian—**tribe Chauliognathini (part)**.

   Elytra short, covering up to three proximal abdominal tergites, exposing most of the wings and abdomen; fronto-clypeal suture straight, barely visible or absent; head shorter, genae short between eyes and base of mandibles; occipital region smooth, without longitudinal furrow; tibial spurs usually absent or only on fore tibia; abdominal pores usually on the apex of tubular projections—**3**.
3. Antennae inserted between the eyes; antennal insertions close to one another, distance shorter than width of scape; lateral arms of discrimen reaching mesocoxae internally; tergite IX of males and females strongly projected posteriorly; sternite IX of males reduced to a narrow blade and concealed by sternite VIII. Pantropical (excluding Australasia), E Palaearctic and Nearctic—**tribe Ichthyurini**.

   Antennae inserted near the anterior margin of eyes; antennal insertions distant from one another, distance longer than width of scape; lateral arms of discrimen reaching mesocoxae externally; tergite IX of males and females not projected posteriorly; sternite IX of males large, oblong and convex. Neotropical, Nearctic—**tribe Chauliognathini (part)**.
4. Abdominal pores barely visible; abdomen of males with nine or 10 visible segments; ventrite VII of males not deeply divided longitudinally; vagina membranous, never sclerified—**5**.

   Abdominal pores large and well visible; abdomen of males with eight visible segments, the ninth and tenth invaginated; ventrite VII of males deeply divided longitudinally into two halves; vagina rather strongly sclerified; styles of gonocoxites reduced. Cosmopolitan—**subfamily Silinae**.
5. Last maxillary palpomere globular and apically pointed—**6**.

   Last maxillary palpomere globular or hatchet-like, apically truncate or sharp, not pointed—**11**.

6. Elytra long, concealing abdomen and wings; discrimen long, lateral arms usually not reaching mesocoxae; wings with radial cell $2R_1$ closed; aedeagus: basal piece reduced to two lateral sclerites covering the base of the lateral lobes; gonocoxites straight and elongate, styles very small or absent. Neotropical—**subfamily Dysmorphocerinae (part)**.
   Elytra short or long; discrimen short and not forked anteriorly; wings with radial cell $2R_1$ completely open; aedeagus: basal piece strongly sclerotized, forming a ventral plaque; styles indistinct, fused to the gonocoxites. Cosmopolitan—**subfamily Malthininae—7**.
7. Gular suture always very close to one another, sometimes confluent; mandibles with a prominent retinaculum; genital segments of females with gonocoxites strongly folded in on themselves; female genitalia always with tubular double spermatheca, located laterally to the vagina—**8**.
   Gular suture distant from one another, rarely confluent; mandibles with slightly prominent retinaculum, equipped with a row of small teeth or smooth; genital segments different; female genitalia always without double spermatheca, not located laterally to the vagina, but with a bulla or a large seminal duct—**9**.
8. Antennae usually filiform to slightly serrate, rarely strongly serrate; anterior corners of pronotum with a distinct glandular pore. Cosmopolitan—**tribe Malthinini**.
   Antennae usually strongly serrate or pectinate (males), rarely filiform (some females); anterior corners of pronotum without glandular pores. Neotropical and Nearctic—**tribe Tytthonyxini**.
9. Mandibles smooth, only the premolar region has a small tooth—**10**.
   Mandibles toothed; if smooth, then without small tooth in the premolar region—**tribe Malthodini (part)**.
10. Pronotum slightly wider than long, showing a flattening on its lateral borders, sometimes barely visible; Elytra completely covering the abdomen; wings with Cu vein entire, with median recurrent branch almost reaching the base; absence of a series of large setae on M3 + 4; aedeagus: basal piece forming a large ventral plaque; genital segments of females with paraproctus, gonocoxites and styles fused, forming a sheath. Palaearctic—**tribe Malchinini**.
    Pronotum transversal with a well-marked lateral lobe; elytra short, never completely covering the abdomen; wings with Cu vein shortened at base, with short median recurrent branch; presence of a series of large setae on M3 + 4; abdominal segments IX and X of males usually developed; aedeagus: basal piece reduced to a ventral sclerified band; genital segments of females with distinct paraproctus and gonocoxites. Holarctic, Afrotropical, Oriental—**tribe Malthodini (part)**.
11. Antennae filiform, simple, without strong modifications, usually with a glabrous stripe in the inner face of median antennomeres; last maxillary palpomeres strongly securiform, hatchet-like; wings with cubital vein divided into two or three branches; gonocoxites elongate. Nearctic including Mexico, Palaearctic, Oriental up to Wallace's line—**subfamily Cantharinae**.
    Antennae of males serrate or filiform, usually with strong modifications, such as torsions, swellings and notches; last maxillary palpomeres elongate, slightly securiform; wings with cubital vein entire, not divided; gonocoxites slender, with or without a small style—**12**.
12. Male antennae simple, filiform, or moniliform, sometimes (*Heteromastix*) with strong modifications in the apical antennomeres. Distribution: Australasian—**subfamily Heteromastiginae**.
    Males antennae most often filiform, sometimes (*Compsonycha* from South Africa) with enlarged, modified proximal and medial antennomeres. Distribution: Neotropical and Afrotropical regions—**subfamily Dysmorphocerinae (part)**.

Checklist of valid genera within the family Cantharidae

Family CANTHARIDAE Imhoff, 1856 (1815): 69. Type genus: *Cantharis* Linnaeus, 1758.

= Telephoridae Leach, 1815: 85 (usage of the younger name Cantharidae Imhoff, 1856 is conserved (ICZN 1999, Art. 40.2).
206 genera (176 extant, 30 fossil).

Subfamily CHAULIOGNATHINAE LeConte, 1861: 186. Type genus: *Chauliognathus* Hentz, 1830 (18 genera).
Tribe CHAULIOGNATHINI LeConte, 1861: 186. Type genus: *Chauliognathus* Hentz, 1830. *Belotus* Gorham, 1881; *Chauliognathus* Hentz, 1830; *Daiphron* Gorham, 1881; *Lobetus* Kiesenwetter, 1852; *Macromalthinus* Pic, 1919; *Malthesis* Motschulsky, 1853; *Malthopterus* Motschulsky, 1853; *Maroniodes* Brancucci, 1981; *Maronius* Gorham, 1881; *Microdaiphron* Pic, 1926; *Paramaronius* Wittmer, 1963; *Psilorrhynchus* Gemminger & Harold, 1869 (12 genera).
Tribe ICHTHYURINI Champion, 1915: 128. Type genus: *Ichthyurus* Westwood, 1848. *Ichthyurus* Westwood, 1848; *Malthoichthyurus* Pic, 1919; *Microichthyurus* Pic, 1919; *Pseudocerocoma* Pic, 1919; *Trypheridium* Brancucci, 1985; *Trypherus* Leconte, 1851 (six genera).

Subfamily MALTHININAE Kiesenwetter, 1852: 239. Type genus: *Malthinus* Latreille, 1806 (18 genera). Incertae sedis: †*Kuskaella* Fanti & Kupryjanowicz, 2017 (one genus).
Tribe MALCHININI Brancucci, 1980: 313. Type genus: *Malchinus* Kiesenwetter, 1863 (= *Macrocerus*). *Macrocerus* Motschulsky, 1845 (one genus).
Tribe MALTHININI Kiesenwetter, 1852: 239. Type genus: *Malthinus* Latreille, 1806. *Caccodes* Sharp, 1885; *Falsomalthinus* Pic, 1924; *Malthinellus* Kiesenwetter, 1874; *Malthinus* Latreille, 1806; *Mimomalthinus* Pic, 1931; *Paramalthinus* Brancucci, 1984; †*Mantimalthinus* Fanti & Castiglione, 2017 (seven genera).

Tribe MALTHODINI Bøving and Craighead, 1931: 48. Type genus: *Malthodes* Kiesenwetter, 1852. *Frostia* Fender, 1951; *Inmalthodes* Pic, 1908; *Malthodes* Kiesenwetter, 1852; *Maltypus* Motschulsky, 1859; *Prosthaptus* Gorham, 1900: *Protomaltypus* Wittmer, 1978 (six genera).
Tribe TYTTHONYXINI Arnett, 1962: 537. Type genus: *Tytthonyx* J. L. LeConte, 1851. *Tytthonyx* J. L. LeConte, 1851 (one genus).
†Tribe MIMOPLATYCINI Kazantsev, 2013: 288. Type genus: *Mimoplatycis* Kazantsev, 2013. †*Mimoplatycis* Kazantsev, 2013 (one genus).
†Tribe NOTHOTYTTHONYCHINI Fanti, 2022: 231. Type genus: *Nothotytthonyx* Li et al., 2022. †*Nothotytthonyx* Li et al., 2022 (one genus).

Subfamily DYSMORPHOCERINAE Brancucci, 1980: 292. Type genus: *Dysmorphocerus* Solier, 1849. *Afronycha* Wittmer, 1949; *Compsonycha* Wittmer, 1949, *Dysmorphocerus* Solier, 1849; *Flabelloontelus* Pic, 1911; *Hansasilis* Pic, 1936; *Hyponotum* Wittmer, 1949; *Micronotum* Wittmer, 1949; *Oontelus* Solier, 1849; *Plectocephalon* Pic, 1928; *Plectonotum* Gorham, 1885; †*Archaeomalthodes* Hsiao, Ślipiński & Pang, 2016 (11 genera).
Remark. Afrotropical genera retained in Dysmorphocerinae based on analysis of the barcode sequence (*Afronycha caffra* (Boheman, 1851)).

Subfamily HETEROMASTIGINAE Motyka, Biffi et Bocak subfam. nov. Type genus *Heteromastix* Boheman, 1858. *Asilis* Broun, 1893; *Heteromastix* Boheman, 1858; *Geigyella* Wittmer, 1972; *Neoontelus* Wittmer, 1972 (four genera).

Subfamily SILINAE Mulsant, 1862: 342 Type genus: *Silis* Charpentier, 1825. *Allocotoma* Gorham, 1895; *Asiosilis* Wittmer, 1977; *Autosilis* Kazantsev, 2011; *Brachysilidius* Pic, 1949; *Cordylocera* Guérin, 1823; *Cordylocerellus* Wittmer, 1969; *Delkeskampia* Wittmer, 1969; *Discodon* Gorham, 1881; *Ditemnomorphus* Champion, 1915; *Ditemnus* LeConte, 1861; *Eusilis* Reitter, 1887; *Fissilanoncodes* Pic, 1912; *Grandesilis* Pic, 1955; *Guineapolemius* Wittmer, 1969; *Incisosilis* Pic, 1908; *Indopolemius* Wittmer, 1969; *Laemoglyptus* Fairmaire, 1886; *Lycopolemius* Pic, 1921; *Macrosilis* Pic, 1911; *Malthinocantharis* Pic, 1914; *Mimopolemius* Pic, 1921; *Neogressittia* Wittmer, 1969; *Onychotelusia* Wittmer, 1969; *Pachymesia* Westwood, 1849; *Pachymesiosilis* Pic, 1911; *Paradiscodon* Wittmer, 1954; *Parasilis* Gorham, 1885; *Peltariosilis* Wittmer, 1952; *Photinomorpha* Champion, 1915; *Podosilis* Wittmer, 1978; *Polemiosilis* Pic, 1921; *Polemius* LeConte, 1851; *Pseudodiscodon* Wittmer, 1969; *Pseudopachymesia* Pic, 1911; *Pseudosilis* Pic, 1911; *Pygodiscodon* Wittmer, 1966; *Silidiscodon* Leng & Mutchler, 1922; *Silidius* Gorham, 1883; *Silis* Charpentier, 1825; *Silisonycha* Wittmer, 1949; *Silvanotelus* Wittmer, 1969; *Socotrasilis* Geiser, 2017; *Sphaerarthrum* Waterhouse, 1884; *Trachelychnus* Kirsch, 1865; *Tylocerus* Dalman, 1823; †*Curche* Alekseev & Kazantsev, 2014; †*Electrosilis* Kazantsev, 2013; †*Markus* Fanti & Pankowski, 2018 (48 genera).

Subfamily CANTHARINAE Imhoff, 1856 (1815) Cantharidae Imhoff, 1856: 69. Type genus: *Cantharis* Linnaeus, 1758 (74 genera). Incertae sedis ("Burmite Cantharinae" sensu Hsiao et al. 2021): †*Brevipterus* Yang, Liu & Zhao, 2022; †*Burmomiles* Fanti, Damgaard & Ellenberger, 2018; †*Cnathrion* Kazantsev & Perkovsky, 2019; †*Cretocantharis* Hsiao et al., 2021; †*Hukawngichthyurus* Fanti & Ellenberger, 2018; †*Myamalycocerus* Fanti & Ellenberger, 2016; †*Ornatomalthinus* Poinar & Fanti, 2016; †*Poinarelektronmiles* Fanti & Damgaard, 2020; †*Sanaungulus* Fanti, Damgaard & Ellenberger, 2018 (nine genera).
Tribe CANTHARINI Imhoff, 1856 (1815): 69. Type genus: *Cantharis* Linnaeus, 1758. *Amphimorphus* Yang et al., 2022; *Ancistronycha* Märkel, 1852; *Armidia* Mulsant, 1862; *Atalantycha* Kazantsev, 2005; *Bactrocantharis* Barovskij, 1926; *Bactronycha* Kazantsev, 2001; *Bisadia* Wittmer, 1972; *Boveycantharis* Wittmer, 1969; *Cantharis* Linnaeus, 1758; *Cantharomorphus* Fiori, 1914; *Cephalomalthinus* Pic, 1921; *Cordicantharis* Švihla, 1999; *Cratosilis* Motschulsky, 1860; *Cultellunguis* McKey-Fender, 1950; *Cyrebion* Fairmaire, 1891; *Cyrtomoptera* Motschulsky, 1860; *Falsopodabrus* Pic, 1927; *Habronychus* Wittmer, 1981; *Islamocantharis* Wittmer & Magis, 1978; *Leiothorax* Wittmer, 1978; *Lycocerus* Gorham, 1889; *Malchinomorphus* Pic, 1922; *Metacantharis* Bourgeois, 1886; *Micropodabrus* Pic, 1920; *Occathemus* Švihla, 1999; *Pacificanthia* Kazantsev, 2001; *Pakabsidia* Wittmer, 1972; *Paracantharis* Wittmer, 1969; *Podabrinus* Fairmaire, 1896; *Podistra* Motschulsky, 1839; *Prothemellus* Švihla, 1992; *Prothemus* Champion, 1926; *Pseudopodabrus* Pic, 1906; *Rambesilis* Pic, 1911; *Rhagonycha* Eschscholtz, 1830; *Rhaxonycha* Motschulsky, 1860; *Sidabia* Švihla, 1994; *Silicantharis* Švihla, 1992; *Simplexonycha* Fanti, 2022; *Sinometa* Wittmer, 1969; *Sogdocantharis* Kazantsev, 1993; *Stenothemus* Bourgeois, 1907; *Taiwanocantharis* Wittmer, 1984; *Taocantharis* Švihla, 2011; *Themus* Motschulsky, 1858; *Walteriella* Kazantsev, 2001; *Yukikoa* Satô, 1976; †*Arturmiles* Fanti, 2021; †*Electronycha* Kazantsev, 2013; †*Juratelacrima* Fanti & Damgaard, 2018; †*Molliberus* Peris & Fanti. 2018; †*Palmnickeneoceras* Fanti & Damgaard, 2018; †*Vitalfranzius* Fanti & Müller, 2022 (53 genera).
Tribe PODABRINI Gistel, 1856: 385. Type genus: *Podabrus* Dejean, 1833. *Asiopodabrus* Wittmer, 1982; *Malthacus* Kirby, 1837; *Hatchiana* Fender, 1966; *Podabrus* Westwood, 1838; *Tibiopodabrus* Švihla, 2004 (five genera).
†Tribe CACOMORPHOCERINI Fanti & Kupryjanowicz, 2018. Type species: *Cacomorphocerus* Schaufuss, 1892. †*Cacomorphocerus* Schaufuss, 1892; †*Electrokleinia* Ellenberger & Fanti, 2019; †*Eridanula* Fanti & Damgaard, 2018; †*Michalskantharis* Fanti, 2017; †*Noergaardia* Fanti & Damgaard, 2018; †*Sucinocantharis* Kuśka & Kania, 2010; †*Sucinorhagonycha* Kuśka, 1996 (seven genera).

Cantharidae incertae sedis: *Ctenophorellus* Silvestri, 1920; *Malthaster* Gorham, 1885; *Porostenus* Motschulsky, 1853 (three genera).

PART V

Studies on neoteny in Coleoptera: phylogenomics, classification, and evolutionary interactions

Dominik Kusý

Phylogenomic and mitogenomic data can accelerate inventorying of tropical beetles during the current biodiversity crisis.

(published manuscript; elife).

RESEARCH ARTICLE

Phylogenomic and mitogenomic data can accelerate inventorying of tropical beetles during the current biodiversity crisis

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Abstract Conservation efforts must be evidence-based, so rapid and economically feasible methods should be used to quantify diversity and distribution patterns. We have attempted to overcome current impediments to the gathering of biodiversity data by using integrative phylogenomic and three mtDNA fragment analyses. As a model, we sequenced the Metriorrhynchini beetle fauna, sampled from ~700 localities in three continents. The species-rich dataset included ~6500 terminals, ~ 1850 putative species delimited at 5% uncorrected pairwise threshold, possibly ~1000 of them unknown to science. Neither type of data could alone answer our questions on biodiversity and phylogeny. The phylogenomic backbone enabled the integrative delimitation of robustly defined natural genus-group units that will inform future research. Using constrained mtDNA analysis, we identified the spatial structure of species diversity, very high species-level endemism, and a biodiversity hotspot in New Guinea. We suggest that focused field research and subsequent laboratory and bioinformatic workflow steps would substantially accelerate the inventorying of any hyperdiverse tropical group with several thousand species. The outcome would be a scaffold for the incorporation of further data from environmental sequencing and ecological studies. The database of sequences could set a benchmark for the spatiotemporal evaluation of biodiversity, would support evidence-based conservation planning, and would provide a robust framework for systematic, biogeographic, and evolutionary studies.

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Editor's evaluation

This manuscript provides clear ideas regarding the usage of next-generation sequencing data, and of more traditional mtDNA markers, to rapidly increase biodiversity inventories. You demonstrate how biodiversity information analyses done in the Metriorrhynchini, a hyperdiverse tropical insect group, can be rapidly expanded via targeted field research and large-scale sequencing. The study sets a benchmark for the spatiotemporal evaluation of tropical biodiversity, supports evidence-based conservation planning, and provides a robust framework for systematic, biogeographic, and evolutionary studies.

Introduction

The number of known insects surpasses that of all other terrestrial groups (***Mora et al., 2011***), and we need much more detailed information to fully understand their diversity. Currently, the available biodiversity data are far from complete, and the majority of insect species remain undescribed (***Novotny et al., 2006***; ***Srivathsan et al., 2019***). In addition, robust phylogenetic hypotheses are lacking for

most lineages, and the genera and tribes are often artificial assemblages which are not relevant to evolutionary and biodiversity research. Therefore, we need to gather new information in order to advance our understanding of evolutionary and genetic relationships, and to build a phylogenetic scaffold for comprehensive taxonomic, biogeographic, and evolutionary studies that would be indispensable for biodiversity management.

Descriptive, morphology-based insect systematics is not keeping pace with the rapid loss and degradation of natural habitats (***Theng et al., 2020***), and with the ongoing decline in insect abundance as a result of human activities and climate change (***van Klink et al., 2020***). The largest taxonomic journal, *Zootaxa*, published almost 30,000 studies describing >60,000 new species and these represent over a quarter of all new animal species reported in 2001–2020 (***Zhang, 2021***). Although these numbers are impressive, they also show how labor-intensive is taxonomic research if, in average, only two new species are reported in a publication. To accelerate the cataloguing of biodiversity, it is vital to gather new material suitable for molecular analyses and to combine available molecular methods with traditional approaches (***Riedel et al., 2013***; ***Srivathsan et al., 2019***; ***Yeo et al., 2020***; ***Sharkey et al., 2021***). DNA data are indisputably a valuable source for modern biodiversity research, and they can address both shallow and deep relationships (***Tautz et al., 2003***; ***Hajibabaei et al., 2007***). There are two principal sources of short-fragment data: voucher-based DNA sequences typically produced by systematists (***Riedel et al., 2013***; ***Yeo et al., 2020***; ***Sharkey et al., 2021***), and DNA sequences produced by an ecosystem-based sequencing that does not associate individual samples with Linnean names (***Andújar et al., 2015***; ***Srivathsan et al., 2019***). It is the responsibility of systematic biologists to assemble the natural system, that is, we need to reliably delimit genus- and tribe-level taxa, to make their ecological and distribution attributes informative. But the short fragments are often unsuitable for the building of deep phylogenies and large genomic datasets need to be used (***McKenna et al., 2019***; ***Baca et al., 2021***). These include transcriptomes, whole genome

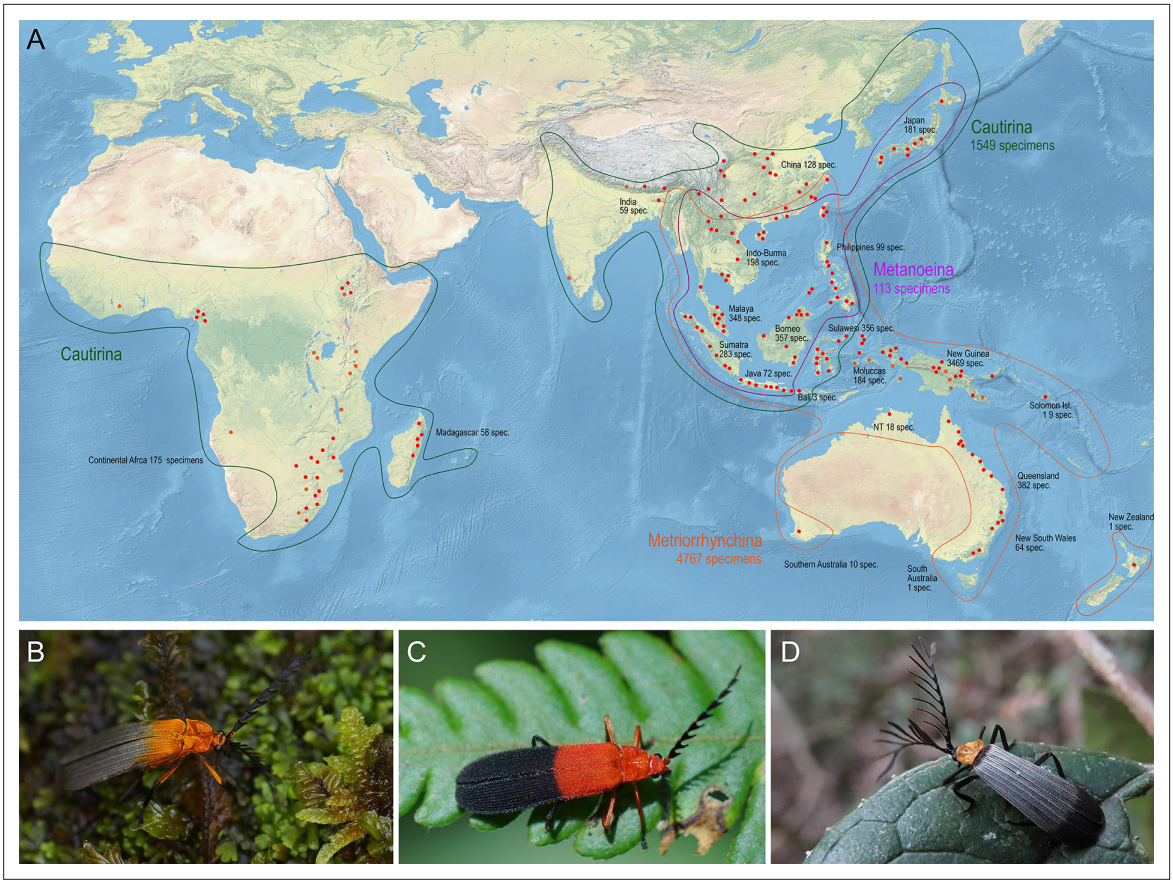

Figure 1. Distribution and appearance of metriorrhynchine net-winged beetles. (**A**) Distribution of Metriorrhynchini with major sampled localities designated by red dots. The numbers of analyzed specimens from individual regions are shown for regions and subtribes. (**B–D**) – General appearance of Metriorrhynchini.

sequences and anchored hybrid capture datasets. Using all information, a robust and stable natural classification will significantly facilitate detailed research into the spatial and temporal distribution of biodiversity (***Morrison et al., 2009***; ***Thomson et al., 2018***). As an ultimate goal, we should attempt to construct a complete tree of life, or at least its backbone, which is invaluable in aiding the selection of groups for more detailed analyses (***Chesters, 2017***; ***McKenna et al., 2019***). With a well-defined high-level classification, it is paramount to exploit all accessible data. We assume that voucher-based molecular phylogenies provide much-needed tools to researchers working on site-based biodiversity assessments (***Andújar et al., 2015***; ***Srivathsan et al., 2019***) and that, in turn, the data produced by environmental and ecosystem-focused sequencing contribute to building the tree-of-life (***Arribas et al., 2016***; ***Bocak et al., 2016***).

We have used hyperdiverse tropical metriorrhynchine beetles (Coleoptera, Lycidae, Metriorrhynchini) as our model. This net-winged beetle tribe contains >1500 recognised species, mostly found in the Old-World tropics (***Figure 1A***), and their classification is complicated by the complex taxonomic history (***Bocak et al., 2020***; see Appendix introductory information). The phenetic plasticity of Metriorrhynchini is relatively high (***Figure 1B–D***), but many distant species resemble each other due to convergent selection in Mullerian rings (***Bocek et al., 2019***; ***Motyka et al., 2020***; ***Motyka et al., 2021***). Therefore, unrelated taxa were often assumed to be closely related due to misleading morphological similarities. Although there are over 40 genera in the tribe, three-quarters of the species have been described in five ambiguously defined genera (*Xylobanus*, *Cautires*, *Trichalus*, *Metriorrhynchus*, and *Cladophorus*). Sometimes a single genus contains species from different subtribes (***Bocak et al., 2020***). In this respect, the Metriorrhynchini is a typical species-rich tropical insect group without well-founded classification and the paucity and inaccuracy of available data (***Letsch et al., 2020***). As a result, unlike vertebrates, these poorly known insect groups have not been considered for use in large-scale, integrative projects and data metanalyses (***Myers et al., 2000***; ***Holt et al., 2013***) and have contributed little to our understanding of global biodiversity patterns.

The principal objective of this study is to demonstrate how biodiversity information for a hyperdiverse tropical group can be rapidly expanded via targeted field research and large-scale sequencing of transcriptomes, genomes, and short mtDNA fragments. Our investigation comprised four distinct steps. First, we assembled material from several hundred localities on three continents (***Figure 1***, ***Table 1***). Second, as hyperdiverse groups are difficult to tackle and the current classification is unreliable, we attempted to compartmentalise diversity using phylogenomics. We then produced a tree,

Table 1. The numbers of sampled localities per region.
Details in ***Appendix 1—table 1***.

Area	Localities	Area	Localities
Australian region	**298**	**Sino-Jap. region**	**79**
Australia	118	China	51
New Guinea & Solomons	179	Japan	28
New Zealand	1		
Wallacea	**49**		
Moluccas	15	**Oriental region**	**206**
Sulawesi	34	S.India & Ceylon	3
		E.India & Burma	12
Afrotropical Region	**64**	E.Indo-Burma	44
West Africa	1	Malay Peninsula	57
Guinean Gulf	11	Sumatra	23
Ethiopia	6	Java & Bali	15
East Africa	10	Philippines	33
South Africa	25		
Madagascar	11	**Total**	**696**

using all available data, to estimate species limits, intraspecific genetic variability, and species ranges. Finally, the tree was pruned and used to estimate shallow phylogenetic relationships, total and regional species diversity, and endemicity, and to define generic ranges and continental-scale range shifts. The applied methods of transcriptome and mtDNA analyses are widely used. The genomic datasets dominate among works focusing on deep relationships (transcriptomes and anchored hybrid capture; ***Misof et al., 2014***; ***Kusy et al., 2019***; ***McKenna et al., 2019***; ***Baca et al., 2021***). The mitochondrial markers have been used mainly to study the phylogeny of restricted clades (***Toussaint et al., 2014***; ***Sharkey et al., 2021***). Until now, the genomic and mitochondrial data have seldom been combined to get simultaneously the phylogenetic backbone for the mid-rank classification (subtribes, groups of genera, generic limits) and the estimations of species diversity (e.g. ***Talavera et al., 2021***). Our information and phylogenetic hypotheses can be a resource for higher level phylogenetics, population genetics, phylogeographic studies, and biodiversity estimation. At the same time, we want to show how limited our taxonomical knowledge is and how this lack is hindering biodiversity research and management (***Thomson et al., 2018***).

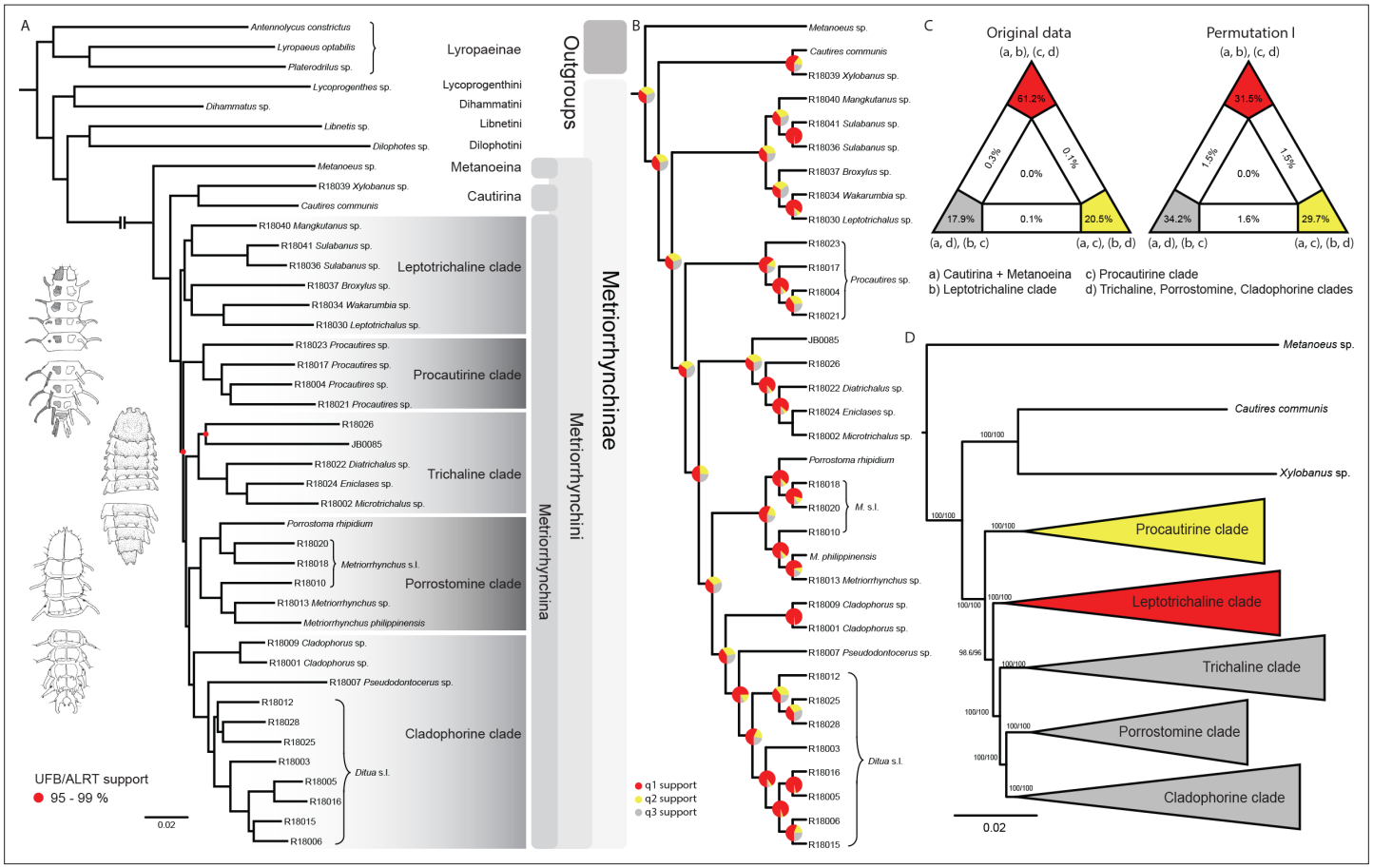

Figure 2. Topologies recovered by phylogenomic analyses. (**A**) Phylogenetic relationships of Metriorrhynchinae based on the ML analyses of the concatenated amino-acid sequence data of supermatrix F-1490-AA-Bacoca-decisive. Unmarked branches are supported by 100/100 UFB/alrt; red circles depict lower phylogenetic branch support. (**B**) Phylogenetic relationships of Metriorrhynchini recovered by the coalescent phylogenetic analysis with ASTRAL when analysing the full set of gene trees (4109 gene trees inferred at the nucleotide level). Pie charts on branches show ASTRAL quartet support (quartet-based frequencies of alternative quadripartition topologies around a given internode). Outgroups taxa are not shown. (**C**) Results of FcLM analyses for selected phylogenetic hypotheses applied at the amino-acid sequence level (supermatrix F). (**D**) Alternative phylogenetic relationships of Metriorrhynchinae based on the ML analyses of the concatenated amino-acid sequence data of supermatrix A-4109-AA. Numbers depict phylogenetic branch support values based on 5000 ultrafast bootstrap replicates.

Results

Sampling of the Metriorrhynchini range

In total, we monitored almost 800 localities, 696 of them with occurrences of the Metriorrhynchini (Tabs. 1, ***Appendix 1—table 1***). The distribution of sampling sites was partly biased due to the large extent of the Metriorrhynchini range, limited time and funds, different goals of various expeditions, and logistic problems (inaccessible regions, legal obstacles). The densest sampling is available from the Sundaland and New Guinea, while India and the Afrotropical region are under-sampled (***Figure 1A***).

Assembly of the phylogenomic tree

The phylogenomic dataset contained 35 Metriorrhynchini terminals (***Appendix 1—table 2***), seven outgroups, and ~4200 orthologs (1.9–5.7 × 10^6 aligned positions; ***Supplementary file 1***;

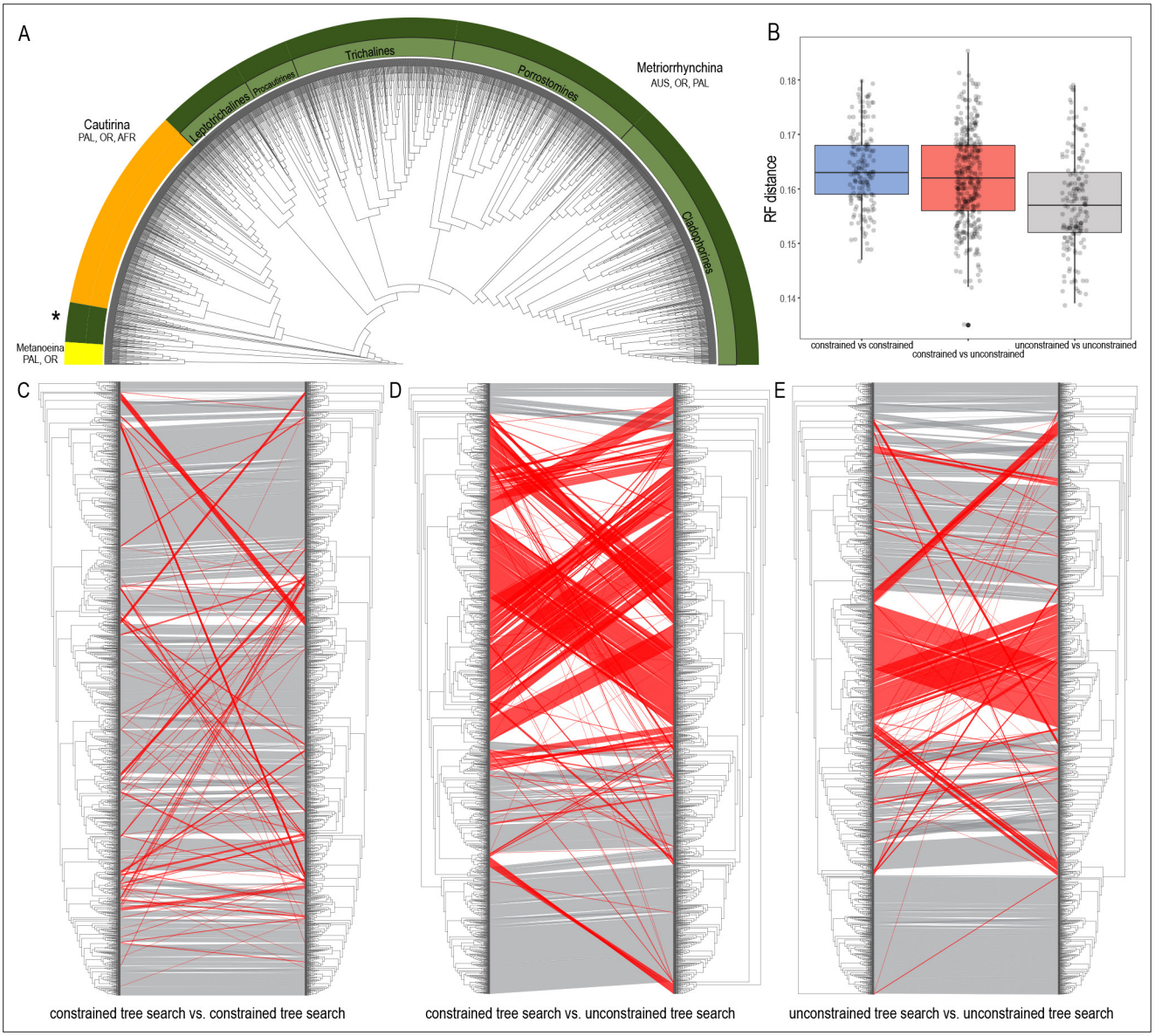

Figure 3. Topologies recovered by mitogenomic analyses. (**A**) Relationships of Metriorrhynchini recovered by the constrained analysis of the pruned dataset at 2% distance. (The full resolution tree is shown in ***Source data 2*** along with a tree recovered from the analysis of a complete dataset of 6429 terminals in ***Source data 1***), asterisk designates a grade of Metriorrhynchina-like taxa found in a position in conflict with their morphology; (**B**) A chart of Robinson-Foulds distances among topologies inferred by repeated runs of the constrained and unconstrained analyses; (**C**) A comparison of the results obtained by two runs of the constrained analysis; (**D**) A comparison of trees inferred with/without the phylogenomic backbone; (**E**) A comparison of results obtained by two runs of the unconstrained analysis. The red lines designate terminals with conflicting positions in compared trees.

Appendix 1—table 3; ***Appendix 1—table 4***). The tree shown in ***Figure 2A*** was produced using maximum likelihood (ML) analyses, whereas the coalescent method produced the topology shown in ***Figure 2B***; additional trees are shown in ***Appendix 1—figures 1–8***. For details on the data sets' characteristics see ***Appendix 1—figures 9–12***. Phylogenomic analyses resolved three subtribes (Metanoeina [Metriorrhynchina, Cautirina]), and five clades were regularly recovered within the Metriorrhynchina, that is, the hereby defined procautirines, leptotrichalines, trichalines, porrostomines, and cladophorines. Different settings (see Materials and methods) produced slightly different topologies and shifted the positions of the leptotrichalines and procautirines (***Figure 3D***). However, the monophyly of major subclades was not affected. The FcLM analysis favored a deeper position for the leptotrichaline clade (61.2%; ***Figure 2C***, ***Appendix 1—figure 13***). The position of the remaining terminals was stable across all analyses. All phylogenomic analyses question the definitions of some species rich genera (***Appendix 1—figures 1–8***) that are either polyphyletic (e.g. *Cladophorus*; 131 described species, most of them recovered in the *Ditua* subclade) or paraphyletic (*Metriorrhynchus* as a grade and *Porrostoma* in the terminal position; 194 species, see ***Figure 2A and B***, ***Appendix 1—figures 1–8***).

Constrained mitogenomics

The mtDNA database contained >11,500 mtDNA fragments (5935 *cox1*, 2381 *rrnL*, and 3205 *nad5*) representing 6429 terminals (2930 aligned positions). Using these data, we inferred additional trees using the constrained positions of 35 terminals whose relationships were determined through

Table 2. The numbers of described species and identified mOTUs (molecular operational taxonomic units) at 2% and 5% thresholds per region and the total number of OTUs identified for subtribes.
Based on morphological identification, the OTUs of the kassemiine and other deeply rooted clades are included in Metriorrhynchina.

Region	Metriorrhynchina described/ analyzed at 2%/5%		Cautirina described/ analyzed at 2%/5%	Metanoeina described/ analyzed at 2%/5%	Metriorrhynchini described/ analyzed at 2%/5%	Ratio Analyzed/ described
Australian region	639/1608/1239			639/1608/1239	2.52–1.93	
Australia	196/167/133			196/167/131	0.85–0.67	
New Guinea	423/1434/1105			423/1434/1105	3.39–2.61	
Solomon Isl.	21/9/9			21/9/9	0.43	
Wallacea	162/174/162	14/10/9		176/184/171	1.05–0.97	
Philippines	51/18/18	45/12/12	8/3/3	104/33/33	0.32	
Continental Asia	43/52/42	331/330/257	30/34/31	404/416/330	1.03–0.82	
Sundaland	36/44/39	201/184/146	24/19/17	261/247/202	0.95–0.77	
Indo-Burma	6/7/7	62/52/42	3/4/4	74/63/53	0.85–0.72	
China, Japan	1/1/1	53/75/58	1/11/11	55/87/70	1.58–1.27	
India		35/19/18	2/0/0	37/19/18	0.51–0.49	
Afrotropical region		231/104/94		231/104/94	0.46–0.41	
Sub-Saharan Africa		178/74/65		178/74/65	0.42–0.37	
Madagascar		53/30/29		53/30/29	0.57	
Total number of OTUs	895/1852/1445	641/456/369	38/37/34	1574/2345/1848	1.50–1.17	

phylogenomic analyses, and the free positions of the other ~6400 terminals (***Source data 1***). The units based on uncorrected pairwise distances represent molecular operational taxonomic units (mOTUs), considered to be putative species, or 'species' for short. Depending on the applied 2% and 5% thresholds, we identified 34–37 mOTUs in the Metanoeina clade and 369–456 mOTUs in Cautirina. The major Metriorrhynchina clade (1376–1763 mOTUs) included procautirines, leptotrichalines, trichalines, porrostomines, and cladophorines. In addition, we identified several deeply rooted lineages, the kassemiines, and another five small clades (69–89 mOTUs in total; ***Source data 2***), each of which comprised a limited number of species. As phylogenomic data for these terminals are still lacking, their positions were determined based only on mtDNA data and they are included in Metriorrhynchina, based on morphological traits (***Table 2***). The number of mOTUs does not include ~50 mOTUs for which *cox1* was unavailable.

Pruned mitogenomic tree with and without constraints

The dataset was subsequently pruned to a single terminal per mOTU based on 2% and 5% distance (see below) and was analyzed both with and without topological constraints (***Figure 3A***; ***Source data 2 and 3***) show the pruned trees at 2% levels to capture the intraspecific genetic variability within the clusters of closely related mOTUs). Repeated runs with different starting seeds identified terminals with unstable positions (***Figure 3A–C***). The major clades were generally stable, whereas small, deeply rooted clades were prone to 'wandering' around the tree, as were distinct singletons. The trees that resulted from each of the seed-specific 19 ML runs differed slightly; tree similarity was thus evaluated using the Robinson-Foulds index, with values ranging from 0.180 (most similar) to 0.147 (most distant; ***Appendix 1—table 4***).

Tree congruence

The degree of incongruence between selected topologies is shown in ***Figure 3C–D***. The unconstrained analysis of mitochondrial data yielded a topology with a high number of terminals that were recovered in positions incongruent with their morphology (***Figure 3D and E***, ***Source data 3***). The same dataset, when analyzed using the constrained position of 35 terminals (based on their relative relationships inferred by prior phylogenomic analyses), produced a topology with a much lower proportion of terminals in dubious positions (***Figure 3C***, ***Source data 2***). The composition of the constituent clades is based on the topology recovered by the constrained mtDNA analyses and the position of genera was validated by morphological comparisons of vouchers with the type species of earlier described genera (all redescribed by ***Bocak, 2002***). The named genera assigned into individual subclades are shown individual clades are characterized in Appendix results.

Species diversity

To investigate the total and regional species diversity of the Metriorrhynchini, we analyzed a dataset comprising 5935 of the 6429 terminals for which the *cox1* mtDNA fragment was available (***Figure 3A***; ***Supplementary file 1***). For the Metriorrhynchini, we identified 1848 and 2345 mOTUs using the 5% and 2% thresholds, respectively (***Appendix 1—figure 14***). We disregarded the presence of ~50 mOTUs (494 terminals) for which *cox1* was missing. The number of mOTUs based on the *cox1* analysis varied by thresholds and the number of delimited OTUs increased relatively slowly with decreasing threshold values from 1% to 10% (***Appendix 1—figure 14***).

Using an earlier published literature review (***Bocak et al., 2020***), we updated species lists for the Cautirina (641 spp. described species), Metanoeina (38 spp.), and Metriorrhynchina by adding taxa described in 2020 and 2021. By analysing DNA data, we identified 34–37 putative spp. of Metanoeina, 369–456 spp. of Cautirina, and 1445–1852 spp. of Metriorrhynchina, depending on the applied mtDNA uncorrected pairwise 5% and 2% mtDNA distance thresholds. The numbers of species per subregion, along with the estimated ratios between formally described and estimated species diversity, are shown in ***Table 2*** for the 2% and 5% threshold (further information in ***Appendix 1—figure 14***). Using both thresholds, 2% and 5%, the numbers of putative species surpass the numbers of species reported in the literature.

We observed very high species turnover even if 5% threshold was applied for delimitation. Only four mOTUs have been recorded in two landmasses separated by a deep-sea (> 200 m). The faunas of Sulawesi and the islands across Wallace's and Weber's lines share two mOTUs, one mOTU was

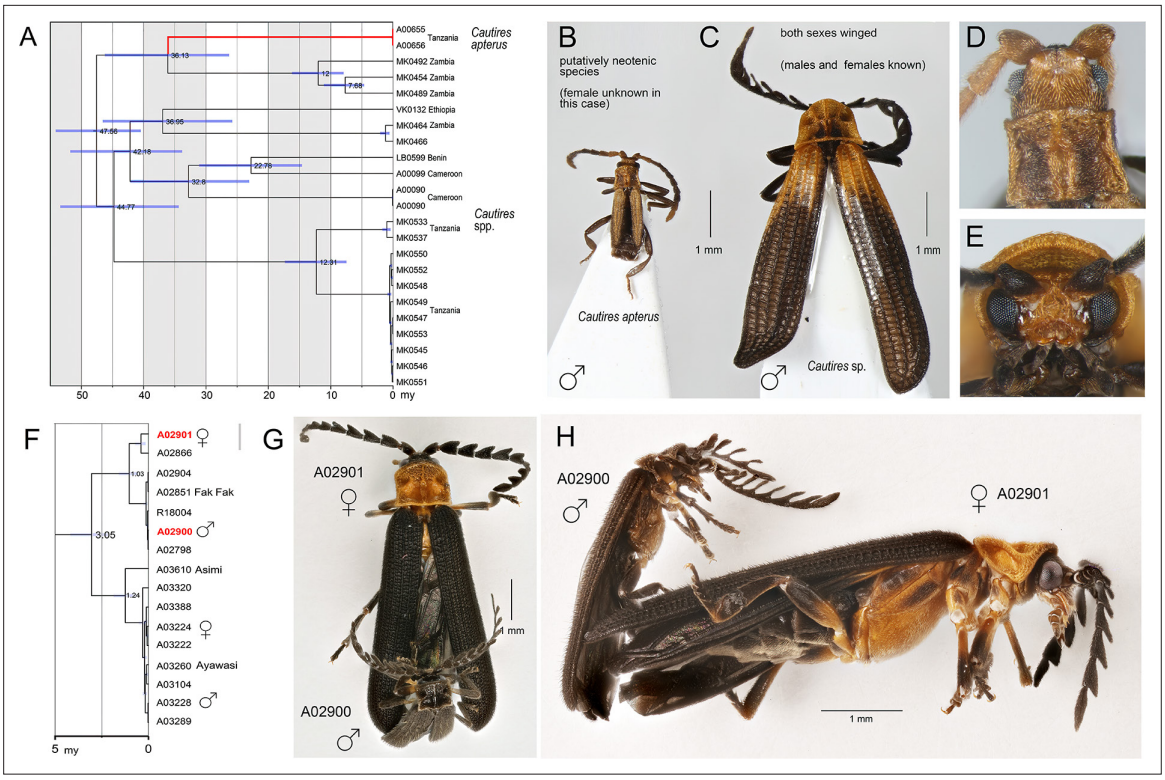

Figure 4. Identification of sexual dimorphism by large-scale biodiversity inventory. (**A**) Relationships of lineages with modified ontogeny, the dated tree; (**B, D**) General appearance and head of *Cautires apterus*, a putative neotenic species; (**C, E**) ditto of the close relative with both sexes winged. Mimetic sexual dimorphism identified during diversity survey. (**F**) The dated tree, red colored terminal labels designate the individuals shown in G and H; (**G**) Dorsal view of individuals in copula; (**H**) Ditto, lateral view. Except of collecting individuals in copula, DNA-based assessment of relationships is the only option as the species are sexually dimorphic and no morphological traits indicate their conspecifity.

simultaneously identified in Laos and Luzon and one species in New Guinea and the Solomon islands. Similarly, only sixteen mOTUs were distributed across two landmasses separated by an inundated shelf (sea depth <100 m). Nine mOTUs were distributed in two or more islands of Southeast Asia and seven species were found in both New Guinea and Australia. The centres of species diversity of the Metriorrhynchini are New Guinea (1,105 putative spp. at 5% threshold) and the seasonally to perennially humid areas of the Sundaland (202 spp. at 5% threshold). The results suggest substantial modifications to the generic limits and ranges for numerous taxa that had been previously delimited (***Appendix 1—figure 15***).

Having the extensive the mtDNA topologies, we looked for examples of the evolution of neoteny and mimetic polymorphism. The detailed inspection of trees identifies the closest available relative of a putative neotenic, *Cautires apterus* (Cautirina). This species is morphologically very distinct (***Figure 4B and D***). The dated subtree indicates the recent origin of morphological divergence (***Figure 4A***). The mtDNA analyses recover some species with pronounced sexual dimorphism, such as an unidentified genus and species of the procautirine clade (***Figure 4F–H***). The origin of the polymorphism is putatively very recent (***Figure 4F***).

Discussion

In the context of the present loss of biodiversity (***Sodhi et al., 2004***; ***Hallmann et al., 2017***; ***Theng et al., 2020***), large-scale genomic resources are urgently needed for biodiversity assessment and conservation (***Hajibabaei et al., 2007***; ***Krehenwinkel et al., 2019***). Molecular data cannot replace morphology-based taxonomy (***Figure 3C–D***; ***Thomson et al., 2018***), but the analyses of our dataset complement and facilitate traditional biodiversity research in several directions. Our first step is to compartmentalize hyperdiverse Metriorrhynchini into manageable natural units (***Figure 2***). The densely sampled phylogeny identifies tribal and generic limits. It provides a useful foundation for

detailed taxonomic research through the identification of weak areas in earlier classifications and points out the clades with undescribed diversity and non-monophyletic genera (e.g. several hundred species of *Ditua* and paraphyletic *Metriorrhynchus*; ***Figure 3***, ***Appendix 1—figure 15***). Furthermore, the analyses of species-rich datasets identify the areas with high species diversity as one of the critical conservation value parameters (***Table 2***; ***Baselga, 2010***; ***Srivathsan et al., 2019***). Traditional taxonomic research costs time and money, and the number of newly described species is relatively low if we consider the enormous diversity of tropical insects (***Novotny et al., 2006***; ***Sangster and Luksenburg, 2015***). Therefore, we use DNA-based units as a provisional descriptor of species diversity (***Hebert et al., 2003***; ***Monaghan et al., 2009***), and subsequently as a source for integrative taxonomy (***Source data 1–3***; ***Srivathsan et al., 2019***). The presented large-scale monitoring project provides information on relationships (***Figures 2 and 3***), genetic divergence (***Source data 1–3***), turnover (***Table 2***), the extent of generic and species ranges (***Appendix 1—figure 15***, ***Source data 1–3***), and on evolutionary phenomena that are usually studied using a few model organisms (***Figure 4***). Using phylogenomics and voucher-based sequencing, we show that taxonomic literature has provided insufficient and sometimes erroneous information, even after the formal consolidation of scattered descriptions (***Bocak et al., 2020***). We show that a taxon-focused continental scale project can effectively assemble comprehensive data for diversity of tropical insects.

Continent-wide taxon-specific monitoring of biodiversity: feasibility and impediments

Tissue and DNA archives have become critical in the assessment of biodiversity status (***Hajibabaei et al., 2007***; ***Blom, 2021***). Although museomics is a potentially valuable source of data (***Gauthier et al., 2020***), in our case, museum collections are insufficient for filling data gaps due to the scarcity of material. For example, the Metriorrhynchini collection deposited in the Natural History Museum in London contains <3000 specimens, whereas there are ~6500 terminals in our dataset. At the beginning of our study, we faced critical absence of primary data. Therefore, we conducted intensive field research to obtain samples for a realistic assessment of the extant Metriorrhynchini diversity. We processed samples from our expeditions (most of which were focused on a range of topics over two decades between 2001 and 2019) and samples obtained through extensive collaboration with other researchers, both local and visiting, and with local naturalists whose contribution has increased with the growing number of citizen science projects (***Jaskula et al., 2021***; ***MacPhail and Colla, 2020***). In such a way, we assembled a Metriorrhynchini tissue collection from almost 700 localities in three continents (***Table 1***, ***Figure 1***). For several reasons our sampling is partly biased. We noted the serious loss of natural habitat in many regions. Previously described species were often collected in vicinity of seaports, but the lowland ecosystems are rapidly disappearing due to human exploitation. Therefore, type localities of many described species could not be sampled during recent expeditions and species known from museum collections are missing in our DNA dataset (***Jiruskova et al., 2019***). The habitat loss in South East Asia also affects other animal groups, and lowland primary forests are seriously endangered in the whole region (***Sodhi et al., 2004***; ***Theng et al., 2020***). Further sampling bias is a consequence of the unsafe conditions and logistic problems in large areas of West Africa, Sahel, and the Congo Basin (***Figure 1A***), where net-winged beetles have not been systematically studied since the 1930 s. Additional data gaps are caused by strict biodiversity research restrictions (***Prathapan, 2018***). Regardless of these limitations, we believe that the assembled dataset is a foundation for a robust classification framework and a soundly based assessment of biodiversity. Our results show the importance of field research for biodiversity studies and systematics (***Basset and Lamarre, 2019***).

Phylogenetic relationships: a scaffold for targeted research

Unresolved taxonomy is a common reason for the exclusion of specific groups from biodiversity research projects and this omission has an effect on conservation policies (***Gutiérrez and Helgen, 2013***). The current phylogenomic and mitogenomic phylogenetic hypotheses (***Figures 2 and 3***; ***Source data 1–3***) supersede the morphology-based topologies (***Bocak, 2002***). The phylogenomic analysis incorporates a large amount of information, and we favor this method over morphological traits and short DNA sequences, both of which contain uncertainties (***McKenna et al., 2019***). Phylogenomics has resolved subtribe relationships and their internal structures. The analyzed 35 transcriptomes and low-coverage genomes were sufficient to identify five major Metriorrhynchina clades with

several hundred putative species each and also to identify the limits of genera, which can be tested using traditional taxonomic methods (***Figures 2 and 3A***; ***Source data 1–3***).

The sampling strategy is critical for building a phylogenomic backbone. Our goal was to cover as many deep lineages as possible and simultaneously to limit the number of sequenced RNA samples to avoid high costs. Therefore, we sequenced RNAlater preserved tissues and conspecific vouchers prior to assigning tissue samples for transcriptomic analyses. In this way, two rounds of sequencing provided us with critical information based on evenly distributed anchor taxa. In the next step, we re-analyzed the short-fragment dataset (***Supplementary file 1***) using constrained positions for taxa whose relationships had already been recovered through phylogenomics (***Figures 2 and 3***). A stabilized phylogenomic backbone is critical for inferring a robust topology because the large-scale analyses of short mtDNA fragments are sensitive, even to the application of starting seeds, and they often produce topologies incongruent with morphological traits (***Figure 3B and E***; ***Sklenarova et al., 2014***). Only several small lineages have remained unanchored by genomic data, owing to a lack of properly fixed samples (***Source data 2 and 3***). For example, four small clades are much more deeply rooted than their morphology suggests (***Figure 3A***, ***Source data 2***) and additional data are needed to place them in a phylogenetic context. Despite some contentious relationships that need further investigation, 35 genomic samples, that is under 2% of species, sufficiently supported relationships among most terminal clades that approximately represent genera, groups of genera and subtribes. ***Talavera et al., 2021*** have shown that only six nuclear markers for 5%–10% of terminals can similarly stabilize the phylogeny of a species rich model group. We assume, that the combination of genomic and short DNA data can be valuable for building of the species-level trees of life.

We identified a substantial conflict between phylogenomic analyses, morphology-based classifications (***Bocak, 2002*** and earlier studies cited therein), and the analyses of a few short DNA fragments (***Sklenarova et al., 2014***, Appendix introductory information). Our analyses confirm the monophyly of the recently described Cautirina and Metanoeina and redefined Metriorrhynchina except several unanchored lineages (***Sklenarova et al., 2013***; ***Sklenarova et al., 2014***), but, for the first time, we can robustly recover subtribal relationships (***Figure 2A and B***). We reject most internal splits suggested by morphological and mtDNA analyses (***Bocak, 2002***; ***Sklenarova et al., 2013***; ***Sklenarova et al., 2014*** and earlier studies cited therein). The present delimitation of five monophyla within Metriorrhynchina resolves the backbone of the subtribe that was contentious due to high levels of homoplasy in Sanger and morphology-based datasets. Similarly, some generic concepts are questioned as they have been mostly based on highly homoplastic traits (***Sklenarova et al., 2014***; ***Kusy et al., 2019***). The taxonomic studies must consider the morphology along with molecular hypotheses. As morphology is not described in this study, we do not discuss the limits of individual genera and report only short information on newly defined subclades (Appendix results).

Our approach yielded a constrained phylogeny with 6429 terminals and almost 2000 mOTUs using 5% mtDNA distance threshold, and this provides the basis for the approximation of species diversity for constituent subclades and geographic regions (***Figure 3***; ***Source data 2 and 3***). Concerning the extent of diversity, phylogenomic and mitochondrial data must be simultaneously analysed to provide a strong foundation for subsequent investigations (***Figure 3C***, ***Talavera et al., 2021***). Phylogenomics cannot deal with thousands of species, and mitogenomic data are insufficient for the construction of robust deep relationships. The final steps are morphological validation of the proposed generic groups and genera (see Appendix results) and, in the future, formal descriptions of biodiversity using the Linnean classification. In such a way, the results of phylogenomic and mitogenomic inventory should be incorporated in the formal classification (***Godfray and Knapp, 2004***).

Species diversity: literature data and reality

Here, we deal with a species rich tropical beetle tribe (> 1500 described species), and therefore we use the uncorrected pairwise distance thresholds for our diversity estimation (***Table 2***, ***Appendix 1—figure 14***). The application of any threshold is a compromise between estimation accuracy, speed, and sequencing costs, taking into account the feasibility of inventorying a hyperdiverse group within a limited time frame (***Hebert et al., 2003***; ***Dupuis et al., 2012***; ***Eberle et al., 2020***). Several taxonomical works on Metriorrhynchini have simultaneously analysed mtDNA fragments, nuclear genes, morphology, and ecology (e.g. ***Bocak and Yagi, 2010***; ***Kalousova and Bocak, 2017***; ***Bocek et al., 2019***; ***Jiruskova et al., 2019***), but their results cannot robustly defend an application of a distance

threshold for the whole tribe. To avoid a possibility of diversity overestimation, we base further discussion on the 5% distance. We assume that such a threshold might be sufficiently cautious. Future taxonomic revisions are surely needed for the validation of here presented data on species diversity.

When analysing the *cox1* mtDNA fragment, we identified 1,848 mOTUs at 5% threshold and the numbers of delimited mOTUs indicate that, the substantial part of species diversity remains undescribed (***Table 2***, ***Appendix 1—figure 14***). Additionally, the slope representing the relationship between the number of mOTUs and distance thresholds was gradual (***Appendix 1—figure 14***) due to a high number of genetically distant, indisputably distinct lineages in the dataset (***Appendix 1—figure 14***).

Our approach provides information about the diversity of the internal lineages. Metriorrhynchina is by far the most diverse group, within which the cladophorines comprise the largest clade (490 mOTUs, e.g, *Ditua* historically has included 2 spp., now ~250 spp.). The porrostomine clade is the next diverse group (373 mOTUs) and contains the speciose *Porrostoma* (126 mOTUs) and a paraphyletic series of lineages whose species have conventionally been placed in *Metriorrhynchus*. The differences between previously published data and our results are substantial and they question any reanalyses of literature data without prior verification (***Bocak, 2002***; ***Bocak et al., 2020*** references therein; ***Source data 2 and 3***).

The numbers of mOTUs must be interpreted in the context of the sampling activity in each region. We did not use standardized protocols due to the long-term character of our research, the incorporation of some samples provided by other researchers, and the necessity to apply appropriate collecting methods to maximize the number of recorded species in various ecosystems and under different weather conditions. We identified only 94 mOTUs from the Afrotropical region, mainly due to the limited number of collecting trips by authors (64 localities) and the inaccessibility of some areas. Despite intensive field research (33 localities), we collected from the Philippines less than one third of the species described (33 mOTUs). Our collection activities in the Philippines were hindered by substantial loss of natural habitats, and this is soon expected to be the case in other regions (***Sodhi et al., 2004***). The number of species known from the Sundaland (114 localities) was approximately equal to the number of sequenced mOTUs despite disproportionately intensive collecting effort by the authors. Even after numerous expeditions to the Sundaland, many regions remain unsampled. As metriorrhynchine species ranges are small (***Jiruskova et al., 2019***; ***Motyka et al., 2020***), the number of species will probably increase in the future. The proportion of new species was regionally ~70% if DNA data and morphology were considered in detailed taxonomic studies (e.g. ***Jiruskova et al., 2019***). While these regions house numerous unknown species, we found New Guinea to be exceptionally diverse, with almost three times the number of species reported in the literature (1,105 mOTUs at 5% threshold; 175 localities, ***Table 1*** and ***Table 2***). Despite the relatively large number of sampled localities, many areas of New Guinea remain unexplored and many places were only superficially sampled by colleagues and never visited by the authors (***Figure 1A***). Additional species were added to the dataset with each batch of sequenced samples from New Guinea and the area possibly houses much higher diversity than documented by the present study.

We observed a high turnover between regions, and few species had ranges which included landmasses separated by shallow seas (seven spp. Queensland / New Guinea, 9 spp. in Southeast Asia; ***Source data 1***). Poorly dispersing lycids generally have very small ranges, except for the few genera that visit flowers and fly in open areas (***Motyka et al., 2021***). A similar small-scale turnover has recently been reported along altitudinal gradients (***Bocek et al., 2019***; ***Motyka et al., 2020***; ***Motyka et al., 2021***). A high turnover indicates a large proportion of hidden diversity, especially in tropical mountains (***Merckx et al., 2015***; ***Mastretta-Yanes et al., 2018***). Mountain fauna is especially vulnerable to climate change and its inventorying is urgently needed.

The Metriorrhynchini has recently received considerable attention in taxonomic studies, and 302 species have been described by several authors over the past three decades, making a total of 1574 formally described species (***Table 1***, ***Kazantsev, 2010***; ***Bocak et al., 2020***; Appendix introductory information). Although the recent 24% increase in described diversity appears substantial, the distance-based analysis indicates the presence of almost 2000 mOTUs (***Appendix 1—figure 14***). An additional ~50 putative species (494 terminals) were identified, but this identification was only based on divergent morphology because of the absence of *cox1*. We assume that our sampling represents only a subset of all known species (< 50%). It means that the dataset contains ~1000 undescribed

species. At the current rate, formal morphological descriptions of an additional 1000 species would take decades or hundreds of years. This is a very long time in the context of the ongoing deforestation and fragmentation of natural habitats, and currently undocumented diversity might be lost long before it can be catalogued (***Brooks et al., 2002***; ***Sodhi et al., 2004***; ***Ceballos et al., 2015***; ***Theng et al., 2020***). The rapid DNA-based inventory is an effective shortcut for obtaining basic information on the true diversity of tropical beetles and for setting a benchmark for future biodiversity re-evaluations.

The results reveal major biodiversity hotspots in New Guinea and the Sundaland. Tropical rainforests currently cover most of New Guinea, a tectonically young island that has not been considered a biodiversity hotspot for vertebrates (***Myers et al., 2000***; ***Hall, 2011***; ***Toussaint et al., 2014***). In the case of net-winged beetles, we show that the New Guinean fauna is phylogenetically diverse, spatially heterogeneous, and extremely rich as regards both the number of species and the endemic genera (***Table 1***). Additionally, the large clades of New Guinean species indicate that the diversification of major lineages preceded the uplift of the islands, and possibly started on the northern margin of the Australian craton and adjacent islands. Southeast Asia is a centre of phylogenetic diversity at the tribal level; its fauna contains all principal lineages and the highest diversity of Cautirina but is smaller than those of New Guinea. The Afrotropical and Palearctic regions represent only recently populated low-diversity outposts.

Impact of biodiversity inventorying on biogeographical and evolutionary research

Detailed data on Metriorrhynchini diversity indicate low dispersal propensity and this makes Metriorrhynchini a promising model for biogeographic studies (***Ikeda et al., 2012***). Our densely sampled phylogeny did not find any long-distance dispersal events, in contrast to many studies of flying beetles (***Balke et al., 2009***; ***Jordal, 2015***). Most recovered overseas dispersal events are limited to distances

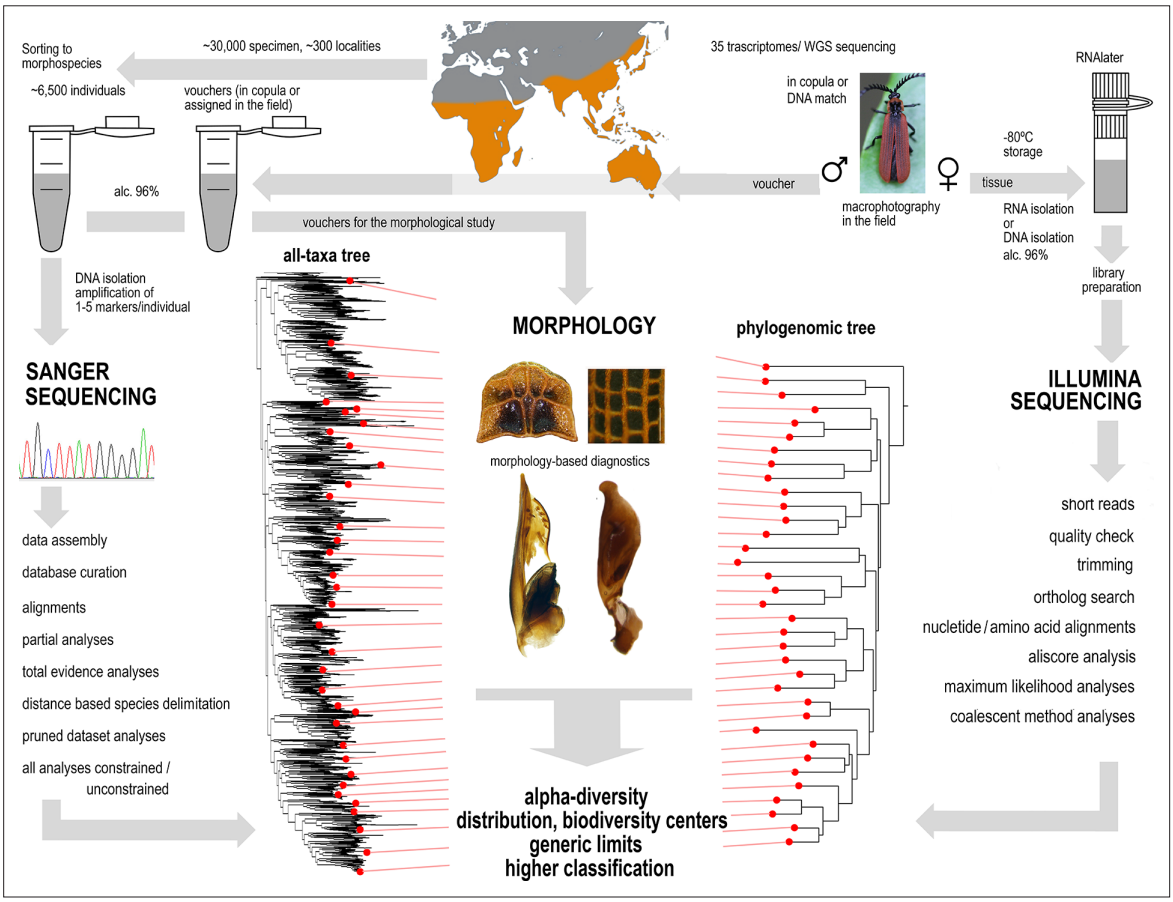

Figure 5. A sequence of applied methods from sampling to hypotheses.

of less than 100 km and are commonly accompanied by speciation (***Source data 2 and 3***). The high proportion of erroneous placement of many taxa (***Appendix 1—figure 15***; ***Bocak et al., 2020***) renders the distribution data cited in previous literature unsuitable for phylogeographic investigations, and revision of the classification is important in order to understand the true distribution of individual taxa. The original and revised ranges of selected genera are compared in ***Appendix 1—figure 15*** as examples.

Intensive biodiversity research has the potential to fill knowledge gaps concerning evolutionary phenomena that are mainly studied using a small number of model species, and the research can identify the unique attributes of other potential models. We document the contribution of a large-scale biodiversity inventory to evolutionary studies with two examples.

Net-winged beetles include several lineages in which females have lost the ability to completely metamorphose (***Bocak et al., 2008***; ***Mcmahon and Hayward, 2016***). If a putative neotenic species is discovered, a comprehensive reference database of the group may identify its closest relatives. We used our data to place the East African *Cautires apterus* in a phylogenetic context, and the results indicated that it may be the youngest neotenic taxon of all net-winged beetles (36.1 my, ***Figure 4***).

Our extensive DNA database of metriorrhynchine diversity may also play an important role in the study of the evolution of mimicry. Our inventory identified an extreme and previously unknown aposematic dimorphism in New Guinean metriorrhynchines (***Figure 4***; ***Figure 5***). The placement of sexually dimorphic species in the phylogeny suggests that the shift to dimorphism was very recent (3.0 mya at the earliest) and began when both sexes were small-bodied. Mimetic sexual polymorphism is well understood in butterflies with non-mimetic males and mimetic females (***Kunte, 2008***), but the advergence of males and females to different aposematic models has only recently been reported in two subfamilies of net-winged beetles (***Motyka et al., 2018***; ***Motyka et al., 2020***; ***Motyka et al., 2021***). Divergent evolution in Müllerian systems appears to be more common in multi-pattern aposematic rings than was previously believed when morphology was the sole source of information.

Conclusion

Priority areas for global conservation have usually been identified based on richness, species endemism and vulnerability of vertebrates (***Myers et al., 2000***; ***Holt et al., 2013***). We assume that different patterns of biodiversity distribution can be revealed if other animal groups are studied. Reliable information on additional groups can focus our conservation efforts on valuable regions (***Morrison et al., 2009***; ***Thomson et al., 2018***). Our model, beetles, is the most speciose group of animals but is much less known than vertebrates. Therefore, new data must be generated, and our research workflow must use innovations to economically produce the large-scale phylogenetic hypothesis for a high number of species. We conducted a worldwide sampling in ~700 localities, analysed transcriptomes, genomes, and mitochondrial markers, and validated our results with morphology. We show that the constrained position of less than 2% terminals increases the stability of tree topology and the congruence of molecular hypotheses with morphological traits (Appendix results). By the simultaneous consideration of genomic and mitochondrial phylogenetic signal, we achieved substantial progress with respect to the development of a Metriorrhynchini tree of life (***Chesters, 2017***; ***Linard et al., 2016***). The voucher-based DNA entries established a framework for classifying samples from other studies, such as environmental sequencing (***Linard et al., 2016***; ***Andújar et al., 2015***; ***Arribas et al., 2016***) and for subsequent morphology-based studies. Despite limited time and funding, we identified almost 2000 mOTUs which indicate that there are at least twice more species than the number reported in the literature. This means that, at a conservative estimate, ~ 1000 species in the dataset were previously unknown to science. Furthermore, we identified New Guinea as a biodiversity hotspot, which is in clear contrast with studies identifying the biodiversity patterns of vertebrates. Our large-scale inventory shows that the literature records of tropical beetles cannot be used for biodiversity conservation and metanalyses without critical revision. We suggest that if focused field research is conducted even by a small research group and subsequent workflow steps are applied to any hyperdiverse tropical group, the results can set a benchmark for future evaluation of spatiotemporal changes in biodiversity.

Materials and methods

Field research

The analyzed individuals had been accumulated by numerous expeditions to various regions of the Metriorrhynchini range (***Figure 1A***, ***Appendix 1—table 1***). The distribution of sampling sites was partly biased, and no samples are available from West Africa, Congo Basin, Sahel, Sri Lanka, and the Lesser Sundas. About 10% of samples were provided by other researchers.

Tissues for transcriptomic analyses were fixed in the field. As field identification is generally unreliable, we preferred to collect pairs *in copula*, then the female was fixed using RNAlater, and the male kept separately in 96% ethanol for Sanger sequencing and the voucher collection. Alternatively, the morphologically similar individual from the same place was fixed in ethanol and the identity of an individual assigned for transcriptomic analysis was confirmed by sequencing *cox1* mtDNA using tissue from the specimen preserved in RNAlater and putatively conspecific voucher (***Figure 2***). About 100 tissue samples were fixed and thirty-five of them were used for sequencing (***Appendix 1—table 2***). Earlier published transcriptomes were added (***McKenna et al., 2019***; ***Kusy et al., 2019***). Due to the inaccessibility of properly fixed tissue, the two critical samples were shotgun sequenced using isolated DNA.

Almost 7000 samples from 696 localities (***Table 1***) were included in the sequencing program to obtain short mtDNA fragments. In total, 6429 yielding at least a single fragment were included in the analysis (***Supplementary file 1***). The analyzed data set contained some previously published sequences (e.g. ***Sklenarova et al., 2013***; ***Bocek and Bocak, 2019***). Voucher specimens are deposited in the collection of the Laboratory of Biodiversity and Molecular Evolution, CATRIN-CRH, Olomouc.

Genomic and transcriptomic sequencing, data analysis

Libraries for thirty transcriptomes were prepared by Novogene Co., Ltd. (Beijing, China) and sequenced on the HiSeq X-ten platform (Illumina Inc, San Diego, CA). The removal of low-quality reads and TruSeq adaptor sequences were performed using fastp v.0.20.0 (***Chen et al., 2018***) with the following parameters: -q 5 u 50 l 50 n 15. All paired-end transcriptomic reads were assembled using SOAPdenovo-Trans-31mer (***Xie et al., 2014***).

Additionally, the total DNA (~33 Gb each) of *Metanoeus* sp. (Voucher code G19002) and an unidentified sample Metriorrhynchina species (Voucher JB0085) was shotgun-sequenced on the same platform. Reads were filtered with fastp using the same settings as above and quality was visualized with FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc). The draft genomes were assembled using SPAdes v.3.13.1 (***Bankevich et al., 2012***), with k-mer sizes of 21, 33, 55, 77, and 99. Obtained contigs were used to train Augustus (***Stanke and Waack, 2003***) for species-specific gene models with BUSCO (***Waterhouse et al., 2018***). Predicted species-specific gene models were then used for ab initio gene predictions in Augustus and predicted protein-coding sequences were used for subsequent analyses. Outgroup taxa were reported in previous studies (***Kusy et al., 2018***, ***Kusy et al., 2019***; ***McKenna et al., 2019***).

The ortholog set was collated by searching the OrthoDB 9.1 (***Zdobnov et al., 2017***) for single copy orthologs in six beetle genomes (***Appendix 1—table 3***). We used Orthograph v.0.6.3 (***Petersen et al., 2017***) with default settings to search in our assemblies for the presence of specified single copy orthologs. From the recovered 4193 orthologs, terminal stop codons were removed, and internal stop codons at the translational and nucleotide levels were masked. The amino acid sequences were aligned using MAFFT v.7.471 with the L-INS-i algorithm (***Katoh and Standley, 2013***). The alignments from each ortholog group were then checked for the presence of outliers. To identify random or ambiguous similarities within amino acid alignments, we used Aliscore v.2.076 with the maximum number of pairwise comparisons –r 10^{27}, option -e. and we masked them using Alicut v.2.3 (***Kück et al., 2010***). Alinuc.pl was then used to apply the Aliscore results to match amino acids to the nucleotide data. MARE v.0.1.2-rc was used to calculate the information content of each gene partition (***Misof et al., 2013***). Partitions with zero information content were removed at both levels. Finally, the remaining 4109 alignments were retained for subsequent multispecies coalescent analyses, and different concatenated datasets were generated for both amino acid and nucleotide levels using FasConCat-G v.1.4 (***Kück and Longo, 2014***; ***Appendix 1—table 4*** and Appendix methods). The degree of missing data and overall completeness scores (Ca) across all datasets were inspected using AliStat v.1.7 (***Wong et al., 2020***).

Compositional heterogeneity tests

To explore the effect of among species compositional heterogeneity and its possible bias to tree reconstruction, we inspected the data with BaCoCa v.1.105 (**Kück and Struck, 2014**) to identify the gene partitions that strongly deviate from compositional homogeneity using relative composition frequency variation value (RCFV). Following **Vasilikopoulos et al., 2019**, we considered compositional heterogeneity among species in a given partition to be high when RCFV ≥0.1. The heterogeneous partitions were excluded from the data to generate a more compositionally homogeneous dataset. We used Maximum Symmetry Test (**Naser-Khdour et al., 2019**) to identify the partitions that strongly deviate from compositional homogeneity at the nucleotide level (p-value cut off <0.05), and partitions below the threshold were excluded. The software SymTest v.2.0.49 (**Ott, 2019**) was used to calculate the overall deviation from stationarity, reversibility, and homogeneity (SRH) (**Ababneh et al., 2006**).

Phylogenomic maximum likelihood analyses

For all datasets, phylogenetic reconstruction was performed using the maximum likelihood (ML) criterion with IQ-TREE 2.1.2 (**Minh et al., 2020**). First, we analyzed all datasets using the original gene partition boundary. The model selection for each gene was performed with ModelFinder (**Chernomor et al., 2016**; **Kalyaanamoorthy et al., 2017**) implemented in IQ-TREE2 (-MFP option). GTR model was considered for nucleotide supermatrices. For the amino acid supermatrices, the substitution models LG, DCMUT, JTT, JTTDCMUT, DAYHOFF, WAG, and free rate models LG4X and LG4M were tested. All possible combinations of modeling rate heterogeneity among sites were allowed (options: -mrate E,I,G,I + G,R -gmedian -merit BIC). We used the edge-linked partitioned model for tree reconstructions (-spp option) allowing each gene to have its own rate. The optimized partition schemes and best-fitting models were inferred for some datasets using -m MFP+ MERGE and the considering same substitution models as above. The fast-relaxed clustering algorithm was used to speed up computation during partition-scheme optimization (**Lanfear et al., 2017**). Ultrafast bootstrap (**Hoang et al., 2018**) and SH-like approximate likelihood ratio test (SH-aLRT) were calculated in IQ-TREE2 (options -bb 5000 and -alrt 5000) to assess nodal supports for focal relationships.

Coalescent analyses and analyses of the confounding and alternative signal

To account for variation among gene trees owing to incomplete lineage sorting and to account for potential gene tree heterogeneity and discordance (**Edwards, 2009**), the data were also analyzed using the coalescent-based species-tree method. For every single gene partition, we calculated an ML gene tree in IQ-TREE2, with 5,000 ultrafast bootstrap replicates (-bb option) and using the same substitution models as predicted by ModelFinder in the above described partitioned analyses. For subsequent coalescent species tree estimation, the Accurate Species Tree Algorithm (ASTRAL-III v.5.7.3; **Zhang et al., 2018**) was used. To account for very poorly resolved branches on gene trees, branches with ultrafast bootstrap ≤10 were collapsed using newick utilities v.1.6 (**Junier and Zdobnov, 2010**) in every ASTRAL analysis. Local posterior probabilities (**Sayyari and Mirarab, 2016**) and quartet frequencies of the internal branches in every species tree were calculated using the parameter '-t = 2'. Pie charts representing quartet scores for the given topology and two alternatives were plotted to the resulting species trees in R using plot_Astral_trees (**Bellot, 2021**).

Additionally, we studied the effect of potentially confounding signals, like non-random distribution of data coverage and violations of SRH conditions, on our phylogenetic reconstructions with the Four-cluster likelihood mapping (FcLM) approach (**Strimmer and Haeseler, 1997**) implemented in IQ-TREE2. Based on the results of our tree reconstructions, we tested the hypotheses about the alternative placement of leptotrichaline and procautirine clades.

Mitochondrial DNA sequencing and data analysis

Total DNA was extracted from the metathorax with a Wizard SV96 kit (Promega Corp., Madison, WI). The yield was measured using a NanoDrop-1000 Spectrophotometer (Thermo Fisher Scientific Inc, Waltham, MA). The PCR settings and cycle sequencing conditions were the same as those used by **Bocak et al., 2008**. Three fragments of mitochondrial genome were sequenced: *cox1*+ tRNA *Leu*+ *cox2* (~1100 bp), *rrnL*+ tRNA *Leu*+ *nad1* (~800 bp), and ~1210 bp of *nad5* and adjacent tRNA-*Phe*, tRNA-*Glu*, and tRNA-*Ser* mtDNA (the mtDNA fragments are further mentioned as *rrnL*, *cox1*, and

nad5). The PCR products were purified using PCRu96 Plates (Merck Millipore Inc, Burlington, MA) and sequenced by an ABI 3130 (Applied Biosystems, Waltham, MA) sequencer using the BigDye Terminator Cycle Sequencing Kit 1.1 (Applied Biosystems, Waltham, MA). Sequences were edited using Sequencher v.4.9 software (Gene Codes Corp., Ann Arbor, MI). Altogether 6476 individuals were analyzed including some previously published (***Sklenarova et al., 2013***; ***Bocek and Bocak, 2019***).

The *cox1* gene fragment was used to OTUs delimitation (***Blaxter et al., 2005***) using CD-hit-est (***Fu et al., 2012***) and different thresholds (from similarity 0.99–0.90 by 0.05 steps). Therefore, we assembled two datasets: (A) the dataset containing all sequenced individuals and (B) all OTUs delineated by 0.98 similarity of the *cox1* gene. The *rrnL* and *tRNAs* were aligned using MAFFT 7.2 with Q-INS-I algorithm (***Katoh and Standley, 2013***), protein-coding genes were eye-checked for stop codons and aligned using Trans-Align (***Bininda-Emonds, 2005***). All fragments were concatenated using FasConCat (***Kück and Longo, 2014***) and analyzed under maximum-likelihood criterium in IQ-TREE v.2.1.2 (***Minh et al., 2020***; ***Appendix 1—table 5***). To assess the branch supports values, we used SH-aLRT test with 1000 iterations. ModelFinder tool implemented in IQ-TREE was used to identify the best fit models using the Bayesian Information Criterion (***Chernomor et al., 2016***). The results of the TSA/WGS analyses were used to constrain basal topology among major clades of Metriorrhynchini in both analyses of datasets A and B. Further, we ran unconstrained analyses of the above-mentioned datasets with identical settings except -g option to compare results. We replicated constrained tree search nineteen-times and compared resulting trees using Robinson–Foulds distances in R package phangorn (***Schliep, 2011***; ***Appendix 1—table 2***). Randomly chosen trees were then compared using cophylo script (phytools; ***Revell, 2012***) with argument rotate = TRUE.

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Author contributions

Michal Motyka, Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review and editing; Dominik Kusy, Conceptualization, Data curation, Formal analysis, Methodology, Resources, Visualization, Writing – original draft, Writing – review and editing; Matej Bocek, Renata Bilkova, Data curation, Investigation, Resources, Writing – review and editing; Ladislav Bocak, Conceptualization, Data curation, Funding acquisition, Project administration, Resources, Supervision, Writing – original draft, Writing – review and editing

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Additional files

Supplementary files

- Transparent reporting form
- Supplementary file 1. Maximum likelihood tree recovered by the analysis of the full dataset (mitochondrial fragments); numbers above branches represent SH-alrt support values.
- Source data 1. Maximum likelihood tree recovered by the analysis of the full dataset (mitochondrial fragments). Depicted numbers above branches represent SH-alrt support values.
- Source data 2. Maximum likelihood tree recovered by the analysis of the reduced dataset (98% similarity OTUs). Depicted numbers above branches represent SH-aLRT support values. The taxa with the constrained position in the tree are marked with the red star.
- Source data 3. Maximum likelihood tree recovered by the analysis of the reduced dataset (mitochondrial fragments, 98% similarity OTUs) with unconstrained backbone. Depicted numbers above branches represent SH-alrt support values.

Data availability

All datasets are deposited in the Mendeley Data repository https://doi.org/10.17632/ntgg6k4fjx.1.

The following dataset was generated:

Author(s)	Year	Dataset title	Dataset URL	Database and Identifier
Motyka M, Kusy D, Bocek M, Bilkova R, Bocak L	2021	Data for: Phylogenomic and mitogenomic data can accelerate inventorying of tropical beetles during the current biodiversity crisis	https://doi.org/10.17632/ntgg6k4fjx.1	Mendeley Data, 10.17632/ntgg6k4fjx.1

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Appendix 1

Appendix introductory information

Overview of Metriorrhynchini systematics and natural history

Lycidae (net-winged beetles) is a diverse, mostly tropical family of Elateroidea and Metriorrhynchini is the largest lycid tribe with almost 1600 species (**Masek et al., 2018**). The history of their classification started with Kleine's proposals of tribes and/or subfamilies Metriorrhynchini/inae, Trichalini/inae, Cladophorini/inae, and Dilolycini/inae (see an overview by **Kleine, 1933**). The supergeneric classification of net-winged beetles was later revised. Conderini were merged with Metriorrhynchini and Trichalini in the widely defined Metriorrhynchinae and Cladophorini and Dilolycini were synonymized to Metriorrhynchini (**Bocak, 2002** and references therein). **Calder, 1998** considered Metriorhynchinae Kleine, 1926 (based on *Metriorhynchus* Guérin-Méneville, 1838) to be a homonym of the crocodilian subfamily Metriorhynchinae Meyer, 1832 and used Dilolycinae Kleine, 1926 as a replacement name. Trichalinae were placed as a tribe Trichalini in Metriorrhynchinae or a subtribe Trichalina in Metriorrhynchini (**Bocak, 2002**). Additionally, Hemiconderina **Bocak et al., 2008** were erected for *Hemiconderis* and related genera. Detailed information on the metriorrhynchine classification history was published in the latest revision of the subtribal classification (**Sklenarova et al., 2014**) and the genera were assigned to subtribes by **Kubecek et al., 2015** and **Bocak et al., 2020**.

The alpha-taxonomy is mostly based on the original, often uninformative descriptions (catalogued by **Kleine, 1933**; **Bocak et al., 2020**). Recently, **Calder, 1998** compiled the catalogue of Australian net-winged beetles and proposed *Metriorhynchus* Guérin-Méneville, 1838 as a junior homonym of *Metriorhynchus* Meyer, 1832. Subsequently, *Porrostoma* Castelnau, 1838 was used as a replacement name for *Metriorhynchus* Guérin-Méneville, 1838 and new combinations and new replacement names were proposed (**Calder, 1998**). **Bocak, 2002** reviewed the status of lycid taxa described by **Guérin-Méneville, 1838** and proposed to replace *Metriorhynchus* Guérin-Méneville, 1838 by *Metriorrhynchus* Gemminger et Harold, 1869. **Bocak, 2002** additionally considered *Porrostoma* Castelnau, 1838 as a valid name for a separate genus in Metriorrhynchini. These studies resulted in repeated transfers of several hundred of species between *Metriorrhynchus* and *Porrostoma* (**Calder, 1998**; **Bocak et al., 2020**).

There are five Metriorrhynchini genera which house the majority of described species: *Xylobanus* (231 spp.), *Cautires* (437 spp.), *Trichalus* (120 spp.) *Metriorrhynchus* (194 spp.), and *Cladophorus* (131 spp.; **Kleine, 1933**; **Calder, 1998**; **Bocak, 2002**; **Bocak et al., 2020**), although their limits have been ambiguous. *Trichalus* was recovered as a much smaller clade than *Microtrichalus* Pic, 1921 (**Bocek and Bocak, 2017**). Australian *Metriorrhynchus* transferred to *Porrostoma* Castelnau, 1838 by **Calder, 1998** were formally returned to Metriorrhynchus by *Metriorrhynchus* by **Bocak et al., 2020**. *Xylobanus* was recovered as a clade that contains most Oriental Cautirina with four primary costae, but Afrotropical *Xylobanus* were recovered as a distantly related terminal clade within Afrotropical *Cautires* (**Sklenarova et al., 2013**; **Sklenarova et al., 2014**). Some Oriental *Xylobanus* belong to Metanoeina and New Guinean species to Metriorrhynchina (**Kubecek et al., 2015**; **Bocak et al., 2020**). An additional typologically defined genus, *Procautires* Kleine, 1925, was reported from Asia, Africa and Australasia (**Kleine, 1933**), but the type species belongs to Metriorrhynchina in contrast to the Afrotropical and Asian species which are close to *Cautires* (Cautirina).

Geographic distribution

The tribe Metriorrhynchini dominates the Australian net-winged beetle fauna with ~85% (196 spp.) of the named taxa. They are similarly common in New Guinea (423 spp.) and the Wallacea (176 spp.). Although highly diversified (261 spp. and 231 spp., respectively), they represent only about 30% of described net-winged beetle species in the Oriental and Afrotropical regions where similar numbers of species belong to Platerodini, Calochromini and Lycini, or several small tribes (**Masek et al., 2018**).

The tribe Metriorrhynchini has a Gondwanan distribution, and the range reaches to the Palearctic East Asia (Russian Far East, Cautirina and Metanoeina). Additionally, the tribe is well represented in the Sino-Japanese realm sensu **Holt et al., 2013** (China, Japan, Cautirina and Metanoeina in the whole area, Metriorrhynchina in southernmost China only) and in the Oriental realm (most of India, Sri Lanka, the lower elevations of the Himalayas, Indo-Burma, Malaya, the

Great and Lesser Sundas, the Philippines). Further, the tribe is widespread in the Oceanian realm, especially in its western part (the Moluccas, New Guinea, the Solomon Islands and a few islands further east) and in the Australian realm (Australia, Tasmania, and one introduced species in New Zealand). The Afrotropical realm houses relatively diversified fauna in forest and savannah habitats, but all species belong to Cautirina and represent only two clades which correspond with putative independent colonization events from drifting India to Madagascar and continental Africa (***Sklenarova et al., 2013***).

The subtribes differ in their distribution. Metriorrhynchina is mostly Oceanian and Australian, relatively rich Metriorrhynchina fauna is known from the Sulawesi, much less species occur in the Philippines and only a few species of *Diatrichalus*, *Leptotrichalus*, *Trichalus*, *Microtrichalus*, and *Metriorrhynchus* in the eastern part of the Oriental realm (***Bocak and Yagi, 2010***; ***Bocek and Bocak, 2019***; ***Bocek et al., 2019***) and southern Yunnan in the Sino-Japanese realm. The Cautirina occurs in the Afrotropical, Oriental, Palearctic, and Sino-Japanese realms, but they do not cross Weber's line that was proposed as a border between Oriental and Oceanian realm (***Holt et al., 2013***). The Metanoeina is Oriental, with a limited number of species in China and Japan. Only *Metanoeus* occurs in the Philippines.

The Metriorrhynchini is the species-rich tribe and the almost 200 years of taxonomic research accumulated data that need serious revision (***Bocak et al., 2020***). In the taxonomy of this group, we face the burden of uninformative descriptions (e.g. the studies by the French entomologist M. Pic in the first half of the 20th century). The careless classification of newly described species, inaccessibility of type-holding collections or their poor organization, and legal regulations limit taxonomic research (***Prathapan, 2018***; ***Bocak et al., 2020***).

Biology

The adults are volatile, but they are poor dispersers due to their soft-bodiedness and many species remain in shaded situations under the canopy, usually sitting on leaves. Only some species (*Porrostoma*, *Metriorrhynchus*, *Leptotrichalus*, and *Trichalus*) visit flowers. The flower frequenting species are more common in semi-dry areas where nectar represents a source of water for adults instead of water on leaves or in rotten wood. All Metriorrhynchini are unpalatable for predators and most are aposematically coloured (***Eisner et al., 2008***; ***Motyka et al., 2021***). The color patterns are usually geographically restricted and the sympatrically occurring species form Müllerian mimicry rings (***Bocak and Yagi, 2010***; ***Motyka et al., 2018***; ***Motyka et al., 2020***; ***Motyka et al., 2021***; ***Bocek et al., 2019***). The larvae of *Metanoeus*, *Cautires*, *Leptotrichalus*, *Metriorrhynchus*, and *Porrostoma* were described by ***Bocak and Matsuda, 2003***. They live in rotten wood including trunks and logs on soil surface or in rotten roots in soil in arid regions.

Appendix results

Metriorrhynchina subclades based on phylogenomic analyses and their morphological characteristics and distribution.

The procautirine clade

The phylogenomic analysis contained four taxa that clustered as the first or second deepest serial branch in Metriorrhynchina. One of the internal clades contains species that are morphologically highly similar to the type-species of *Procautires*. They have reduced secondary elytral costae in the middle part of the elytra. Other procautirine species have fully developed secondary costae unlike *Procautires*. The only morphological trait supporting the monophyly of the procautirine clade is a setose patch in the internal sac of the male genitalia.

The leptotrichaline clade

The phylogenomic backbone identified close relationships of three endemic genera from the Sulawesi (*Mangkutanus*, *Wakarumbia*, and *Broxylus*), and two genera, *Sulabanus*, *Leptotrichalus*, known from Sulawesi and the Philippines (***Bocak et al., 2020***). The mtDNA dataset identified as closely related the Australian and New Guinean *Synchonnus* and New Guinean *Falsolucidota*. Two lineages of the clade putatively colonized the Oriental region: *Leptotrichalus* (the Philippines, Sundas, Indo-Burma and southernmost China) and *Sulabanus* (only the Philippines, 2 spp. from Mindanao and Sibuyan identified herein, either undescribed species or currently placed in Cautirina: *Xylobanus*). We have not found any reliable external diagnostic morphological character

which would support the relationships of these genera. Only *Sulabanus* and *Mangkutanus* have a complete set of lateral pronotal carinae. Other genera have simplified of absent carinae except those that form the median lanceolate areola (**Bocak, 2002**). The group contains genera with the highly diverse arrangement of elytral costae.

The trichaline clade

The genomic dataset contained representatives of three genera: *Diatrichalus*, *Microtrichalus*, and *Eniclases*, all of them sharing the characteristic morphological traits of the trichaline clade, that is a single median areola in the pronotum and the shortened first primary elytral costa. We identified as close relatives two lineages of small-bodied Metriorrhynchina with conspicuous median pronotal areola and either absent or simplified lateral carinae, but with the full-length elytral costa 1. The structure of elytral costae is diverse in the clade: most trichalines sensu stricto have well-developed secondary costae, that is, nine costae at humeri, only a few *Diatrichalus* have only four primary costae in the humeral part. All genera of the here delimited trichaline clade, except *Diatrichalus*, share similar male genitalia (**Bocak, 2002**). The deepest branches of the trichaline clade contain mostly New Guinean species and several lineages of the trichalines colonized the Sulawesi, Philippines, Greater Sundas, Malaya, Indo-Burma, and southernmost China.

The porrostomine clade

Six transcriptomes were analyzed to recover the backbone of the clade. *Porrostoma* and *Metriorrhynchus* are distantly related and easy to distinguish with the genital morphology (**Bocak, 2002**). Numerous small bodied, morphologically diverse Metriorrhynchina species were identified as their close relatives. Most *Metriorrhynchus* and their closest relatives come from New Guinea, but several species of a terminal clade crossed Lydderker's, Weber's, and Wallace's lines and colonized Sulawesi, the Philippines, Greater Sundas, Malaya, and Indo-Burma (**Bocak and Yagi, 2010**).

The cladophorine clade

Altogether eleven taxa were included in the genomic analysis, and we found *Cladophorus* as a sister to *Pseudodontocerus* (=*Carathrix*) and *Ditua*. Most genera have seven pronotal areoles, flabellate to pectinate male antennae, and nine well developed costae in the elytra. Mitochondrial dataset recovered this clade as the most speciose group. The characteristic large-bodied species resembling the type-species of *Cladophorus* were found as a terminal subclade among several lineages of small-bodied Metriorrhynchina which do not resemble them in general appearance. Further splits contain the *Pseudodontocerus* clade and a large clade containing representatives of earlier described genera *Ditua* and *Cautiromimus*. The species of the *Cladophorus* clade are most diverse in New Guinea, but some species of the *Ditua* clade were recorded from the Philippines and Sulawesi, a limited number of species is native to continental Australia.

Impact of molecular phylogenetics on the assessment of morphological evolution

With the robust, data-rich phylogeny, we can test earlier hypotheses on morphological evolution. Relationships among subtribes have been based on the similarity of larvae and ambiguously supported by Sanger data (**Sklenarova et al., 2013**; **Sklenarova et al., 2014**). Our results imply that bipartite larval terga can be inferred either as a synapomorphy of Metriorrhynchini with reversal to entire tergites in Cautirina or we must alternatively consider their independent origin in Metanoeina and Metriorrhynchina. The mapping of the character on the phylogenomic tree needs two instead of a single step as earlier supposed (**Sklenarova et al., 2014**). When most larvae are unknown and their morphology variable, the phylogenetic hypotheses based on incomplete morphological data and uncertain polarity urgently need validation from independent sources of information.

Due to quite high morphological disparity and ambiguous interpretation of some morphological characters, there were proposed subfamilies, tribes, and/or subtribes for various subsets of genera now placed in the Metriorrhynchina (Metriorrhynchinae/ini, Cladophorinae/ini, Trichalinae/ini, Dilolycinae/ini, Hemiconderina, and possibly Melanerotini, now in Lycidae *incertae sedis*; **Bocak, 2002**; **Kazantsev, 2010**; **Kusy et al., 2019**). Phylogenomics reject the possibility to assign a high rank to Trichalini and Hemiconderina as numerous coordinated taxa would have to be erected to make all groups reciprocally monophyletic and keep the earlier proposed names valid.

The phylogenomic analyses reinforce the previously identified monophyly of the trichaline clade (=earlier Trichalini part; **Bocek and Bocak, 2017**), but simultaneously suggest their delayed origin as a terminal clade and the presence of some 'non-trichaline' lineages as serial sister-groups to the trichalines in the earlier sense. The cladophorine clade contains numerous taxa which had been placed until recently into *Cautires* (Cautirina), a distant lineage in respect to cladophorines. Herein, we further delimit the porrostomine clade which contains *Metriorrhynchus* and *Porrostoma*, but along with them several deeply rooted lineages which have been incorrectly placed to various genera.

For the first time, we define the leptotrichaline clade that contains the genera earlier placed into the trichalines (*Leptotrichalus*), Hemiconderina (*Falsolucidota*), Lycinae: Calopterini (*Broxylus*), and Cautirina (some *Xylobanus*). The procautirine clade is also a newly defined group. *Procautires* had been for long considered as related to *Cautires*, but it is one of earliest Metriorrhynchina.

Until recently, the classification had been based almost exclusively on the arrangement of pronotal carinae, elytral costae and the shape of antennae. These traits are easily observable and sometimes define a clade, but in many cases their presence and/or absence is misleading as they are highly plastic even within restricted clusters of species (**Sklenarova et al., 2014**; **Bocek and Bocak, 2017**; **Kusy et al., 2019**). Before the availability of molecular data, the homoplasy of external morphological characters could not be tested. As a result, the earlier studies coped with uncertain relationships of newly described species at the levels from a tribe down to a genus (**Kleine, 1933**; **Calder, 1998**; **Bocak et al., 2020**).

The list of Metriorrhynchini genera with complete synonymy:

Subtribe Metanoeina **Sklenarova et al., 2014**
Matsudanoeus **Sklenarova et al., 2014**
Metanoeus Waterhouse, 1878
Ochinoeus Kubecek et al., 2015
Xylometanoeus Sklenarova et al. 2014
Subtribe Cautirina Sklenarova et al. 2014
Subtribe Cautirina Sklenarova et al. 2015
Caenioxylobanus Waterhouse, 1878
Cautires Waterhouse, 1878
Prometanoeus Kleine, 1925
= *Tapromenoeus* Bocak et Bocakova, 1989
Paracautires Kazantsev, 2012
Spartoires Kazantsev, 2012
Tricautires Kazantsev, 2006
Xylobanus Waterhouse, 1878
Subtribe Metriorrhynchina Kleine, 1926
Procautirine clade
Procautires Kleine, 1925b
Xylothrix Kazantsev, 2015
Leptotrichaline clade
Falsolucidota Pic, 1921
= *Hemiconderis* Kleine, 1926
Broxylus Waterhouse, 1879
= *Samanga* Pic, 1921
Mangkutanus Kubecek et al., 2011
Sulabanus Dvorak & Bocak, 2007
Leptotrichalus Kleine, 1925
Synchonnus Waterhouse, 1879
= *Achras* Waterhouse, 1879
= *Enylus* Waterhouse, 1879
= *Strophicus* Waterhouse, 1879
Wakarumbia Bocak, 1999
Cladophorine clade
Cautiromimus Pic, 1926
Cladophorinus Kleine, 1926
Cladophorus Guérin-Méneville, 1830
= *Odontocerus* Guérin-Méneville, 1838

= *Spacekia* Strand, 1936
subgenus *Cladophorus* s. str.
subgenus *Falsocautires* Pic, 1926
Ditua Waterhouse, 1879
Marena Kazantsev, 2007
Pseudodontocerus Pic, 1921
= *Carathrix* Kleine, 1926
Trichaline clade
Diatrichalus Kleine, 1926
= *Mimoxylobanus* Pic, 1921
Eniclases Waterhouse, 1879
= *Trichalolus* Pic, 1923
Flabellotrichalus Pic, 1921
= *Stereotrichalus* Kleine, 1926
= *Villosotrichalus* Pic, 1921
subgen. *Flabellotrichalus* s. str.
subgen. *Maibrius* Bocek et Bocak, 2017
Lobatang Bocak, 1998
subgenus *Lobatang* s. str.
subgenus *Spinotrichalus* Kazantsev, 2010
Microtrichalus Pic, 1921
= *Falsoenylus* Pic, 1926
Schizotrichalus Kleine, 1926
Trichalus Waterhouse, 1877
= *Xantheros* Fairmaire, 1877
Porrostomine clade
Metriorrhynchus Gemminger et Harold, 1869
= *Metriorhynchus* Guérin-Méneville, 1838
= *Dilolycus* Kleine, 1926
= *Flabelloporrostoma* Pic, 1923
Porrostoma Laporte, 1838
Oriomum Bocak, 1999
Metriorrhynchoides Kleine, 1926
Stadenus Waterhouse, 1879
Metriorrhynchina *incertae sedis*
Malacolycus Kleine, 1943
Xylobanomimus Kleine, 1926
Xylobanomorphus Kleine, 1935
Kassemia Bocak, 1998

Appendix methods

Genomic and transcriptomic sequencing, data analysis, individual datasets

Libraries for thirty transcriptomes were prepared by Novogene Co., Ltd. (Beijing, China) and sequenced on the HiSeq X-ten platform (Illumina Inc, San Diego, USA). The removal of low-quality reads and TruSeq adaptor sequences were performed using fastp v.0.20.0 (***Chen et al., 2018***) with the following parameters: -q 5 u 50 l 50 n 15. All paired-end transcriptomic reads were assembled using SOAPdenovo-Trans-31mer (***Xie et al., 2014***).

Additionally, the total DNA (~33 Gb each) of *Metanoeus* sp. and an unidentified sample JB0085 was shotgun-sequenced on the same platform by Novogene Co., Ltd. (Beijing, China) for 150 bp paired-end reads. Read were filtered with fastp using same setting as above and quality was visualized with FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc).

The draft genomes were assembled using SPAdes v.3.13.1 (***Bankevich et al., 2012***), with all parameters set to default values and k-mer sizes of 21, 33, 55, 77 and 99. Obtained contig sequences were used to train Augustus (***Stanke and Waack, 2003***) for species specific gene models with BUSCO v.3 (Waterhouse et al., 2018), -long option, Endopterygota set of conserved genes (n = 2442) and -sp tribolium2012 as the closest relative. Predicted species specific gene models were then used for *ab initio* gene predictions in Augustus and predicted protein coding sequences were used for subsequent analyses. Seven outgroup taxa, *Porrostoma* and

Mangkutanus transcriptomes were reported in previous studies (**Kusy et al., 2018**, Kusy et al., 2019; **McKenna et al., 2019**). The filtering and assembling methods for these samples have been described by **Kusy et al., 2019**.

The ortholog set was collated by searching the OrthoDB v.9.1 database (**Zdobnov et al., 2017**) for single copy orthologs in six beetle genomes *Onthophagus taurus* (**Poelchau et al., 2015**), *Tribolium castaneum* (**Shelton et al., 2015**; **Richards et al., 2008**) *Dendroctonus ponderosae* (**Keeling et al., 2013**), *Anoplophora glabripennis* (**McKenna et al., 2017**), *Leptinotarsa decemlineata* (**Poelchau et al., 2015**) and *Agrilus planipennis* (**Poelchau et al., 2015**; **Appendix 1—table 3**). OrthoDB v.9.1 predicted 4225 single copy orthologs for beetle species and Coleoptera (Polyphaga) reference node. We used Orthograph v.0.6.3 (**Petersen et al., 2017**) with default settings to search in our assemblies for the presence of specified single copy orthologs. From the recovered 4193 orthologs, terminal stop codons were removed, and internal stop codons at the translational and nucleotide levels were masked using the Perl script summarize_orthograph_results.pl (**Petersen et al., 2017**). The amino acid sequences were aligned using MAFFT v.7.471 with the L-INS-i algorithm (**Katoh and Standley, 2013**). The alignments from each ortholog group were then checked for the presence of outliers using the script checker_complete.1.3.1.2.pl, according to previously published methods (**Misof et al., 2014**; **Peters et al., 2017**). Identified outlier sequences were removed from amino acid and nucleotide alignments. After this step, the sequences of reference taxa and all gap-only sites were pruned. Corresponding multiple sequence alignments of nucleotides were generated using Pal2Nal (**Suyama et al., 2006**). To identify random or ambiguous similarities within amino acid alignments, we used Aliscore v. 2.076 with the maximum number of pairwise comparisons –r 1027, a special scoring approach for gap-filled amino acid sites option -e, and other parameters set to default values. Any random or ambiguous similarities were masked using Alicut 2.3 (**Kück et al., 2010**). Alinuc.pl was then used to apply the Aliscore results to match amino acids to the nucleotide data (**Peters et al., 2017**). Additionally, in each gene alignment, the short randomly aligned fragments were replaced with gaps and sequences with ≥80% missing data (calculated as percentage of '-' and 'X' in the amino acid alignments and '-' and 'N' in the nucleotide alignments) were removed using Python scripts (**Zhang, 2021**). MARE v. 0.1.2-rc was used to calculate the information content of each gene partition in terms of amino acid coding (**Misof et al., 2013**). Partitions with zero information content (IC0) were removed at both amino acid and nucleotide level.

Finally, the remaining 4,109 alignments were retained for subsequent multispecies coalescent analyses, and different concatenated datasets were generated for both amino acid and nucleotide levels using FasConCat-G v.1.4 (**Kück and Longo, 2014**). With all 4109 genes, we generated datasets designated as A-4199-AA and B-4199- NT (name-number of partitions-level). To reduce the effect of saturation on phylogenetic reconstruction (**Breinholt and Kawahara, 2013**) we created the dataset C-4199-NT12 and E-4109-NT2, with third-codon or third and first-codon positions excluded. The degree of missing data and overall completeness scores (Ca) across all datasets were inspected using AliStat v.1.7 (**Wong, 2021**) and heatmaps of pairwise completeness scores for all analysed datasets were generated (see **Appendix 1—table 3**). Further Information content and saturation values (the overall degree of data coverage with respect to gene presence or absence) were calculated with MARE v.0.1.2-rc (MAtrix REduction) (**Misof et al., 2013**) for each dataset.

Compositional heterogeneity tests

To explore the effect of among species compositional heterogeneity and its possible bias to tree reconstruction, we inspected the data with BaCoCa v.1.105 (**Kück and Struck, 2014**) to identify the gene partitions that strongly deviate from compositional homogeneity using relative composition frequency variation value (RCFV). Following **Fernández et al., 2016** and **Vasilikopoulos et al., 2019**, we considered compositional heterogeneity among species in a partition to be high when the overall RCFV value was ≥0.1. The compositionally heterogeneous partitions were excluded from the dataset A-4109-AA to generate more compositionally homogeneous dataset D- 3370-AA. To create more homogenous and decisive dataset, we reduced the dataset D-3370-AA to contain only partitions with all taxa and created dataset F-1490-AA. Additionally, we used MARE to increase information content of dataset D-3370-AA and default output supermatrix is designed as dataset J-2129-AA_MARE. We used tree matched-pairs

tests of homogeneity the MPTS (matched- pairs test of symmetry), MPTMS (matched-pairs test of marginal symmetry), and MPTIS (matched- pairs test of internal symmetry) (***Naser-Khdour et al., 2019***) implemented in IQ-TREE2 to identify the partitions that strongly deviate from compositional homogeneity at nucleotide level (P-value cutoff <0.05) and partitions below the threshold were excluded. And following datasets generated: G-NT-1767_MaxSymTest, H-NT-1645_MaxSymTestmarginal and I-NT-3905_MaxSymTestInternal.

The software SymTest v.2.0.49 (https://github.com/ottmi/symtest) was used to calculate the overall deviation from stationarity, reversibility, and homogeneity (SRH) (***Ababneh et al., 2006***) between the amino acid and nucleotide sequences. Heatmaps were generated for all datasets to visualize the pairwise deviations from SRH conditions.

Phylogenetic maximum likelihood analyses

For all datasets phylogenetic reconstruction was performed using maximum likelihood (ML) criterion with IQ-TREE 2.1.2 (***Minh et al., 2020***). First, we analyzed all datasets using original gene partition boundary. The model selection for each gene was performed with ModelFinder (***Chernomor et al., 2016***; ***Kalyaanamoorthy et al., 2017***) implemented in IQ-TREE2 (-MFP option). GTR model were considered for nucleotide supermatrices. For the amino acid supermatrices, the substitution models LG, DCMUT, JTT, JTTDCMUT, DAYHOFF, WAG and free rate models LG4X and LG4M were tested. All possible combinations of modeling rate heterogeneity among sites were allowed (options: -mrate E,I,G,I + G,R -gmedian -merit BIC). We used the edge-linked partitioned model for tree reconstructions (-spp option) allowing each gene to have its own rate. The optimized partition schemes and best-fitting models were inferred for dataset A-4109-AA using -m MFP+ MERGE and considering same substitution models as above. The fast-relaxed clustering algorithm was used to speed up computation during partition-scheme optimization (***Lanfear et al., 2017***). The top 10% of partitions schemes `--rclusterf` 10 and maximum 12 327 partitions pairs (three times the number of partitions) `--rcluster-max` 12 327 were considered. Dataset A-4109-AA was also analyzed without any prior partitioning and under best fitting JTT + F + R9 substitution model. Ultrafast bootstrap (***Hoang et al., 2018***) and SH-like approximate likelihood ratio test (SH-aLRT) were calculated in IQ-TREE2 (options -bb 5000 and -alrt 5000) to assess nodal supports for focal relationships (***Appendix 1—table 3***).

Analyses of the confounding and alternative signal with four-cluster likelihood mapping (FcLM) and sequence data permutations

Additionally, we studied the effect of potentially confounding signal, like non-random distribution of data coverage and violations of SRH conditions, on our phylogenetic reconstructions with the Four- cluster likelihood mapping (FcLM) approach (***Strimmer and Haeseler, 1997***) as described by ***Misof et al., 2014*** and implemented in IQ-TREE2. Based on the results of our ML tree reconstructions we tested the hypotheses about alternative placement of the leptotrichaline and procautirine clades. Permutation of data were executed as described in ***Misof et al., 2014*** Our approach only differs in the use of the LG substitution matrix (***Le and Gascuel, 2008***) for permuting the supermatrices instead of WAG substitution matrix. For the analysis of the permuted supermatrices we used the option -q for the partition model in IQ-TREE2 and we used the LG model (for amino-acid alignments) and GTR model (for the nucleotide alignments). With original data, we performed the FcLM analyses by applying previously estimated substitution models (edgelinked models, -spp) with IQ-TREE2.

For estimation of *Cautires apterus* and sexually dimorphic *Metriorrhynchus* sp. splits were used subsets of the samples which represent the most common lineages. Dating of forementioned diversification was analyzed in BEAST v.1.8.1 (***Drummond et al., 2012***) applying HKY + I + G substitution model, uncorrelated relaxed clock model and birth–death process tree prior and 0.0115 rate for *cox1* gene as applied earlier by ***Bocak and Yagi, 2010***. The stationary phase was checked in Tracer v.1.5 (http://beast.bio.ed.ac.uk/Tracer). The consensus tree was computed with TreeAnnotator v.1.8.2 (http://beast.bio.ed.ac.uk/treeannotator) after eliminating a part of trees as a burn-in after evaluating ESS in tracer v.1.5. The final trees were visualised in Figtree v.1.4.4.

Data deposition: The additional supporting data and all the analysed supermatrices are deposited in the Mendeley Data repository DOI:https://doi.org/10.17632/ntgg6k4fjx.1.

PART VI

Studies on neoteny in Coleoptera: phylogenomics, classification, and evolutionary interactions

Dominik Kusý

How Do Genomic, Mitochondrial, and Morphological Data Contribute to the Linnean Classification of the Porrostomine Net-Winged Beetles (Coleoptera, Lycidae)?

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Research

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How Do Genomic, Mitochondrial, and Morphological Data Contribute to the Linnean Classification of the Porrostomine Net-Winged Beetles (Coleoptera, Lycidae)?

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Abstract

The Lycidae genera have seldom been tested with phylogenetic analyses. Therefore, we assembled genomic data to estimate the phylogenetic backbone of the porrostomines, one of Metriorrhynchina's major clades. Further, mtDNA and morphology were employed to assign 352 analyzed species to genera. We present evidence for the paraphyly of *Metriorrhynchus* and terminal position of *Porrostoma*, revise the generic classification, and describe eight genera: *Maraiakoreus* **gen. nov.**, *Kuarhynchus* **gen. nov.**, *Riedelrhynchus* **gen. nov.**, *Bundikanus* **gen. nov.**, *Yamarhynchus* **gen. nov.**, *Bekorhynchus* **gen. nov.**, *Sundarhynchus* **gen. nov.**, and *Isuarhynchus* **gen. nov.** We synonymize *Stadenus* Waterhouse, 1879, **syn. nov.**, *Metriorrhynchoides* Kleine, 1923, **syn. nov.**, and *Oriomum* Bocak, 1999a, **syn. nov.**, to *Porrostoma* Castelnau, 1838. Next, we propose 75 new combinations and four new species: *Bundikanus styskalai* **sp. nov.**, *Kuarhynchus sisrangensis* **sp. nov.**, *Maraiakoreus argenteus* **sp. nov.**, and *Yamarhynchus sinopassensis* **sp. nov.** We identified repeated origins of several external morphological traits earlier used to delimitate genera. Therefore, we prefer concordant evidence from the densely sampled mitochondrial phylogenies and male genitalia. The analyses identify high phylogenetic diversity and species richness in New Guinea, much lower phylogenetic diversity of the Australian continental fauna, and the limited permeability of the Wallacea that resulted in a single porrostomine genus in Asia. We point to the common acceptance of paraphyletic and polyphyletic taxa in the current classification. As a result, taxonomy has not provided expected support for any state-of-the-art evolutionary and zoogeographic studies. The phylogeny, species inventory, and classification of porrostomines set the basis for future evolutionary and zoogeographical studies.

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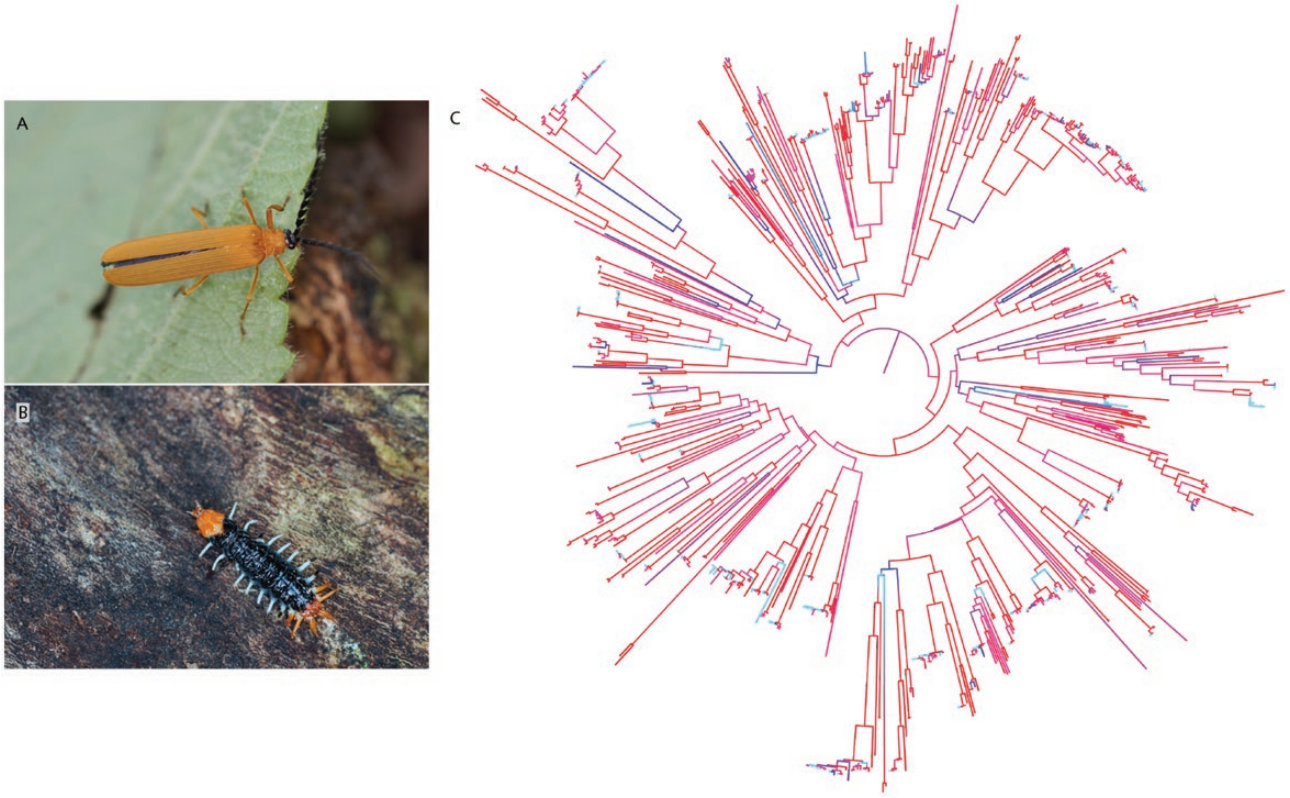
Graphical Abstract



Key words: diversity, integrative taxonomy, Australian, Oriental, Metriorrhynchini

The highest diversity of beetles occurs in tropical rain forests that are unexplored yet threatened by the growing human population (Butchart et al. 2010, Theng et al. 2020, van Klink et al. 2020, Castle et al. 2021). Lycidae is one of the major elateroid lineages with over 4,200 described species, most of them described from humid tropics (Kleine 1933a, Masek et al. 2018). The most diverse lycid tribe, Metriorrhynchini (Fig. 1), is no exception, and the recent molecular survey has locally shown up to three times higher species diversity than earlier expected (Motyka et al. 2021a). Although the group has recently attracted attention (Bocak 1999a, b, 2000; Kazantsev 2015, 2016, 2020; Bocek and Bocak 2017, etc.), the classification remains contentious in many aspects. Ambiguously defined genera hamper the progress of taxonomic research and, as a result, also signal false records from distant regions outside the actual range (Bocak et al. 2020). Therefore, we try to ameliorate the situation by using our earlier published genomic and mitochondrial data (Motyka et al. 2021a) as an information source for integrative systematics (Will et al. 2005, Packer et al. 2009, Schmidt et al. 2015, Eberle et al. 2020).

Genomic analyses are changing phylogenetics. Yet with relatively high costs, the recent studies have been mainly focused on the high classification (Misof et al. 2014, McKenna et al. 2019), less often on relationships among families, tribes, and species (e.g., Vasilikopoulos et al. 2019, Price et al. 2022). Yet, a few well-chosen terminals with sequenced several thousand orthologs can robustly solve long-standing taxonomic problems and recover the phylogenetic backbone also at a low level. Then, a constrained topology can be inferred from large-scale mtDNA datasets and high-resolution phylogenetic trees can be used to investigate the evolution of various morphological diagnostic traits. In such a way, large chunks of diversity can be economically analyzed in a short time and with already available sources of appropriately preserved tissues for DNA analyses. Here, we focus on the classification as, like many other tropical groups, our model group is insufficiently known, and most species are concentrated in a single 'garbage-bin' genus (Table 1).

We revise the generic classification of one large sublade of Metriorrhynchini Kleine, 1926a that contains ~350 of 1,850 putative species treated by Motyka et al. (2021a). The supergeneric classification of Metriorrhynchini beetles has substantially changed in recent decades. Kleine (1926a, 1928a, 1933a) established a tribal system for the net-winged beetles of the world with 15 tribes or subfamilies, among them Metriorrhynchini/inae, Trichalini/inae Kleine, 1929, Cladophorini/inae Kleine, 1929, and Dilolycini/inae Kleine, 1926a for the genera now placed in Metriorrhynchini (Bocak and Bocakova 1990a, 2008; Bocak et al. 2008; Kazantsev 2005, 2012, 2013). Bocak and Bocakova (1990a) challenged Kleine's (1933a) tribal classification and merged Metriorrhynchini and Trichalini into the widely defined Metriorrhynchini and synonymized Cladophorini and Dilolycini (Table 1). Since then, the monophyly of the tribe has been questioned neither with molecular nor morphological analyses (Kazantsev 2005, 2013; Bocak and Bocakova 2008; Sklenarova et al. 2013; Masek et al. 2018; Kusy et al. 2019). The relationships with other net-winged beetles had been unclear. The group was given subfamily rank or assigned to Lycinae and Calochrominae, respectively (Bocak and Bocakova 1990a, 2008; Kazantsev 2005, 2013). Recently, phylogenomics provided evidence for the relationships

Fig. 1. Metriorrhynchina net-winged beetles in nature. A—*Metriorrhynchus* sp., male; B—*Cladophorus tricoloratus* Kleine 1943; C—*Sundarhynchus mindanaoensis* Bocak, Matsuda et Yagi 2006, the last instar larva; D—Metriorrhynchina, the porrostomines, unidentified larvae.

Table 1. Overview of the classification of metriorrhynchine net-winged beetles

Classifications of Metriorrhynchini			Classification of Metriorrhynchina	Generic classification of the porrostomine clade as defined by Motyka et al. 2021a			
Kleine 1933a	Bocak and Bocakova 1990a, Bocak 2002	Sklenarova et al. 2014, Motyka et al. 2021a	Motyka et al. (2021a) informal groups (=clades)	Earlier generic classifications	Named spp.	Revised generic classification in the present study	Analyzed spp.
Metriorrhynchini			unnamed clades			*Maraiakoreus*	15
						unnamed clade	6
	Metriorrhynchini	Metriorrhynchini	procautirines	*Metriorrhynchus*	194		
						Kuarhynchus	42
	Metriorrhynchina	Metriorrhynchina	leptotrichalines			*Bundikanus*	19
Cladophorini	=Cladophorini		(=Hemiconderina)			*Riedelrhynchus*	2
	=Dilolycini					*Yamarhynchus*	23
		Cautirina	trichalines	*Porrostoma*	26	*Bekorhynchus*	28
Trichalini	Trichalina		(=Trichalina)	*Stadenus*	8	*Sundarhynchus*	29
				Metriorrhynchoides	1	*Metriorrhynchus*	50
Dilolycini	Hemiconderina	Metanoeina	porrostomines	*Oriomum*	4	*Isuarhynchus*	27
			cladophorines			*Porrostoma*	111

between four Oriental tribes and Metriorrhynchini, and these tribes constitute Metriorrhynchinae *sensu* Kusy et al. (2019).

The first metriorrhynchine phylogeny based on the morphological cladistic analysis recovered topologies of weak support despite a relatively high number of analyzed characters (Bocak 2002). Sklenarova et al. (2014) sampled ~150 Metriorrhynchini species and established the subtribal system inferred from rRNA and mtDNA data as well as adult and larval morphology. Recently, Motyka et al. (2021a) assembled a dataset for ~2,000 species and defined the informal generic groups in Metriorrhynchina, including the porrostomines (Table 1). DNA-based

intratribal relationships challenged the morphology-based classification. Hemiconderina and Trichalina did not warrant the subtribal rank and were synonymized with Metriorrhynchina (Sklenarova et al. 2014). Additionally, the tribal classification was affected by nomenclatural uncertainty. Calder (1998) considered Metriorhynchinae Kleine 1926a nec Meyer 1830 (type genus *Metriorrhynchus* Guérin-Méneville, 1838) to be a junior homonym and used Dilolycinae Kleine 1926s as a replacement name. Metriorrhynchinae Kleine, 1926a (type-genus *Metriorrhynchus* Gemminger et Harold, 1869) is an older available synonym (Bocak 1998). Therefore, the Dilolycinae has not been used as a valid name again.

The Oriental origins of all relatives in the subfamily: Libnetini Bocak et Bocakova, 1990a, Dilophotini Kleine, 1929, Dihammatini Bocak et Bocakova, 2008, and Lycoprogenthini Bocak and Bocakova, 2008 (Masek et al. 2018, Kusy et al. 2019) and the earliest subtribe of Metriorrhynchini (Metanoeina Sklenarova et al., 2014; Sklenarova et al. 2013, Motyka et al. 2021a) reject the earlier hypothesis of Gondwana as the cradle of the whole Metriorrhynchini (Kazantsev 2012). Metriorrhynchina is the only extant Australian metriorrhynchine lineage after molecular studies confirmed that the subtribe does not occur in Africa and that Oriental species are terminals in much larger Australian clades (Sklenarova et al. 2013, Masek et al. 2018, Bocek and Bocak 2019, Motyka et al. 2021a). There is no fossil evidence for the age estimation of Metriorrhynchini. The recent report on *Prototrichalus* Molino-Olmedo, Ferreira, Branham et Ivie 2020, as a metriorrhynchine net-winged beetle from Kachin amber was rejected, and the genus was transferred to Tenebrionoidea (Molino-Olmedo et al. 2020; Bocak et al. 2022). Using the molecular evolution rates, external calibration, and tectonic information, Sklenarova et al. (2013) dated the earliest splits within the tribe at ~100 million yr ago (mya), 30–50 million yr after the first split within net-winged beetles (McKenna et al. 2015, 2019; Bocak et al. 2016; Toussaint et al. 2017).

The porrostomine clade contains 233 described species (Bocak et al. 2020) ranging over Australia, New Guinea, the Solomon Islands, Wallacea, Southeast Asia, and eastern Indo-Burma, but the tribe reaches the highest phylogenetic and species diversity in Australia and New Guinea (Kleine 1933a, Masek et al. 2018, Motyka et al. 2021a). The number of named species markedly decreases from New Guinea to the Wallacea, the Greater Sundas, and continental Asia as the northernmost outpost of the range (Kleine 1926a, 1933a, 1935a, b, 1939; Bocak et al. 2020). Although the earlier studies identified high species diversity in New Guinea, the structure of the fauna and its relationships to Australian species have not been studied (Masek et al. 2018, Bocek and Bocak 2019).

The subtribe Metriorrhynchina Kleine, 1926a represents ~20% of the Lycidae diversity (Masek et al. 2018, Bocak et al. 2020). Several authors have recently studied the alpha-taxonomy, and > 200 species have been described over the past three decades, bringing the total to 895 Metriorrhynchina species (e.g., Bocak and Bocakova 1990b; Bocak 1999a, b; Kazantsev 2015, 2016, 2020; Kusy et al. 2018). These possibly represent only a fraction of the actual diversity, as has been shown by several studies that reviewed local faunas and discovered high numbers of endemic species in such relatively well-studied areas as Sulawesi (Dvorak and Bocak 2007, 2009; Kazantsev 2020), the Malay Peninsula (Jiruskova et al. 2019) and the Philippines (Bocak et al. 2006). The field research on net-winged beetles has recently shown that the Australian continental fauna is also incompletely known (Kusy et al. 2018). Similarly, the taxonomists described many new species from New Guinea and the Moluccas (Bocak and Bocakova 1990b; Kazantsev 2007, 2010, 2015, 2016; Kalousova and Bocak 2017; Bocek and Bocak 2017; Bocek and Adamkova 2019). Although the number of named species grows relatively quickly, the recently published analysis of the metriorrhynchine species diversity indicated the presence of additional ~1,000 species new to science in the assembled DNA database (Motyka et al. 2021a).

We explore a large-scale DNA-based biodiversity inventory (Motyka et al. 2021a) to address the classification of Metriorrhynchini at the generic level. We aim to show how sparsely sampled genomic data can robustly solve the reclassification of a paraphyletic assemblage that survived almost two centuries in taxonomic literature (Guérin-Méneville 1830, 1838). All earlier studies failed in the delimitation of *Metriorrhynchus* which comprised hundreds of species mainly in Australia and New Guinea (Kleine 1933a, Calder 1998). Only the analysis of several thousands of orthologs robustly recovered its paraphyly. When the genomic backbone is available, we employ an extensive mtDNA dataset to infer monophyla with dozens of terminals. Morphological investigations discover the diagnostic value of various characters. We strive not only for a classification that will be robustly based on phylogeny, but still user-friendly to future students from various fields. Therefore, we translate the results into the delimitation of reciprocally monophyletic genera only if morphological traits are available for routine identification. We show a pitiful current state of the beetle classification in the tropics (New Guinea) but also in the southern temperate zone (Australia) where diversity is manageable. Most species are incorrectly placed in genera and most genera are poorly defined by some apomorphies without fulfilled reciprocal monophyly. Our goal is to get closer to the complete species-level phylogeny. We suggest that the well-classified groups can serve for evolutionary and biogeographic studies. Then, the hyperdiverse insects can be used as models for extensive biosurveillance and conservation projects that have been a domain of vertebrates (Castle et al. 2021).

Material and Methods

Sampling

The authors collected the material during several research expeditions across the range of Metriorrhynchina between 2001 and 2019, and local and visiting entomologists provided an additional ~10% of samples. Four hundred localities were sampled across the whole range of Metriorrhynchina; 349 of them yielded Metriorrhynchina samples. For DNA and RNA isolation, the specimens were preserved in 96% ethyl alcohol or RNAlater. Most specimens were collected by hand or by sweeping and beating vegetation. Individuals from each locality were sorted into morphospecies, and 3–5 individuals per species were included in molecular analyses. Altogether, ~1,030 porrostomine samples were sequenced for at least the *cox1* + tRNA-*Leu* + *cox2* (~1,100 bp) mtDNA fragment. After preliminary analyses, *rrnL* (~780 bp) and *nad5* (~970 bp) mtDNA fragments were sequenced for at least one individual per species and a distant locality (detailed methods given by Motyka et al. 2021a). Each specimen was assigned a voucher code and used to label the appropriate terminal in the phylogenetic trees and illustrations. The geographic origins of samples are given in Supp Fig. 1 and 2 (online only). All voucher specimens were dry mounted for subsequent morphological studies.

Phylogenetic Analyses

We take analyses of transcriptomic and whole genome data from the earlier published study on the Metriorrhynchini that defined the porrostomine clade as one of five major internal lineages (Motyka et al. 2021a). The mtDNA sequences for the porrostomine clade

were extracted from the 6,200 terminal mtDNA Metriorrhynchini dataset (Motyka et al. 2021a), and we conducted new mtDNA analyses with porrostomine species and five cladophorines. When ~2,000 Metriorrhynchini species were analyzed, the topologies were unstable as the computing of large phylogenetic trees from data with weak phylogenetic signals can be affected even by starting seeds (Bocak et al. 2014). Therefore, morphology must be considered to identify possibly misplaced taxa in the subdataset. The preliminary analyses and morphological investigation identified six non-porrostomine taxa incorrectly recovered in the porrostomine clade when the whole Metriorrhynchini were simultaneously analyzed (Motyka et al. 2021a). The porrostomine data containing a single representative per molecular operational taxonomic unit (mOTU) were newly aligned and analyzed. The putative species were delimited based on earlier mtDNA analyses considering 2 and 5% pairwise distance, external appearance, and the morphology of male genitalia. The morphology's unsupported mOTUs were excluded from further analyses if their uncorrected pairwise distance did not reach more than 5%. The pruned dataset of morphologically verified mOTUs contained 352 porrostomine terminals, each representing one putative species. Due to the phenotypic similarity of unrelated species, the data volume, and alpha-taxonomic problems, we refrain from species-level identification unless the sample was compared with the primary type (see *Yamarhynchus* gen. nov. species for an example of highly similar members of the mimetic ring).

The *rrnL* and tRNA-Leu were aligned using MAFFT v.7.2 with the Q-INS-I algorithm (Katoh and Standley 2013), protein-coding genes aligned using Trans-Align (Bininda-Emonds 2005) and were eye-checked for stop codons. The fragments were concatenated using FasConCat-G v.1.4 (Kück and Longo 2014) and analyzed under the maximum-likelihood (ML) criterion in IQ-TREE v.1.6.12 (Nguyen et al. 2015). The model selection for each gene was performed with ModelFinder (Chernomor et al. 2016, Kalyaanamoorthy et al. 2017; for detailed information, see Supp Table 1 [online only]). Ultrafast bootstrap (Hoang et al. 2017), SH-like approximate likelihood ratio test (SH-aLRT; Guindon et al. 2010), and a Bayes factor (Anisimova et al. 2011) were calculated in IQ-TREE (options -bb 10,000; -alrt 10,000 and -abayes) to assess nodal supports for focal relationships.

Then, the internal porrostomine backbone was constrained by TSA/WGS relationships among six terminals representing five subclades as they are defined in the present study (*Metriorrhynchus*, *Sundarhynchus*, *Bekorhynchus*, *Isuarhynchus*, and *Porrostoma*) using -g option and the free position of other terminals for which genomic data remain unavailable. To assess the stability of the tree searches, we replicated the analysis a hundred times with different seeds.

Additionally, the reconstruction of the ancestral areas was analyzed using the ML framework in BIOGEOBEARS (Matzke 2013) in RASP v.4.0 (Yu et al. 2020). We compared all alternative models of possible transition (simple analysis and with the + J parameter; Matzke 2014; Supp Tables 2 and 3 [online only]) that tests founder-event speciation. The localities were assigned to respective taxa and coded for geographical origin analyses. Papua, Australia, the Wallacea, Sundaland, Philippines, and Indo-Burma were coded as separate regions.

Morphological Analysis

We dissected multiple representatives from all genera of the porrostomine clade to study the morphological traits in the male genitalia. The voucher specimens were relaxed in 50% ethyl alcohol for an hour, and then detached abdomens were shortly treated with hot 10% KOH solution to remove muscles and fat body. The external characters and genital morphology were observed under an Olympus SZX-16 microscope and photographed by an attached digital camera. We used Helicon Focus (www.heliconsoft.com) and Photoshop 13.0 to arrange stacked photographs in figures showing the habitus, pronotum, the basal part of antennae, structure of elytral costae, and male genitalia in various aspects. Illustrated 41 species document the morphological disparity of the porrostomines. We preferably designated type species from those included in the phylogenomic analysis to ensure nomenclatural stability.

We use the following abbreviation for the measurements of type specimens: BL—the body length measured from the tip of the head to the apex of the elytra, Ediam—the maximum diameter in the lateral view, Edist—the minimum frontal distance between eyes, PW—the maximum pronotal width, usually measured at the base of the pronotum, PL—the pronotal length at midline, WH—the width at humeri, EL—the length of elytra, PhL—the length of the phallus.

Depository of Material

All voucher specimens are deposited in the Laboratory of Biodiversity and Molecular Evolution voucher collection, CATRIN-CRH, Olomouc, Czech Republic (LMBC). We studied museum collections to obtain information on earlier described species and the distribution of Metriorrhynchina: Museum national d'Histoire naturelle, Paris (coll. M. Pic, J. M. Bourgeois, L. Fairmaire); Natural History Museum, London (species described by C. O. Waterhouse, R. Kleine); Muzeum i Instytut Zoologii, PAN, Warszawa (coll. R. Kleine), Museum d'Histoire naturelle, Bruxelles (coll. J. M. Bourgeois).

Nomenclature

The study represents a published work according to the International Commission on Zoological Nomenclature (ICZN 1999, 2012), and the new names are published under that Code from the electronic edition. The work and the nomenclatural acts have been registered in ZooBank under LSID urn:lsid:zoobank.org:pub:45FAAD64-1ED5-444D-AE64-575F30129FB3 and LSIDs are listed with all nomenclatural acts.

Results

Tree Assembly

Transcriptomics

The phylogenomic dataset contained six porrostomine terminals, 26 Metriorrhynchina outgroups, and ~4,200 orthologs (1.9–5.7 x 10^6 aligned positions). The tree in Fig. 2A shows ML topology for the Metriorrhynchini (see Motyka et al. 2021a for complete results). Phylogenomic analyses resolved five major clades, i.e., the procautirines, leptotrichalines, trichalines, cladophorines, and porrostomines.

The monophyly of porrostomines and their sister relationships with cladophorines were stable across all analyses. The porrostomines included one sample of *Porrostoma* and five samples morphologically identified as *Metriorrhynchus sensu* Kleine (1926a). The relationships among porrostomine terminals were robustly supported and consistently indicated the paraphyly of *Metriorrhynchus* in the conventional sense (Fig. 2A).

Constrained Mitogenomics

The mtDNA Metriorrhynchini database contained data for 6,429 individuals, of them 1,030 porrostomines (2,930 aligned positions). The pruned dataset represented 352 species, and this dataset was

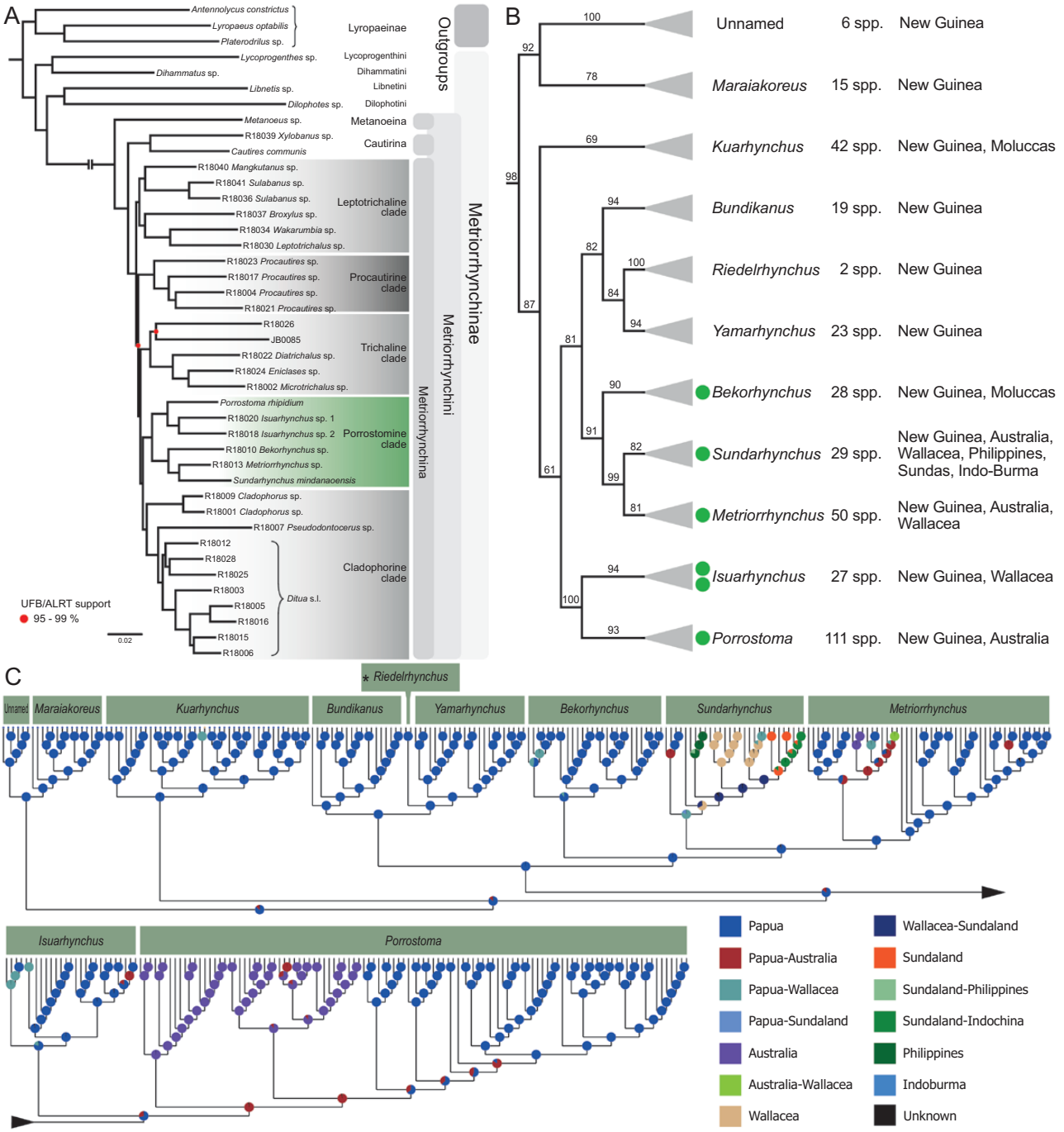


Fig. 2. A—The phylogenomic hypothesis on Metriorrhynchini recovered by maximum likelihood analysis of 4,200 orthologs by Motyka et al. (2021a). B—Generic relationship with the best likelihood value among 100 runs of the pruned constrained mitogenomic analysis; green dots designate anchor taxa. The numbers of species and ranges refer to redefined genera. C—The BIOGEOBEARS analysis of ancestral regions.

used for all analyses presented. Fig. 2C and Supp Fig. 1 (online only) illustrate the best likelihood constrained tree; Fig. 2B shows its backbone with the numbers of species and ranges. The alternative topology is illustrated in Supp Fig. 2 (online only). We recovered the following porrostomine clades treated as genera: *Maraiakoreus* **gen. nov.** (15 spp.) *Kuarhynchus* **gen. nov.** (42 spp.), *Bundikanus* **gen. nov.** (19 spp.), *Riedelrhynchus* **gen. nov.** (2 spp.), *Yamarhynchus* **gen. nov.** (23 spp.), *Bekorhynchus* **gen. nov.** (28 spp.), *Sundarhynchus* **gen. nov.** (29 spp.), *Metriorrhynchus* (50 spp.), *Isuarhynchus* **gen. nov.** (27 spp.), and *Porrostoma* (111 spp.). In addition, we recovered one deeply rooted lineage (6 spp.) in the porrostomine clade whose morphology is aberrant: all six species have a characteristic phallus resembling those of *Metriorrhynchus* (10G–I) but do not resemble their putative sister clade, *Maraiakoreus* in external characters. All species are small-bodied, and slender, with seven pronotal areoles and nine elytral costae.

The recovered tree of the porrostomines was stable. We did not recover any competing topology substantially questioning the composition of genera if we re-run the analyses with/without constraints and with different starting seeds. Nevertheless, the genus-rank

terminal clades sometimes obtained low support (Supp Fig. 2 [online only]). A pair of *Bekorhynchus* species was sometimes incorrectly recovered in *Porrostoma* (Supp Figs. 1 and 2 [online only]; the terminals are highlighted with red arrows in Supp Fig. 2 [online only]). These trees show topological instability due to stochastic factors in the computing of large trees (all settings identical except random starting seed).

Distribution and the Reconstruction of Ancestral Ranges

The porrostomines occur in the whole range of Metriorrhynchina, and New Guinea harbors the highest number of species (Fig. 2C, Supp Figs. 1 and 2 [online only]). The program BIOGEOBEARS reconstructed the ancestral area states of critical nodes in the porrostomine phylogeny. The analyses strongly supported for the New Guinean origin of the group (Fig. 2C, Supp Fig. 3 [online only]). The Oriental ad Sulawesi species are represented only by *Sundarhynchus*. A limited number of genera crossed the Lydekker's and Weber's lines westward. The deep open sea proved to be an effective barrier to dispersal, as only a few lineages crossed the open seas (Fig. 2C). The Australian continental fauna is phylogenetically much less diverse than New Guinea's. Only two extensive clades of *Porrostoma* are known from the Australian continent; several other Australian species belong to *Metriorrhynchus* (3 spp.) and *Sundarhynchus* (1 sp.).

Taxonomy

Metriorrhynchinae Kleine, 1926a
Metriorrhynchini Kleine, 1926a
Metriorrhynchina Kleine, 1926a

Metriorrhynchina Kleine, 1926a: 97

Type species. *Metriorhynchus* Guérin-Méneville, 1838, cited as *Metriorrhynchus* in the original publication; *Metriorrhynchus* Gemminger and Harold, 1869 is a junior objective synonym of *Metriorhynchus* Guérin-Méneville, 1838, see Bocak (1998) for details.

=Cladophorinae Kleine, 1928a: 222.

Type genus. *Cladophorus* Guérin-Méneville, 1830); Bocak and Bocakova (1990a: 646).

=Dilolycinae Kleine, 1926a: 186.

Type genus. *Dilolycus* Kleine, 1926a; Bocak and Bocakova (1990a: 646).

=Haplothoracinae Kleine, 1926a: 95, *nomen nudum*.

Type genus. *Haplothorax* Kleine, 1926a, *nomen nudum*.

=Trichalinae Kleine, 1928a: 222.

Type genus. *Trichalus* Waterhouse, 1877.

=Trichalini: Kleine (1933a: 69); Bocak and Bocakova (1990a: 646).

=Trichalina: Sklenarova et al. (2014: 47).

=Hemiconderina Bocak and Bocakova, 1990a: 645; Sklenarova et al. (2014: 47).

Type species. *Hemiconderis* Kleine 1926a.

Diagnoses of Metriorrhynchini and Metriorrhynchina

The tribe is defined by the unique circular phallobase, absent parameres (in contrast with Lycinae: Conderini that can have similar pronotal carinae), and the characteristic shape of the pronotum and its carinae that form a slender lanceolate areola in the middle and 2–6 lateral areolae (Figs. 1, 3–14; Bocak 2002, Weiszenstein and Bocak 2011, Kazantsev 2012, Jiruskova et al. 2019). Most individuals can be intuitively assigned to the tribe with external morphological traits, and recent students of net-winged beetles have agreed with the limits of Metriorrhynchini (Calder 1998, Kazantsev 2012).

Unlike the whole tribe, the Metriorrhynchina is impossible to define by a single morphological trait. Most genera have characteristic sharply defined seven areoles on the pronotal disc and the compact pronotum without elevated lateral edges (Figs. 3–14). However, some species have pronota resembling Cautirina Sklenarova et al., 2014 and Metanoeina (Bocak 2002, Sklenarova et al. 2014) (Cautirina, some Metanoeina) or have indistinct pronotal ridges (*Metanoeus* Waterhouse, 1879, Weiszenstein and Bocak 2011). Several modifications of pronotal carinae, unknown or seldom observed in other subtribes, were identified in the terminal lineages of Metriorrhynchina. Some small-bodied species have considerably reduced pronotal carinae. Either all carinae are lost, and the surface of the pronotum is rugose with an unclear median keel, or a slender, poorly defined lanceolate areola is present and lateral carinae are lost. The trichaline and several leptotrichaline and porrostomine genera have lanceolate, well-defined median areole and lateral and fronto-lateral carinae are absent (Bocek and Bocak 2017). The absence of lateral carinae resembles some *Cautires* Waterhouse, 1879 (the species earlier placed in *Bulenides* Waterhouse, 1879), but the pronota of trichalines have conspicuous frontal angles. Rhomboidal areola with distinct lateral carinae is known in *Falsolucidota* Pic, 1921 (=*Hemiconderis* Kleine, 1926a), *Wakarumbia* Bocak, 1999b, and resembles the distantly related Conderini Bocak and Bocakova, 1990a (Lycinae) and Dictyopterini Houlbert, 1922 (Erotinae Leconte, 1881), which substantially differ in their trilobate male genitalia (Bocak and Bocakova 1990a, 2008; Kazantsev 2005, 2020; Dvorak and Bocak 2009). The shape of male genitalia is also variable, and we cannot define a single trait that all Metriorrhynchina would share and be absent in other lineages. Most species have an asymmetrical base of the phallus, sometimes broad, unlike Cautirina. The unique phallic types in Metriorrhynchina include the phalli with various characteristic sclerotized structures (Figs. 3–11). In contrast, most species of *Porrostoma* share simple tube-like phalli with an exposed membranous internal sack that never has thorns or sclerotized rods (Figs. 12–14). The phalli of Metanoeina are similarly variable (Weiszenstein and Bocak 2011), those of Cautirina are uniformly lanceolate, and the internal sac of most species bears a pair of thorns (Bocak 2002, Jiruskova et al. 2019).

Metriorrhynchina and related Cautirina and Metanoeina occur sympatrically only in the Oriental region and Sulawesi (Kubecek et al. 2011, Masek et al. 2018). In these areas, it is possible to misidentify the Metriorrhynchini tribes. Still, most species belong to clearly defined genera, and, in case of uncertainty, they can be reliably identified after the dissection of male genitalia.

Diversity and Distribution

Most Metriorrhynchina occur in the Australian region, namely in New Guinea (423 spp.) and continental Australia (196 spp.), where they dominate the local faunas with ~80% of the named net-winged beetle taxa. The group also occurs in the Wallacea (176 spp.), but the numbers of species decrease with the distance from the Australian continental shelf. The Metriorrhynchina of the Moluccas shares with New Guinea the genera but differs in species. Similarly, the Sulawesi fauna is relatively species-rich but differs in the presence of several endemic genera (*Sulabanus* Dvorak et Bocak 2007, *Wakarumbia* Bocak 1999b, *Mangkutanus* Kubecek et al. 2011). Less Metriorrhynchina occurs in the eastern part of the Oriental region, i.e., the Sundaland, Philippines, Malaya, and Indo-Burma,

Fig. 3. *Maraiakoreus* **gen. nov.** A—Distribution; B—Simplified phylogenetic hypothesis of species-level relationships with information on the position of illustrated individuals (dark green dots with the voucher code of the illustrated specimen) and with information on species distribution (terminal dots, see legend); C–AF Illustrations of habitus, head, and pronotum, the basal part of the antenna, the middle part of elytron and male genitalia in various aspects; all males, the codes are voucher codes of displayed individuals. The figures W–Z and AE–AG show the morphology of *M. argenteus* **sp. nov.**, the type species of *Maraiakoreus*. The voucher codes in parentheses designate the conspecific individual shown in Supp Figs. 1 and 2 (online only). Scales 0.5 mm, for habitus 1.0 mm.

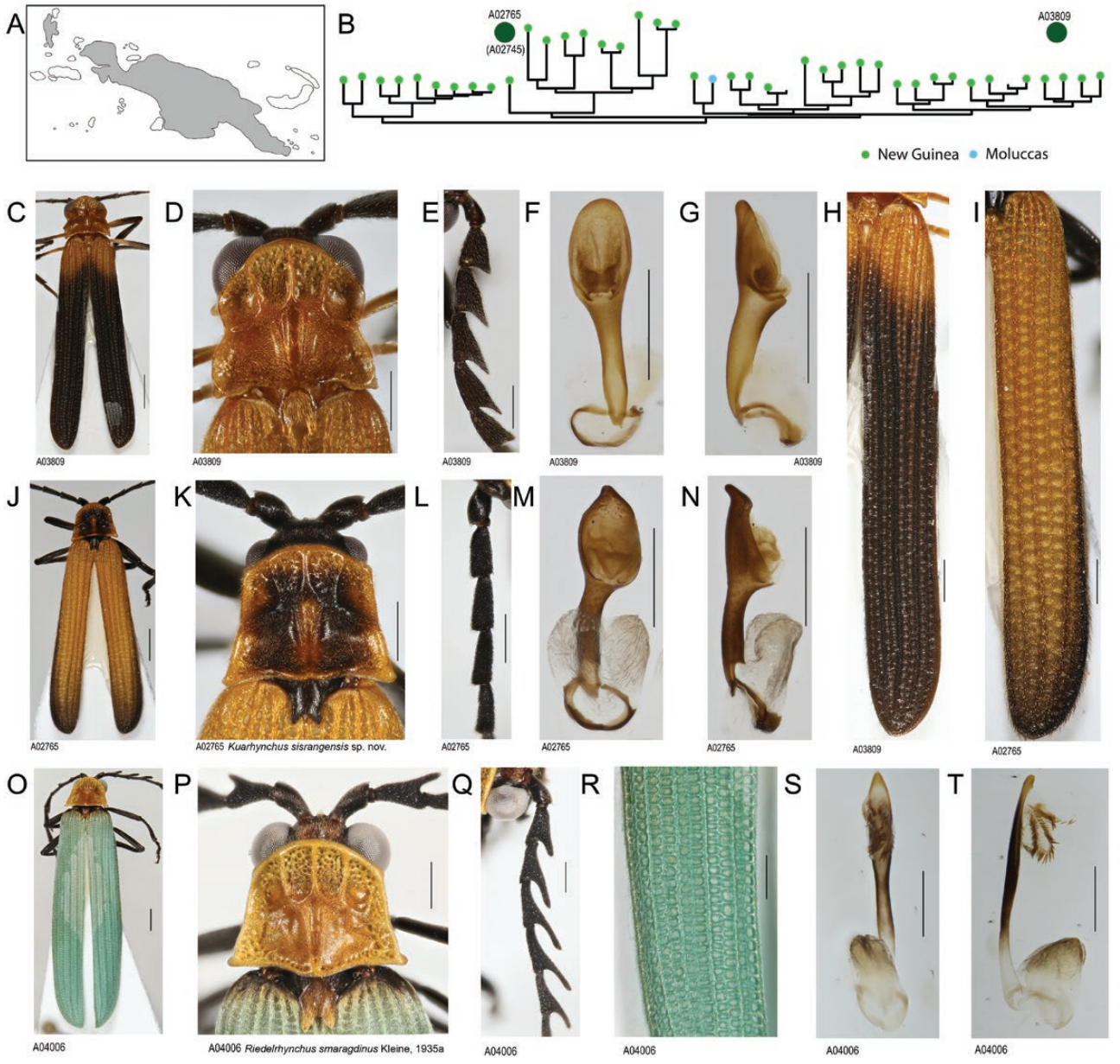


Fig. 4. *Kuarhynchus* **gen. nov.** A—Distribution of *Kuarhynchus*; B—Simplified phylogenetic hypothesis of species-level relationships with information on the position of illustrated *Kuarhynchus* individuals (dark green dots with the voucher code of the illustrated specimen) and with information on species distribution (terminal dots, see legend); C–N *Kuarhynchus*, Illustrations of habitus, head, and pronotum, the basal part of the antenna, the middle part of elytron and male genitalia in various aspects. Figures C–H show the morphology of *K. sisrangensis* **sp. nov.**, the type species of *Kuarhynchus*; O–T ditto, *Riedelrhynchus smaragdinus* Kleine 1935; all males. The codes designate voucher codes of displayed individuals. The voucher codes in parentheses designate the conspecific individual shown in Supp Figs. 1 and 2 (online only). Scales 0.5 mm, for habitus 1.0 mm.

where they represent a small fraction of described net-winged beetles (<5%; Sklenarova et al. 2013; Masek et al. 2018; Bocek et al. 2019, 2020). The earlier records of Metriorrhynchina outside of the here described range regions refer to misplaced taxa (Kleine 1933a, Bocak et al. 2020).

Biology

The adults are regularly fully winged, but many species are inactive during the day and remain in shaded situations sitting on leaves (Motyka et al. 2018, 2020, 2021b; Bocek et al. 2019). Some *Porrostoma*, *Metriorrhynchus*, *Leptotrichalus* Kleine, 1925a, and *Trichalus* visit flowers they pollinate (Forster 1989, Kleine 1926b). The flower frequenting species are diurnal and are the most common Metriorrhynchina in semi-dry areas where nectar represents a potential source of water and energy for adults. All Metriorrhynchina are unpalatable for potential predators, and most of them are aposematically colored (Moore and Brown 1981, 1989; Eisner et al. 2008). The color patterns are usually geographically restricted, and the sympatrically occurring species often form several Müllerian rings (Bocak and Yagi 2010; Motyka et al. 2018, 2020, 2021b; Bocek et al. 2019).

Bocak and Matsuda (2003) and Levkanicova and Bocak (2009) described the larvae of *Cautires*, *Leptotrichalus*, *Metriorrhynchus*, and *Porrostoma*, and some unidentified larvae were collected in New Guinea (Fig. 1C and D). Most Metriorrhynchina larvae have

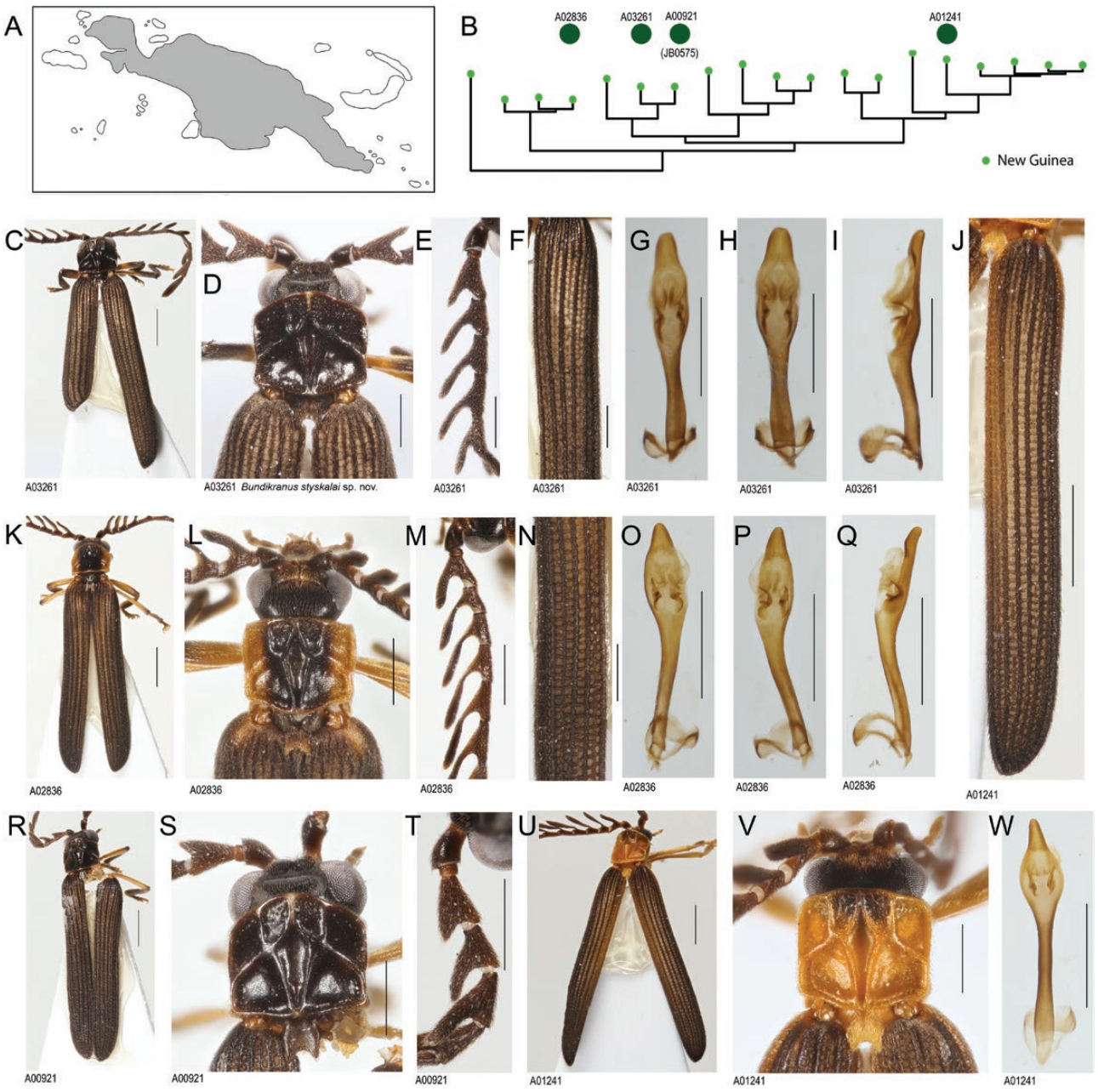


Fig. 5. *Bundikanus* **gen. nov.** A—Distribution; B—Simplified phylogenetic hypothesis of species-level relationships with information on the position of illustrated individuals (dark green dots with the voucher code of the illustrated specimen) and with information on species distribution (terminal dots, see legend); C–V Illustrations of habitus, head and pronotum, the basal part of the antenna, the middle part of elytron and male genitalia in various aspects; all males, the codes are voucher codes of displayed individuals. Figures K–R show the morphology of *B. styskalai* **sp. nov.**, the type species of *Bundikanus*. The voucher codes in parentheses designate the conspecific individual shown in Supp Figs. 1 and 2 (online only). Scales 0.5 mm, for habitus 1.0 mm.

bipartite terga like Metanoeina, but many do not have free lateral sclerotized processes (Bocak and Matsuda 2003). The larvae live in rotten wood, including trunks and logs on soil and plant debris. Larvae suck water rich in microbial life from decaying plant material, and we suppose that humid tropics set conditions supporting the high abundance and diversity of net-winged beetles.

The Porrostomine Clade

Diagnosis

The porrostomine clade contains metriorrhynchines that usually have a tubular, basally sclerotized phallus and an internal sac that can be membranous or bearing spines, but never with paired rods or thorns deeply set close to the base of the phallus. Most species can be reliably assigned to the porrostomines and individual genera after dissection of the male genitalia. However, we also encountered an aberrant genital morphology, such as simplified phallus or lost sclerotized structures of the internal sac (e.g., Fig. 11N, O, and AB). The comparison with a related species with unmodified genitalia can solve such cases. The external morphology is variable, and the group contains the species with serrate to flabellate male antennae, usually with seven pronotal areoles (seldom reduced to five or three areoles) and four or nine longitudinal elytral costae. Although most species are medium- to large-bodied (7–20 mm), the clade also contains

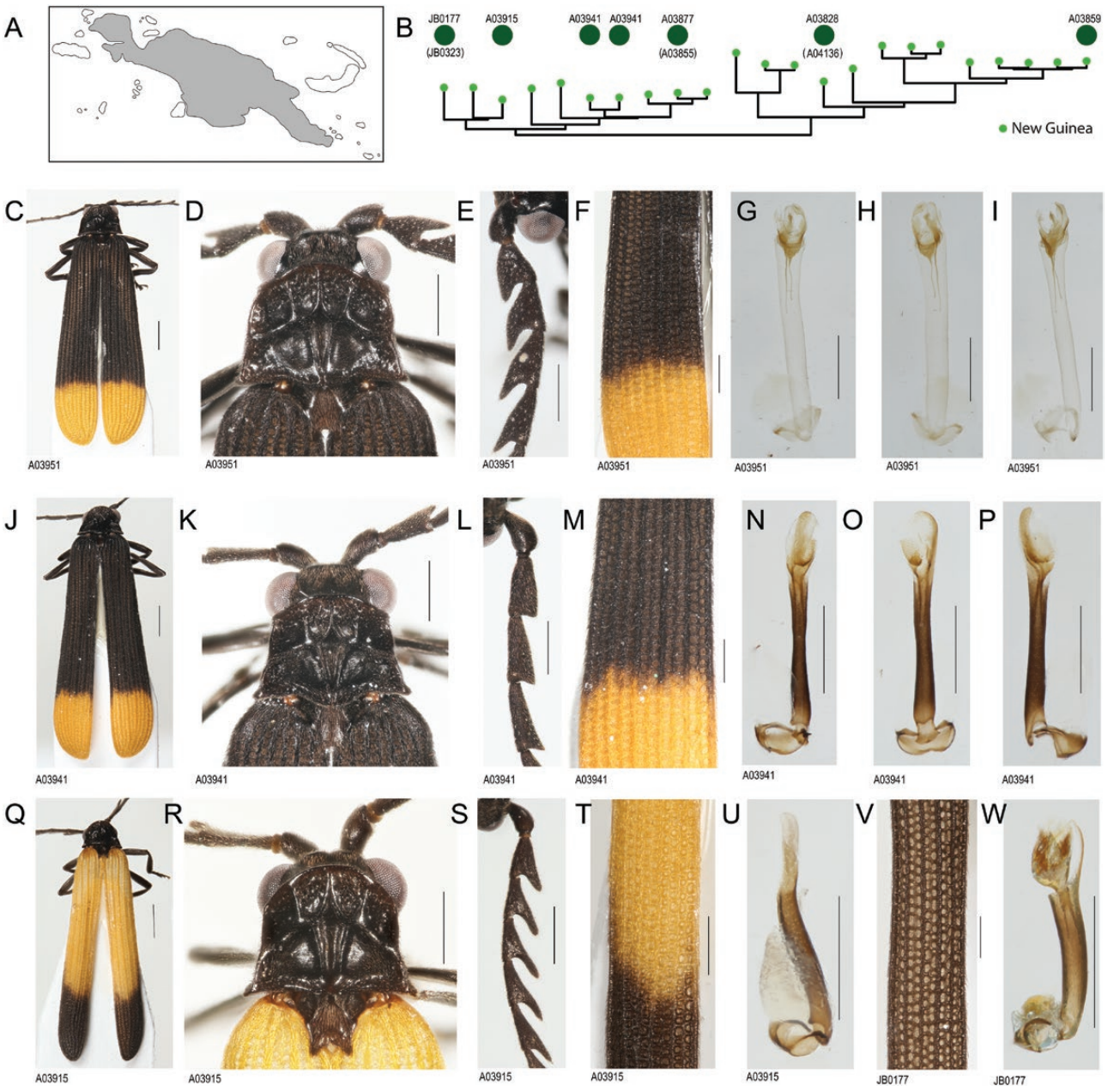


Fig. 6. *Yamarhynchus* **gen. nov.** A—Distribution; B—Simplified phylogenetic hypothesis of species-level relationships with information on the position of illustrated individuals (dark green dots with the voucher code of the illustrated specimen, incl. individuals illustrated in Fig. 7) and with information on species distribution (terminal dots, see legend); C–V Illustrations of habitus, head, and pronotum, the basal part of the antenna, the middle part of elytron and male genitalia in various aspects; all males, the codes are voucher codes of displayed individuals. The voucher codes in parentheses designate the conspecific individual shown in Supp Figs. 1 and 2 (online only). Scales 0.5 mm, for habitus 1.0 mm.

some small-bodied representatives (~4 mm) that have reduced costae and pronotal carinae. Five earlier described genera have been included in the porrostomine clade: *Metriorrhynchus* Gemminger et Harold, 1869 (194 spp.), *Stadenus* Waterhouse, 1879 (8 spp.), *Porrostoma* Castelnau, 1838 (26 spp.), *Metriorrhynchoides* Kleine, 1926a (4 spp.), *Oriomum* Bocak, 1999a (1 spp.), but three of them are synonymized in the present study (see below).

Diversity and Distribution

Altogether 233 spp. have been assigned to the porrostomine clade (Bocak et al. 2020). They occur in Australia, Tasmania, New Zealand (introduced), New Guinea, New Britain, Bougainville, the Solomon Islands, the Moluccas, Sulawesi, the Philippines, the Greater Sundas, the Malay Peninsula, Indo-Burma (Laos, Thailand; Supp Figs. 1 and 2 [online only]). No record is available from the Lesser Sundas, although an occurrence of some species is probable (Bocak et al. 2020). We identified the highest phylogenetic and alpha diversity of the porrostomines in New Guinea, where occur all known genera. The porrostomine clade is the second concerning species diversity, and we identified 352 putative species by the mtDNA analysis and morphological validation in the analyzed dataset.

Fig. 7. *Yamarhynchus* **gen. nov.** A–U Illustrations of habitus, head, and pronotum, the basal part of the antenna, the middle part of elytron, and male genitalia in various aspects; all males, the codes are voucher codes of displayed individuals. Figures O–U show the morphology of *Y. sinopassensis* **sp. nov.**, the type species of *Yamarhynchus*. The voucher codes in parentheses designate the conspecific individual shown in Supp Figs. 1 and 2 (online only). Scales 0.5 mm, for habitus 1.0 mm.

Maraiakoreus Kusy, Motyka, Bilkova, and Bocak, gen. nov.

(Fig. 3A–AG)

urn:lsid:zoobank.org:act:6294B1D0-2390-40F2-A00B-7BBB1399DFA0

Type Species

Maraiakoreus argenteus **sp. nov.**

Diagnosis

Maraiakoreus is characterized by a single pronotal areola (Fig. 3D, L, T, AB, and AF) unknown in other porrostomines except in some *Porrostoma* and by the slender phallus with two thorns located in the basal third of the phallus. If only the median areola is present in some Australian *Porrostoma*, it is much longer and broader, and the phallus has an entirely membranous internal sac. The known species of *Maraiakoreus* are small-bodied (~6 mm), dorsoventrally compressed, with posteriorly widened elytra (Fig. 3C, K, S, AA, and AE). The shape of male genitalia strongly supports the monophyly of the genus as recovered by the molecular analysis. The male antennae can be serrate to flabellate (Fig. 3E, M, U, AC, and AG), and if flabellate, then the lamella of the antennomere 3 is the same length as the lamella of the antennomere 5 (Fig. 3M, AC, and AG). See the description of *M. argenteus* **sp. nov.** for further information.

Material Examined

Maraiakoreus **gen. nov.** was unavailable for the phylogenomic analysis; the mtDNA dataset contained 15 spp. No earlier described species resembling the proposed genus has been found in net-winged beetle collections.

Diversity and Distribution

There have been identified 15 spp., all occurring in New Guinea (Fig. 3A and B).

Etymology

The generic name is a patronym in honor of Heveakore Maraia (Binatang Research Centre, Papua New Guinea), our colleague who took part in the field research in Papua New Guinea. Gender masculine.

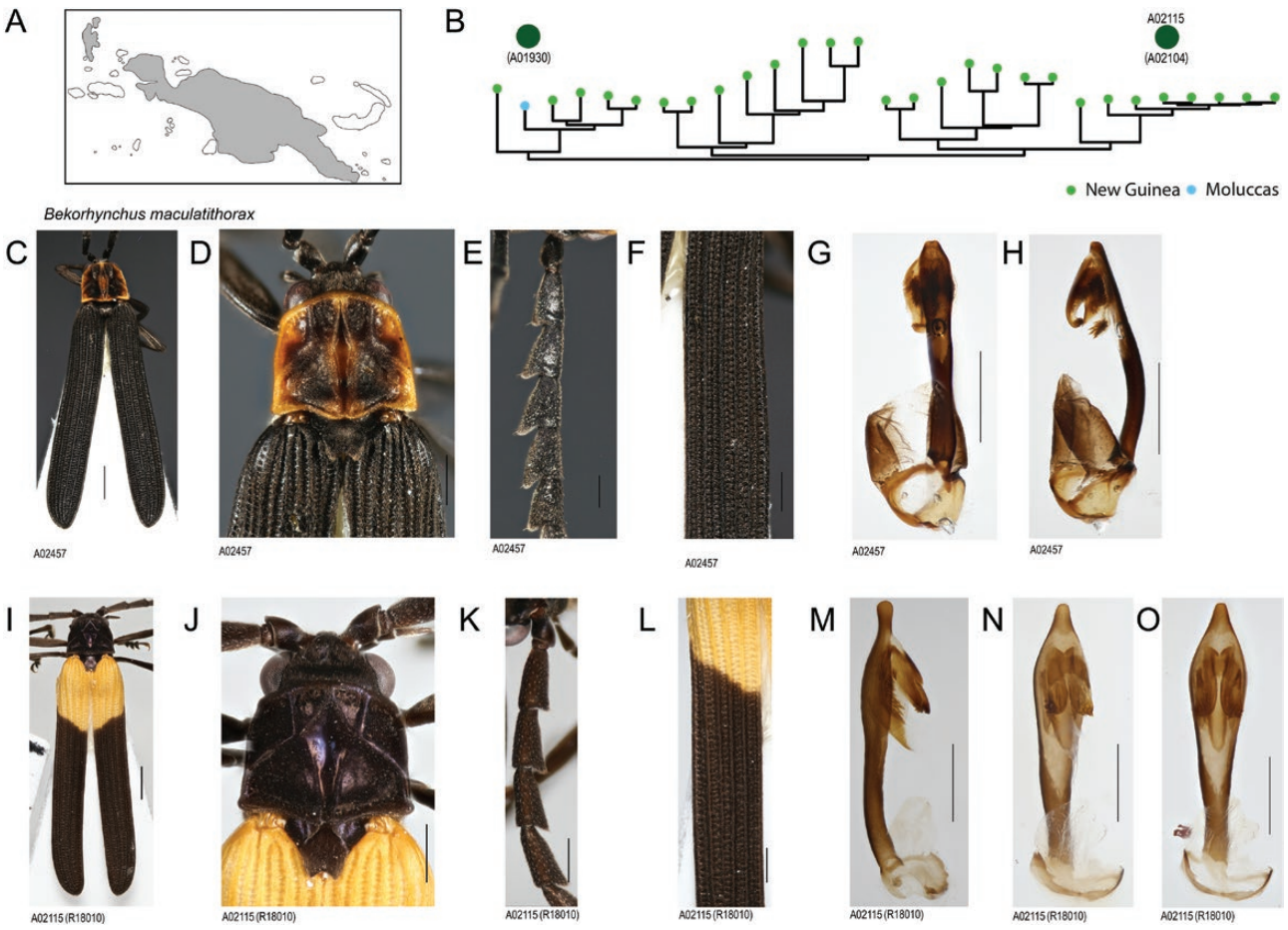


Fig. 8. *Bekorhynchus* **gen. nov.** A—Distribution; B—Simplified phylogenetic hypothesis of species-level relationships with information on the position of illustrated individuals (dark green dots with the voucher code of the illustrated specimen) and with information on species distribution (terminal dots, see legend); C–V Illustrations of habitus, head, and pronotum, the basal part of the antenna, the middle part of elytron, and male genitalia in various aspects; all males, the codes are voucher codes of displayed individuals. The voucher codes in parentheses designate the conspecific individual shown in Supp Figs. 1 and 2 (online only). Scales 0.5 mm, for habitus 1.0 mm.

Remark

The clade was recovered as a part of the porrostomines, either as a sister to *Isuarhynchus* (Motyka et al. 2021a) or as a sister of other porrostomine genera (Fig. 2B). However, the external morphology differs from other porrostomines, and the male genitalia do not equivocally support the genus's placement either (Fig. 3). After comparison with cladophorine genera that differ in the short, more robust phallus and thorns regularly located deeper in the basal part of the internal sac, we prefer to keep the genus in the porrostomines till genomic data are available for the confirmation of relationships.

Some species are brightly colored but seldom resemble other net-winged beetles. Only one species of *Diatrichalus* Kleine, 1926a and several *Yamarhynchus* are putative co-mimics (Motyka et al. 2021b).

Maraiakoreus argenteus Kusy, Motyka, Bilkova, and Bocak, sp. nov.

(Fig. 3W–Z, AE–AG)

urn:lsid:zoobank.org:act:037AEC73-D074-4677-B23C-36DFDC81E63E

Type Material

HOLOTYPE: male, PAPUA NEW GUINEA, Mt Hagen, Paya, 5°50′S 144°7′ E, 1,910 m, 17-IV-2018 (voucher code A03797, LMBC). PARATYPES: 2 males, same data; 1 female, PAPUA NEW GUINEA, Sino Pass, Bundi vill., 5°45′S 145°11′E, 2,015 m, 16-VII-2017 (LMBC).

Diagnosis

At present, *M. argenteus* **sp. nov.** is the only named species of *Maraiakoreus*. It belongs to a group of species with long male antennal lamellae (Fig. 3M, AC, and AG) and differs from other species in the coloration (Fig. 3C, L, S, and AA); the phallus differs slightly from related species (Fig. 3).

Male

Body small-sized, 7.05 mm long, dorsoventrally flattened, robust. Body black colored, pronotum light testaceous and wholly covered with dense silver pubescence (Fig. 3AE, and EF), elytra, except small part of humeri and apical tenth orange-yellow, otherwise black (Fig. 3AE). Head small, only partly hidden by pronotum, densely pubescent, with large, hemispherically prominent eyes, interocular distance 1.29 times eye diameter. Antennae flat, flabellate, antennomere 2 very short, antennomeres 3 with lamella about same length as antennomere trunk, lamellae shorter from antennomere 6 to 10, apical antennomere slender,

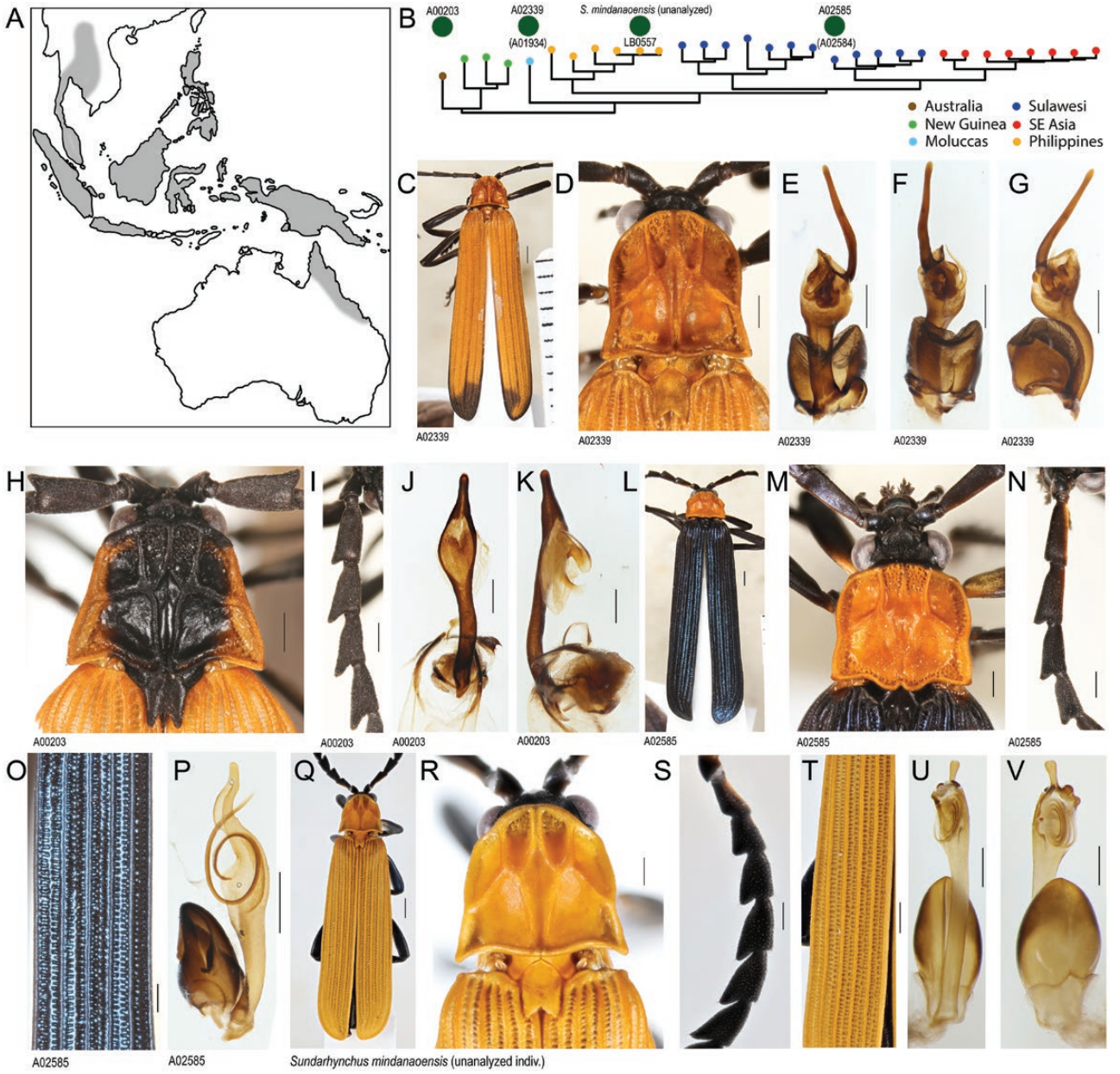


Fig. 9. *Sundarhynchus* **gen. nov.** A—Distribution; B—Simplified phylogenetic hypothesis of species-level relationships with information on the position of illustrated individuals (dark green dots with the voucher code of the illustrated specimen) and with information on species distribution (terminal dots, see legend); C–V Illustrations of habitus, head, and pronotum, the basal part of the antenna, the middle part of elytron and male genitalia in various aspects; all males, the codes are voucher codes of displayed individuals. The illustrations of *S. mindanaoensis* Bocak et al. 2006 show an unanalyzed individual of the species included in the whole genome sequencing and transcriptomic analyses. The voucher codes in parentheses designate the conspecific individual shown in Supp Figs. 1 and 2 (online only). Scales 0.5 mm, for habitus 1.0 mm.

parallel-sided (Fig. 3AG). Pronotum transverse, widest at basal margin, narrower frontally, 1.45 times wider than long at midline, apical margin widely rounded, lateral margins elevated, slightly concave in anterior two-thirds, projected in bulge in basal third, posterior angled acute, surface with dense pubescence. Elytra 3.1 times longer than width at humeri, wide apically, with primary and secondary costae 1–3 of similar strength, costa 4 strong (Fig. 3Z), all costae developed in almost whole elytral length, elytral cells tiny, rounded to slightly transverse. Phallus slender, almost parallel-sided, with open ventral part, internal sac with two small thorns, basally with small sclerite (Fig. 3W–Y). Female. Like male, antennae serrate.

Measurements

BL 7.0 mm, Ediam 0.45 mm, Edist 0.58 mm, WH 1.9 mm, PW 1.42 mm, PL 0.98 mm, EL 5.9 mm, PhL 1.14 mm.

Distribution

M. argenteus has only been reported from two localities in the high mountains of eastern New Guinea (Mt Hagen and Mt Wilhelm).

Etymology

The species epithet '*argenteus*' refers to the silver-colored pubescence of the pronotum (Fig. 3AF).

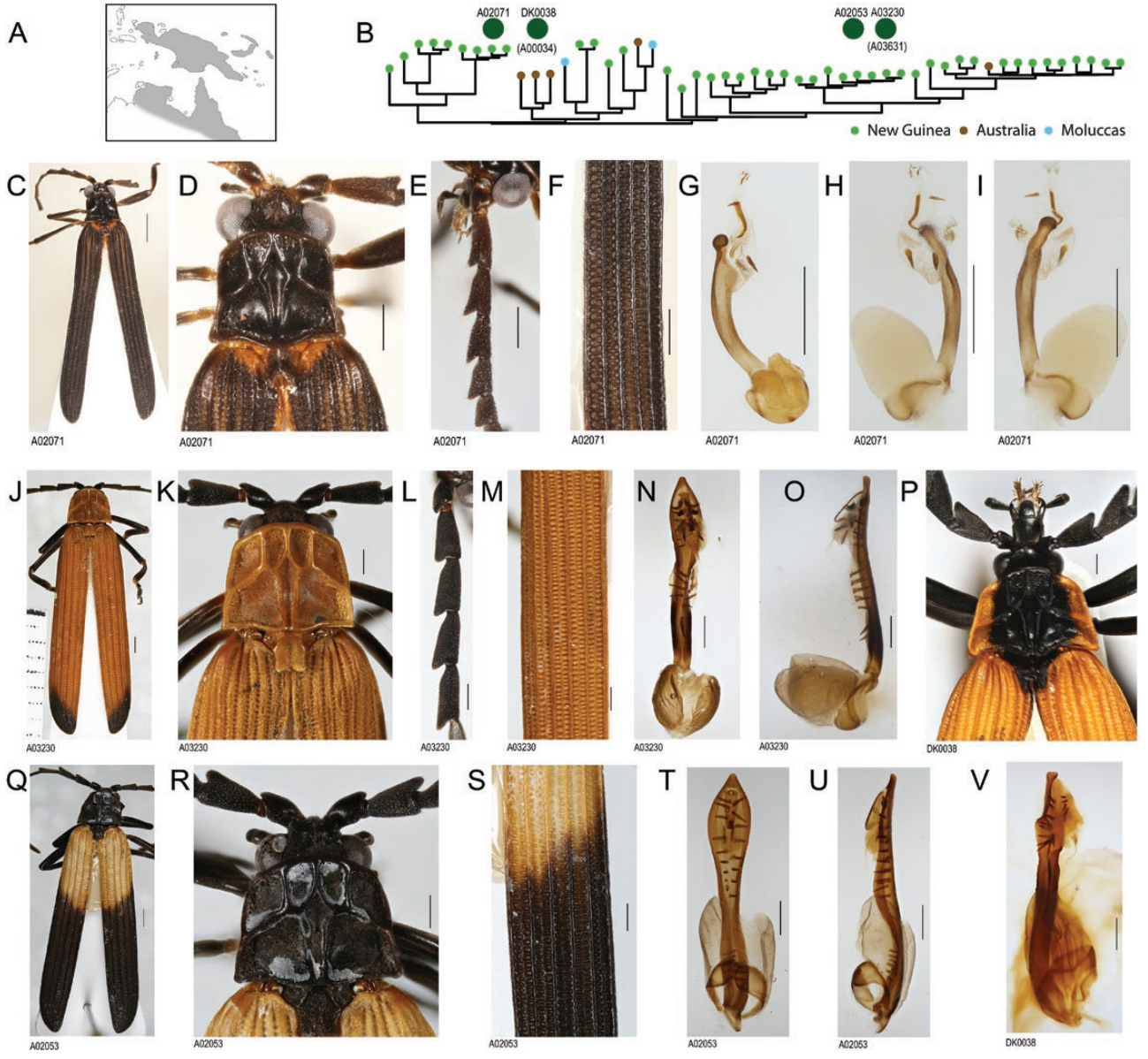


Fig. 10. *Metriorrhynchus* Guérin-Méneville. A—Distribution; B—Simplified phylogenetic hypothesis of species-level relationships with information on the position of illustrated individuals (dark green dots with the voucher code of the illustrated specimen) and with information on species distribution (terminal dots, see legend); C–V Illustrations of habitus, head and pronotum, the basal part of the antenna, the middle part of elytron and male genitalia in various aspects. The voucher codes in parentheses designate the conspecific individual shown in Supp Figs. 1 and 2 (online only). Scales 0.5 mm, for habitus 1.0 mm.

The *Metriorrhynchus* Grade

Metriorrhynchus Gemminger et Harold, 1869 (=*Metriorrhynchus* Guérin-Méneville, 1838, Bocak 1998) has served as a collective taxon for mid- and large-bodied Metriorrhynchina with a short- or non-rostrate head, slender body, seven pronotal areoles, and nine elytral costae (Kleine 1926a, 1933a; Bocak 2002; Kazantsev 2015). The phylogenomic and subsequent constrained mtDNA analyses recovered at the deep part of the tree a succession of branches whose species have conventionally been assigned in *Metriorrhynchus* (Fig. 2A, B, Supp Figs. 1, 2 [online only]). When three genera are synonymized to *Porrostoma* (see below), only two generic names remain available: *Porrostoma*, an extensive terminal clade (Fig. 3C), and *Metriorrhynchus* in the traditional sense as a paraphylum. A taxonomic action is needed to reflect the paraphyly. Therefore, we delimit and assign names to eight constituent clades: *Kuarhynchus* **gen. nov.**, *Bundikanus* **gen. nov.**, *Riedelrhynchus* **gen. nov.**, *Bekorhynchus* **gen. nov.**, *Yamarhynchus* **gen. nov.**, *Sundarhynchus* **gen. nov.**, *Metriorrhynchus* s. str., and *Isuarhynchus* **gen. nov.** We describe seven of them as new genera and propose the revised concept of *Metriorrhynchus sensu stricto* (Fig. 3C).

Kuarhynchus Kusy, Motyka, Bilkova, and Bocak, gen. nov.

(Figs. 4A–N)

urn:lsid:zoobank.org:act:87385D57-0D2D-4F75-9722-024CCCB28C55

Type Species

Kuarhynchus sisrangensis **sp. nov.**

Fig. 11. *Isuarhynchus* **gen. nov.** A—Distribution; B—Simplified phylogenetic hypothesis of species-level relationships with information on the position of illustrated individuals (dark green dots with the voucher code of the illustrated specimen) and with information on species distribution (terminal dots, see legend); C–AB Illustrations of habitus, head, and pronotum, the basal part of the antenna, the middle part of elytron and male genitalia in various aspects. The voucher codes in parentheses designate the conspecific individual shown in Supp Figs. 1 and 2 (online only). Scales 0.5 mm, for habitus 1.0 mm.

Diagnosis

Kuarhynchus is characterized by the basally slender phallus with an extended, bulb-like apical part. The membranous internal sac is packed in the widened apical part of the phallus; the internal sac has basally an extensive pigmented area (Fig. 4F, G, M, and N)). The species placed in the genus are morphologically diverse (Fig. 4C–N), but all share more or less shortened elytral costa 3 (Fig. 4H and I). The costa 3 typically reaches one-third of the elytral length but can be much longer if only four longitudinal costae are present in the middle of the elytra (Fig. 4I). All species have

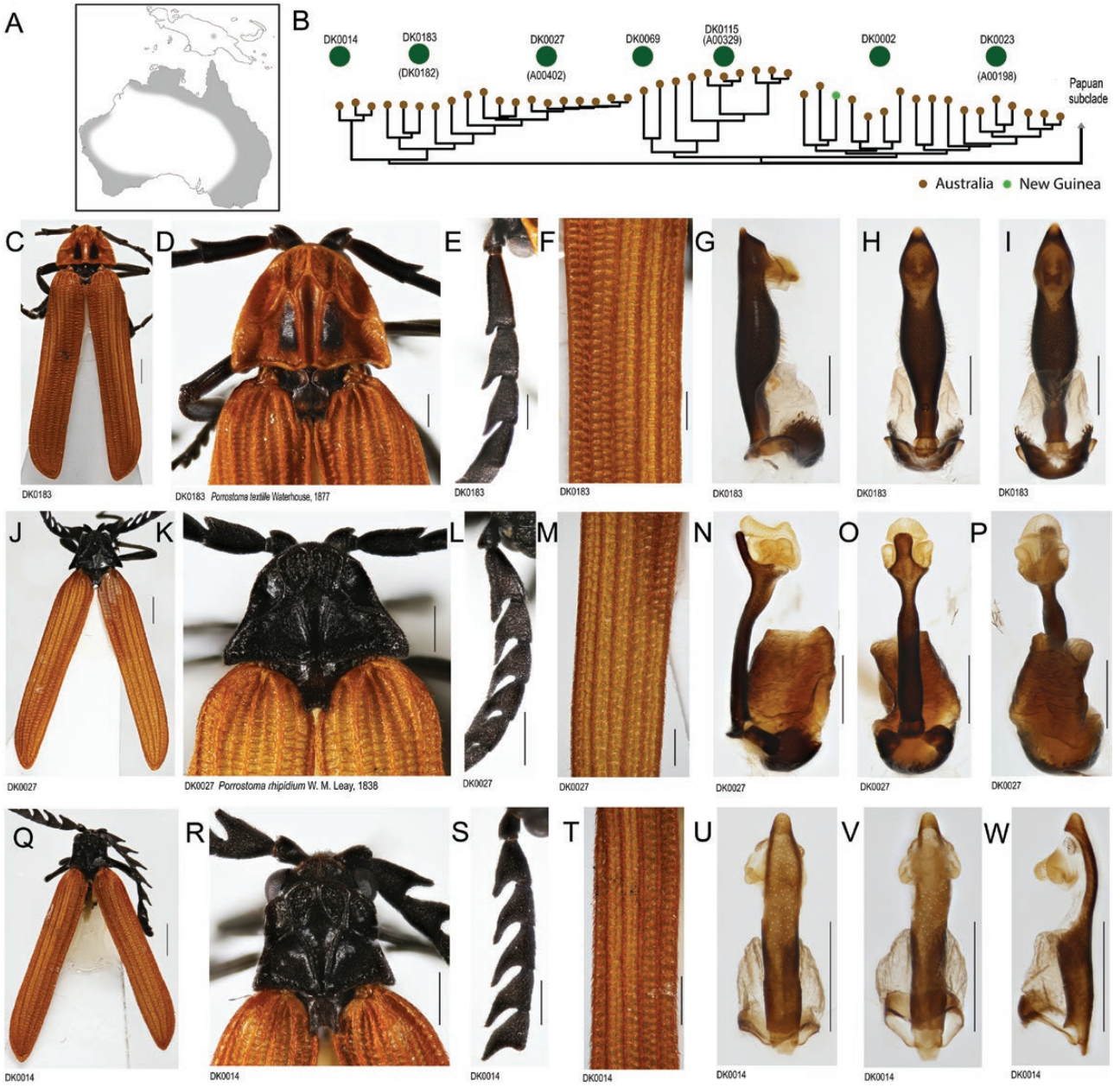


Fig. 12. *Porrostoma* Castelnau; Australian grade. A—Distribution; B—Simplified phylogenetic hypothesis of species-level relationships with information on the position of illustrated individuals (dark green dots with the voucher code of the illustrated specimen) and with information on species distribution (terminal dots, see legend); C–V Illustrations of habitus, head and pronotum, the basal part of the antenna, the middle part of elytron and male genitalia in various aspects. The voucher codes in parentheses designate the conspecific individual shown in Supp Figs. 1 and 2 (online only). Scales 0.5 mm, for habitus 1.0 mm.

a slender body, filiform to serrate antennae, and seven pronotal areoles (Fig. 4). See the description of *K. sisrangensis* **sp. nov.** for further information.

Material Examined

Kuarhynchus gen. nov. was unavailable for the phylogenomic analysis; mtDNA analyses contained 42 spp.

Diversity and Distribution

In contrast with the 42 species in the analyzed dataset, we have not found any earlier described species belonging to *Kuarhynchus* in museum collections. The genus occurs in New Guinea and the Moluccas (Fig. 4A and B).

Etymology

The generic name is a patronym in honor of Joseph Kua (Binatang Research Centre, Madang, and Mu village, Kundiawa, Papua New Guinea), and the Latin '*rhynchus*' refers to the similarity of some species to *Metriorrhynchus sensu* Kleine 1926a. Gender masculine.

Remark

Kuarhynchus is a newly proposed genus for the deepest clade of the *Metriorrhynchus* grade. The molecular analysis recovered as a monophylum the group of species with very similar male genitalia but quite variable external morphology (Fig. 4). Some species are relatively small-bodied, and, possibly because of miniaturization,

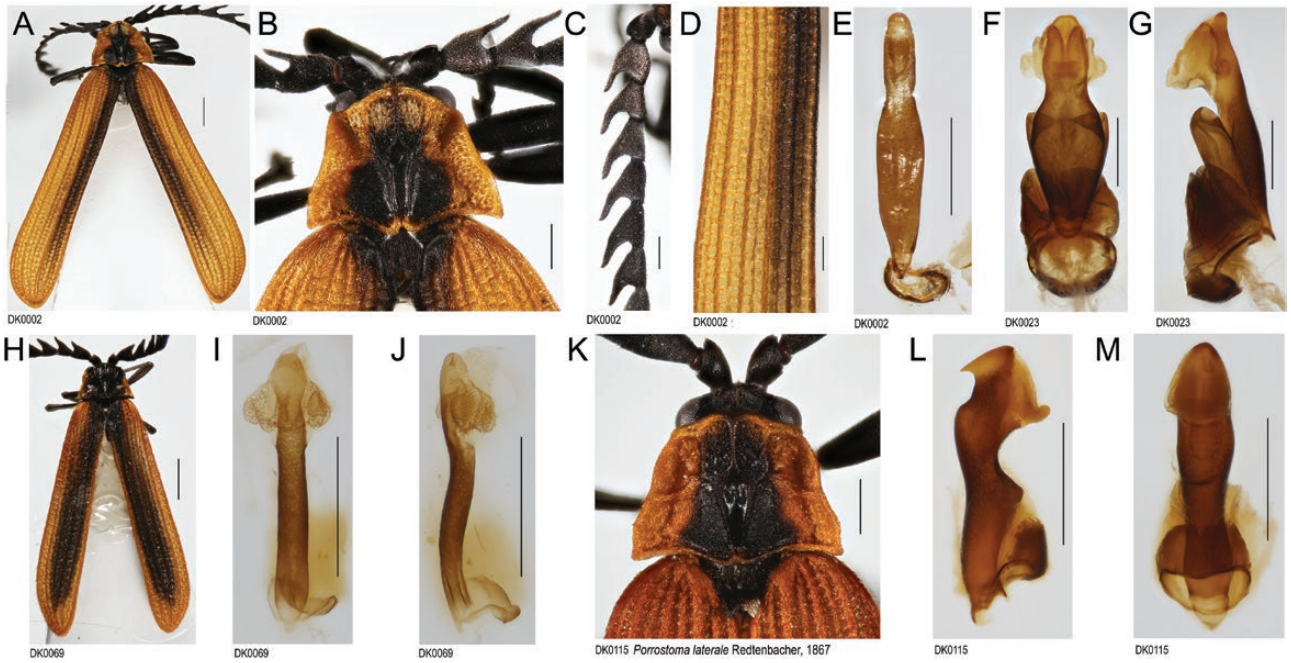


Fig. 13. *Porrostoma* Castelnau; Australian grade, continued. A–M Illustrations of habitus, head and pronotum, the basal part of the antenna, the middle part of the elytron, and male genitalia in various aspects. The voucher codes are mapped on the tree in the previous figure. Scales 0.5 mm, for habitus 1.0 mm.

they entirely lost the secondary elytral costae in the middle of the elytra (Fig. 4G). Although the shape of the male antennae has often been used as a diagnostic character, *Kuarhynchus* contains species with slender, parallel-sided to acutely serrate antennomeres 3–10 (Fig. 4E and L). Neither the structure of secondary elytral costae nor the male antennae define morphologically distinct subgroups that would be reciprocally monophyletic. Identifying the genus is unreliable with external characters. The dissection of male genitalia should verify the assignment based on the shortened costa 3.

Kuarhynchus sisrangensis Kusy, Motyka, Bilkova, and Bocak, sp. nov.

(Fig. 4I–N)

urn:lsid:zoobank.org:act:3151AFEA-F1DF-43ED-B975-D4C9B8D29D11

Type Material

HOLOTYPE: male, INDONESIA, West Papua, Sisrang, 25 km NW Ransiki, 1° 21′ N 134°0′ E, 1,840 m (Voucher code A02765, LMBC). PARATYPES: 3 females, same data (LMBC).

Diagnosis

At present, *K. sisrangensis* **sp. nov.** is the only named species of *Kuarhynchus*. It belongs to a group of species with almost filiform antennae (Fig. 4L) and differs from other species in the coloration (Fig. 4J) and the bent apical part of the phallus (Fig. 4N).

Male

Body small-sized, 6.5 mm long, relatively slender. Whole body dark brown colored, only substantial part of pronotal disc, median areola, and most of elytra yellow, elytral apex, and apical half of their lateral margin dark brown, infuscate apical part very gradually changes in yellow-colored area, appendages brown to black (Fig. 4C–E). Head small, hidden by pronotum, sparsely pubescent, with small but hemispherically prominent eyes, interocular distance 1.94 times eye diameter (Fig. 4K). Antennae flat, filiform, antennomere 2 very short, antennomeres 3–10 slender, compressed, parallel-sided, apical antennomere pointed. Pronotum widest at basal margin, with acutely projected posterior angles, narrower frontally, basally 1.31 times wider than long at midline, apical margin slightly projected, lateral margins concave, frontal part coarsely punctuated. Elytra 3.65 times longer than width at humeri, very slightly widened posteriorly, with secondary costae much weaker than primary ones, costa 3 shorter, secondary costae absent in middle part, elytral cells variable tiny at humeri, large and quadrate in middle of elytra. Phallus parallel-sided basally, bulbous apically, widest in apical third, pointed apically, apex bent in lateral view, open ventrally; internal sac partly pigmented, without any conspicuous sclerotized structure (Fig. 4M and N). Female. Body slightly larger, length 7.0–7.8 mm, elytra completely yellow.

Unlike the holotype, all paratypes have uniformly yellow elytra and the dark patch in the pronotum is limited to a small area basally to the median areola.

Measurements

BL 6.5 mm, Ediam 0.31.0 mm, Edist 0.60 mm, PW 3.36 mm, PL 1.04 mm, WH 1.44 mm, EL5.25 mm, PhL 1.02 mm.

Distribution

K. sisrangensis **sp. nov.** has only been reported from the type locality.

Etymology

The species epithet ‘sisrangensis’ refers to ‘Sisrang,’ the type locality of this species.

Fig. 14. *Porrostoma* Castelnau; New Guinean clade. A—Distribution; B—Simplified phylogenetic hypothesis of species-level relationships with information on the position of illustrated individuals (dark green dots with the voucher codes of the illustrated specimen) and with information on species distribution (terminal dots, see legend); C–V Illustrations of habitus, head, and pronotum, the basal part of the antenna, the middle part of elytron, and male genitalia in various aspects; all males if not otherwise stated. The voucher codes in parentheses designate the conspecific individual shown in Supp Figs. 1 and 2 (online only). Scales 0.5 mm, for habitus 1.0 mm.

Riedelrhynchus Kusy, Motyka, Bilkova, and Bocak, gen. nov.

(Fig. 4O–T)

urn:lsid:zoobank.org:act:F9AECB98-8476-46CC-8149-9F37E41FB7D5

Type Species

Metriorrhynchus smaragdinus Kleine, 1935a.

Diagnosis

Riedelrhynchus is characterized by the exposed, membranous, and densely setose internal sac (Fig. 4S and T). It substantially differs in the male genitalia, body shape, and coloration from both closely related genera, *Kuarhynchus* and *Bundikanus* (Figs. 4C–N and 5). Both species assigned to the genus are brightly colored with the elytra's unusual blue to green-blue coloration (Fig. 4O). All primary costae reach the apex of elytra, but costae 1 and 3 are weak (Fig. 4R). Two species with blue and blue colored elytra form a clade deeply rooted in the tree as a sister to the *Bundikanus* + *Yamarhynchus* clade.

Male

Body medium-sized, 9–12 mm long, slender. Body dark colored, elytra green or blue (Fig. 4O). Head small, hidden by pronotum, densely pubescent, with large, hemispherically prominent eyes. Antennae flat, shortly flabellate, antennomere 2 very short, antennomere 3 almost triangular, antennomeres 4–10 with gradually shorter lamellae, apical antennomere slender, parallel-sided (Fig. 4Q). Pronotum wide, widest at basal margin, only slightly narrower frontally, 1.35 times wider than long at midline, apical margin widely rounded, lateral margins concave, margins shallowly punctuated, surface almost bare (Fig. 4P). Elytra 4.2 times longer than width at humeri, parallel-sided, with strong primary costae 2 and 4, costae 1 and 3 weak, elytral cells tiny, variable in shape, mostly rounded. Phallus slender, widest in apical quarter, pointed apically, with open ventral part, internal sac membranous with numerous pigmented setae (Fig. 4S and T). Female. Unknown.

Material Examined

Unavailable for the phylogenomic analysis, mtDNA analysis 2 spp. (Fig. 2C, Supp Figs. 1 and 2 [online only]).

Diversity and Distribution

Kleine (1935a) described two species belonging to this clade. *Riedelrhynchus smaragdinus* Kleine, 1935a, **comb. nov.** and *R. tricolor* Kleine, 1935a, **comb. nov.**, occur in the high mountains of eastern New Guinea.

Etymology

The species is named in honor of Alexander Riedel (Karlsruhe), a specialist in curculionid beetles and prominent New Guinean beetle diversity student. The second part of the name, '*rhynchus*,' refers to the earlier placement of the type species in *Metriorrhynchus*. Gender masculine.

Bundikanus Kusy, Motyka, Bilkova, and Bocak, gen. nov.

(Fig. 5A–W)

urn:lsid:zoobank.org:act:2D95E51A-EFE4-41D2-8BAB-A4879BA220A1

Type Species

Bundikanus styskalai **sp. nov.**

Diagnosis

Bundikanus has a very characteristic arrangement of elytral costae. The primary costae reach the apex of the elytra except for the slightly shorter costa 3 and the secondary costae are absent in the interspaces between primary costae 1, 2, and 3 in the middle part of the elytra (Fig. 5). There are usually no traces of secondary costae in that part of the elytra, but we have found one large-bodied female with incomplete secondary costae. Further, *Bundikanus* differs from other genera of the *Metriorrhynchus* grade in the slender basal part of the phallus and with two thorns in the internal sac (Fig. 5G–I, O–Q, and W). Description as *B. styskalai* **sp. nov.**

Material Examined

Bundikanus was unavailable for the phylogenomic analysis, the mtDNA dataset contains 19 spp. (Fig. 5A, Supp Figs. 1 and 2 [online only]).

Diversity and Distribution

Bundikanus **gen. nov.** was represented in the dataset by 19 species and is known only from New Guinea. We identified among earlier described *Metriorrhynchus* one *Bundikanus* species, *B. angustulus* (Waterhouse, 1879), **comb. nov.** from New Guinea. The holotype of this species deposited in the Natural History Museum London is a female. Therefore, we prefer to designate *B. styskalai* **sp. nov.** as the type species of the genus.

Etymology

We name the genus in honor of Marcus Bundikana (Degenumbu, Mt Wilhelm), who provided support during our stay in the Degenumbu village. Gender masculine.

Bundikanus styskalai Kusy, Motyka, Bilkova, and Bocak, sp. nov.

(Fig. 5C–I)

urn:lsid:zoobank.org:act:6EDFF5AF-D41F-4597-87BF-01B938585F8C

Type Material

HOLOTYPE: male, INDONESIA, West Papua, Aywasi, 142 km E of Sorong, 490 m, 1°13′S 132°28′E (Voucher code A03261, LMBC). PARATYPE: same data (A03001, LMBC).

Diagnosis

Presently, *B. styskalai* **sp. nov.** is one of two named species of *Bundikanus*. It belongs to a group of species with flabellate male antennae (Fig. 5E and N) and differs from other species in the uniformly dark-colored dorsum (Fig. 5D) and the shape of the phallus (Fig. 5G–I).

Male

Body small-sized, 6.1.0 mm long, relatively slender, whole body dark brown colored, ventral part of thorax, and partly legs light brown, scutellum black (Fig. 5C and D). Head small, hidden by pronotum, densely pubescent, with large, hemispherically prominent eyes (Fig. 5E),

eye diameter 1.07 times interocular distance. Antennae flat, flabellate, antennomere 2 very short, antennomeres 3–10 flabellate, lamella of antennomere 3 slightly longer than trunk, lamellae longest in middle antennomeres (Fig. 5E), apical antennomere slender, parallel-sided. Pronotum very slightly transverse, 1.11 times wider than long at midline, almost parallel-sided in posterior half, widest in frontal third, apical margin almost straight, frontal part shallowly punctuated, surface without apparent pubescence. Elytra 3.63 times longer than width at humeri, parallel-sided, with primary and secondary costae of similar strength, all primary costae developed in whole length of elytra except costa 3 that is slightly shorted, secondary costae apparent only at humeri and apex, elytral cells variable in shape, mostly rounded (as in Fig. 5J). Phallus basally slender, widest in apical third, widely rounded apically, with open ventral part, internal sac membranous, with two conspicuous thorns (Fig. 4G–I). Female. Similar in body size, body length 9.6 mm, antennae acutely serrate, with flat, broad antennomeres 3–9; females head differs in presence of robust antennal sockets and deep depression posteriorly of them. The female (paratype) with testaceous postero-lateral pronotal carinae and bottoms of lateral areolae.

Measurements

BL 6.1.0 mm, Ediam 0.45 mm, Edist 0.48 mm, PW 1.1.0 mm, PL 0.99, WH 1.46 mm, EL 5.3 mm.

Distribution

B. styskalai **sp. nov.** has only been reported from the type locality in Bird's Head Peninsula (New Guinea).

Etymology

The specific epithet '*styskalai*' is a patronym in honor of Jakub Stýskala (Olomouc, Czech Republic).

Yamarhynchus Kusy, Motyka, Bilkova, and Bocak, gen. nov.

(Figs. 6A–W and 7A–U)

urn:lsid:zoobank.org:act:86FDAFCC-6E74-45E8-A58C-FB7D598DD748

Type Species

Yamarhynchus sinopassensis **sp. nov.**

Diagnosis

Yamarhynchus **gen. nov**. is defined based on the slender phallus and the unique shape of the internal sac with a pair of thin rods at the base (Figs. 6 and 7). The rods are sometimes less conspicuous, but the phallus is always characteristically slender (Fig. 6U). All known species have bicolored elytra. The typical lycid pattern with a brightly colored humeral portion of elytra is present (Fig. 6Q). Still, some species form unique aposematic patterns with the reversed position of the bright part (Fig. 6C and J) or with a transverse black band in the middle of elytral length (Fig. 7A, H and O). Other characters cannot be used for the generic diagnosis: all species share the pronotum with seven areoles, four or nine costae in elytra (Figs. 6 and 7D), and the species can have serrate to flabellate male antennae (Figs. 6 and 7). Distantly related species are morphologically similar, putatively due to the convergent evolution of the aposematic signaling (Fig. 7). Description as *Yamarhynchus sinopassensis* **sp. nov.**

Material Examined

Yamarhynchus was unavailable for the phylogenomic analysis; the mtDNA analysis contained 25 spp. (Figs. 2C, 6B, Supp Figs. 1 and 2 [online only]). We have not identified any earlier described *Yamarhynchus* species in the dataset.

Diversity and Distribution

We delimited 25 species in the present dataset, all recorded from New Guinea (Fig. 6B).

Etymology

The generic name is a patronym in honor of Simon Yama (Sino Pass, Mt Wilhelm), and '*rhynchus*' refers to the earlier placement of the type species in *Metriorrhynchus*. Gender masculine.

Yamarhynchus sinopassensis Kusy, Motyka, Bilkova, and Bocak, sp. nov.

(Fig. 7O–U)

urn:lsid:zoobank.org:act:936677FC-85E7-456B-B1B3-D618C504CF00

Type Material

HOLOTYPE: male, PAPUA NEW GUINEA, Madang Prov., Sino Pass, Bundi area, 15-VII-2017, 5°46′S, 145°11′E, 2,050–2,080 m (voucher code A03828, LMBC). PARATYPES: male, female, PAPUA NEW GUINEA, Madang Prov., Bruno's Sawmill, 14-VII-2017, 5°45′S 145°09′E, 2,450 m (A03850, A03854, LMBC); 2 males, female spec., locality data as the holotype (A04136, A03825, A03830, LMBC).

Diagnosis

Currently, *Y. sinopassensis* **sp. nov.** is the only named species of *Yamarhynchus*. It belongs to a group of species with flabellate antennae (Fig. 7Q), and it is the only species with flabellate antennae and the yellow elytra with a black transverse band (Fig. 7O). The phallus of this species is relatively robust (Fig. 7S–U).

Male

Body medium-sized, 10.3 mm long, robust, with posteriorly widened elytra. Whole body black colored, with only humeral and apical elytral two-fifths of elytra brightly orange (Fig. 7O). Head small, hidden by pronotum, densely pubescent, with large, hemispherically prominent eyes, eye diameter equal to interocular distance. Antennae flat, serrate, antennomere 2 very short, antennomere 3 flabellate, lamella 3 about twice longer than trunk, antennomeres 4–10 with only slightly shorter lamellae, apical antennomere slender, parallel-sided (Fig. 7Q). Pronotum transverse, much broader basally, narrow frontally, 1.58 times wider than long at midline, apical margin slightly projected, lateral margins convex, frontal part shallowly punctuated, surface shortly pubescent (Fig. 7P). Elytra 3.60 times longer than width at humeri, widest in apical third, with primary and secondary costae of similar strength, all costae developed in whole length of elytra except costa 3 that is shortened by one-tenth of its length, elytral cells variable in shape, mostly rounded. Phallus slender, widest in apical quarter, widely rounded apically, with open ventral part, internal sac partly pigmented, with two inconspicuous thorns (Fig. 7S–U and G). Female. Antennae serrate.

Measurements

BL 10.3 mm, Ediam 0.35 mm, Edist 0.35 mm, PW 2.28 mm, PL 1.44, WH 2.5 mm, EL 9.0 mm PhL 1.90 mm.

Distribution
Y. sinopasensis sp. nov. has only been reported from the Mt. Wilhelm area, Madang Province, New Guinea.

Etymology
The specific epithet '*sinopassensis*' refers to the type locality, the Sino Pass village in the Mt Wilhelm area plus the Latin suffix '*-ensis*.'

Remark
Y. sinopassensis **sp. nov.** is one of several similarly colored and sympatrically occurring species of *Yamarhynchus*. The males can be reliably identified by the shape of male antennae, genitalia, and sometimes also by the differences in the structure of elytral costae. The females of *Yamarhynchus* are very similar and often cannot be assigned to conspecific females without DNA sequencing.

Bekorhynchus Kusy, Motyka, Bilkova, and Bocak, gen. nov.

(Fig. 8A–O)

urn:lsid:zoobank.org:act:1FB41FB8-9283-4541-A36E-2C1042D916B1

Type Species
Metriorrhynchus maculithorax Kleine, 1926a

Diagnosis
Bekorhynchus **gen. nov**. is characterized by slender, only slightly widened phallus and the internal sac with a large, c-shaped structure, often accompanied by a group of basal densely packed thorns (Fig. 8G, H, M–O). There are no clear diagnostic characters available in the external morphology. All species share serrate antennae in both sexes, a complete set of pronotal carinae, four primary and five secondary elytral costae. They are mid- to large-bodied, often brightly colored, and integrated into local mimetic rings; the ventral part of the body often has metallic shine, and sometimes also dark-colored pronotum is metallically shining (Fig. 8J).

Body small- to medium-sized, 6–14 mm, slender, parallel-sided (Fig. 8C and I); most species brightly colored at least in humeral part of elytra. Head small, partly hidden by pronotum (Fig. 8D and J), most species with short, stout rostrum. Labrum about as long as wide, simply rounded frontally. Mandibles small, slightly curved, without teeth. Antennae serrate in both sexes (Fig. 8E and K). Both palpi with parallel-sided to slightly securiform apical palpomeres. Pronotum usually wider than long, with seven distinct areoles (Fig. 8D and J). Elytra parallel-sided, 3.4–4.2 times longer than width at humeri. Each elytron with four primary costae and five secondary longitudinal costae, which differ slightly in robustness (Fig. 8F and L). Transverse costae dense, elytral areolae rounded to transverse. Ventral part of body regularly with metallic blue shine. Male genitalia with c-shaped phallobasal membrane and batch of strong setae in most species (Fig. 8G, H, M–O).

Material
The phylogenomic analysis contained one species (voucher code R18010), and the mtDNA analysis 28 spp. of *Bekorhynchus* (Fig. 8, Supp Figs. 1 and 2 [online only]).

Diversity and Distribution
Bekorhynchus is a species-rich genus. We identified 28 species in the mtDNA dataset and transferred 17 species from *Metriorrhynchus*, including the type species *M. maculithorax* Kleine, 1926a identified in the analyzed material. *Bekorhynchus* occurs across New Guinea and the Moluccas. The following species are transferred from *Metriorrhynchus* to *Bekorhynchus*: *B. amplikefalus* (Kazantsev, 2015), **comb. nov.** – New Guinea; *B. brunneoflavus* (Kazantsev, 2015), **comb. nov.** – New Guinea; *B. chamaeleon* (Kazantsev, 2015), **comb. nov.** – New Guinea; *B. chimaera* (Kazantsev 2015), **comb. nov.** – New Guinea; *B. formosus* (Kleine, 1926a), **comb. nov.** – New Guinea; *B. immersus* (Waterhouse, 1879), **comb. nov.** – New Guinea; *B. lateanticus* (Kazantsev, 2015), **comb. nov.** – New Guinea; *B. maculithorax* (Kleine, 1926a), **comb. nov.** – New Guinea; *B. pallidipes* (Kazantsev, 2015), **comb. nov.** – New Guinea; *B. platypus* (Kazantsev, 2015), **comb. nov.** – New Guinea; *B. stenus* (Kazantsev, 2015), **comb. nov.** – New Guinea; *B. telnovi* (Kazantsev, 2015), **comb. nov.** – New Guinea; *B. turbinatus* (Kazantsev, 2015), **comb. nov.** – New Guinea; *B. weigeli* (Kazantsev, 2015), **comb. nov.** – New Guinea.

Etymology
The generic name is a patronym in honor of John Beko, the lecturer at the Papua New Guinea University of Technology, Forestry College in Bulolo, and '*rhynchus*' refers to the earlier placement of the type species in *Metriorrhynchus*. Gender masculine.

Remarks
Bekorhynchus is a sister to the *Sundarhynchus* + *Metriorrhynchus* clade. As the latter two genera are morphologically different (Figs. 9 and 10), we describe *Bekorhynchus* as a separate genus. Alternatively, widely defined *Metriorrhynchus* with three subgenera could be defined, but we prefer to give all three clades the genus rank as they are robustly supported in the molecular topology and by the morphology of male genitalia. The three genera now contain > 100 species, potentially increasing if we collect samples in other localities.

Sundarhynchus Kusy, Motyka, Bilkova, and Bocak, gen. nov.

(Fig. 9A–V)

urn:lsid:zoobank.org:act:5E35BE79-1852-4AE3-8CD6-47A169DC7F85

Type species. *Metriorrhynchus mindanaoensis* Bocak, Matsuda et Yagi, 2006.

Diagnosis
The hook-like to spirally coiled internal sac defines *Sundarhynchus* **gen. nov.** (Fig. 9E–G, J, K, P, U, and V). All species have fully developed primary and secondary elytral costae, seven pronotal areoles, and the robust phallus, broadest in the middle part or apical third, is ventrally opened and has at least partly sclerotized internal sac. There are three principal types of the internal sac in *Sundarhynchus*: simple hook (Fig. 9J and K), strait rod-like part attached to the basal hook (Fig. 9E–G), and the spirally coiled internal sac (Fig. 9P, U, and V).

Male
Body small- to medium-sized, 6–19 mm long parallel-sided (Fig. 9C and Q); most species brightly colored at least in humeral part of elytra. Head small, partly hidden by pronotum (Fig. 9D, H, and R), most species with short, stout rostrum. Labrum about as long as wide, simply rounded frontally. Mandibles small, slightly curved, without teeth. Antennae serrate in both sexes (Fig. 9I, N,

and S). Maxillary palpi 4-segmented, labial palpi 3-segmented, apical palpomeres parallel-sided to slightly securiform in both palpi. Pronotum usually wider than long, with seven distinct areoles, sometimes ridges in middle of pronotal disc inconspicuous (Fig. 9D, H, M, and R). Elytra parallel-sided, 3.6–4.9 times longer than width at humeri. Each elytron with four primary costae and five secondary longitudinal costae, which differ slightly in robustness (Fig. 9O and T). Transverse costae dense, elytral areolae strongly transverse in most species. Ventral part of body regularly with metallic blue shine. Male genitalia with sclerotized phallobasal membrane, strong straight phallus widened at middle or apical part, internal sac sclerotized, spirally coiled (Fig. 9).

Material Examined

The phylogenomic analysis used the whole genome sequence and the transcriptome of *S. mindanaoensis*; the mtDNA dataset contained 25 spp.

Diversity and Distribution

Sundarhynchus is a genus with an extensive range covering Australian and Oriental regions. Unlike other genera, the identified ancestral region of the genus covers the combined area of the Wallacea and Papua, which means the northernmost margin of the Australian craton and the islands north of it. Only one species occurs in continental Australia, and more species occur in the Wallacea than in New Guinea. Along with *Cautiromimus* Pic, 1926 and *Microtrichalus*, this genus' range covers the areas west and east of Lydekker's, Weber's, and Wallace's lines (Bocak and Matsuda 1998; Bocak et al. 2006, 2019; Bocak 2007). *Sundarhynchus* is the only representative of the porrostomines in continental Southeast Asia.

Altogether 25 spp. were identified in the mtDNA dataset, some of them undescribed. We estimate the total diversity to be ~40 species. The twenty-six species are transferred from *Metriorrhynchus* to *Sundarhynchus*: *S. boettcheri* (Kleine, 1926b), **comb. nov.** – Philippines; *S. cribripennis* (Waterhouse, 1879), **comb. nov.** – Moluccas; *S. croceus* (Kleine, 1926b), **comb. nov.** – Philippines; *S. doleschali* (Redtenbacher, 1867), **comb. nov.** – Moluccas; *S. elongaticollis* (Pic, 1925), **comb. nov.** – Philippines: Mindanao; *S. flavoabdominalis* (Kleine, 1926a), **comb. nov.** – Moluccas: Bacan; *S. forcipatus* (Kleine, 1926b) – **comb. nov.** – Philippines; *S. inaequalis* (Fabricius, 1801), **comb. nov.** – Sumatra, Borneo, Malaya; *S. intricatus* (Kleine, 1928b), **comb. nov.** – Moluccas: Bacan; *S. isarogensis* (Bocak, Matsuda et Yagi, 2006), **comb. nov.** – Philippines; *S. laosensis* (Bocak, 2007), **comb. nov.** – Laos; *S. lineatus* (Kirsch, 1875), **comb. nov.** – Sumatra, Borneo, Malaya; *S. lobatus* (Bocak and Matsuda, 1998), **comb. nov.** – Sulawesi; *S. menieri* (Bocak, Matsuda et Yagi, 2006), **comb. nov.** – Philippines; *S. mindanaoensis* (Bocak, Matsuda et Yagi, 2006), **comb. nov.** – Philippines; *S. moluccanus* (Kleine, 1926a), **comb. nov.** – Moluccas: Bacan; *S. newbataanensis* (Bocak, Matsuda et Yagi, 2006), **comb. nov.** – Philippines; *S. ochii* (Bocak, Matsuda et Yagi, 2006), **comb. nov.** – Philippines; *S. palawanensis* (Bocak, Matsuda et Yagi, 2006), **comb. nov.** – Philippines; *S. philippinensis* (Waterhouse, 1879), **comb. nov.** – Philippines; *S. sericans* (Waterhouse, 1879), **comb. nov.** – Indo-Burma; *S. sericeus* (Waterhouse, 1879), **comb. nov.** – Java; *S. taoi* (Bocak and Matsuda, 1998), **comb. nov.** – Sulawesi; *S. thoracicus* (Fabricius, 1801), **comb. nov.** – Sulawesi, Moluccas; *S. yoshioi* (Bocak, Matsuda et Yagi, 2006), **comb. nov.** – Sulawesi. Note. *M. longissimus* (Pic, 1922) is provisionally transferred to *Sundarhynchus* too. The species has a membranous internal sac and absent secondary elytral costae, unlike typical *Sundarhynchus* but differs even more from *Metriorrhynchus* s. str. The species was not available for molecular study.

Etymology

The generic name is derived from the word 'Sundas,' associating the new genus with the Greater Sundas, and '*rhynchus*' refers to the earlier placement of the type species in *Metriorrhynchus*. Gender masculine.

Remark

The only Australian species in the analysis was inferred as a sister to the New Guinean subclade (3 spp.; Fig. 9, Supp Figs. 1 and 2 [online only]). Although the species was regularly found in that position (Sklenarova et al. 2013, Figs. 1 and 2 [online only] in the present study), it differs from other *Sundarhynchus* in the shape of male genitalia (Fig. 9J and K) that resemble those of *Bekorhynchus* (Fig. 8). We keep the species in *Sundarhynchus* clade till more data are available.

Metriorrhynchus Gemminger and Harold, 1869

(Fig. 10A–V)

Metriorrhynchus Gemminger et Harold,1869: 1629.

Type species. *Lycus parallelus* Guérin-Méneville, 1835; by subsequent designation.

=*Metriorhynchus* Guérin-Méneville, 1838 (Type species *Lycus parallelus* Guérin-Méneville, 1838; by subsequent designation).

=*Flabelloporrostoma* Pic, 1923: 35, hors-texte (Type species *Porrostoma mirabilis* Pic, 1923; by monotypy); as the subgenus of *Porrostoma*; Bocak 2002: 340.

=*Dilolycus* Kleine, 1926a: 186 (Type species *D. lamellatus* Kleine, 1926a; by original designation); Bocak 2002: 340.

Diagnosis

The redefined *Metriorrhynchus* represents a clade characterized by an exposed internal sac, ventrally strengthened by a sclerotized rod and a pair to a high number of lateral spines (Fig. 10G–I, N, O, T–V). Most *Metriorrhynchus* are large-bodied, often with a gibbous pronotum (Fig. 10C, J, and Q); their elytra have fully developed primary and secondary costae and dense transverse cells (Fig. 10F, M, and S). The male antennae are variable in shape, serrate to shortly flabellate (Fig. 10E and L).

Male

Body small- to medium-sized, 6–18 mm long, parallel-sided (Fig. 10C, J, and Q), variably colored, light yellow to black, elytra often bicolored. Head small, partly hidden by pronotum (Fig. 10D, K, P, and R), with short to moderately long rostrum (Fig. 10P). Labrum about as long as wide, simply rounded frontally. Mandibles small, slightly curved, without teeth. Antennae slightly serrate in both sexes (Fig. 10E, L, and Q). Both palpi with parallel-sided to slightly securiform apical palpomeres. Pronotum usually wider than long, only slightly wider at base than at frontal margin, with seven distinct areoles (Fig. 10D, K, P, and R). Elytra parallel-sided, 3.6–4.5 times longer than the width at humeri. Each elytron with four primary and five secondary longitudinal costae, all primary costae similar in strength (Fig. 10F, M, and S). Transverse costae dense, elytral areolae strongly transverse in most species (Fig. 10F, M, and S). Ventral part of body usually with metallic blue shine. Male genitalia with partly sclerotized phallobasal membrane, strong straight phallus only slightly widened at apex, internal sac with slender ventral rod and lateral thorns sometimes present in most of the internal sac length (Fig. 10G–I, N, O, T–V).

Material Examined
Phylogenomic analysis: 1 spec., voucher code R18013, mtDNA analysis: 50 spp.

Diversity and Distribution
Altogether 194 spp. have been assigned to the genus, but many of them, especially from Australia, do not belong there. The name *Metriorrhynchus* was conventionally designating Metriorrhynchina without a long rostrum. Therefore, the distribution is defined based on the present dataset. *Metriorrhynchus* is endemic to the Australian region and occurs in continental Australia, New Guinea, Biak, Solomon Isl. and the Moluccas. Most species occur in New Guinea, which is hypothesized as the ancestral area (Fig. 2C). The genitalia of the following seven species confirm the placement in *Metriorrhynchus*: *M. lorentzi* Kleine, 1926a—New Guinea, New Britain, Moluccas: Halmahera; *M. olivieri* Kazantsev, 2015—New Guinea; *M. parallelus* Guérin-Méneville, 1835—New Guinea; *M. puncticollis* Waterhouse, 1879—New Guinea; *M. stenothorax* Kazantsev, 2015—New Guinea; *M. tuzovi* Kazantsev, 2015—New Guinea; *M. wallacei* Kleine, 1933b –Mysol isl. Many species were described based on females, are unrelated to the porrostomine genera, or the types were inaccessible, and their relationships could not be considered with available information.

Isuarhynchus Kusy, Motyka, Bilkova, and Bocak, gen. nov.

(Fig. 11A–AB)

urn:lsid:zoobank.org:act:A8AD60ED-37B8-4D3C-9C4D-4AC4EDC530C0

Type Species
Metriorrhynchus impudens Kleine, 1926a.

Diagnosis
The clade representing *Isuarhynchus* is characterized by the shape is a sclerite in the internal sac of the male genitalia. The sclerite has a form of a letter 'G' in a constricted position and can be as deep as two-thirds of the phallic length or very small, located in the apical part of the phallus (Fig. 11E–G, N, O, X–AB).

Male
Body small- to medium-sized, 6–18 mm long, parallel-sided (Fig. 11C, K, P, and V), variably colored, light yellow to black, elytra seldom bicolored. Head small, partly hidden by pronotum (Fig. 11D, L, Q, and W), without rostrum. Labrum about as long as wide, simply rounded frontally. Mandibles small, slightly curved, without teeth. Antennae parallel-sided, serrate in both sexes (Fig. 11J, M, R, and U).

Both palpi with apical parallel-sided to slightly securiform palpomeres. Pronotum usually wider than long, only slightly wider at base than at frontal margin, with seven distinct areoles (Fig. 11D, L, Q, and W). Elytra parallel-sided, 3.4–4.4 times longer than the width at humeri. Each elytron with four primary and five secondary longitudinal costae, all primary costae similar in strength (Fig. 9O and T). Transverse costae dense, elytral areolae slightly transverse in most species (Fig. 9H, J, T, and S). Ventral part of body without metallic blue shine. Male genitalia with membranous phallobasal membrane, strong straight phallus sometimes conspicuously widened at apex; internal sac partly sclerotized, G-shaped in most species (Fig. 11E–G, N, O, X–AB).

Material Examined
The phylogenomic analysis contained two species with voucher codes R18018, R18020; mtDNA analysis identified 27 spp.

Diversity and Distribution
Altogether, 27 species of *Isuarhynchus* were recovered by molecular analysis and morphological verification. We transfer the following species from *Porrostoma to Isuarhynchus*: *Isuarhynchus angustifasciatus* (Kazantsev, 2015), **comb. nov.** – New Guinea; *I. echidna* (Kazantsev, 2015), **comb. nov.** – New Guinea; *I. fasciapiculatus* (Kazantsev 2015), **comb. nov.** – New Guinea; *I. halmaheraensis* (Kazantsev, 2015), **comb. nov.** – Halmahera; *I. tigroides* (Kazantsev, 2015), **comb. nov.** – New Guinea; *I. flavus* (Kleine, 1926a), **comb. nov.** (=*Metriorrhynchus flavus* Kleine, 1926a; *Porrostoma flavum*: Kazantsev 2015). Further, *Isuarhynchus ater* (Waterhouse, 1879), **comb. nov.** – New Guinea, Aru Isl.; *I. caesareus* (Kleine, 1935), **comb. nov.** – Solomon Isl.; *I. impudens* (Kleine, 1926a), **comb. nov.** – New Guinea are transferred from *Metriorrhynchus*. *Isuarhynchus* occurs in Australia, New Guinea, the Solomon Islands, and Moluccas (Fig. 11A and B). New Guinea is preferred as the ancestral region (Fig. 5C).

Etymology
The generic name is a patronym in honor of Brus Isua (Binatang Research Centre, Madang and Ohu village, Papua New Guinea), and '*rhynchus*' refers to the earlier placement of the type species in *Metriorrhynchus*. Gender masculine.

The *Porrostoma* Clade

***Porrostoma* Castelnau, 1838**

(Figs. 12A–V, 13A–M, and 14A–AB)

Porrostoma Castelnau, 1838: 26.

Type species. *Lycus rufipennis* Fabricius, 1801; by monotypy.

Invalid designation. *Porrostoma erythropterum* Erichson, 1842; C. O. Waterhouse, 1879: 44.

= *Stadenus* Waterhouse, 1879: 61, **syn. nov.**

Type species. *P. dichroum* Waterhouse, 1877: 86; by original designation.

= *Metriorrhynchoides* Kleine, 1926a: 118, **syn. nov.**

Type species. *M. helleri* Kleine, 1926a: 119; by original designation.

=*Oriomum* Bocak 1999a: 111, **syn. nov.**

Type species. *O. femoralis* Bocak, 1999a: 111; by original designation.

Diagnosis
All species currently placed in *Porrostoma* have similar male genitalia characterized by a relatively slender, parallel-sided phallus and a membranous, although sometimes pigmented internal sac without any spines or rods (Figs. 12G–I, M–O, T–V, 13E–G, L, M and 14H, I, N, O, T, U, Z–AB) and slender maxillary palpi, with a parallel-sided apical palpomere. Some species of *Porrostoma* have a long rostrum and sharp, straight pronotal carinae forming seven areoles (Figs. 12 and 13). Other species can be identified as *Porrostoma* only after the dissection of male genitalia. The external morphological variability includes the body shape (from the slender, parallel-sided body to large-bodied species with a wide, apical part of elytra, earlier placed in *Metriorrhynchoides*) and serrate to flabellate male antennae (Figs. 12E, L, R, 13C and 14F, L, R, X). The mandibles are minute in rostrate species but longer and curved in the species without a rostrum. Elytra always have

complete primary and secondary longitudinal costae; transverse costae are primarily regular, dense cells predominantly transverse in large-bodied species (Figs. 12 and 13).

Male

Body small- to medium-sized, 5–20 mm long, parallel-sided, slightly widened apically (Figs. 12 and 13) to extremely wide in apical third of elytra, most species brightly colored at least in the humeral part of elytra, some species with blue colored elytra, ventral part of body regularly with metallic blue shine. Head small, partly hidden by pronotum, most species with short, stout rostrum, some species with long, slender rostrum. Labrum about as long as wide, simply rounded frontally. Mandibles small, slightly curved, without teeth. Antennae serrate to flabellate in males, serrate females (Figs. 12 and 13). Maxillary palpi 4-segmented, labial palpi 3-segmented, apical palpomeres parallel-sided in both palpi. Pronotum usually wider than long, with seven distinct areoles, mostly delimited by sharp and straight ridges, sometimes lateral ridges absent, then fewer areoles present. Elytra parallel-sided to expanded apically, 3.0–4.5 times longer than width at humeri. Each elytron with four primary costae and five secondary longitudinal costae, which differ slightly in robustness, sometimes secondary costae interrupted; transverse costae dense in large-bodied species, with strongly transverse elytral areolae, small-bodied species with lower number of transverse costae. Male genitalia with sclerotized phallobasal membrane, phallus straight, mostly parallel-sided, sometimes very robust. Internal sac membranous, small, apically exposed. Female. Antennae without lamellae, slender to serrate.

Diversity and Distribution

Bocak et al. (2020) assigned only 26 species to the genus, and we added additional eleven species in the present study. Out mtDNA analyses identified much higher diversity and delimited 111 species in the dataset (Supp Figs. 1 and 2 [online only]). The Metriorrhynchina from the Australian continent were traditionally placed in *Porrostoma*, but the New Guinean species in *Metriorrhynchus* and *Metriorrhynchoides* (Kleine 1926a, 1933a). The range and species diversity are much higher after many New Guinean species are included in *Porrostoma* (Figs. 2C and 12B). The genus occurs in continental Australia, Tasmania, New Guinea, the Solomon Islands, and one introduced, *P. rufipennis* (F.), species from New Zealand (Figs. 12A and 14A).

The following eleven species are transferred from *Metriorrhynchus* to *Porrostoma*: *P. diffusimaculatus* (Kleine, 1928b), **comb. nov.** – Australia: Queensland, *P. explanatum* (Kleine, 1926a), **comb. nov.** – New Guinea, *P. franklinmuelleri* (Kleine, 1928b), **comb. nov.** – Australia: Queensland, *P. hackeri* (Kleine, 1928b), **comb. nov.** – Australia: Queensland, *P. lunatum* (Kleine, 1926a), **comb. nov.** – New Guinea, *P. nigricauda* Kleine, 1928b, **comb. nov.** – Australia: Queensland, *P. nigripes* (Macleay, 1872), **comb. nov.** – Australia, *P. occidentalis* (Blackburn, 1892), **comb. nov.** – Australia: West Australia, *P. ordinarium* (Lea, 1909), **comb. nov.** – Australia: Victoria, *P. paradoxa* (Blackburn, 1900), **comb. nov.** – Australia: Victoria, *P. uniforme* Waterhouse, 1877, **comb. nov.** – Australia: Queensland, *P. vittatum* (Blackburn, 1888), **comb. nov.** – Australia.

Material Examined

P. rhipidium was included in the phylogenomic analysis; McKenna et al. (2019) reported original data; we identified 111 spp. in the mtDNA analysis (Fig. 12A, Supp Figs. 1 and 2 [online only]).

Nomenclatural Remarks

Porrostoma was sometimes considered a junior synonym of *Metriorrhynchus* either due to their supposed similarity or as the replacement name (Kleine 1933a, Calder 1998). Bocak (1998) based the revised concepts of these genera on differences in the male genitalia and restored *Porrostoma* from synonymy. When *Porrostoma* was treated as a junior synonym of homonymous *Metriorhynchus* Guérin-Méneville 1838, all Australian *Metriorrhynchus* were transferred into *Porrostoma sensu* Calder (1998). The species originally placed in *Metriorhynchus* Gemminger et Harrold 1869 were returned to the genus by Bocak et al. (2020).

The Internal Structure of the *Porrostoma* Clade

Relationships among Australian and New Guinean *Porrostoma* remain dubious, but all analyses indicate, although with low support for the backbone, that *Porrostoma* in the traditional sense was a paraphyletic taxon (Kleine 1933a, Bocak et al. 2020). We recovered three or four major clades at the basal split (Supp Figs. 1 and 2 [online only]). If three clades are present, two deeper clades contain continental species, including the nominative *Porrostoma rufipennis* (Supp Fig. 1 [online only]). Recently described *P. viridum* Kazantsev, 2015 from New Guinea was found within this clade but with low bootstrap support (Fig. 12B, Fig. S1). The terminal clade is formed by > 60 New Guinea species and one or two species from the continent (Northern Queensland, Fig. 13B, Supp Fig. 2 [online only]). Alternatively, the New Guinea clade breaks in two: the first (9 spp. and one Australian species) recovered as the sister taxon to the remaining *Porrostoma*, and the second (56 spp. New Guinean and one Australian species, Supp Fig. 2 [online only]) in the terminal position as earlier. Two large clades of dominantly Australian species and the extensive New Guinean clade indicate an ancient split between faunas and rare dispersal events between the continent and adjacent islands north of it. Concerning the similarity of male genitalia, the uncertainty of subclade delimitation, and the absence of diagnostic traits, we define *Porrostoma sensu lato* including New Guinean species (*Metriorrhynchoides* and *Oriomum*) and continental *Stadenus*.

Synonymy and New Combinations

Porrostoma's only distinct diagnostic trait is the membranous, usually exposed internal sac. The external characters are highly variable (Figs. 12 and 13). Already, Bocak (2002) noted that morphology does not support the reciprocal monophyly of *Porrostoma* and *Metriorrhynchoides*. We found out that the New Guinean clade contains species closely related to *P. helleri* (the type species of *Metriorrhynchoides*) and that Australian species of *Porrostoma* represent two clades (Supp Figs. 1 and 2 [online only]). Therefore, we cannot retain *Metriorrhynchoides* as a valid name unless the genus rank is given to all deeper subclades defined based on the molecular analysis but unsupported by morphology (Supp Figs. 1 and 2 [online only]). Hence, we propose synonymizing *Metriorrhynchoides* Kleine, 1926a to *Porrostoma* Castelnau, 1838, and the following species are newly combined with *Porrostoma*: *P. eminens* Kleine, 1926a, **comb. nov.**, *P. flavofasciatus* Kleine, 1926a, **comb. nov.**, *P. helleri* Kleine, 1926a, **comb. nov.**, and *P. pulcher* Kleine, 1926a, **comb. nov.**; all species occur in New Guinea.

Stadenus has been defined based on reduced pronotal carinae. The species with similar morphology were found in the *Porrostoma* clade. Other characters of *Stadenus* are shared with *Porrostoma* as delimited here. The following species are transferred from *Stadenus* to *Porrostoma*: *P. appositum* (Kleine, 1933b), **comb. nov.**, *P. atricornis* (Lea, 1909), **comb. nov.**, *P. dichroum* Waterhouse, 1877, **comb. nov.**,

P. inquilinum Waterhouse, 1877, **comb. nov.**, *P. nigrovittatum* (Lea, 1909), **comb. nov.**, *P. obscuripennis* (Lea, 1909), **comb. nov.**, *P. puncticollis* (Kleine, 1933b), **comb. nov.**, *P. triareolatum* (Lea, 1909), **comb. nov.**; all from Australia and Tasmania (Bocak et al. 2020).

Oriomum was characterized by several unique traits not known from other Metriorrhynchini: opisthognathous head with extremely long rostrum reaching mesothoracic coxae, parallel-sided antennomeres 1–5, slightly serrate antennomere 6, and short, triangular antennomeres 7–9; elytra with entire but apically weak primary costa 1, secondary costae distinct only at humeri, posterior margins of fore and mid femora armed by sharp spines, and hind legs with acutely projected triangular trochanters (Bocak 1999a). Further, *Oriomum* has seven areoles in the pronotum, the slender, parallel-sided phallus, with an exposed, densely pubescent apical part of the internal sac. Based on male genitalia, *Oriomum* is a member of the porrostomine clade. Still, despite being well-defined by apomorphies, the shared traits suggest that it is a modified *Porrostoma,* and its genus rank is unjustified. Therefore, *Oriomum* is synonymized with *Porrostoma* and the new combination *P. femoralis* (Bocak, 1999a), **comb. nov.** is proposed.

Discussion

Sampling and Data Completeness

As with other tropical insects, the actual diversity of the porrostomines has not yet been recognized (Novotny et al. 2002, Mora 2011). Recent estimations of beetle diversity came to ~1.5 million species (Hamilton et al. 2010, Stork et al. 2015). Our porrostomine dataset contains 352 species compared to 233 named species (Bocak et al. 2020), and at least some belong to other Metriorrhynchina clades (e.g., *Metriorrhynchus dilutus* Waterhouse, 1879 is a cladophorine). Additionally, we supposedly sequenced less than half of described species mainly due to the sparse sampling of lowlands and especially the type localities close to ports. As a result, some ~250 species in the present dataset have not yet been described, and the actual diversity of the porrostomines is at least twice that expected. The proportion of undescribed species is lower than the mean estimate for beetles (Stork et al. 2015). Still, many regions remain undersampled, and a small team conducted the project for only ~20 yr. Regardless of such limitations, this dataset is the most comprehensive one available to date. Therefore, we assume that generic diagnoses reflect the adequate evaluation of morphological disparity (Figs. 3–14).

Robustness of the Analysis

The backbone nodes are poorly supported if topologies are inferred from a few loci (Bocak et al. 2014, Young and Gillung 2020). As the monophyly of *Metriorrhynchus* is the principal question of the porrostomine classification, we inferred the deep relationships from 4,200 orthologs that provide a much higher volume of data (Fig. 2A, Letsch et al. 2021, Motyka et al. 2021a, Talavera et al. 2021). The phylogenomic results confirm the paraphyly of *Metriorrhynchus* in the traditional sense and show that *Porrostoma* is an extensive terminal clade (Fig. 2B and C, Supp Figs. 1 and 2 [online only]; Motyka et al. 2021a). Similar relationships were recovered earlier (Sklenarova et al. 2013, 2014) but the data provided low support, and no morphological evidence could be found for the clades. Hence, the relationships among *Metriorrhynchus*, *Sundarhynchus*, *Bekorhynchus*, *Isuarhynchus*, and *Porrostoma* are based on phylogenomics, and the positions of further five genera (*Maraiakoreus, Kuarhynchus*, *Riedelrhynchus*, *Bundikanus*, and *Yamarhynchus*) are recovered based on mtDNA data only (Fig. 2, Supp Figs. 1 and 2 [online only]). We delimit genera that are reciprocally monophyletic and simultaneously supported by at least some morphological trait. If a DNA-based relationship obtained low support and we have not found any morphological evidence for the placement, we refrain from the formal description till more data is available (see 'unnamed clade' in Supp Figs. 1 and 2 [online only]).

Molecular Phylogeny, Morphology, and Classification

This study comes two decades after the comprehensive generic revision of Metriorrhynchini that was based on the detailed study of all type species (Bocak 2002) and after numerous alpha-taxonomic and molecular studies that revealed the species diversity and morphological disparity of the metriorrhynchines (e.g., Bocak et al. 2006, Bocak and Yagi 2010, Sklenarova et al. 2014, Kazantsev 2015, 2016). Informed by the molecular phylogeny, we focus on the delimitation of porrostomine genera, but, for several reasons, we postpone the alpha-taxonomic revisions. First, other metriorrhynchine groups have not yet been revised. Therefore, many valid genera are poly- or paraphyletic (Motyka et al. 2021a), and if some species are transferred into them, their placement would be only tentative. Further, the alpha-taxonomic revision of >200 porrostomine types is beyond this project's scope. Although we have studied all European collections housing primary types of the Metriorrhynchina, we transfer species only within the porrostomine clade and propose new species only if necessary for the definition of new genera. Many species of Metriorrhynchina have been described based on single female specimens that do not possess critical diagnostic traits, and the entire group appears to be in a somewhat nebulous state of alpha-taxonomy. The revisions will also have to solve the inaccessibility of some types and the low information content of their original descriptions.

Although the changes to the higher-level classification primarily rely upon molecular analyses, we do not a priori reject morphological data as evidence of relationships. Merely, we critically consider the congruence between morphological and molecular phylogenetic signals and test if DNA-based clades correspond with the groups sharing morphological traits. The earlier discussion on the role of molecular and morphological approaches in systematics oscillated between acceptance and refusal (Tautz et al. 2003, Will et al. 2005, Riedel et al. 2013, Srivathsan et al. 2019, Sharkey et al. 2021). At the level of generic classification, integrating morphology and molecular phylogeny can rapidly consolidate the classification of a hyperdiverse tropical beetle lineage. Additionally, we can evaluate molecular and morphological data informativeness with independent signal sources.

The earlier studies suggested alternative classifications of the porrostomines. *Metriorrhynchus* was synonymized to *Porrostoma* (Kleine 1933a, Calder 1998), or both genera were accepted as valid (Bocak 1998, 2002; Kazantsev 2015). If *Metriorrhynchus* is synonymized, most species of the porrostomine clade would be placed in *Porrostoma* containing over 300 spp. and more in the future. Widely delimited *Porrostoma* would be morphologically very heterogeneous and very difficult to define even with a large set of various combinations of characters (Figs. 4–14). Further, it would be the largest genus in the subfamily, with an evolutionary history already starting in the late Cretaceous (Sklenarova et al. 2013, Bocak et al. 2020). Therefore, we prefer the acceptance of *Metriorrhynchus* as a separate genus (Bocak 1998, 2002; Kazantsev 2015). Paraphyletic taxa cannot be accepted. Consequently, we define several genera using molecular phylogeny and a subjective degree of morphological

uniformity for the species earlier assigned in *Metriorrhynchus* (Fig. 2B; Taxonomy section). The morphological diagnoses often depend on genital morphology as external morphology is less informative. The proposed generic classification splits the diversity into manageable units, each genus with 2–111 putative species (mean = 32, median = 27; Fig. 2B).

Our analyses recovered intrageneric disparity in several traditionally used diagnostic characters. The high similarity of phylogenetically distant species is possibly caused by convergent morphological evolution in mimetic rings that includes the body shape and size with corresponding modifications of pronotal ridges and elytral costae (Bocek et al. 2019, Motyka et al. 2021a). Other traits might be affected by the shared biology. For example, the shape of the male antennae often delimited lycid genera (Kleine 1926a), but recently split species have serrate or flabellate male antennae (e.g., (*Maraiakoreus* Fig. 3E and M; *Kuarhynchus* Fig. 5E and L; *Yamarhynchus* Fig. 6L and S; *Porrostoma* Fig. 14E and L). The antennae bear sensillae involved in pheromonal communication, and the shape of the antennae reflects their function (Bohacz et al. 2020). Similarly, some *Porrostoma* and all *Maraiakoreus* independently lost some pronotal carinae (Fig. 3). The elytral costae may also provide a misleading phylogenetic signal. Most Metriorrhynchina have nine costae (four strong primary and five much weaker secondary costae, Bocak 2002), but closely related species often display distinct forms. The primary elytral costa 3 can be weak or shortened, as in *Kuarhynchus* (Fig. 4). Secondary costae can be absent in the whole length of the elytra, in the middle part only, or the entire elytron except humeri (Fig. 7D and K). The absent secondary costae in the middle part have defined the polyphyletic *Procautires* Pic, 1925b (Bocak et al. 2020, Motyka et al. 2021a). The analogous reduction was found in some *Kuarhynchus* and *Bundikanus* (Figs. 4H, I and 5J). To sum up, different structures can evolve in closely related species more often than expected.

The 19th and early 20th century authors did not dissect male genitalia and diagnosed the genera with overreliance on these highly variable external characters (Guérin-Méneville 1838, Waterhouse 1879, Lea 1909, Pic 1923, etc.). Kleine (1926a, b, 1935a, b, etc.) illustrated the male genitalia. Still, Still, at the time, so few species were known that it was impossible to generalize the differences and define the genera using the genital morphology. Similarly, the revision of the generic classification of Metriorrhynchini and alpha-taxonomic studies were not detailed enough to justify the substantial modifications in generic limits and diagnoses (Bocak 2002, Kazantsev 2015). Only the large-scale mtDNA sequencing and the phylogenomic data provide additional information on relationships that, compared with the morphology, enabled the reclassification of the porrostomine genera.

Distribution and Species Diversity

The present study produces a high volume of geospatially linked data for ~350 porrostomine net-winged beetle species (Supp Figs. 1 and 2 [online only]) that are poor dispersers due to their limited flight ability, poor desiccation resistance, and low salinewater tolerance (Bocak and Matsuda 2003, Masek et al. 2018). The porrostomine genera are endemic to the Australian region, incl. Wallacea. Only *Sundarhynchus* presumably colonized the Philippines, Greater Sundas, and the eastern part of Indo-Burma (Fig. 2C). *Maraiakoreus*, seven genera belonging to the Metriorrhynchus grade, and one or two subclades of *Porrostoma* are predominantly New Guinean. Considering a phylogenetic context (Fig. 2A; Kusy et al. 2019, Motyka et al. 2021a) and the geographic ranges of all deep porrostomine lineages (Fig. 2C, Supp Fig. 3 [online only]), the identification of the northern part of the Australian region as the cradle of porrostomine generic diversity is robustly supported. The high diversity of the New Guinean porrostomines stands in contrast to the recent origin of New Guinea that evolved to the present form in the last 15 million yr (Hall 2011, Baldwin et al. 2012, Toussaint et al. 2014). The southern part of the island represents the northern margin of the Australian tectonic plate. The Central Cordillera and northern lowlands contain uplifted forearc of oceanic islands, accreted continental fragments, and microplates. Some northern terrains were possibly subaerial in the Eocene to Oligocene and the earliest lineages perhaps inhabited these Arafura High landmasses (Pigram and Symonds 1991, Quarles van Ufford and Cloos 2005). The mountain ranges where many endemic species were identified are only ~8 million yr old. Therefore, one of the prerequisites for the evolution of high diversity, the long-term ecological and tectonic stability (Carnaval et al. 2005), is missing.

Nevertheless, New Guinea and its mountain ranges house all deeply split clades and high species diversity (Supp Figs. 1 and 2 [online only]; Motyka et al. 2021a). Deep rooting and the extent of New Guinean clades suggest that they are older than the present-day terrane, but the high mountain fauna probably recently evolved in situ as no similar mountain habitats were close enough to serve as a source area (Merckx et al. 2015, Holt et al. 2013). The origin of Metriorrhynchina has been dated to the Late Cretaceous (Sklenarova et al. 2013). Therefore, we assume that the porrostomines originally inhabited the low-lying northern margin of the Australian craton and oceanic islands north of Australia that putatively persisted since the Early Tertiary (Hall 2011, Toussaint et al. 2014).

Our field research was time-limited, and we could not visit all mountain ranges and islands that putatively house an endemic fauna (Riedel et al. 2010, Toussaint et al. 2014, Merckx et al. 2015, Mastretta-Yanes et al. 2018). Nevertheless, even incomplete data show a very high turnover at the species level. The analysis of ~1,030 terminals shows that less than 30% of putative species have been collected in two or more localities with >50 km distance. Although porrostomines are flying, they are seldom widely distributed. Our data show that some species with supposedly large ranges can be the complexes of genetically differentiated lineages deserving the species status (e.g., the *Porrostoma rhipidium* complex, Supp Figs. 1 and 2 [online only]). Small ranges make the porrostomines vulnerable to extinction (Myers et al. 2000, Pimm and Raven 2000, Dirzo and Raven 2003). Although the earlier predicted high extinction rates were occasionally questioned (Pereira et al. 2010, Stork et al. 2015), the identified endemism of most porrostomines calls for small-scale conservation management.

The porrostomine clade dominates the Australian net-winged beetle fauna (125 of 196 named species, 48 analyzed). Nevertheless, only three out of ten porrostomine genera, *Porrostoma*, *Sundarhynchus*, and *Metriorrhynchus* (Fig. 2A, Supp Figs. 1 and 2 [online only]), are known from Australia, and of them, only two deep branches of *Porrostoma* are extensive clades formed by Australian species (17 and 24 spp.; Supp Figs. 1 and 2 [online only]). Besides these, *Metriorrhynchus* is represented by a single clade of three species, and a single species marks the first split of the *Sundarhynchus* clade (Supp Figs. 1 and 2 [online only]). The narrow and shallow Torres Strait is the only barrier between New Guinea and Australia (Sun et al. 2000, Hall 2011). New Guinean ancestral terranes were either a part or close to the Australian craton for the most time (Hall 2011, Toussaint et al. 2014). Therefore, it is intriguing that the Australian fauna is phylogenetically less diverse, and we had

not identified any common species for these two regions when we simultaneously considered morphology and genetic differentiation. Metriorrhynchina prefers perhumid forests (Bocak and Matsuda 2003). Therefore, the seasonally dry open woodlands supposedly covering the exposed continental shelf were unfavorable for them, and specific ecological requirements substantially decreased the dispersal of the porrostomines to the south (Whittaker and Fernandes-Palacios 2007). As a result, extant Australian species are mostly endemics of the northeastern mountains affected by monsoons (89 spp.). We have not found any indication of the early diversification of the porrostomines in the higher southern latitudes of the Australian continent (Mcphail 2007). Merely, the southern species are nested in the clades dominated by the species from the north. The only New Zealand species, *P. rufipennis* (Fabricius, 1801), is confirmed as recently introduced (Kuschel 1990). Unfortunately, our dataset does not contain the conspecific individuals from the continent, only a closely related species from Western Australia (Supp Figs. 1 and 2 [online only]).

Sundarhynchus dominates the Moluccan and Sulawesian porrostomine faunas (Fig. 2C). Although putatively of Australian/New Guinean origin, this genus is uncommon in Australia (1 sp.) and New Guinea (3 spp.). The species that colonized the Greater Sundas and continental Asia are nested in the Sulawesi grade (Supp Figs. 1 and 2 [online only]; Bocak et al. 2006, Bocak and Yagi 2010). This pattern indicates an ancestral area on the oceanic islands in the contact zone between the Australian and Philippine plates that drifted westward due to tectonic processes. These terranes include Halmahera, the northern peninsula of Sulawesi, and the southern Philippines except the Zamboanga peninsula (Hall 2011). The route was also hypothesized for other beetles (Sklenarova et al. 2013, Toussaint et al. 2015, Bocek and Bocak 2019, Letsch et al. 2020). Only a few species from the other three genera have been recorded west of Lydekker's line (*Bekorhynchus*, *Kuarhynchus*, and *Isuarhynchus*), and they did not colonize the islands west of Weber's line (Figs. 2C, 3–14). Overall, spatial patterns indicate that Lydekker's, Weber's, and Wallace's lines are permeable, but the porrostomines rarely cross a large sea expanse. The low dispersal capacity of lycids is common (Li et al. 2015, Masek et al. 2015, Kusy et al. 2021) and stands in contrast with findings on weevils (Toussaint et al. 2015, Letsch et al. 2020) or some dytiscids (Balke et al. 2009). Unlike lycids, these beetles are well sclerotized and possibly resistant to high salinity.

Conclusion

Taxonomic research on net-winged beetles copes with enormous species diversity and ambiguous delimitation of genera. Therefore, we test the relationships of porrostomine lineages with independent character systems to build the new phylogenetic classification. We show how incomplete is our inventorying of tropical beetles and how generic concepts that do not reflect relationships limit the taxonomic and evolutionary research (Tautz et al. 2003, Riedel et al. 2013, Stork et al. 2015). The study highlights the high portion of species, ~70%, placed in artificial taxa (Table 1). The redefined *Metriorrhynchus* remains the second largest genus of the porrostomines but shrinks to less than a quarter of the earlier extent. Now, *Porrostoma* is the largest Metriorrhynchina (111 analyzed species), but half of its diversity is newly reported from New Guinea, where almost all species have been reported as *Metriorrhynchus*. The novel generic concepts also affect hypotheses on centers of origin, generic ranges, and a different evolutionary scenario for the Australian continental fauna. Porrostomines are another amazing example of species radiation in the New Guinean mountains (Toussaint et al. 2014, Pujolar et al. 2022). These beetles also are an exciting model for studies on mimicry (Fig. 7; Motyka et al. 2021b) and phylogeography (Fig. 2C, Bocak et al. 2006, Sklenarova et al. 2013, Bocek and Bocak 2019). We assume that the compiled DNA database and updated classification provide the basis for more detailed future studies on the origin of tropical species diversity.

Supplementary Data

Supplementary data are available at *Insect Systematics and Diversity* online.
urn:lsid:zoobank.org:pub:45FAAD64-1ED5-444D-AE64-575F30129FB3

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Author Contributions

Conceptualization, L.B., D.K., M.M.; formal analyses, M.M. (Sanger data), D.K. (phylogenomic data); investigation, R.B., D.K., L.B, M.M.; writing, original draft preparation, L.B., M.M., D.K.; writing, review, and editing, L.B., M.M., D.K., R.B.; visualization, L.B., M.M., D.K.; funding acquisition, L.B., M.M., D.K. All authors have read and agreed to the published version of the manuscript. This research was funded by The Czech Science Foundation, grant numbers 18-14942S, 22-35327S, and private funds of the authors. D.K. was partly supported by the Internal Grants (PrF-2021-026, PrF-2022-024). The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Data Availability

The analyzed supermatrices are deposited in the Mendeley Data repository DOI: 10.17632/wyyktthv6.1.

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PART VII

Studies on neoteny in Coleoptera: phylogenomics, classification, and evolutionary interactions

Dominik Kusý

Sexually dimorphic characters and shared aposematic patterns mislead the morphology-based classification of the Lycini (Coleoptera: Lycidae).

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Sexually dimorphic characters and shared aposematic patterns mislead the morphology-based classification of the Lycini (Coleoptera: Lycidae)

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The Lycini (Elateroidea: Lycidae) contains > 400 species placed in four typologically based genera and numerous subgenera. We assembled a mito-ribosomal dataset representing ~100 species from the whole range and recovered a phylogeny rejecting *Lycus* and *Lycostomus* as polyphyletic assemblages. The male-specific wide elytra and elytral thorns are identified in unrelated *Neolycus* and *Lycus*. The morphological similarity based on sexual dimorphism and aposematic patterns defined terminal clades and misled the genus-rank classification. We delimit *Neolycus*, *Rhyncheros* reinst. name (= *Thoracocalon* syn. nov. = *Lyconotus* syn. nov.), *Lipernes Lycostomus*, *Haplolycus* and *Lycus*. *Demosis* and six subgenera of *Lycus* are synonymized with *Lycus*. *Celiasis* Laporte, 1840 is kept in the classification as a *nomen dubium* until any specimen is available. The deep lineages are known from the Americas and Asia. Africa was colonized by *Lycus* and *Haplolycus*. Each specific aposematic pattern occurs in a limited range, and the similar body shape and coloration evolved in unrelated sympatrically occurring lineages. High intraspecific polymorphism is putatively a result of the adaptation of various populations to local mimetic assemblages. Therefore, the delimitation of many phenotypically diverse species should be investigated.

ADDITIONAL KEYWORDS: ancestral areas – divergence dating – net-winged beetles – taxonomy – zoogeography.

INTRODUCTION

The Lycini is a species-rich net-winged beetle tribe distributed across all continents but Antarctica (Figs 1, 2; Masek *et al.*, 2018). These beetles are very common in the Afrotropical region (~320 species), and a further ~130 species have been described from the eastern Palaearctic, Oriental, Nearctic, northern Neotropical and western part of the Australian region (Kleine, 1933; Zoological Records Database; https://clarivate.libguides.com/webofscienceplatform/zr). Despite the availability of large museum collections and common occurrence in nature, no studies have examined their generic relationships, mainly owing to their extraordinary diversity and the chaotic and neglected taxonomy produced by earlier researchers (e.g. Laporte, 1836, 1840; Motschulsky, 1861; Waterhouse, 1879; LeConte, 1881; Bourgeois, 1883; Pic, 1913, 1922, 1923, 1930; Kleine, 1933; Green, 1949; Marie, 1968). As a result, the Lycini has been perceived as a group with widely distributed *Lycus* Fabricius, 1787 and *Lycostomus* Motschulsky, 1861 and a few species-poor endemic genera scattered across the whole range of the tribe (Fig. 2; Supporting Information, Table S1; Kleine, 1933; Pérez-Hernández *et al.*, 2019). The robust phylogeny of this diverse clade is a necessity for the stable natural classification and further studies of general interest.

The tribe Lycini belongs to Lycinae, the recently redefined subfamily, which additionally contains Thonalmini, Leptolycini, Calopterini and Platerodini as their closest relatives (Bocak & Bocakova, 2008; Masek *et al.*, 2018; Kusy *et al.*, 2019). The

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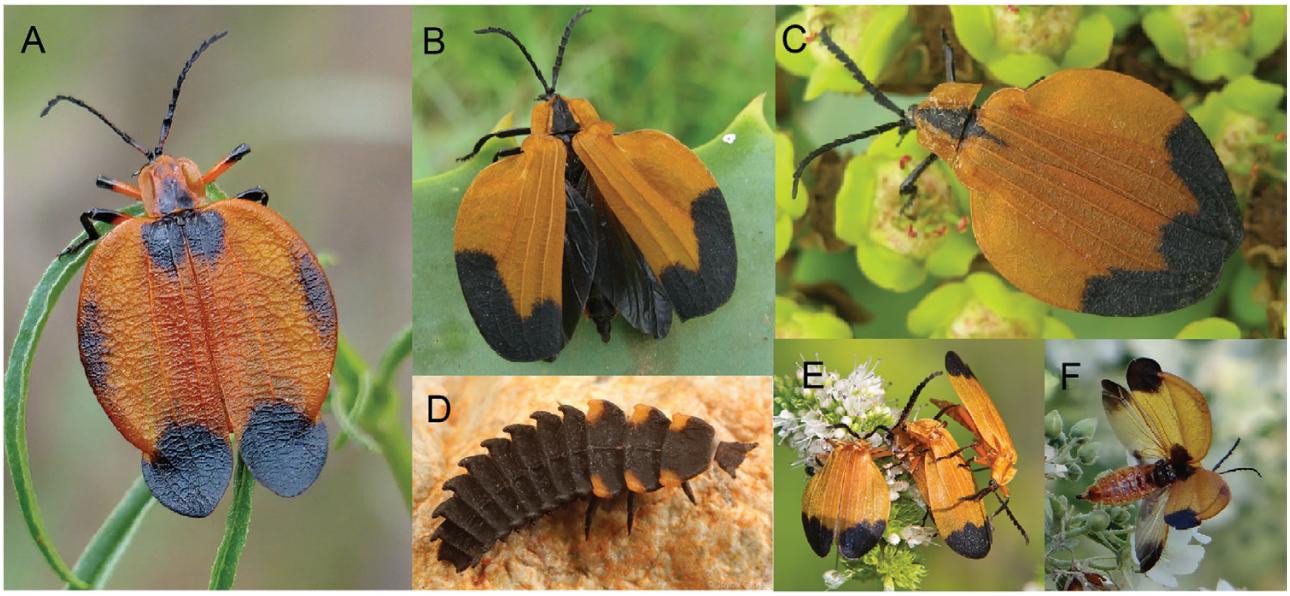

Figure 1. The Lycini in nature. A, *Lycus trabeatus* from South Africa (photograph B. Dupont, CC BY-SA 2.0). B, *Lycus* sp. (photograph T. Rulkens, CC BY-SA 2.0). C, *Lycus melanurus* from Mozambique, (photograph T. Rulkens, CC BY-SA 2.0). D, *Lycus* sp., larva (photograph © Joyce Gross). E, *Neolycus* sp. (photograph CC BY-NC 4.0 California Academy of Sciences, San Francisco). F, *Lycus* sp. (photograph P. Erb, CC BY-NC 4.0).

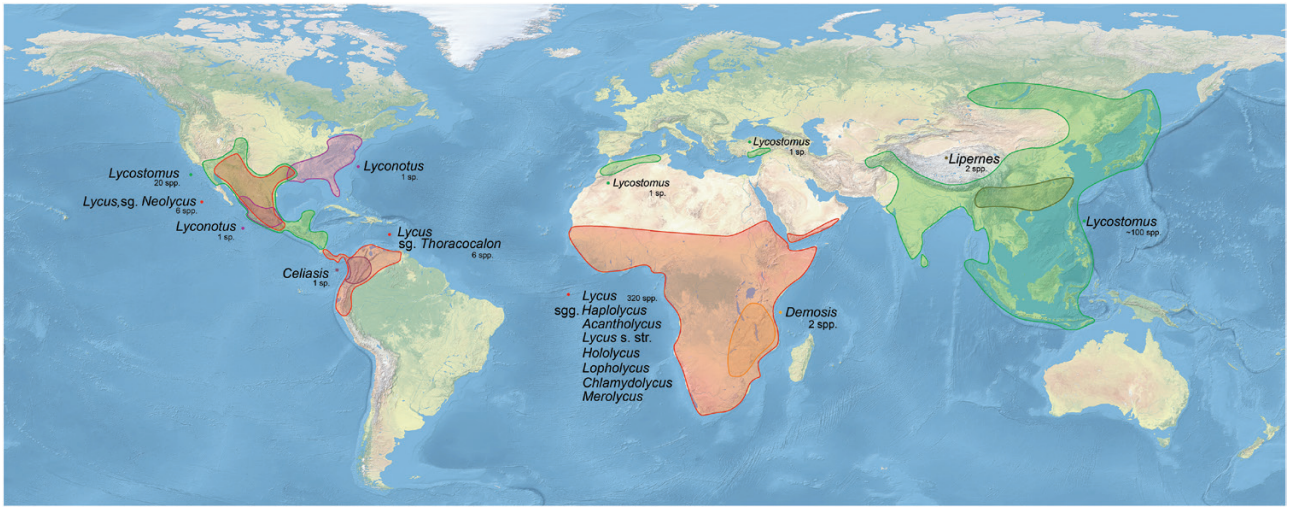

Figure 2. The distribution and alpha diversity of the Lycini as defined until the present analysis.

Lycini is morphologically well defined by several morphological traits, and their monophyly has never been questioned. Unlike most relatives, they are less common in humid tropical forests than in savannah ecosystems; the adults fly during daylight hours in open situations and are not restricted to shaded places (Kleine, 1933; Bocak & Bocakova, 2008; Jiruskova *et al.*, 2019). Owing to high adult mobility, many species are widespread. The highest alpha diversity and common occurrence are known from Afrotropical savannahs (Masek *et al.*, 2018), and they are likewise common but less diverse in semiarid habitats of the south-western USA, Mesoamerica and Southeast Asia (Green, 1949). The Lycini larvae are often collected because of their aposematic coloration but seldom identified to a species level (Fig. 1D; Bocak & Matsuda, 2003). They suck juices in the crevices of dead tree trunks and remain on

the surface or under loose bark. The larvae diapause during the dry season and pupate on trunks, often in large aggregations, in a similar manner to the closely related Calopterini (Young & Fischer, 1972; Hall & Branham, 2008).

The history of the taxonomic research in Lycini could be a textbook example of a historical burden in beetle systematics and impediments affecting the studies of highly diverse tropical insect groups (Riedel *et al.*, 2013). We struggle with ambiguous morphological delimitations of genera and subgenera, the widespread usage of some genera as waste baskets without any attempt to consider phylogenetic relationships, uninformative alpha-taxonomic descriptions and often absent differential diagnoses and inaccessible, destroyed and lost type material. As an example, we can cite the complete description of *Lycostomus testaceipes* by M. Pic (1923): '*Oblongus, testaceous, tarsis brunnescentibus, antennis nigris, ad basis testaceis*. Long 10 mill. Mexique.–A place près de *loripes* Chev. dont il n'est peut-être qu'une variété'. Such problems are common in insect taxonomy, and the net-winged beetles are no exception (Motyka *et al.*, 2017; Grebennikov, 2019), but the Lycini, and specifically *Lycus*, present all problems concentrated within a single highly diverse group.

The first lycine species were described in the second half of the 18th century because the Lycini are very common in the whole of Sub-Saharan Africa and they are very apparent in the field owing to their size, aposematic coloration and common aggregations on flowers (Fig. 1C, E, F). In the beginning, all species were placed in *Pyrochroa* Müller, 1764 and later in *Lycus* Fabricius, 1787 (Linné, 1767; Fabricius, 1775, 1787). Further genus-rank taxa were proposed by Laporte (1836, 1840), Motschulsky (1861), Waterhouse (1879), LeConte (1881), Bourgeois (1883), Green (1949) and Marie (1968). The generic and subgeneric concepts were originally based on external characters clearly present in the respective type species. Unfortunately, with hundreds of later described species, the generic limits became controversial. Although taxonomic revision was greatly needed, the limits of 16 valid genus-group taxa have never been studied in detail, and their monophyly has not been tested rigorously (Supporting Information, Table S1). Bourgeois (1883) announced an upcoming comprehensive study on *Lycus* when he delimited a number of subgenera. Thereafter, despite almost three decades of his intensive taxonomic research, such a study was never published. Green's (1949) revision of North American fauna is the only relatively modern study dealing with some genera of Lycini. Unfortunately, it was restricted to the USA. As a result, the generic classification of Lycini has remained unstable. Either the Lycini were divided into four genera, i.e. *Lycus*, *Celiasis* Laporte, 1840 (one sp.), *Demosis* Waterhouse, 1878 (two spp.) and *Lipernes* Waterhouse, 1879 (two spp.), and the first genus contained a number of subgenera and 99% of species (Kleine, 1933; Blackwelder, 1945; Green, 1949) or some subgenera of *Lycus* were treated as genera, e.g. *Lycostomus* (~110 spp.) and *Lyconotus* (ten spp.; Gorham, 1881; Nakane, 1969; Kazantsev, 1993, 2018; Zaragoza-Caballero, 1996; Pérez-Hernández *et al.*, 2019).

J. Bourgeois, M. Pic and R. Kleine described a large number of species in the late 19th century and in the 1920s and 1930s, but they never re-evaluated the limits of genera and subgenera and seldom compared the proposed new species with name-bearing types of those described by their fellows (e.g. Bourgeois, 1883; Kleine, 1926, 1937; Pic, 1913, 1922, 1923, 1930, etc.). A major part of the present chaos was caused by Maurice Pic's anecdotic and usually uninformative descriptions without any illustration of the general appearance or male genitalia. As a result, the intensive taxonomic research of the Lycini has never been resurrected after these authors ceased their activity. Only two restricted studies were published in the 1960s (Alves, 1962; Marie, 1968) and a few additional descriptions and zoogeographical records later (Turzanski, 1989; Chown & Stamhuis, 1992; Kazantsev, 1993, 2018; Zaragoza-Caballero, 1996; Matojo, 2014; Pérez-Hernández *et al.*, 2019). Currently, the bulk of ~300 Afrotropical species is placed in *Lycus* (Table 1). The Neotropical and Nearctic subgenera *Thoracocalon* Bourgeois, 1883 and *Neolycus* Bourgeois, 1883 comprise approximately ten species combined (Green, 1949; Pérez-Hernández *et al.*, 2019). *Lycostomus* contains ~80 Oriental, five Australian (Sulawesi and the Lesser Sundas, only), 20 Palaearctic, 20 Nearctic and five Neotropical species (~30% of the Lycini diversity; Supporting Information, Table S1).

Besides bad practice (e.g. Pic, 1913, 1923, etc.; Matojo, 2014) and historical methodological limitations, the problems also arose from some specific characteristics of the Lycini. The earlier studies used as diagnostic characters the morphological peculiarities in the male morphology, which might be affected by the sexual selection for pronounced sexual dimorphism (Emlen & Nijhout, 2000; Jennions *et al.*, 2001). These traits include the widely expanded, flat elytra in *Acantholycus* Bourgeois, 1883 and *Chlamydolycus* Bourgeois, 1883 (Fig. 1A–C), the considerably dilated convex elytra in *Lycus s.s.* and *Neolycus* (Fig. 1E), prominent humeral thorns (*Acantholycus*) or rounded flat elytra with elevated humeri (*Merolycus* Bourgeois, 1883).

Another factor affecting the lycine morphology is their unpalatability, advertised by the bright and black coloration (Linsley *et al.*, 1961; Selander *et al.*, 1963; Moore & Brown, 1981; Eisner *et al.*, 2008).

Table 1. The proposed classification of the Lycini Laporte, 1836

Genus/subgenus	Type species	Number of species	Distribution
Celiasis Laporte, 1840	*Celiasis mirabilis* Laporte	1	Columbia
Neolycus Bourgeois, 1883	*Lycus schoenherri* Chevrolat	11	Central America, Southern USA
Rhyncheros LeConte, 1881, reinst. name	*Lycus sanguinipennis* Say	9	USA, Central America
= *Thoracocalon* Bourgeois, 1883, syn. nov.	*Lycus adumbratus* Bourgeois	–	Northern South America
= *Lyconotus* Green, 1949, syn. nov.	*Lycus lateralis* Melsheimer	–	–
Lipernes Waterhouse, 1879	*Lycus perspectus* Waterhouse	~40	East and Southeast Asia
Haplolycus Bourgeois, 1883	*Lycus congener* Gerstaecker	~20	Sub-Saharan Africa
Lycostomus Motschulsky, 1861	*Lycus similis* Hope	~40	Asia, North Africa
Lycus Fabricius, 1787	Pyrochroa palliata Fabricius	~320	Sub-Saharan
= *Acantholycus* Bourgeois, 1883, syn. nov.	*Lycus praemorsus* Dalman	Africa	–
= *Hololycus* Bourgeois, 1883, syn. nov.	*Lycus intermedius* Bourgeois	South Arabian	–
= *Lopholycus* Bourgeois, 1883, syn. nov.	*Lycus raffrai* Bourgeois	Peninsula	–
= *Chlamydolycus* Bourgeois, 1883, syn. nov.	*Lycus trabeatus* Guérin-Méneville	–	–
= *Merolycus* Bourgeois, 1883, syn. nov.	*Lycus rostratus* Linnaeus	–	–
= *Demosis* Waterhouse, 1878, syn. nov.	*Demosis peltatus* Waterhouse	–	–
= *Concavolycus* Marie, 1968, syn. nov.	*Lycus maublanci* Pic	–	–
= *Alycus* Rafinesque, 1815	*Pyrochroa palliata* Fabricius	–	–

Similar to other net-winged beetles, the lycine species form mimetic rings, resembling each other in a single locality, and are mimicked by other insects, usually some moths and longhorn beetles (Eisner *et al.*, 2008; Motyka *et al.*, 2018). The structures with signalling function are often positively selected, and multiple parallel origins of similar structures should be considered as a possible mode of their evolution (Motyka, 2019; Bocek *et al.*, 2019a). Additionally, their colour patterns have the potential to evolve rapidly (Badyaev, 2002; Eberhard, 2004). Therefore, the origins of such modifications should be investigated rigorously before they are accepted as diagnostic traits for the delimitation of the natural genus- and species-rank taxa.

The Lycini has the potential to be a model group for evolutionary studies owing to their high diversity, wide distribution and morphological plasticity. But, unless the genus-rank classification is revised, further taxonomic, phylogeographical and evolutionary studies would be difficult to conduct and this interesting group would remain neglected. Our aim is to construct the first molecular phylogeny of Lycini using a broad representation of species. We try to elucidate the origins of morphological traits earlier used for the classification, i.e. those connected to the evolution of mimetic patterns and sexual dimorphism. Molecular data can provide an insight into the evolution of such traits, and we intend to propose a revised classification, in which we strive to circumscribe DNA-based genera using diagnostic morphological traits for reliable identification without the necessity to obtain molecular data. Furthermore, we reconstruct a time-calibrated tree to identify important dispersal routes and the centres of origins and radiation (Myers *et al.*, 2000; Masek *et al.*, 2018).

MATERIAL AND METHODS

Material, sequencing and phylogenetic analyses

The ingroup contained 465 Lycini terminals, representing ~100 species from all geographical regions (Supporting Information, Table S2). The species are not formally identified except for a few cases in which we are sure that misidentification is improbable. There is a high chance that many species have been described several times, and without detailed study of hundreds of primary types in numerous collections we are not able to identify most species. As a result, the species are numbered within each redefined genus. The outgroup was represented by 74 Platerodini and Calopterini terminals whose sequences were reported in previous studies (Bocak *et al.*, 2008; Masek *et al.*, 2018).

We extracted DNA from the thoracic muscles of ethanol-preserved specimens using a Wizard SV96 kit (Promega Corp., Madison, WI, USA) following the manufacturer's protocol. Three mitochondrial DNA (mtDNA) fragments [i.e. *rrnL* (~800 bp), *cox1-tRNA-Leu-cox2* (~1000 bp) and *nad5-tRNAs* mtDNA (~1200 bp)] and two ribosomal RNA fragments (rRNA; complete 18S rRNA and D2 region of 28S rRNA) were

amplified using the primers listed and PCR settings described in the Supporting Information (Table S3). A voucher number was assigned to each specimen in the format UPOL XX1234, and the sequences were submitted to GenBank under the accession numbers MN936181–MN937192 (Supporting Information, Table S2; Fig. S1).

Chromatograms were edited using the SEQUENCHER v.4.8 software package (Gene Codes Corp., Newark, NJ, USA). All sequences were aligned using MAFFT v.7.407 (Katoh & Standley, 2013), and individual gene alignments were concatenated into supermatrix using FASCONCAT-G v.1.04 (Kück & Longo, 2014). The concatenated supermatrix was partitioned by the genes and analysed using the maximum likelihood (ML) criterion and Bayesian inference (BI). The analyses were performed in a ML framework using Q-TREE v.1.6.12 (Nguyen *et al.*, 2015), with the best substitution model selected by MODELFINDER (Chernomor *et al.*, 2016; Kalyaanamoorthy *et al.*, 2017) implemented in IQ-TREE. The IQ-TREE analyses were run with the -spp parameter to allow each partition to have its own evolutionary rate. All models and partitions are listed in the Supporting Information (Table S4). The ultrafast bootstrap (UFboot) option was set to 5000 bootstrap iterations (Hoang *et al.*, 2018).

Additionally, we assembled the pruned dataset using QIIME, with a single representative per genetically distinct cluster of individuals (Caporaso *et al.*, 2010). As a threshold, we used 2% of DNA pairwise uncorrected distance in the *cox1* fragment, and a single individual with the highest fragment coverage represented each genetically distinct cluster. The reduced dataset contained 118 ingroup (~100 spp.) and 11 outgroup terminals. The low delimitation threshold was intentionally chosen to keep in the subsequent analyses all putative species and the representatives of genetically and geographically distant populations; therefore, the number of terminals is higher than the estimated number of species. The pruned dataset was analysed under the ML criterion as above and using BI as implemented in MRBAYES v.3.2.6 (Huelsenbeck & Ronquist, 2001). The Markov chain Monte Carlo (MCMC) was set with independent parameters for 16 partitions (Supporting Information, Table S4) under the general time-reversible model with a category of invariant sites and gamma-distributed rates (GTR+I+G). Four chains were run for 5×10^7 generations, with trees sampled every 10 000 generations. Adequate sampling, mixing and convergence to the stationary distribution, i.e. the effective sample size values > 200 for all parameters, were checked using TRACER v.1.6 (Rambaut *et al.*, 2014). The first 20% of generations were discarded as burn-in. A 50% majority-rule consensus tree was constructed to determine the posterior probabilities (PP) from the remaining trees. Posterior probabilities ≥ 95% indicate strong statistical support. The resulting phylogenetic trees were visualized using FIGTREE v.1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

GEOGRAPHICAL DISTRIBUTION AND ANCESTRAL STATE RECONSTRUCTION

The age of principal splits was estimated using BEAST v.1.8.1 (Drummond *et al.*, 2012), the mtDNA pruned dataset and the fixed topology inferred from the previous analysis. The HKY model, Yule process and lognormal uncorrelated relaxed clock, as proposed in the BEAST v.1.8.1 manual, were set in the BEAST analysis after application of the GTR+I+G model did not reach convergence (Drummond & Bouckaert, 2015). Given that no fossils of Lycini are available, we used a secondary calibration point taken from Bocak *et al.* (2016), where authors inferred the split among *Dictyoptera* sp. and *Lycus* sp. using a whole mitochondrion dataset and fossil calibrations of Elateriformia. The MCMC parameters were set to 100 million generations, with sampling every 10 000 generations. The pre-stationary phase and the effective sample size > 250 were identified using TRACER v.1.6 (Rambaut *et al.*, 2014). The initial 30 million generations were discarded as burn-in.

Additionally, the ancestral areas were inferred using the ML framework in BIOGEOBEARS (Matzke, 2013) implemented in RASP v.4.0 (Yu *et al.*, 2020), using the dataset containing 118 terminals (a single terminal per species as described above). We compared all alternative models of colonization, all also with +J (Matzke, 2014), which tests founder-event speciation. The localities were assigned to respective taxa and coded for geographical origin analyses. South America, Central America, North America, China, Japan, Indo-Burma, Sundaland, Sulawesi, India, Sub-Saharan Africa and Turkey were coded as separate regions in the analyses.

We applied BAMM v.2.5.0 (Rabosky, 2014a) to test how diversification rates (speciation, extinction and net diversification) have evolved throughout the history of the Lycinae tribe. Priors were set to the scale of the tree using the setBAMMpriors function in BAMMTOOLS v.2.1.6 (Rabosky, 2014b) in R v.3.4.3 (R Core Team, 2013). Although the expected NumberOfShifts parameter was identified as one, we explored ranges of priors for diversification by setting shifts to 0.1, 1, 5, 10 and 50, respectively. For each, we ran 100 million generations, sampling every 10 000 generations with four chains. The segLength parameter was set to default value = 0.02. All results

were analysed and interpreted according to BAMM guidelines (http://bamm-project.org).

Morphology

Morphological characters were observed for all sequenced specimens. We evaluated the sexual dimorphism and colour pattern within the clusters of individuals with similar mitochondrial sequences. Additionally, male genitalia were dissected for putative species assignation. The male genitalia were photographed using an Olympus SZX-16 binocular microscope and a Canon EOS digital camera, and the photographs of general appearance were taken with the same camera and Canon EOS MP-65 lenses. The shots were assembled in Helicon Focus v.6 (www.heliconsoft.com). The scale bars were derived from measurements taken with an ocular scale.

RESULTS

Phylogenetic relationships and morphological diversity

The molecular phylogeny robustly corroborated the monophyly of the Lycini, and the ML and Bayesian analyses recovered fully resolved, similar trees, with a few inconsistencies in the topology of terminal branches (Figs 3, 4; Supporting Information, Figs S1–S3). A lower nodal UFboot support was inferred for the relationships among some genus-rank branches (UFBoot 35–56%), but high posterior probabilities were recovered for these clades by the BI analysis using the pruned dataset (PP 0.99–1.0). The support for the monophyly of genera and earlier defined subgenera was robust, but the subgenera represent only terminal species groups nested in more extensive clades (Supporting Information, Fig. S1). We recovered the Nearctic and Neotropical genera *Neolycus* and *Rhyncheros* as the deepest clades, and they formed a clade in the sister position to other Lycini (Fig. 3; Supporting Information, Figs S1–S3). All analyses split the earlier defined Asian *Lycostomus* into two independent and distant lineages. The deeply rooted *Lipernes* was recovered as sister to all Afrotropical Lycini and *Lycostomus* in the here redefined sense (Fig. 3). *Lipernes* is known from the south-eastern part of the Palaearctic and Oriental regions. The newly delimited *Lycostomus* is a terminal branch with Asian distribution and is nested in the large, dominantly Afrotropical *Haplolycus* + *Lycus* clade. *Lycostomus* is diverse in the Himalayas and the Sundaland; two species occur in North Africa and Turkey, and some species occur in China, Korea, Japan, the Russian Far East, Siberia and Sulawesi (Fig. 5A, B).

The dated tree indicates the delayed origin of the Lycini, with the deepest split dated to the Eocene–Oligocene boundary (33.58 Mya; Supporting Information, Fig. S4). The variability of the diversification rate was investigated using the BAMM analysis, but no apparent changes in rates were identified in the evolution of the Lycini (Supporting Information, Fig. S5). The diversification was highest at the beginning of the diversification in the Lower Oligocene and with continually decreasing rates up to the present (Supporting Information, Figs S5, S6).

The Afrotropical fauna consists of *Haplolycus* and *Lycus*, and the latter comprises a number of typologically defined historical subgenera (Fig. 2). The general appearance of species that characterized earlier described subgenera of *Lycus*, both males and females if sexually dimorphic, and the male genitalia are shown in the taxonomic section and on the molecular phylogeny in the Supporting Information (Fig. S1). Most traditional subgenera can be defined clearly by morphological traits, but a set of deeply rooted species cannot be assigned to any such defined subgenus using the morphology.

The intraspecific and intrapopulation polymorphism were evaluated for the species that were represented multiple individuals (usually more than ten specimens). We found that the black elytral patches can be absent or differently shaped in some individuals; the pronotum can be either red or black within a single species. Likewise, we found high variability in the shape of the elytra and the strength of humeral costae. The variability of sexually dimorphic traits is usually found in males, but a similar series of morphological modifications were observed also in some females of *Lycus* sp. 51 (see taxonomic section for details).

Distribution and diversification

The Lycini occur in five major zoogeographical regions. *Neolycus*, *Rhyncheros* and *Celiasis* are found in the New World. *Neolycus* has been recorded from the northern part of the Neotropical region (Mesoamerica, Northern South America, i.e. Columbia, Ecuador, Peru, French Guiana and north-eastern Brazil) and the southern part of the Nearctic region (Northern Mexico, California, Arizona, Utah, Colorado, New Mexico and Texas). *Rhyncheros* occur sympatrically with *Neolycus* and additionally from Tennessee to Maine (Fig. 5A). *Celiasis* was reported from Columbia when described and never reported later (Laporte, 1840). Two genera occur in the Eastern Palaearctic and Oriental regions: *Lipernes* in southern China, India, the Himalayas,

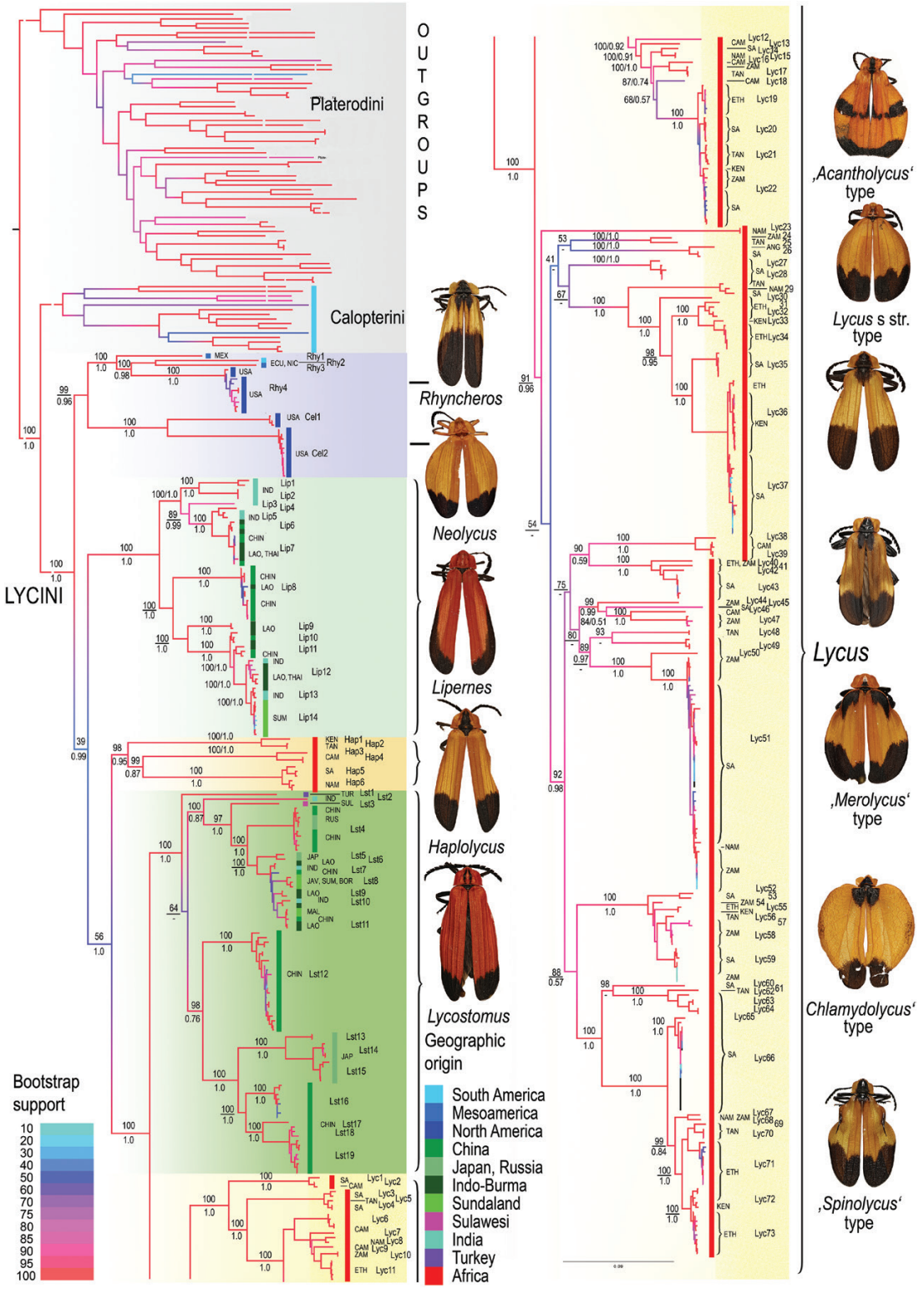

Figure 3. Phylogenetic hypothesis of the Lycini relationships resulting from the maximum likelihood analysis of the mito-ribosomal dataset. Upper numbers represent ultrafast bootstrap, lower numbers posterior probabilities obtained by the Bayesian analysis of the pruned dataset. The support values for terminal branches are omitted.

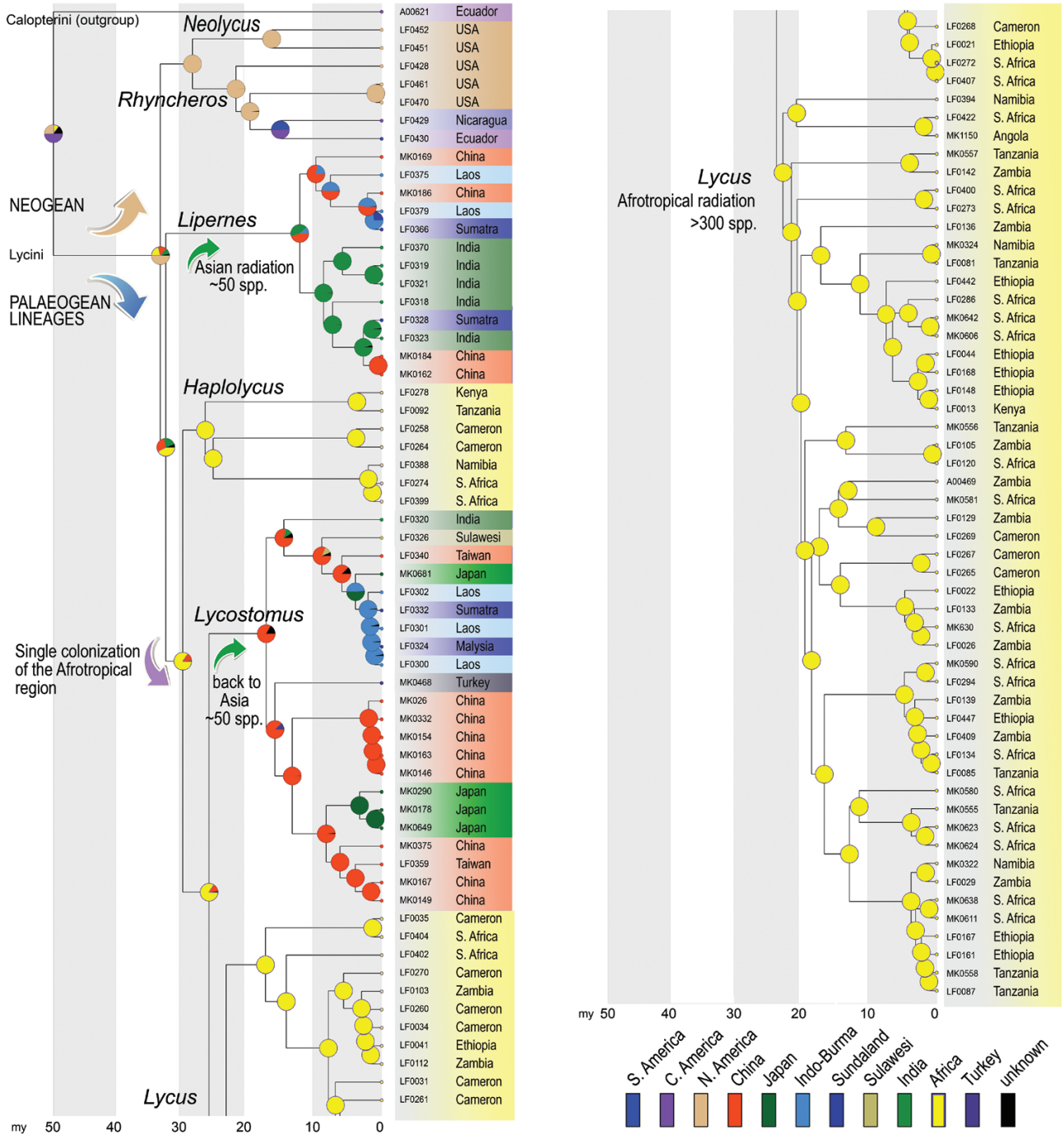

Figure 4. The dated phylogenetic tree and ancestral area reconstruction recovered by the analysis of the pruned dataset.

Indo-Burma, Sundaland, the Philippines and Sulawesi. *Lycostomus* is more widespread and, except for the range of *Lipernes*, it occurs also in northern China, the Russian Far East, Siberia, Japan, Korea, Afghanistan (one sp.), Turkey (one sp.) and northern Africa (one sp.). *Lycus* and *Haplolycus* occur in the whole of Sub-Saharan Africa, and their range reaches the southernmost part of the Arabian Peninsula (only *Lycus*).

The reconstruction of ancestral areas located the early splits in the Southern part of the Nearctic region and Mesoamerica when Calopterini is considered as the sister group of Lycini on the basis of previous transcriptomic analyses. The early dispersal event

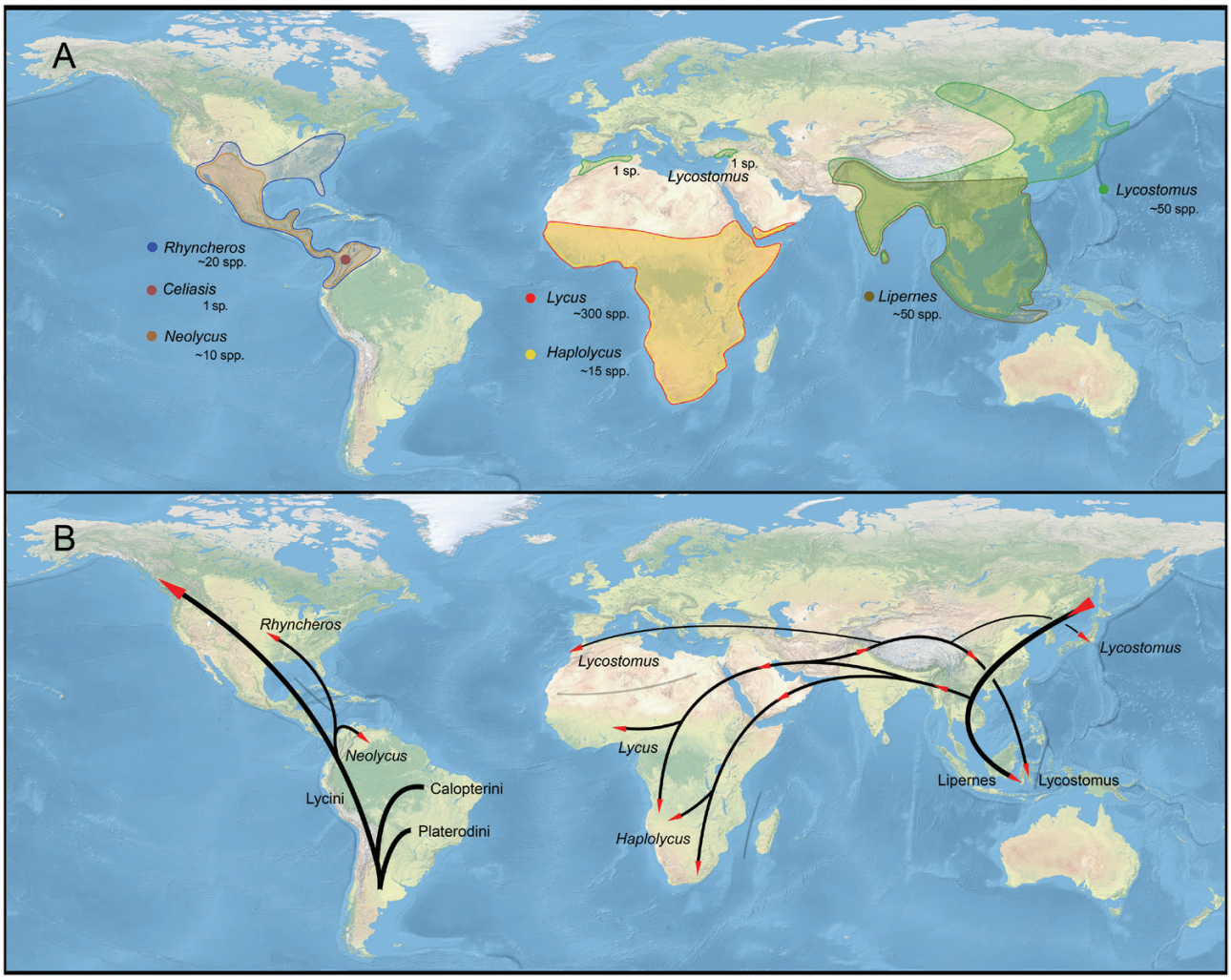

Figure 5. A, the revised distribution of genera and their alpha diversity. B, the putative dispersal routes recovered by the analysis of the pruned dataset. The grey lines designate dispersal barriers that were never crossed by the Lycini.

brought the Lycini to Eastern Asia. *Lipernes* remained there, and further dispersal events brought two separate lineages, i.e. *Lycus* and *Haplolycus*, to Sub-Saharan Africa. *Lycostomus* is a part of this dominantly Afrotropical clade as the sister of *Lycus*. Dozens of *Lycostomus* species occur in a large part of the eastern Palaearctic region and reach, in the continuous range, to Afghanistan. Large open sea straits prevent dispersal of the Lycini. No species colonized the Great Antilles, Madagascar and the Moluccas despite their relative geographical closeness of a few hundred kilometres. Likewise, no species crossed the arid areas of Northern Africa and the Arabian Peninsula (Fig. 5B). The Lycini occur on some shelf islands (Sri Lanka, Sumatra, Borneo, Java and Bali), on the islands of the continental origin (Japan, Sakhalin and Taiwan) or oceanic islands in the proximity of a continent (the Philippines, Lesser Sundas and Sulawesi; Fig. 5A). The details of the RASP analysis are given in the Supporting Information (Table S5).

TAXONOMY

LYCINI LAPORTE, 1836

(FIG. 6A–R)

Lycini Laporte, 1836: 25.

Type genus: Lycus Fabricius, 1787.

Diagnosis

The Lycini is defined by the presence of the rostrum (Fig. 6D, E), flat, serrate to parallel-sided antennomeres 3–10 (Fig. 6C), absent pronotal carinae (Fig. 6F), weak longitudinal and absent or irregular

transverse costae in the elytra (Fig. 6A, S–X), tubular mesothoracic spiracles (Fig. 6H), the spoon-shaped phallobase, short parameres, a long and slender phallus (Fig. 6O, AB–AG), large lateral glands in the female sexual duct, short valvifers and the short spermaduct (Fig. 6P, Q). Their closest relative is Calopterini, which has well-developed parameres, apparent transverse costae in the elytra and commonly has flabellate male antennae (Bocakova, 2003, 2005).

Redescription

Adults: Length 5–26 mm. Weakly sclerotized beetles, with a flexible cuticle. Body elongate, dorsoventrally flattened; elytra slightly to substantially wider than abdomen, often globuliform (Fig. 6A). Most species are aposematically coloured: uniformly red, dark red and yellow or with variable dark patches on a yellow or red background (e.g. Figs 6S–X, 7A–N). Vestiture dense and short.

Head small, rostrate, much narrower than prothorax, highly movable; hypognathous to opistognathous in resting position (e.g. Fig. 6A, D, Z, AA). Eyes small, their diameter never larger than frontal eye distance, hemispherically prominent (Fig. 6D). Frontoclypeal suture absent, anterior edge of frontoclypeus slightly concave; labrum rounded, small (Fig. 6D). Antennal insertions close; antennae with 11 antennomeres, slightly serrate in both sexes, sometimes almost parallel sided, never flabellate (Fig. 6C). Mandibles tiny. Maxilla with prolonged stipes, galea and lacinia fused, membranous, setose; maxillary palpi four-segmented, slender. Labium small, praementum slender, mentum reduced, transverse, ligula reduced; labial palpi short, three-segmented (Fig. 6B).

Pronotum usually with elevated lateral edges, much narrower than elytra in most species. Anterior edge rounded, anterior angles mostly obtuse. Posterior angles only slightly projected, never acute (e.g. Fig. 6F, Y). Prosternum transverse (Fig. 6F); scutellum triangular to parallel-sided (e.g. Fig. 6Y). Mesoventrite widely emarginate anteriorly (Fig. 6H). Metaventrite broad, with incomplete discrimen (Fig. 6G). Metendosternite robust, short (Fig. 6G). Spiracles simple, mesothoracic spiracles tubular (Fig. 6I). Elytra only slightly widened posteriorly, flat; alternatively wide, rounded or globular; elytral longitudinal costae weak, transverse costae irregular to absent (Fig. 6A, S–X), elytra rarely shortened (*Lycus sanguinipennis* Say, 1823 from Arizona). Hindwings well developed if elytra cover whole abdomen. Legs slender, flattened; coxae separate, globular to slightly elongate; trochanters prolonged (Fig. 6E), femora slender, long, seldom robust in some males; tarsi with five tarsomeres, claws simple (Fig. 6R).

Abdomen with eight and seven visible sternites in males and females, respectively. Sclerites weakly sclerotized, connected by membranes (Fig. 6J, K), abdomen shorter and much narrower than elytra in most species (Fig. 6A). Male terminal abdominal segments slender, terminal ventrite spoon-like, slender basally, long; terminal tergite straight and wide at base, penultimate sternite with short processes attaching to base of terminal ventrite (Fig. 6N); last visible female sternite with long spiculum ventrale (Fig. 6L). Phallus slender, parameres absent, short or fused with basal part of phallus, phallobase spoon-like, internal sac inconspicuous, membranous (Fig. 6O, AB–AG). Ovipositor with plate-like coxites, short styli and with rod-like paraproctal baculi (Fig. 6P). Vagina sack-like, membranous, with two lateral accessory glands attached distally and small ventral gland; spermathecal duct short; spermatheca simple, moderately sclerotized, globular, apically bearing Y-shaped gland (Fig. 6Q).

Distribution

Afrotropical, Palaearctic, Oriental, Australian (Sulawesi and Timor only), Nearctic and Neotropical (northern part only) regions (Fig. 5A; Kleine, 1933; Masek *et al.*, 2018).

NEOLYCUS BOURGEOIS, 1883

Neolycus Bourgeois, 1883: 61.

Type species: Lycus schoenherri Chevrolat, 1834.

Diagnosis

Neolycus is the only New World genus with two thorns in the middle part of the phallus (Fig. AB–AD). Additionally, it differs from *Rhyncheros* in having wide male elytra (Fig. 6S, T). There is no character available to distinguish *Neolycus* and *Celiasis*, and the latter might be congeneric with *Neolycus*.

Redescription

Adults: Body slender. Head mostly concealed by pronotum, with long, slender rostrum (Fig. 6Z); pronotum widest at base, with projected posterior angles (Fig. 6Y). Sexually dimorphic; male with considerably dilated and convex elytra, female with moderately dilated elytra; both sexes with four indistinct costae in each elytron (Fig. 6S, T); male genitalia with a pair of ventral thorns around their mid-length (Fig. 6AB–AD).

Distribution

USA (south and southwest), Mexico and Peru (Fig. 5A; Kleine, 1933; Bocakova *et al.*, 2015).

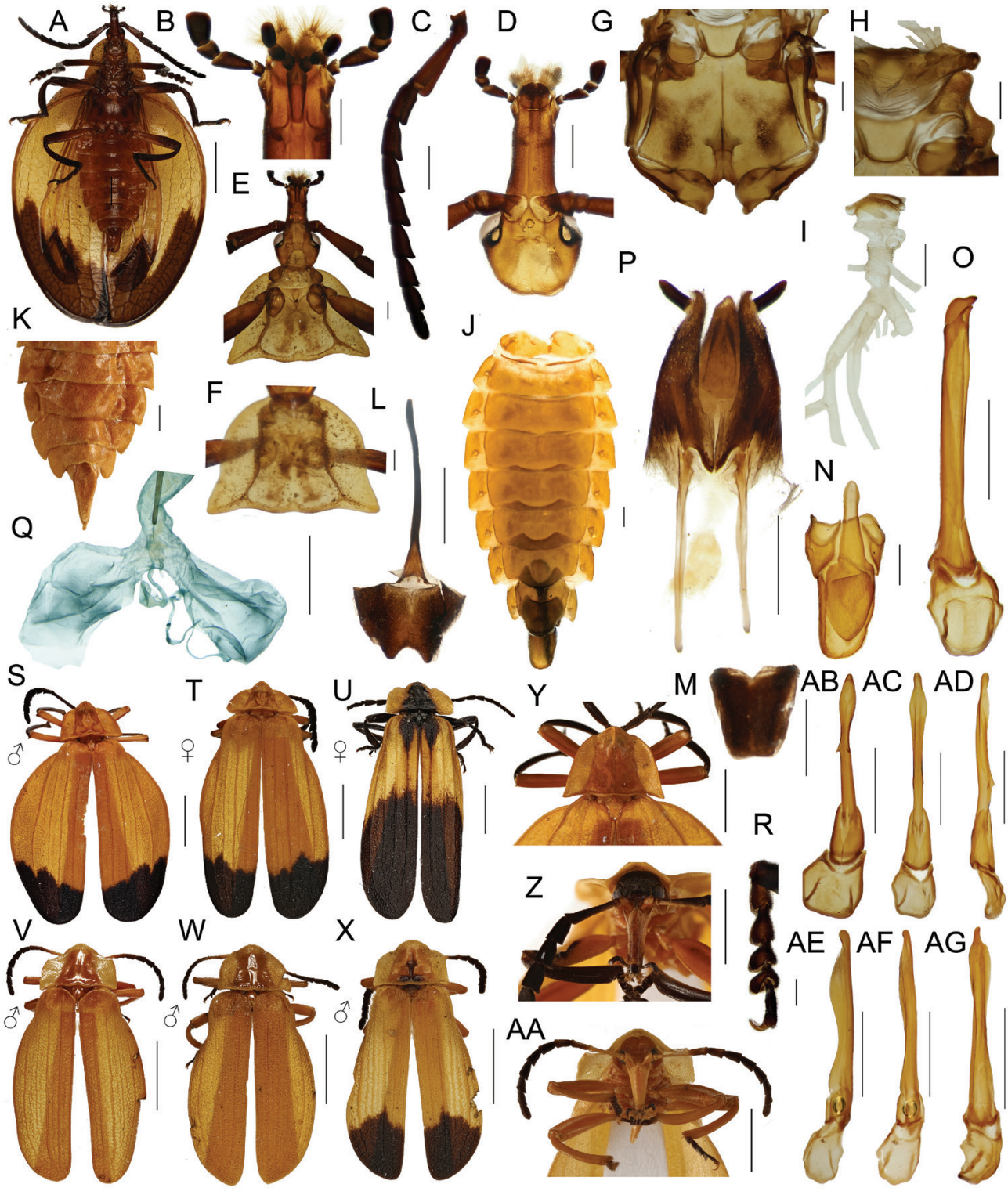

Figure 6. *Lycus pallidus* (F.), male unless stated otherwise. A, general appearance, ventral view. B, apical part of the rostrum, ventral view. C, antenna. D, head, dorsal view. E, prothorax and head, ventral view. F, pronotum, dorsal view. G, meso- and metasternum, ventral view; mesosternum. H, ventral view. I, mesothoracic spiracle with attached trachea. J, abdomen. K, abdomen of *Neolycus fernandezi* (Dugès), male. L, female terminal ventrite. M, female terminal tergite. N, terminal abdominal segments. O, male genitalia. P, ovipositor. Q, female internal genital duct. R, metatarsus. S, *N.*

Remarks

Since its proposal, *Neolycus* has been cited only in the catalogues of North American and Mexican net-winged beetles and usually placed as a subgenus of *Lycus* (Kleine, 1933; Green, 1949; Zaragoza-Caballero, 1996; Pérez-Hernández *et al.*, 2019). As *Neolycus* was recovered in a distant position from *Lycus*, it has to obtain the genus rank, and no *Lycus* occurs in the Nearctic and Neotropical regions after the proposed changes in the Lycini classification (Fig. 5A).

RHYNCHEROS LECONTE, 1881

Rhyncheros LeConte, 1881: 17.

Type species: Lycus sanguinipennis Say, 1823.
= *Thoracocalon* Bourgeois, 1883, syn. nov.

Type species: Lycus adumbratus Bourgeois, 1877.
= *Lyconotus* Green, 1949, syn. nov.

Type species: Lycus lateralis Melsheimer, 1846 (not *Lycus semiustus* Chevrolat, 1834, designated by Zaragoza-Caballero, 1996).

Diagnosis

Rhyncheros contains all New World Lycini without the sexually dimorphic shape of elytra (Fig. 6U–X). Besides, it differs from *Neolycus* in the absence of thorns in the middle part of the phallus (Fig. 6AE–A G).

Redescription

Adults: Body slender. Head mostly concealed by pronotum, rostrate. Rostrum long. Pronotum widest at base, with projected posterior angles. Elytra without sexual dimorphism, moderately dilated posteriorly in both sexes; four indistinct costae in each elytron (Fig. 6U–X). Male genitalia without any thorns around, with membranous ventral part apically and simple apex (Fig. 6AE–AG).

Distribution

Nearctic region and the northern part of the Neotropical region (Fig. 5A); Mesoamerica, Columbia (two spp.), Ecuador (two spp.), and French Guiana and north-eastern Brazil (one sp.) (Kleine, 1933).

Remarks

Rhyncheros, a long-overlooked genus, was placed in Dictyopterini by Kleine (1933) and later transferred into the Lycini as a synonym of *Lycus* (Green, 1949). The type species, *Rhyncheros sanguinipennis*, was placed in the subgenus *Lycostomus*. The current analysis recovered that *Lycus* occurs only in the Afrotropical region and *Lycostomus* in the Palaearctic and Oriental regions (Fig. 5A). *Rhyncheros*, *Celiasis* and *Neolycus* are valid names for the Nearctic and Neotropical Lycini, and further available genus-rank names are *Lyconotus* and *Thoracocalon*. We compared the structure of genitalia of *R. sanguinipennis*, the type species of *Rhyncheros*, and *Lycus lateralis*, the type species of *Lyconotus*, and they indicate close relationships of these species. Based on recovered relationships of Nearctic species (Fig. 2), we propose that *Lyconotus* should be considered a younger subjective synonym of *Rhyncheros*. *Thoracocalon* is the name last used in the Lycini classification in the late 19th century, and the type species was unavailable for the present study. The genus differs from other Lycini in having widened lateral margins of the pronotum (Bourgeois, 1883). The slightly wider pronotum can also be observed in some *Rhyncheros* (Fig. 6U). Therefore, we propose that *Thoracocalon* should be synonymized with *Rhyncheros*.

LIPERNES WATERHOUSE, 1879

Lipernes Waterhouse 1879: 9.

Type species: Lipernes perspectus Waterhouse, 1879.

Diagnosis

Lipernes is similar to *Lycostomus* in general appearance (Fig. 7A–O), and these genera differ in the shape of male genitalia. *Lipernes* has a robust phallus with simple apex or vertical cleft (Fig. 7AA–AR).

Redescription

Adults: Body slender. Head mostly concealed by pronotum, rostrate. Rostrum short to moderately long, often very stout. Pronotum widest at base, with rectangular to slightly projected posterior angles, variable in shape. Elytra without sexual dimorphism, moderately dilated posteriorly in both sexes, with four indistinct costae in each elytron (Fig. 7A–O, R–U).

fernandezi (Dugès), general appearance, male. T, ditto, female. U, *Rhyncheros* sp., general appearance, male. V, *Rhyncheros loripes* (Chevrolat), general appearance, male. W, *Rhyncheros* sp. 1. X, *Rhyncheros* sp. 3, male. Y, *N. fernandezi*, head and prothorax, dorsally. Z, ditto, ventrally. AA, *Rhyncheros loripes* (Chevrolat); head and prothorax, ventrally. AB–AG, male genitalia. AB, *N. arizonensis* (Green), ventrally. AC, *N. fernandezi* (Dugès), dorsally. AD, ditto, laterally. AE, *Rhyncheros* sp. 1, laterally. AF, ditto, ventrally. AG, *Rhyncheros* sp. 3, dorsally. Scale bars: 3 mm (A, S, X); 1 mm (C); 0.5 mm (B, D–R, Y–AG).

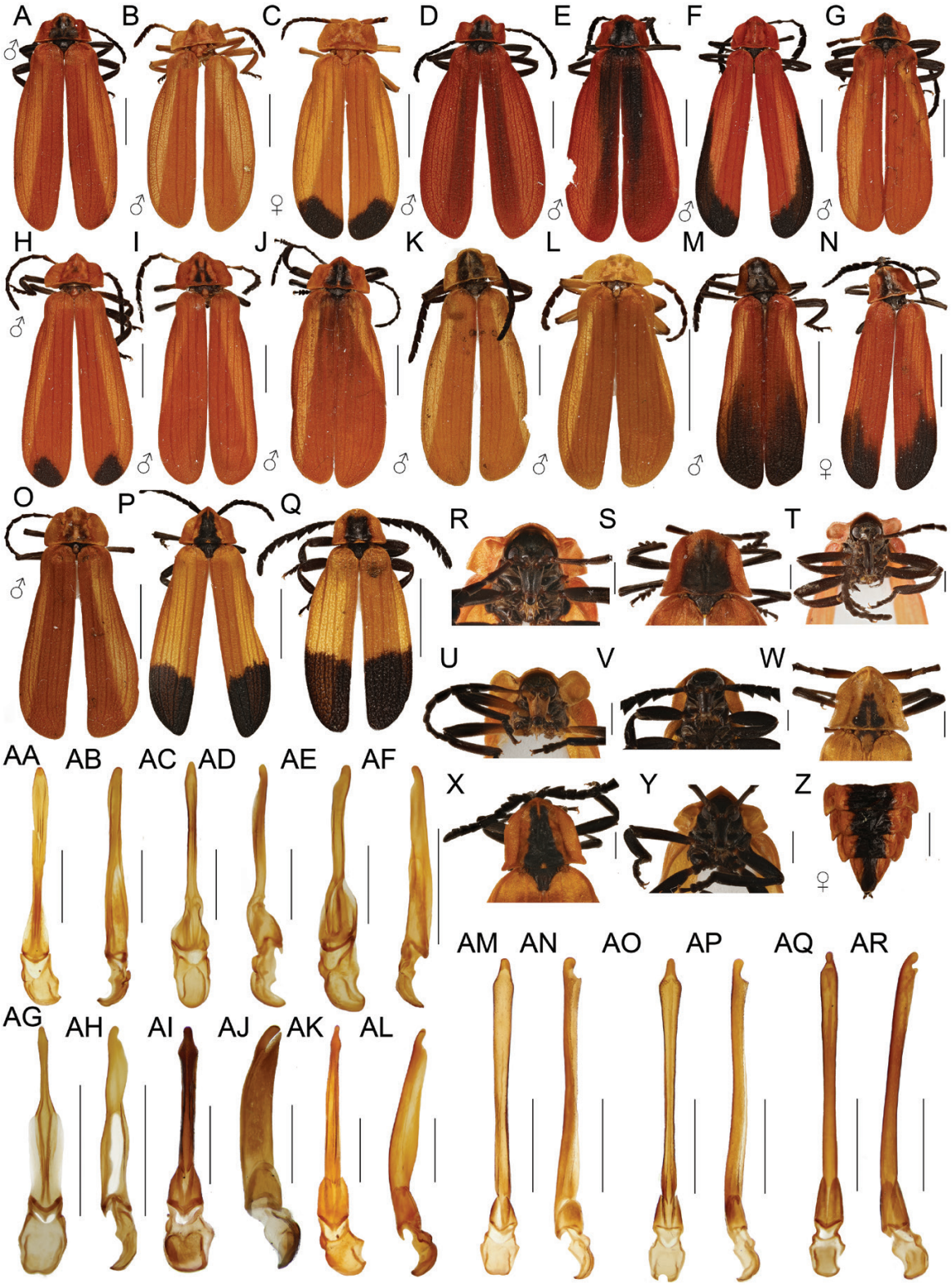

Figure 7. General appearance. A, *Lipernes* sp. 3, male. B, *Lipernes* sp. 1, male. C, *Lipernes* sp. 1, female. D, *Lipernes* sp. 2, male. E, ditto, female. F, *Lipernes* sp. 4, male. G, *Lipernes* sp. 5, male. H, *Lipernes* sp. 6, male. I, *Lipernes* sp. 7, male. J, *Lipernes* sp. 8, male. K, *Lipernes* sp. 9, male. L, *Lipernes* sp. 13, male. M, *Lipernes* sp. 14, male. N, *Lipernes* sp. 14, male. O,

Male genitalia without any thorns, with membranous ventral part apically; simple apex and in most species dilated basally, with translucent windows at base and ventral sclerotized keel (Fig. 7AA–AR).

Distribution

East Palaearctic and Oriental regions (Fig. 5A).

Remarks

The concept of *Lycostomus* has been very wide and merged all Nearctic, Neotropical, Palaearctic and Oriental Lycini without pronounced sexual polymorphism in the shape of elytra (Figs 6U–X, 7A–Q). The Neotropical and Nearctic species have to be transferred from *Lycostomus* to *Rhyncheros*. Palaearctic and Oriental species represent two independent lineages and must be placed in redefined *Lipernes* and *Lycostomus*. We found that the species of the deeply rooted Asian clade (Fig. 2) have genitalia similar to those of *Lipernes perspectus* Waterhouse, 1879. Therefore, we use the name *Lipernes* for the designation of this clade. *Lipernes* and *Lycostomus* occur sympatrically, and they can be distinguished only using male genitalia (Fig. 7AA–AR). Their general appearance is similar; most species are red or red and black in colour (Figs 7A–Z, 8A–O). The length of the apical palpomere, which was originally used as a diagnostic character, is variable in *Lipernes*, as has been noted already by Kazantsev (1993).

HAPLOLYCUS BOURGEOIS, 1883

Haplolycus Bourgeois, 1883: 62.

Type species: Lycus congener Gerstaecker, 1871.

Diagnosis

Haplolycus is an African endemic, but resembles in the shape of elytra Oriental *Lycostomus* and *Lipernes* (Fig. 7P, Q). It differs in the very long, slender, straight phallus with weakly emarginate apex (Fig. 7AM–AR). Unlike *Lycus*, the males and females of *Haplolycus* are similar and do not differ in the shape of elytra.

Redescription

Adults: Body slender. Head rostrate. Rostrum short to moderately long, often very stout. Pronotum widest at base, variable in shape. Elytra without sexual dimorphism, moderately dilated posteriorly in both sexes, with four indistinct costae in each elytron (Fig. 7P, Q, V–Y). Male genitalia without thorns, very slender, only weakly emarginate at apex and without membranous ventral part apically (Fig. 7AM–AR).

Distribution

Afrotropical region (Fig. 5A).

Remarks

The identification of *Haplolycus* is complicated by the unavailability of the type of *Haplolycus congener*. Using the information in the original description and identified specimens in collections localized by J. Turzanski, we assign to *Haplolycus* the species without sexual dimorphism and with a very long, slender, straight phallus with weakly emarginate apex (Fig. 7AM–AR). *Haplolycus* and *Lycostomus* share a similar shape of elytra, and they differ only in the form of male genitalia. These genera are allopatrically distributed and differently coloured owing to membership of specific local mimetic complexes. Therefore, investigation of male genitalia is not needed for the generic identification.

LYCOSTOMUS MOTSCHULSKY, 1861

Lycostomus Motschulsky, 1861: 136.
Type species: Lycus similis Hope, 1831.

Diagnosis

Lycostomus is similar to *Lipernes* in general appearance, and these genera differ in the shape of the male genitalia. *Lycostomus* has a very slender and long phallus with an emarginate apex and ventro-apical membranous part.

Lipernes sp. 12, female. P, *Haplolycus* sp. 6, male. Q, *Haplolycus* sp. 1, male. R–Y, prothorax and head. R, *Lipernes* sp. 5; ventrally. S, *Lipernes* sp. 3, dorsally. T, *Lipernes* sp. 2, ventrally. U, *Lipernes* sp. 12, ventrally. V, *Haplolycus* sp. 1, ventrally. W, X, *Haplolycus* sp. 6, dorsally. Y, ditto, ventrally. Z, *Lipernes* sp., abdominal terminal segments, female. AA–AR, male genitalia, dorsal and lateral views. AA, AB, *Lipernes* sp. 1. AC, AD, *Lipernes* sp. 5. AE, *Lipernes* sp. 6. AF, *Lipernes* sp. 14. AG, AH, *Lipernes* sp. 12. AI, AJ, *Lipernes perspectus* Waterhouse. AK, AL, *Haplolycus* sp. 6. AM, AN, *Haplolycus* sp. 1. AO, AP, *Haplolycus* sp. 2. AQ, AR, *Haplolycus* sp. 4. Scale bars: 3 mm (A–Q), 1 mm (O–AR).

Redescription

Adults: Body slender. Head rostrate. Rostrum short to moderately long, often very stout. Pronotum widest at base, variable in shape. Elytra without sexual dimorphism, moderately dilated posteriorly in both sexes, with four indistinct costae in each elytron (Fig. 8A–O). Male genitalia without thorns, very slender, emarginate at apex and sometimes with membranous ventral part apically (Fig. 8P–R).

Distribution

Eastern part of the Palaearctic region (Russian Far East, southern Siberia, Korea, China, northern Pakistan and Afghanistan), western part of the Palaearctic region (Fig. 5A; Turkey, one sp. and Algeria, one sp.).

Remarks

Lycostomus is phenotypically very similar to *Lipernes*, and both genera occur sympatrically in southern and Southeast Asia. The genera differ only in the shape of male genitalia, and both of them need revision to solve the placement of the species earlier placed in *Lycostomus*.

LYCUS FABRICIUS, 1787

Lycus Fabricius, 1787: 163.

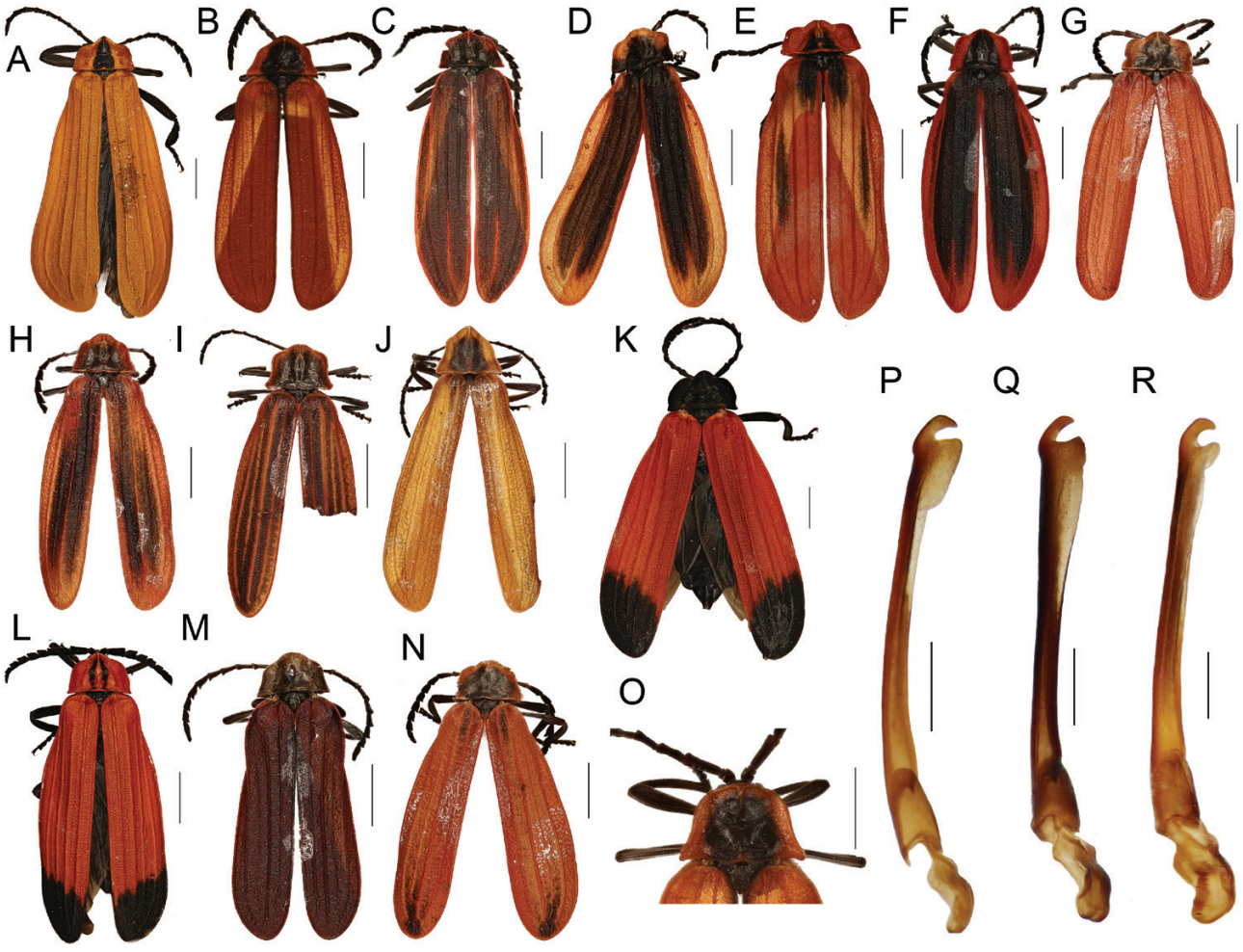

Figure 8. General appearance. A, *Lycostomus* sp. 3. B, C, *Lycostomus* sp. 4. D–F, *Lycostomus* sp. 12. G, H, *Lycostomus* sp. 16. I, *Lycostomus* sp. 18. J, *Lycostomus* sp. 19. K, L, *Lycostomus* sp. 8. M, *Lycostomus modestus* (sp. 13). N, *Lycostomus* sp. 19. O, pronotum and head, *Lycostomus* sp. 4. P–R, male genitalia. P, *Lycostomus* sp. 10. Q, *Lycostomus* sp. 9. R, *Lycostomus* sp. 8. Scale bars: 3 mm (A–O); 1 mm (P–R).

Type species: Pyrochroa palliata Fabricius, 1775.
= *Acantholycus* Bourgeois, 1883, syn. nov.

Type species: Lycus praemorsus Dalman, 1817.
= *Demosis* Waterhouse 1879, syn. nov.

Type species: Demosis peltatus Waterhouse, 1879.
= *Hololycus* Bourgeois, 1883, syn. nov.

Type species: Lycus intermedius Bourgeois, 1884.
= *Lopholycus* Bourgeois, 1883, syn. nov.

Type species: Lycus raffrai Bourgeois, 1877.
= *Chlamydolycus* Bourgeois, 1883, syn. nov.

Type species: Lycus trabeatus Guérin-Méneville, 1835.
= *Merolycus* Bourgeois, 1883, syn. nov.

Type species: Lycus rostratus Linnaeus, 1767.
= *Concavolycus* Marie, 1968, syn. nov.

Type species: Lycus maublanci Pic, 1933.
= *Alycus* Rafinesque, 1815 (objective synonym).

Type species: Pyrochroa palliata Fabricius, 1775.

Diagnosis

Lycus is highly variable in the shape of elytra (e.g. Figs 9–11), and most shapes are unique in the whole family. Most *Lycus* have, unlike *Haplolycus*, a short, robust phallus (Figs 9P–AM, 10T–AA). If the phallus is slender, then its apex is simple and without any cleft (Fig. 11R–T).

Redescription

Adults: Body slender. Head rostrate. Rostrum short to moderately long. Pronotum widest at base, with rectangular to slightly projected posterior angles, variable in shape. Elytra exhibit sexual dimorphism in most species, eventually moderately dilated posteriorly, with four indistinct costae in each elytron (Figs 9A–O, 10A–S, 11A–P). Male genitalia without thorns; phallus often with terminal processes (Figs 9P–AM, 10T–AA, 11R–T).

Morphological diversity of Lycus: There are several conspicuous types of elytra in *Lycus* that can be described in detail. The '*Acantholycus*-type' clade (Supporting Information, Fig. S1) contains species with the widened male elytra and elevated costa 3, which is sometimes projected at humeri in a thorn (Fig. 9E, I, J). The female elytra have a strong costa 3 but never any thorn, and their elytra are slender (Fig. 9D, H, L). The males display another trait, which has not been identified in other Lycini: the apical part of the elytra is projected in a posterolateral thorn, which marks the obtusely 'cut' apex (Fig. 9E, G). These elytra resemble the silhouette of resting moths, which commonly resemble Lycini and Calopterini (L. Bocak, field observation). We do not know whether the mimetic moths are palatable and how commonly they co-occur in the range of these species of *Lycus*. The Müllerian mimics should resemble each other, meaning that the unique shape of the *Acantholycus*-like elytron is a result of coevolution (either convergence or advergence, depending on the number of individuals and numerical dominance of co-mimics; Sherratt, 2008).

The '*Merolycus* type' is represented by *Lycus* sp. 51, with flat, leaf-shaped male elytra (Fig. 10K–M) and inconspicuously modified female elytra (Fig. 10N–Q).

The '*Chlamydolycus* type' is a clade of several closely related species that have extremely flat elytra with an apical process (Fig. 9J, K). These species have weak but apparent irregular transverse costae in elytra, and these resemble the venation of small, yellowish leaves of some shrubs in African savannahs (L. Bocak, field observation in Ethiopia). Although they are potentially inconspicuous when sparsely distributed on leaves, these beetles aggregate on flowers and are clearly visible from a distance.

The '*Lycus* type' contains species with globular elytra (Fig. 9C). As in other species, the females have much narrower elytra (Fig. 9D).

The terminal '*Lopholycus*-type' clade contains variable species, some of them with posteriorly widened male elytra and some with a thorn in the middle of the costa (Fig. 11A–F). A protective function can be expected if a thorn is short and sharp (Fig. 11B), but long, curved and weakly sclerotized thorns in some species can hardly have such a function (Figs 10R, 11Q).

Distribution

Afrotropical region (Fig. 5A).

Remarks

Most species can be assigned to subgenera described by earlier authors using external morphological characters and with the shape of male genitalia. For example, the males of *Acantholycus* have the humeral part of costa 3 projected in a thorn, which can be long and acute (Fig. 9E, I, K) and simultaneously, the species with such modified costa have the phallus with two finger-like processes (Fig. 9Z–AI). We could delimit a subgenus with

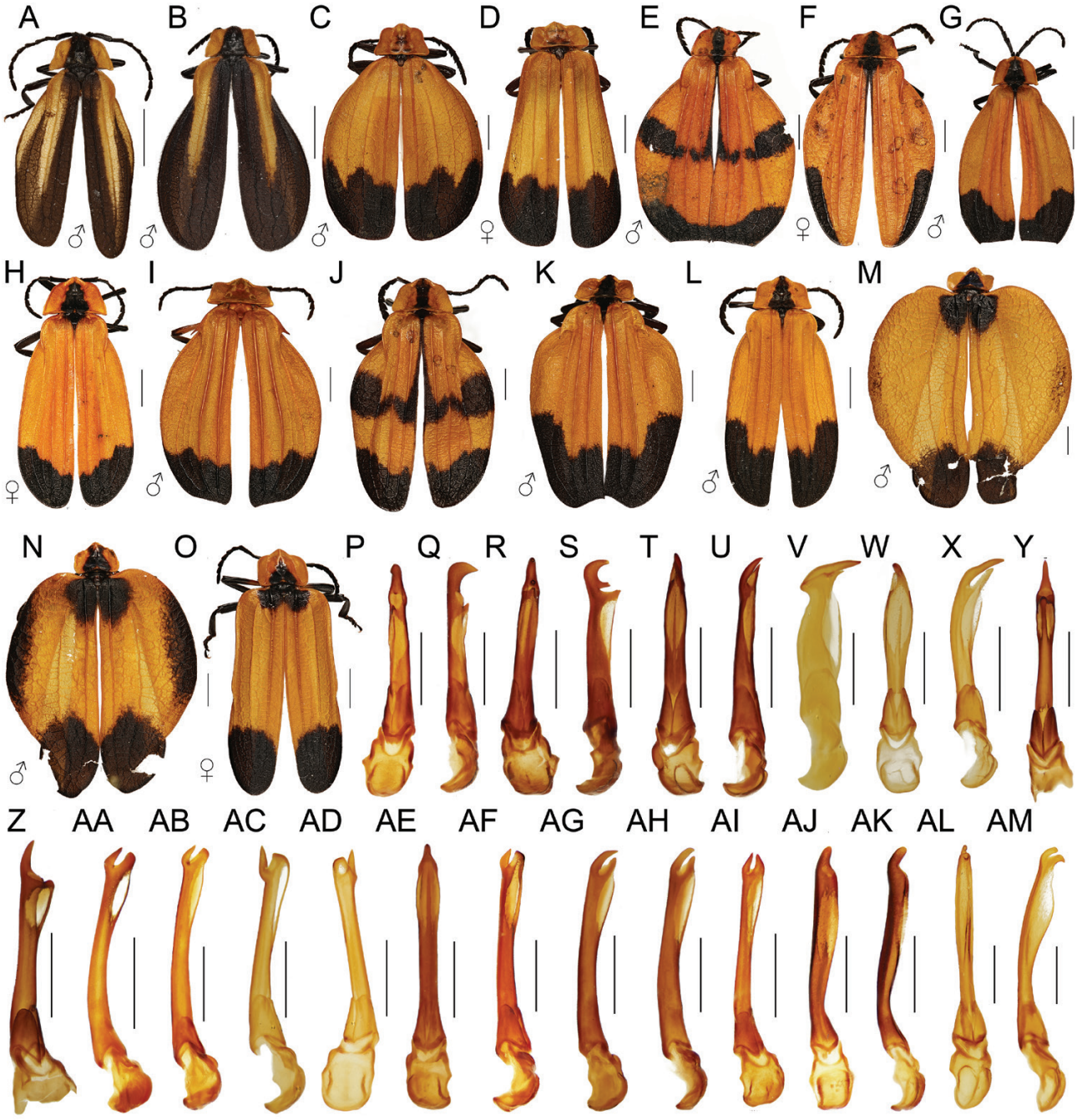

Figure 9. General appearance. A, B, *Lycus* sp. 32. C, D, *Lycus* sp. 36. E, *Lycus* sp. 6. F, *Lycus* sp. 8. G, H, *Lycus* sp. 10. I, *Lycus* sp. 15 J, *Lycus* sp. 6. K, L, *Lycus* sp. 22. M–O, *Lycus* sp. 68. P–AM, male genitalia. P, Q, *Lycus* sp. 33. R, S, *Lycus* sp. 34. T, *Lycus* sp. 36. U, V, *Lycus* sp. 29. W, X, *Lycus* sp. 32. Y, *Lycus* sp. 5. Z, *Lycus* sp. 20. AA, *Lycus* sp. 21. AB, AC, *Lycus* sp. 15. AD, *Lycus* sp. 6. AE, *Lycus* sp. 22. AF, *Lycus* sp. 6. AG, *Lycus* sp. 9. AH, *Lycus* sp. 6. AI, AJ, *Lycus* sp. 49. AK, AL, *Lycus* sp. 68. AM, Lycus sp. 59. Scale bars: 3 mm (A–O); 1 mm (P–AM).

these characters if we did not find several related species which do not have either the thorn in the elytron or the widened apical part of the phallus or both these characters (Fig. 9A–D, P–Y). We face a similar problem in the delimitation of other subgenera, and therefore, we prefer to discard all these subgenera from the formal classification. If subgenera were to be accepted in the current form, it would be necessary to merge unrelated, deeply rooted species in a polyphyletic subgenus that lacks

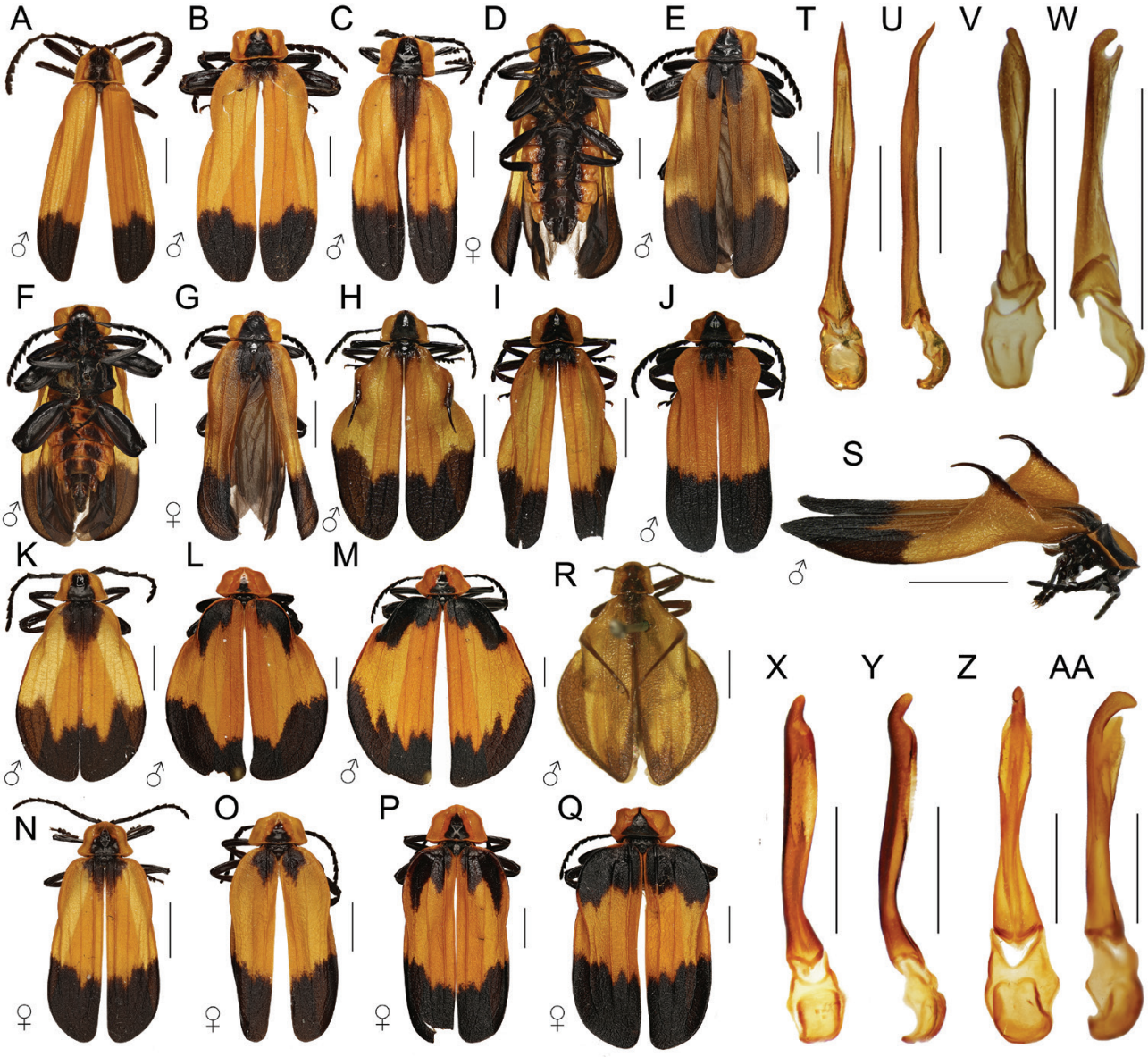

Figure 10. General appearance. A, *Lycus* sp. 39. B, *Lycus* sp. 42. C, *Lycus* sp. 40. D–G, *Lycus* sp. 43. H, I, *Lycus* sp. 47. J, *Lycus* sp. 49. K–Q, *Lycus* sp. 51;. R, *Lycus* sp. (absent in the analysis). S, *Lycus* sp. 47, lateral view. T–AA, male genitalia. T, U, *Lycus* sp. 47. V, W, *Lycus* sp. 23. X, Y, *Lycus* sp. 40. Z, AA, *Lycus* sp. 51. Scale bars: 3 mm (A–S); 1 mm (T–AA).

the distinct characters of the members of terminal lineages.

Demosis peltatus Waterhouse, 1879 was described from Botswana, and its genitalia indicate the relationships with *Lycus*. We propose that *Demosis* Waterhouse 1879 should be synonymized with *Lycus* Fabricius, 1801.

Celiasis Laporte, 1840

Celiasis Laporte, 1840: 263.

Type species: Lycus mirabilis Laporte, 1840.

Remarks

The genus name *Celiasis* has not been used except in old literature and catalogues (Bourgeois, 1883; Kleine, 1933; Blackwelder, 1945). This genus was proposed in a short note attached to the description of *Lycus mirabilis* Laporte, 1840 from Columbia. Laporte (1840) characterized it as an insect with a triangular head terminating in a point, an almost rectangular pronotum and dilated, convex elytra. The original specimen was in Laporte's collection destroyed in 1843 (Evenhuis, 2012), and we do not have access to any Columbian species resembling *Celiasis mirabilis* Laporte, 1840

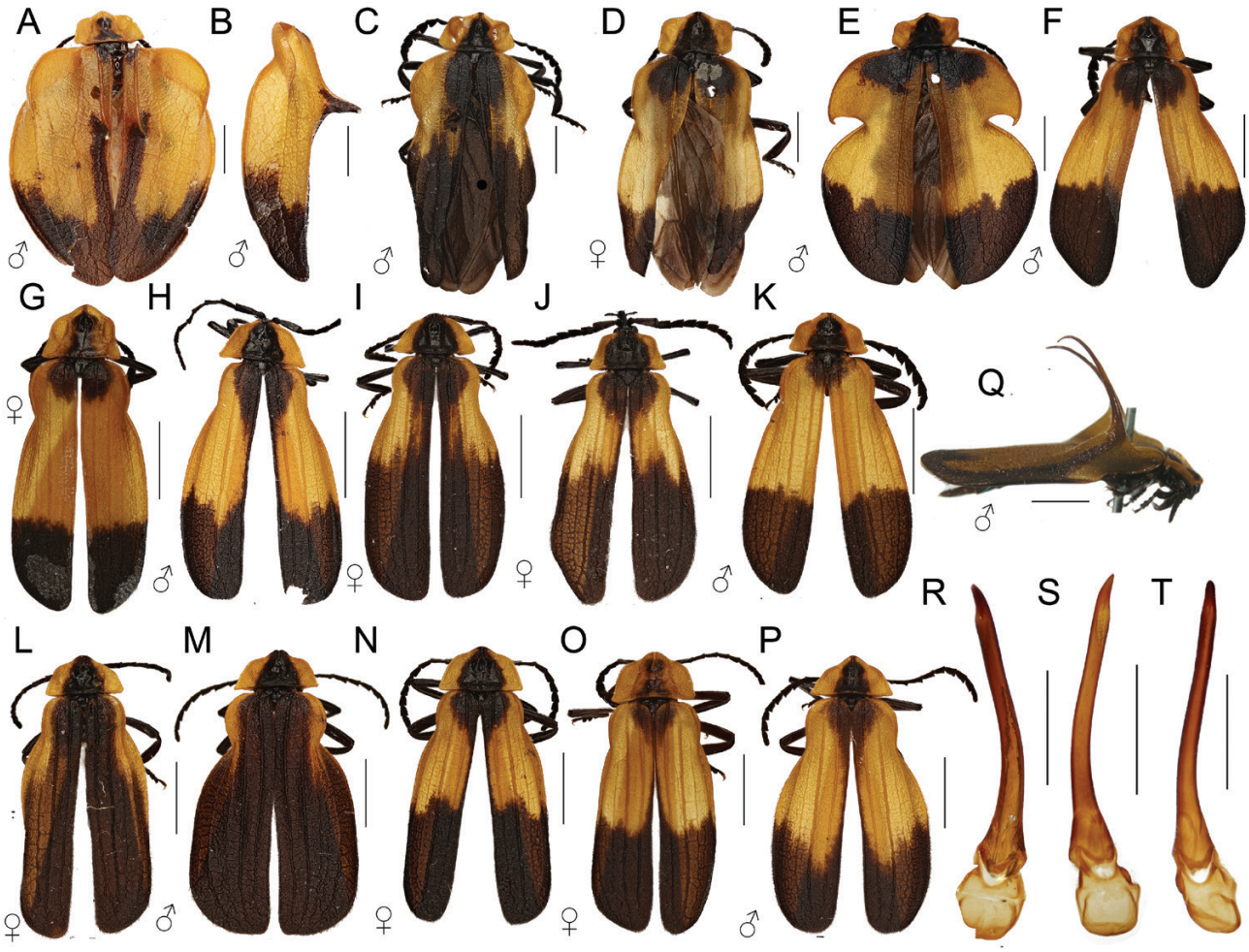

Figure 11. General appearance. A, *Lycus* sp. 60. B, ditto, elytron in the lateral view. C, *Lycus* sp. 61. D, *Lycus* sp. 62. E, *Lycus* sp. 62. F, G, *Lycus* sp. 66. H–J, *Lycus* sp. 71. K, L, *Lycus* sp. 72. M–P, *Lycus* sp. 73. Q, *Lycus* sp., lateral view (absent in the analysis). R–T, male genitalia. R, *Lycus* sp. 73. S, *Lycus* sp. 68. T, *Lycus* sp. 69. Scale bars: 3 mm (A–Q); 1 mm (R–T).

because this genus has never been reported again. Its distribution and phenetic similarity raise the possibility that *Neolycus* and *Celiasis* are congeneric. Given that we cannot designate the neotype, which would be necessary for the stability of the nomenclature, we have to keep this taxon as a *nomen dubium* in the classification. Only new specimens from Columbia can provide further information about its relationships to other Neotropical genera.

DISCUSSION

Relationships and classification

Here, we confirm the morphologically based delimitation of the tribe (Bocak & Bocáková, 1990; Masek *et al.*, 2018) and present the first attempt to reconstruct DNA-based generic relationships of Lycini (Fig. 3). The lycines commonly exhibit sexual dimorphism and are unpalatable; putatively, their morphology is affected by the complex evolution of affected traits (Motyka *et al.*, 2018; Jiruskova *et al.*, 2019; Bocek *et al.*, 2019a). Our molecular markers are not directly connected to phenotypes and can identify the convergent evolution of similar morphological traits. The present ribosomal and mitochondrial phylogeny is based on a large number of sequenced individuals and species from the whole range (Supporting Information, Table S2); nevertheless, 100 sequenced species in the dataset means that only one-quarter of species were studied (Table 1; Supporting Information, Table S2).

We identified six main lineages whose monophyly was supported by all analyses and we compared

KEY TO GENERA OF LYCINI

1. Rostrum very slender and long as in Figure 6Z, AA; terminal maxillary palpomere parallel-sided; all species known from Americas 2

   Rostrum wide basally, short; terminal maxillary palpomere widest apically; all species known from Asia, Africa and the western Wallacea 3

2. Male genitalia with pair of thorns in middle part of phallus (Fig. 6AB–AD); male with wide elytra which are only ~1.2 times longer than wide in the middle part (Fig. 6C) ..... *Neolycus*

   Male genitalia without pair of thorns in middle part of phallus (Fig. 6AE–AG); without sexual dimorphism; elytra widest usually apical third, even when widest in middle part, they are ≥ 1.5 times longer than wide in the widest part (Fig. 6U–X) ..... *Rhyncheros*

3. Phallus relatively short, with the part between tips of parameres and tip of phallus at most twice the combined length of parameres and phallobase; if phallus long, then very simple, extremely slender and apically pointed (Figs 9P–AM, 10T–10AA, 11R–T) 4

   Phallus long, with the part between tips of parameres and tip of phallus at least three times longer than parameres and phallobase combined (Figs 7AM–AR, 8P–R) 5

4. Phallus mostly robust, with simple apex, seldom cleft apically (Fig. 7AA–A L); all species known from Asia and western Wallacea ..... *Lipernes*

   Phallus with various processes apically (Figs 9P–AM, 10V–AA), if apical part simple, then phallus very slender and pointed (Figs 10T, U, 11R–T); all species known from Sub-Saharan Africa and the southernmost Arabian Peninsula ..... *Lycus*

5. Apical part of phallus simple or shallowly emarginate (Fig. 7AM–AR); Sub-Saharan Africa .... *Haplolycus*

   Apical part of phallus deeply emarginate (Fig. 8P–R); Asia, western Wallacea, northern Africa .... *Lycostomus*

Celiasis cannot be distinguished from *Neolycus* and, given that no specimen is available, this genus was not included in the key.

our results with the limits of morphology-based genera and subgenera. The lycine classification and morphology-based diagnoses have been presented in the taxonomy section above. We assigned the genus rank to the following lineages: *Neolycus*, *Rhyncheros*, *Lipernes*, *Haplolycus*, *Lycostomus* and *Lycus* (*Celiasis* is kept as *nomen dubium* in the classification because no specimen is available and Laporte's type was lost). The modifications of elytra are very apparent and distinct in some species, but they are absent, or their presence is contentious, in a number of closely related species. All modifications except elytral thorns have unique origins (Figs 9I, 11A, E, Q), but if characters such as the shape of elytra or coloration were accepted as diagnostic characters, many species would have to be merged in collective paraphyletic taxa, which cannot be accepted in the phylogenetic classification (Fig. 3; Supporting Information, Figs S1–S3). Therefore, the traditional subgenera do not deserve their subgenus rank and are synonymized to *Lycus s.s.*

PHYLOGEOGRAPHY

The Lycini have already been considered as close relatives of the dominantly Neotropical Calopterini, but the evidence was based on a few morphological characters and weakly supported by Sanger data analyses (Bocak & Bocáková, 1990, 2008; Bocakova, 2003, 2005; Kazantsev, 2018; Masek *et al.*, 2018). The phylogenomic analysis by Kusy *et al.* (2019) robustly supported the relationships of these two groups and additionally assigned the Platerodini and Leptolycini to the same clade. The study was based on ~4200 orthologues with several million positions for each lineage of Lycinae, and the clade of Platerodini, Leptolycini, Calopterini and Lycini was robustly supported. The Calopterini are endemic to the New World, and Leptolycini and Thonalmini are endemics of the Caribbean islands (Masek *et al.*, 2018). There are several platerodine genera known from the Neotropical region, and the Platerodini could start their diversification in the same region (Bocáková, 2001). Based on these results, we analysed

the area of the origin and dispersal routes of Lycini with Platerodini and Calopterini as their sister lineages (Fig. 4; Supporting Information, Fig. S4). The analyses recovered the common ancestor of the Lycini + Calopterini in the New World and gave ~50% probability to the origin of the Lycini in the Nearctic region. Our results point to southwestern Laurasia or the northern part of South America as putative ancestral areas of the Lycini + Calopterini clade. The early phase of the diversification is estimated to the Lower Oligocene, ~33 Mya, which is known for extensive connections between landmasses and a drop in the global sea level (34–30 Mya; Haq *et al.*, 1987; Zachos *et al.*, 2001; Hall, 2002). We suppose that these conditions enabled the rapid expansion of the range of the Lycini (Fig. 5A) and a slightly higher diversification rate identified in this phase of their evolution (Supporting Information, Fig. S5). The period of low Early Oligocene sea levels was independently inferred as crucial for the dispersal of the neotenic net-winged beetles, which are well known for their limited dispersal propensity (Masek et al., 2014; Bray & Bocak, 2016). The modern diversity of this tribe evolved recently, with the majority of species dated to the last 20 Myr (Supporting Information, Figs S4, S7).

The deepest lycine split separates two New World genera, i.e. *Neolycus* and *Rhyncheros*, and the clade of Asian and Afrotropical genera, i.e. *Lipernes*, *Lycostomus*, *Haplolycus* and *Lycus*. The analyses suggest a single westward dispersal event from Asia to Africa in the Mid to Upper Oligocene, but owing to the absence of the Lycini in a large span between western India and western Yemen, we can hardly estimate in which area a single species, which gave the origin to *Lycostomus*, reversed the direction of dispersal to the east (Fig. 4). The absence of *Lycostomus* in the Afrotropical region and the delayed start of the radiation of *Lycostomus* compared with *Haplolycus* and *Lycus* (*Lycostomus* 17.19 Mya, *Haplolycus* 26.89 Mya and *Lycus* 23.78 Mya) support a possibility that the ancestor of *Lycostomus* never reached Africa and returned to Southern and Eastern Asia from some area west of India, which has now a depauperate beetle fauna owing to very dry conditions. We have not found any evidence in our analyses for the separate colonization of Africa by *Lycus* and *Haplolycus*, which could be considered as an alternative scenario owing to their terminal position in the tree (Fig. 3; Supporting Information, Figs S1–S3).

Almost three-quarters of the Lycine species have been described from Africa (~320 spp.), and most of them are members of the *Lycus* clade (Fig. 2). Nevertheless, our speciation rate analysis did not indicate any change in speciation rate connected to the colonization of the Afrotropical region (Supporting Information, Fig. S5). The analysis of the speciation rate is complicated by highly unreliable information on the number of species in each region and the insufficient sampling, which represents only one-quarter of the described diversity of the group. Further taxonomic and zoogeographical studies should clarify whether the speciation rate in *Lycus* increased after it colonized semiarid Africa compared with their relatives, which remained in Southern and eastern Asia, where they lived in very different ecosystems and competed with other net-winged beetle lineages. Despite the limitations mentioned, the present results support the earlier finding that the Lycini fauna of Africa is relatively young and that some lineages that colonized Africa possibly used the route opened by the presence of wet forests along the northern coast of the Tethys Sea (Kosuch *et al.*, 2001; Yu *et al.*, 2014). The Lycini supposedly colonized the continent simultaneously with the Calochromini (a single dispersal event 29 Mya; Motyka *et al.*, 2017), but much later than Metriorrhynchini: Cautirina (65 Mya; Sklenarova *et al.*, 2013).

Presence of sexual dimorphism

The origins of the sexually dimorphic width and shape of elytra have never been investigated in Lycini, and they can be connected with intrasexual competition and mate choice, as in other beetles (Emlen & Nijhout, 2000; Jennions *et al.*, 2001). We found that most deeply rooted clades, i.e. *Rhyncheros*, *Lipernes*, *Haplolycus* and *Lycostomus*, have a similar shape of elytra in both sexes (Figs 6T–X, 7A–Q, AR, 8O). Although the humeral part of the elytral costa 3 is also conspicuous in these genera, it never has a form, which would substantially alter the silhouette of the elytron (Figs 6T–X, 7A–Q, 8A–O). The pronounced sexual dimorphism is present only in distantly related *Neolycus* and *Lycus*, but within *Lycus* there are numerous non-dimorphic subclades, such as *Lycus* sp. 32 (Fig. 9A, B) and *Lycus* spp. 39–43 (Fig. 10A–G). The non-dimorphic species are dispersed among the restricted terminal clades with the very pronounced sexual dimorphism (Supporting Information, Fig. S1). If sexual dimorphism is present, the males have wide either convex or strongly flattened elytra (e.g. Figs 9C, E, M, N, 10L, M). Additionally, some peculiar structures can be present. The modifications include elevated humeral costae (e.g. Figs 9B, J, 10J, L, 11G, M), thorns at the humeri (Figs 9E, I, K, 10R, S, 11E, Q) and a thorn in the middle of the elytron (Fig. 11A, B). The estimated phylogeny suggests that a similar shape, in addition to various thorns, evolved multiple times in Lycini and *Lycus*, respectively (Supporting Information, Fig. S1). Although we observe the common origin of sexual dimorphism only in *Neolycus* and *Lycus*, we must consider besides the phylogenetic

structure of this trait also a possibility that its origin is ecologically driven. North American *Neolycus*, in addition to Afrotropical *Lycus*, occur commonly in masses, and their large numbers and aggregations on flowers might support the evolution of widened elytra producing a conspicuous aposematic signal (Riipi *et al.*, 2001).

We found a continuous intraspecific variation in the width of elytra and the strength of humeral thorns in some *Lycus* (e.g. Figs 10K–Q, 11M, P). The evolution of such sexually selected structures is supposed to be costly (Emlen & Nijhout, 2000; Jennions *et al.*, 2001), and we observed an allometric growth of some processes and the expansion of elytra (Figs 10K–M, 11C, E). The small-bodied males can be substantially different and might be identified incorrectly as separate species (e.g. Fig. 10K, M; Chown & Stamhuis, 1992). The phenotypic variability is not limited to males. Although limited if compared with males, similar differences were found in females of *Lycus* sp. 51. The aberrant females have parallel-sided elytra (Fig. 10Q) and differ substantially from conspecific females that are morphologically similar to other *Lycus* species (Fig. 10N–P).

Mimicry, morphological variability and alpha taxonomy

Non-lycid prey tends to resemble local unpalatable Lycini (Linsley *et al.*, 1961; Rowe *et al.*, 2004). We identified the occurrence of distinct aposematic patterns in various regions, and the Lycini clearly form a part of local mimetic complexes. As a result, almost all Oriental Lycini are coloured red and similar in body shape (Figs 7A–O, 8A–N). In contrast, all Afrotropical species are coloured yellow and black, and many of them have considerably expanded elytra (Figs 7P, Q, 10A–Q, 11A–P). Simultaneously, we found that a similar colour pattern can evolve in unrelated taxa and distant regions (Figs 6S, 7P, Q, 9I, 10A, 11O, P). Using the general appearance as a diagnostic character, the Nearctic species of *Neolycus* have been placed, owing to superficial similarity, in *Lycus* as a subgenus (compare Figs 6S, 9I) or an Arabian *Lycus* was placed in *Lycostomus* (Kazantsev, 2018). The levels of colour and shape variation have not been analysed by previous taxonomists, and only Chown & Stamhuis (1992) described intraspecific variability in two putative species and synonymized one of them. We observed both local intrapopulation variability and the geographically structured distribution of similar Müllerian phenotypes, as has been documented in other net-winged beetles (Eisner *et al.*, 2008; Motyka *et al.*, 2018). The Afrotropical fauna, in particular, is very diverse, and high numbers of species co-occur in a single area and resemble each other.

We showed that the aposematic elytral patterns and the colour of the pronotum can be highly variable within a species, and the transverse dark bands often disintegrate in separate patches (e.g. Figs 8K, L, 10K–Q, 11M–P). Likewise, we recovered high intraspecific variability in the shape of elytra (Fig. 10K–Q). The evaluation of intraspecific diversity shows that in some cases, intrapopulation phenotypic variability might be higher than the distinctiveness used earlier for the delimitation of separate species and questions the earlier definitions based on such plastic traits. When the genital morphology was ignored, as in all taxonomic work by M. Pic (1922, 1923, 1930) and recently by Matojo (2014), aberrant intraspecific forms might have been described as separate species. Among researchers working on Lycini, only Kleine (1926, 1937) illustrated male genitalia, but the very low diversification of male genitalia in some closely related species (e.g. Fig. 11R–T) limits their diagnostic value. The differences are often so subtle that the delimitation of a species can be contentious.

We do not revise the alpha taxonomy of Lycini because we are unable to locate all types, some of them being females without any applicable diagnostic characters. Additionally, more information is needed to discriminate between genetically isolated populations and the lineages deserving species status if they differ only in the external characters that are strongly affected by the selection for similarity and sexual dimorphism (Rowe *et al.*, 2004).

Here, we present an attempt to solve the present situation by the DNA-based taxonomy that, at least at a genus-rank level, can provide the basis for the phylogenetic classification (Tautz *et al.*, 2003). We are not able to say whether the numbers of species are inflated, but over-description and confused taxonomic scenarios are highly probable. A similar situation is common in net-winged beetles (Bocek *et al.*, 2019b), and the simultaneous application of multiple methods and evaluation of the congruence of variously based species limits are highly desirable in further studies (Carstens *et al.*, 2013; Sanchez & Cassis, 2018). We suggest that further sequencing of the diversity of Lycini will be needed before the alpha taxonomy of this group reaches some acceptable state.

Conclusion

No phylogenetic studies have focused on relationships within the Lycini tribe, and our aim was to propose the natural genus-rank classification of the Lycini. We redefine the six genera and synonymize two genera and seven subgenera. One genus, *Celiasis*, is kept as a *nomen dubium*. Based on the molecular phylogeny,

we recovered that the North and South American lineages *Rhyncheros* and *Neolycus* originated early in the evolution of Lycini. Asian *Lipernes* is a sister to the clade of Afrotropical and Asian *Haplolycus*, *Lycostomus* and *Lycus*. The previous classifications were strongly affected by the superficial morphological similarity, which can be a result of convergent evolution in mimetic complexes and multiple origins of sexual dimorphism.

The range of the Lycini covers five continents, but the distribution of all genera is limited to a restricted part of the tribal range, usually a continuous continental landmass. We note the difference between the areas where Lycini are most common and where they are represented by the highest number of species, i.e. the Afrotropical region, and the areas where the deepest lineages occur, but a much lower number of species, i.e. Nearctic region and East to Southeast Asia. The African fauna is represented by two terminal clades, and the colonization of Africa was a late event in the evolution of Lycini.

The search for all type specimens, including the female holotypes, which do not have any applicable diagnostic characters, the preparation of a catalogue, the critical evaluation of species limits and the identification of putative repeated descriptions of some highly variable and widespread species is highly desirable but beyond the scope and extent of the present study. As mentioned earlier, the upcoming revision of *Lycus* was announced as early as the late 19th century, but never published (Bourgeois, 1883). As an immediate next step, we should try to obtain more extensive molecular data, which would cover unsampled or undersampled regions, such as Brazil, Colombia, Ecuador, Mesoamerica, Sri Lanka, India, West Africa, Congolian Basin and East African Highlands (Supporting Information, Table S2). Unfortunately, some regions are 'protected' from taxonomic research by strict national laws based on the Nagoya Protocol (Prathapan *et al.*, 2018) and some are inaccessible for other reasons. As soon as possible, the new, densely sampled molecular phylogenies should be combined with a thorough investigation of primary types in Pic's, Kleine's and Bourgeois' collections. With the described limitations in sampling, the results presented here can be used for taxonomic, zoogeographical and evolutionary studies, because the morphological diversity has already been connected with DNA-based relationships (Figs 6–11). The study shows the importance of integrative DNA taxonomy (Tautz *et al.*, 2003); the present data can be expanded, and some clades can be studied separately. As a result, we should be able gradually to build the species-level fully labelled phylogenetic tree of this highly diverse tribe.

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CONFLICT OF INTEREST

We have no competing interests.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. The original classification of Lycini.
Table S2. Taxa included in the analysis, with geographical origins, voucher and GenBank accession numbers.
Table S3. Primers and conditions used for polymerase chain reaction amplification.
Table S4. Characteristics of concatenated supermatrices and used models of DNA evolution.
Table S5. Characteristics and results of the BioGeoBEARS analysis implemented in RASP.
Figure S1. Maximum likelihood tree recovered by the analysis of the full dataset. Outgroups were omitted from the figure and are shown in the main text (Figure 2).
Figure S2. The maximum likelihood tree recovered by the analysis of the reduced dataset.
Figure S3. Bayesian tree recovered by the analysis of the reduced dataset.
Figure S4. Time-calibrated maximum clade credibility tree computed in BEAST from the reduced dataset, with the topology constrained to the results of the maximum likelihood analysis.
Figure S5. Results of the macroevolutionary rate analysis in BAMM representing changes in diversification dynamics through time. Branch colours indicate estimated diversification rates, with warmer colours assigned to lineages with higher values.
Figure S6. Speciation-through-time plots recovered using BAMM. The red line represents median values with 95% confidence intervals.
Figure S7. Lineage-through-time (LTT) plot averaged over 20 000 posterior trees from the Bayesian analysis.

PART VIII

Studies on neoteny in Coleoptera: phylogenomics, classification, and evolutionary interactions

Dominik Kusý

Enigmatic Campyloxenus: Shedding light on the delayed origin of bioluminescence in ancient Gondwanan click beetles.

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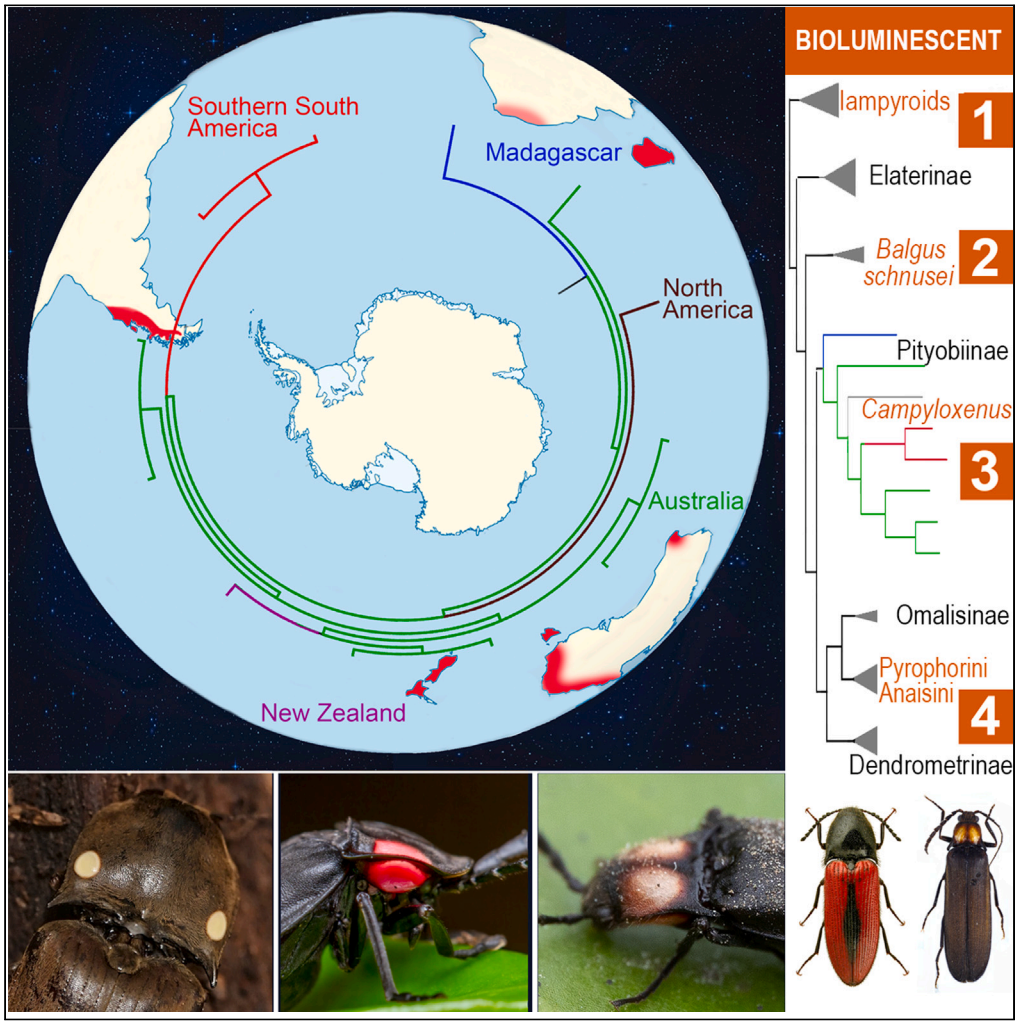

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Highlights

We studied bioluminescence and relationships among Gondwanan click beetles

The newly hypothesized clade of elaterid Gondwanan lineages contains ancient groups

Campyloxenus unveils a separate, recent origin of bioluminescence in elateroids

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Enigmatic *Campyloxenus*: Shedding light on the delayed origin of bioluminescence in ancient Gondwanan click beetles

Michal Motyka,[1,4] Dominik Kusy,[1,4] Elizabeth T. Arias-Bohart,[2] Seth M. Bybee,[3] and Ladislav Bocak[1,5,*]

SUMMARY

Gondwanan elaterids, previously thought to be unrelated, include bioluminescent *Campyloxenus* earlier placed in bioluminescent Pyrophorinae. Genomic data suggest close relationships between Gondwanan groups. We maintain Morostomatinae and Hapatesinae and redefine Pityobiinae with Nearctic Pityobiini, Gondwanan Parablacini stat. nov., Campyloxenini stat. nov., and Tibionemini trib. nov. Their ancestors putatively underwent differentiation in Gondwana during the Cretaceous separation of southern continents. In contrast with their age, extant groups are species poor. *Campyloxenus* represents a recent origin of bioluminescence, no older than ~53 my. Its large pronotal lanterns differ from Pyrophorini and resemble color patches of sympatric beetle co-mimics. This discovery highlights the fourth or fifth origin of bioluminescence in Elateroidea, alongside the lampyroid clade, click beetles Pyrophorini, *Alampoides* and *Coctilelater* in Anaissini (Pyrophorinae), and *Balgus schnusei* (Thylacosterninae). While our phylogenetic findings illuminate the phylogenetic aspects, the complete story awaits further field observations and in-depth genomic analyses of biochemical pathways used by bioluminescent elateroids.

INTRODUCTION

Elateridae stands as the largest family within the Elateroidea, boasting over 10,000 species distributed worldwide. Approximately twenty elaterid subfamilies have been recognized.[1–6] The subfamilies Elaterinae, Agrypninae, and Dendrometrinae hold thousands of species and have truly global distribution. In contrast, some subfamilies are somewhat limited in their diversity, being monogeneric or containing only a few genera with a handful of species. In this study, our focus centers on five such species-poor, morphologically diverse, and predominantly Gondwanan subfamilies: Hapatesinae, Pityobiinae, Morostomatinae, Campyloxeninae, and Parablacinae.[1,2] Our research delves into the origins, relationships, and zoogeography. Additionally, our investigation aims to shed light on the evolutionary origins of bioluminescence in click beetles as one of the Gondwanan lineages, *Campyloxenus*, possesses pronotal light-producing lanterns.

Phylogenetic relationships and classification

The actual classification stems from a prolonged period of taxonomic instability, a result of discrepancies between morphological and, later, short-fragment molecular analyses.[1,2,7–9] Morostomatinae was established by Dolin[10] and encompasses several originally dendrometrine genera from Madagascar. Hapatesinae, proposed recently by Kusy et al.,[11] groups two initially dendrometrine genera.[12,13] Costa[14] created the monogeneric Campyloxeninae, excluding *Campyloxenus* Fairmaire & Germain from Agrypninae. While its exact position relative to other click beetle subfamilies remained unclear, its status as a separate subfamily was rarely questioned.[7] Throughout the years, the concepts of these subfamilies have remained clear, and their generic composition has shown stability.

On the contrary, the boundaries of Pityobiinae have undergone substantial shifts over time. Initially, Hyslop[15] established the monogeneric Pityobiini as a Nearctic group within Agrypninae. It was only much later that the group began to expand, with additional taxa altering the perception of pityobiines and their placement.[7,16] Crowson[17] hypothesized a connection between *Metablax* Candèze from New Zealand and North American *Pityobius* LeConte. Subsequently, Dolin[18] expanded the pityobiines to include Australian *Parablax* Schwarz and South American *Tibionema* Solier. Calder[16] added further Australian genera, and, more recently, Arias-Bohart & Elgueta[19] described *Sharon* Arias-Bohart & Elgueta from Chile. Eventually, the parablacine genera were transferred to a separate subfamily, Parablacinae,[2] causing Pityobiinae to shrink to only *Pityobius* and *Tibionema*. Nonetheless, *Pityobius* and *Tibionema* exhibit distinct morphological characteristics, warranting further investigation into their relationship.[3]

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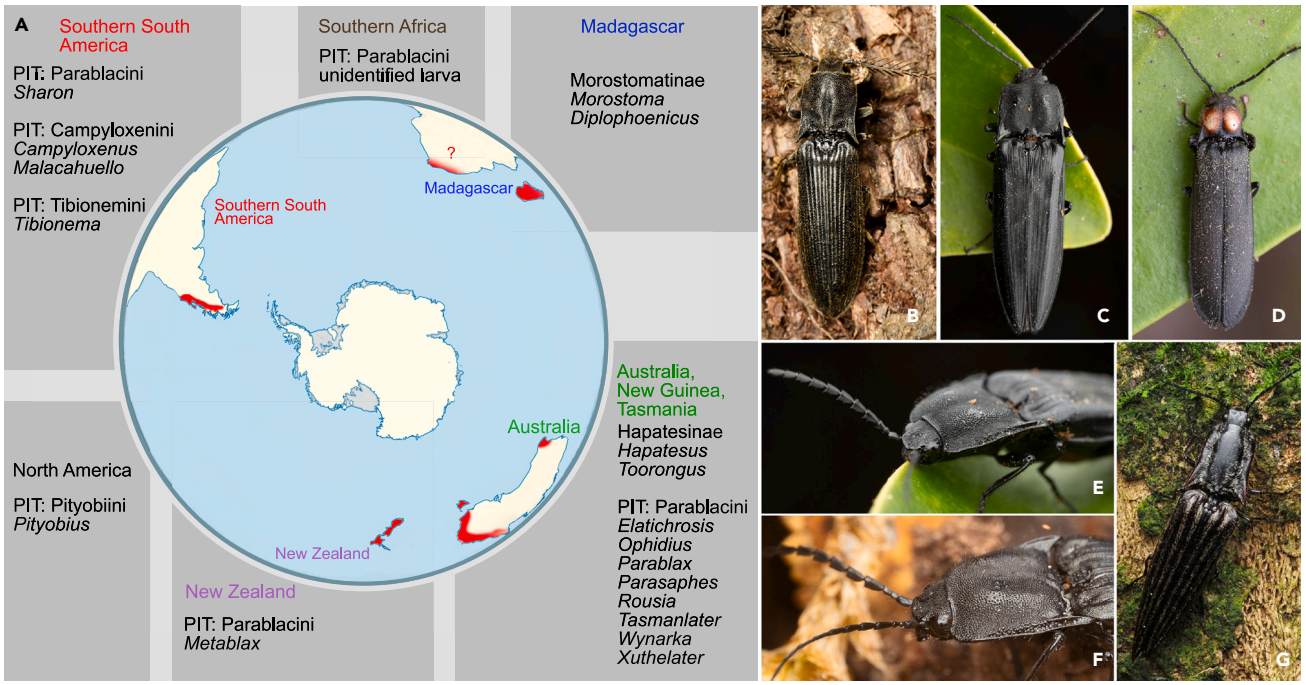

Figure 1. Distribution and diversity of focal groups

(A) The modern distribution of focal subfamilies in the Southern Hemisphere (distribution of Nearctic *Pityobius* not shown). Representatives of pityobiine lineages in nature: (B) *Pityobius anguinus* (photo by Chris Rorabaugh, USA, FL), (C, E, and F) *Tibionema abdominalis* (photo by Matías Gargiulo, Chile), (D) *Campyloxenus pyrothorax* (photo by Matías Gargiulo, Chile), and (G) *Metablax* sp. (photo by Lance Wakefield, New Zealand). Abbreviation. PIT – Pityobiinae; constituent tribes spelled in full.

Based on morphology (Figures 1B–1E), the relationships among these subfamilies remained uncertain. Previous studies proposed various positions for Pityobiinae, suggesting it as a sister to the rest of Elateridae (including Eucnemidae and Throscidae), closely related to Agrypninae or Oxynopterinae (now Dendrometrinae: Oxynopterini), and potentially included within Dendrometrinae.[7,9,16,20,21] Molecular studies on Gondwanan groups were limited initially. Morostomatinae was placed as a sister to Agrypninae,[22] a sister to Dendrometrinae, Negastriinae, and Cardiophorinae,[2] or as the next branching event after the separation of Hapatesinae + Pityobiinae.[11] Kundrata et al.[2] proposed Parablacinae as a separate subfamily based on its redefinition. *Pityobius* was hypothesized as a sister to the Lissominae + Thylacosterninae clade, and Parablacinae formed a clade of four genera following Hemiopinae + Oestodinae and preceding *Panspoeus guttatus* Sharp. A mitogenomic analysis merged *Hapatesus*, *Parasaphes*, and *Tibionema* into a single clade,[11] and the latest phylogenetic study on click beetles reaffirmed these relationships when other taxa were unavailable.[3] Thus, all analyses support the distinctiveness of these click beetles and their early origin, although their exact mutual relationships remain contentious.

Zoogeography

The poor representation of rare and endemic Gondwanan lineages in phylogenetic studies has been a long-standing problem of beetle phylogenetics.[23–25] Concerning click beetles, Douglas et al.[3] also called for including the Southern Hemisphere's fauna in the analyses. The Gondwanan groups are species poor, yet they are essential for understanding the click beetle evolution due to their ancient origins and the high taxonomic ranks in the earlier studies.[1]

Apart from *Pityobius*, all genera within these families exhibit a Gondwanan distribution (Figure 1A). Morostomatinae is endemic to Madagascar and comprises eight genera, including *Morostoma* Candèze and *Diplophoenicus* Candèze. Hapatesinae, with two genera, is predominantly found in Australia, with some species recorded in New Guinea.[9,11–13] Parablacinae is mainly distributed in Australia and New Zealand (Figure 1A), with eight genera, while one genus, *Sharon,* was recently described from South America[2,9,26] (Figure 1G). Pityobiinae *sensu* Douglas et al.[3] includes two genera, *Pityobius* from North America (Figure 1B) and *Tibionema* from South America (Figures 1C, 1E, and 1F). Two monotypic Campyloxeninae genera are known from Chile and Argentina[26] (Figure 1D).

Various earlier published topologies suggested that the Gondwanan groups are distantly related. It kept open the possibility that the Gondwanan fauna is highly phylogenetically diverse. Elaterids are indeed ancient enough to exist before the Gondwana breakup. The abundant fossil record can be traced back to the Triassic of Britain,[27] the Jurassic of Kyrgyzstan and China,[28,29] the Upper Cretaceous of the Jinju Formation,[30–33] Cretaceous deposits in Russia,[34] and Mid-Cretaceous amber.[35] Although some Mesozoic elaterids were transferred to other families, it is widely accepted that click beetles were diverse already in the Late Jurassic/Early Cretaceous and predate the split of Gondwana.

Pityobiinae is also present in the fossil record,[36] and *Cretopityobius* Otto was reported from Cenomanian Burmese amber, which contains both Gondwanan and Laurasian fauna. Therefore, pityobiines are ancient enough to consider the hypothesis of the ancestor in Gondwana when it separated from the northern continents.[37]

Bioluminescence

Recently, the bioluminescence of elaterids has been a hotly debated topic.[38–41] The luciferin-luciferase reaction, interactions in predator-prey systems, chemoecology of protective compounds, and genes critical in beetle bioluminescence were studied by various authors.[42–46] However, disagreements persist among systematists regarding the phylogenetic relationships of crucial lineages.[3,4,47,48] Notably, some recent studies discussed only the origin of bioluminescence in Pyrophorini and did not comment on non-pyrophorine groups.[38,41] We assert that a comprehensive exploration of the phylogenetic origins of bioluminescence is imperative to provide a robust foundation for further genomics research.

All bioluminescent elateroids are concentrated in the elaterid-lampyroid clade proposed by Kusy et al.[4] Over 2,000 bioluminescent species form the lampyroid group consisting of Sinopyrophoridae, Lampyridae, Phengodidae, and Rhagophthalmidae. According to Kusy et al.'s 4,200 ortholog phylogeny,[4] this clade emerges as a sister to elaterids. Alternatively, the clade Lampyridae + Phengodidae + Rhagophthalmidae occupies the terminal position in elaterids when *Sinopyrophorus* was absent in the analysis.[3] Under both scenarios, it is an ancient group with no later than an early Cretaceous origin of a bioluminescent most recent common ancestor.[39] The subsequent clade of bioluminescent taxa encompasses Pyrophorini in Agrypninae and eventually Anaissini (only *Alampoides* Schwarz and *Coctilelater* Costa). The latter tribe involves *Alampoides* known for bioluminescent larvae,[49] yet its relationship to Pyrophorini necessitates further confirmation. The age of the Pyrophorini clade would be notably younger compared to the lampyroid clade if lampyroids are regarded as elaterids' sister group,[4] while it could approximate contemporaneity if the lampyroid clade is positioned terminally within click beetles.[3] *Balgus schnusei* (Heller) stands as the solitary bioluminescent species within the otherwise non-luminescent *Balgus* Fleutiaux of the Lissominae-Thylacosterninae clade.[50,51] *Balgus schnusei* possibly represents the most recent origin of bioluminescence in Elateroidea.

Campyloxenus, with its orphan position in the elaterid classification,[14] is the last potential candidate for an independent bioluminescence origin. The sole known species within *Campyloxenus* is named "*pyrothorax*."[52] Although it is unlikely that the species epithet's creators had observational data and no subsequent information on its luminescence has been reported in the literature, *C. pyrothorax* Fairmaire & Germain has always been designated a bioluminescent click beetle. Its bioluminescence initially contributed to its placement within Agrypnini, a tribe that houses most bioluminescent genera of Elateridae.[7,52,53] Costa,[14] upon revising pyrophorine genera, identified morphological distinctions warranting establishing a distinct subfamily. However, the closest relatives of Campyloxeninae have never been hypothesized.[1] A non-luminescent *Malalcahuello* Arias-Bohart was recently described within Campyloxeninae.[26]

In this study, we undertake the first assessment of the placement of bioluminescent *Campyloxenus*, which has, at times, been linked closely with other bioluminescent elaterids.[7,54] With the newly shotgun-sequenced elaterid genomes from Gondwanan continents, our objective also addresses fundamental zoogeographic inquiries, including the possibility of a shared ancestor among Gondwanan elaterid lineages and their estimated age. The analyses also aim to improve click beetle classification as relationships of Gondwanan lineages are poorly understood. The incomplete and highly unstable click beetle classification negatively affects further research in bioluminescence and other interesting evolutionary phenomena.

RESULTS

Molecular analyses

We shotgun-sequenced eight click beetle taxa and extracted orthologs for phylogenetic analyses. The AliStat completeness of the 66-ortholog dataset was 0.79 (Figure S1). The SymTest analyses unveil a high compositional heterogeneity among click beetle in the nucleotide (NT) dataset but low in the amino acid (AA) dataset (Figure S2). The aligned orthologs were analyzed with maximum likelihood (ML) and coalescent approaches to identify relationships among Gondwanan subfamilies, *Pityobius*, and other click beetles. Half of the analyses (i.e., ML analyses at the AA level, partitioned and unpartitioned, and the ASTRAL analysis at the AA level) suggest the clade of Nearctic *Pityobius* (Pityobiinae: Pityobiini) and four traditional subfamilies with Gondwanan distribution—*Diplophoenicus* (Morostomatinae) *Tibionema* (currently Pityobiinae), *Hapatesus* (Hapatesinae), *Elatichrosis*, *Parasaphes*, *Rousia* (currently Parablacinae), and *Campyloxenus* (currently Campyloxeninae) (Figures 2A and S3–S6). This clade is deeply rooted, and it is a sister to the Omalisinae + Dendrometrinae + Agrypninae clade in most analyses. Alternatively, *Diplophoenicus* (Morostomatinae) shifts from the first split of the previously recovered clade to the base of its sister, i.e., the Omalisinae, Agrypninae, and Dendrometrinae clade (both ML analyses at the NT level; Figures 2A and S3–S5) or to the base of the *Diplophoenicus* + Dendrometrinae clade (the coalescent analysis at the NT level; Figure S5), when the base of the whole clade (local posterior probability, LPP 99) is recovered as a series of very short branches with low support (LPP values 0.5 and 0.41, the coalescent analysis at the NT level; Figure S5).

The first lineage to split was *Diplophoenicus*, and its inclusion in the recovered clade consisting of Gondwanan subfamilies and *Pityobius* was generally weakly supported in both hypothesized positions (bootstrap support, BS 53%–100%; Figures S3–S5). Given the poor support in ML and coalescent analyses, we performed a quartet likelihood mapping analysis using all 66-gene datasets at AA and NT levels to determine the most probable position of Morostomatinae (Figures 2B–2D). Both analyses preferred the sister relationships of Morostomatinae and the *Pityobius*/Gondwanan subclade, but the differences between the numbers of quartets supporting alternative topologies are low (44% versus 32% at the AA level and 42% versus 35% at the NT level). Additional tests also did not provide

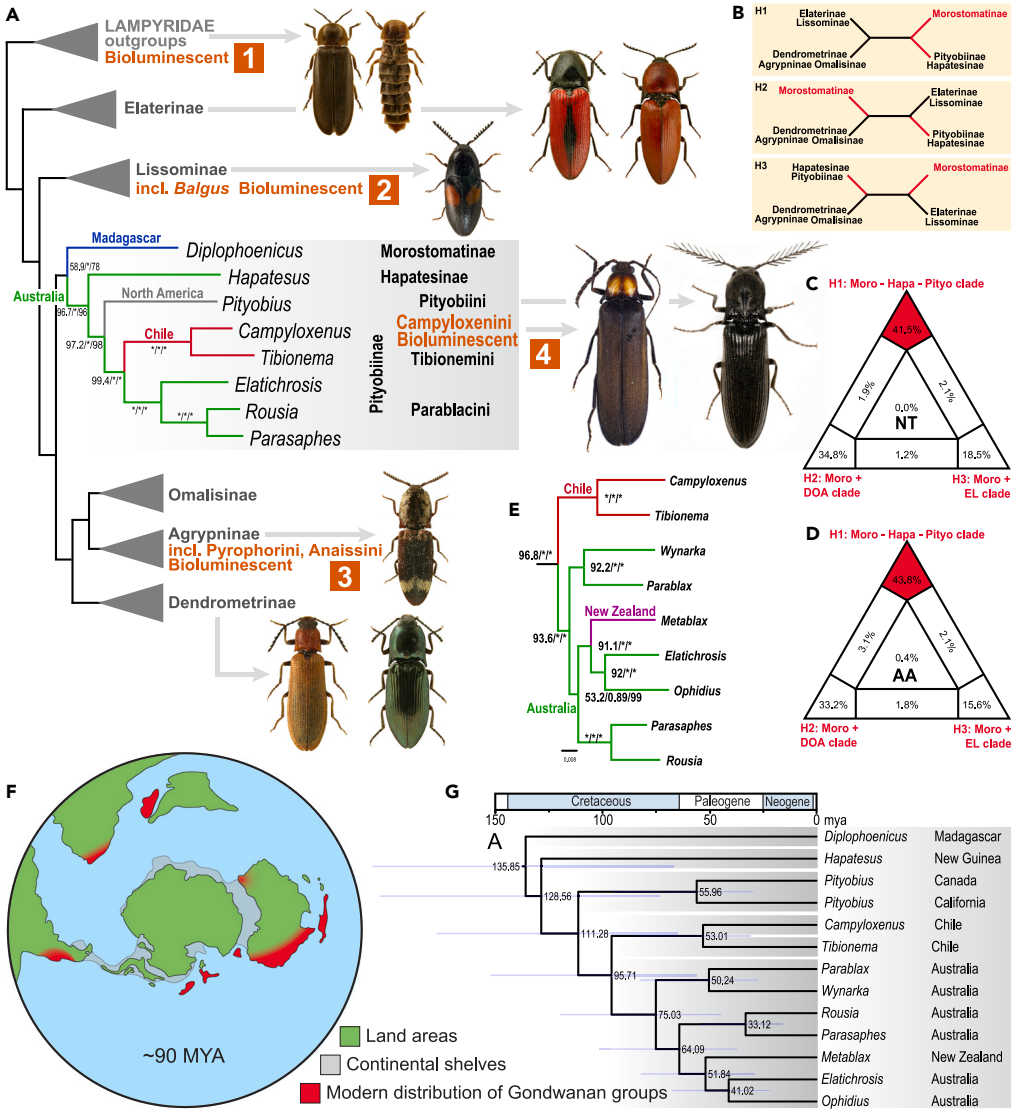

Figure 2. The results of phylogenetic analyses

(A) Topology recovered by the maximum likelihood (ML) analysis of the 66-gene dataset at the amino acid (AA) level. The numbers separated by slashes designate SH-aLRT, aBayes, and UFBoot, respectively, asterisks indicate the maximum support in Figures A and E; (B) The tested four-cluster likelihood mapping (FcLM) hypotheses; (C) The support of alternative hypotheses recovered by the nucleotide analysis of the 66-gene dataset; (D) ditto at the AA level; (E) The relationships of *Campyloxenus*, *Tibionema*, and Pityobiini genera recovered by the ML analysis of the four-fragment dataset (node numbers as in Fig. A); (F) The positions of southern continents ~90 mya with the extant distribution of Gondwanan subfamilies marked; the map courtesy of J. P. Klages/AWI; (G) Dated tree of the Gondwanan groups and *Pityobius* (all numbers designate million years ago). The light blue bars designate the 95% highest posterior density interval. Full versions of all trees are presented in supplemental information). Photographs of focal taxa were provided by L. Borowiec (European species) and C. Rorabaugh (*Pityobius*).

significant support. The p values were insignificantly higher for the alternative with Morostomatinae as a part of the clade of Gondwanan subfamilies (Table S4).

Almost all subsequent splits were recovered with full support in all analyses at the NT and AA levels and under various settings (Figures 2 and S3–S7). Only one analysis suggested the shift between serial splits of *Hapatesus* and *Pityobius* (Figure S5; the coalescent analysis at the AA level). The aberrant position got low support (LPP 55%), unlike the alternative topology.

We also expanded the earlier published 4-gene dataset[2,8,22,55] by additional samples of *Diplophoenicus*, *Pityobius*, *Hapatesus*, *Elatichrosis*, *Parasaphes*, *Rousia*, *Tibionema*, and *Campyloxenus*. The ML analyses (partitioned/unpartitioned) did not recover all focal taxa as a single clade (Figure S7). Two *Pityobius* species were recovered as a sister to the Lissominae + Thylacosterninae clade in the partitioned analysis, and other focal lineages as a sister to the Dendrometrinae + Agrypninae clade (Figure S7). The unpartitioned analysis of the same dataset yielded

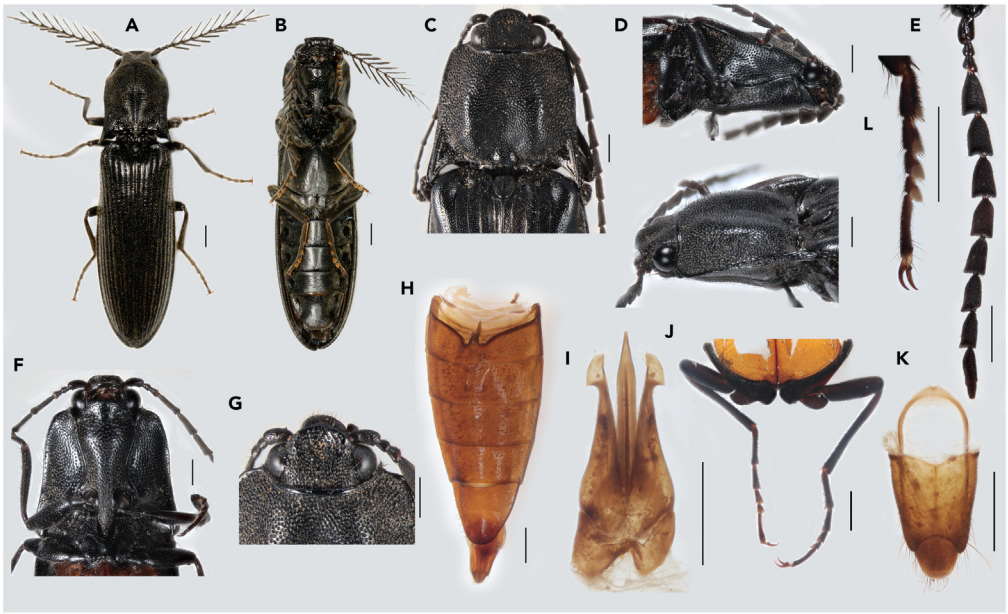

Figure 3. The morphology of Pityobiini and Tibionemini

Pityobius anguinus. (A, B) habitus dorsally and ventrally. *Tibionema abdominalis*. (C–F) Pronotum in various aspects, the arrow designates mesosternal keel; (G) Head, dorsal view; (H) antenna, male; (I) abdomen, ventral view; (J) male genitalia, (K) abdominal terminal segments, male; (L) hind legs; (M) tarsus, prothoracic leg. Scales 1 mm (A–H, J), 0.5 mm (I, K).

a large clade of focal lineages and several other subfamilies. The first subclade contained Hemiopinae, *Pityobius* spp., *Hapatesus*, and Oestodinae, while the second subclade was formed by parablacine genera, *Tibionema* and *Campyloxenus* (Figure S7). The BS values of the deepest splits were as low as 46%. The Campyloxenini + Tibionemini + Parablacini clade was recovered, and the internal relationships agree with the 66-gene topology (Figure 2A).

We estimate the first split in the clade of Gondwanan subfamilies and *Pityobius* at 136 million years ago (mya) and the subsequent splits between Australian Hapatesinae and Pityobiinae at 129 mya (Figures 2F, 2G, and S8). The separation of North American *Pityobius* from other Pityobiinae predates the continental breakup of Gondwana. North American *Pityobius* is the only taxon of these subfamilies that occurs outside the Gondwanan continents, but, with available sampling, the separation of *Pityobius* from its relatives is very ancient. There is no evidence of active dispersal routes from Australia to southeastern Asia or between Madagascar and Africa. If the dated topology is analyzed without Morostomatinae as the sister of the hapatesine-pityobiine clade (due to uncertain position), similar ages were estimated for shallower splits (Figures S8D and S8E).

Taxonomy

Subfamily Pityobiinae Hyslop, 1917, new sense.

Pityobiini Hyslop, 1917: 249.[15]

Pityobiinae Hyslop, 1917: various authors.[17,18]

Type genus: *Pityobius* LeConte, 1853.

Diagnosis

Several morphological characters support the relationships of pityobiine lineages. Notably, these features include short ball-like antennomeres 2 and 3 (Figures 3 and 4), a pronotal disc with parallel bulges (Figures 3 and 4), and the relatively short tarsomere 4 (Figures 3 and 4). Yet, these characters are not shared by all members. For example, Campyloxenini exhibits differences in the structure of antennomeres[14] (Figure 1D). Additionally, certain Protelaterinae and Agrypninae have similar relative lengths of basal antennomeres.

Remark

We uphold the classification of Morostomatinae and Hapatesinae as separate subfamilies based on their distinct morphological characteristics and congruency with earlier classifications.[1,3,11] We propose merging Pityobiini, Parablacini, Campyloxenini, and Tibionemini trib. nov. into a redefined subfamily Pityobiinae. This clade is robustly supported by phylogenomic analyses (Figures 2A and 2E), although the support from morphology is less clear. Pityobiinae retains its current rank, with Pityobiini as a nominotypical tribe comprising only *Pityobius*. Meanwhile, Parablacinae and Campyloxeninae are downranked to tribes within Pityobiinae. To address the non-monophyly of Pityobiinae *sensu*

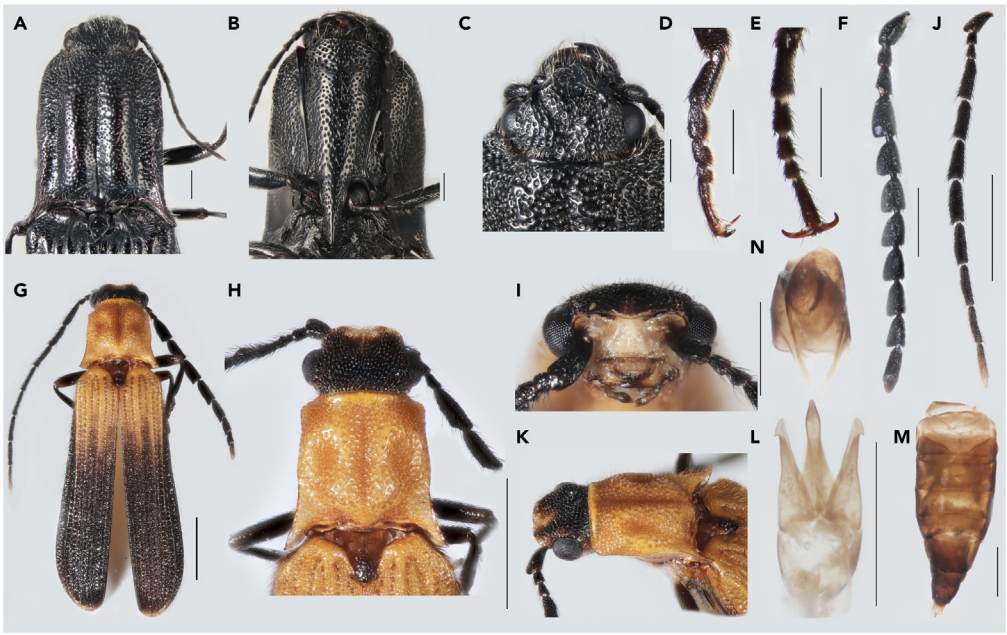

Figure 4. The morphology of Parablacini

Elatichrosis sp. (A, B) pronotum, dorsal, and ventral view; (C) head, dorsally; (D, E) tarsus, prothoracic leg, lateral and ventral view; (F) antenna, female. *Rousia* sp. (G) habitus; (H, K) pronotum, dorsal, and dorsolateral views; (I) head, frontal view, (J) antenna, male; (L) male genitalia; (M) abdomen, ventral view. Scales 1.0 mm (Figures A–K, M), 0.5 mm (Figures L, N).

Kundrata et al.[2] and Douglas et al.[3] (Figures 2A and 2E), we introduce a new tribe, Tibionemini trib. nov. The generic composition and species richness of genera are given in Table 2.

Tribe Pityobiini Hyslop, 1917: 249,[15] **new sense**.

Type genus: *Pityobius* LeConte, 1853.

Remark

Pityobius and *Tibionema* are morphologically distinct (Figures 1A, 1C, and 4), have a long evolutionary history (*Pityobius* split from relatives at 111 mya; Figure 2G), and occur in distant regions (Figure 1A). We propose to render Pityobiini into a monogeneric tribe and handle *Tibionema* in a tribe of its own (see in the following).

Parablacini Kundrata, Gunter, Douglas & Bocak, 2016, **stat. nov.**

Parablacinae Kundrata, Gunter, Douglas & Bocak, 2016: 299.[2]

Type genus: *Parablax* Schwarz, 1906.

Remark

The phylogenomic analyses revealed that three parablacine genera form a subterminal monophyletic group within the Gondwanan clade. This clade diverges after *Pityobius* but before the *Campyloxenus* + *Tibionema* clade (Figures 2A and 2E). As a result of these findings, we propose downranking Parablacinae to Parablacini stat. nov.

Based on the genomic analysis and shared morphological characters, we propose transferring *Rousia* and *Elatichrosis* from Dendrometrinae to Pityobiinae: Parablacini. The decision to make these transfers is supported by the presence of similar ball-like antennomeres 2 and 3 (Figures 3 and 4) and concealed labrum (Figures 3 and 4) in these genera, akin to the Chilean Parablacini and at least one group of *Metablax* from New Zealand. *Rousia* also shares certain features with other Parablacini, such as a poorly defined anterior margin of the scutellum. Notably, Calder[9,56] did not include *Elatichrosis* and *Rousia* in the widely defined Pityobiini, encompassing other parablacine genera. The potential reason for Calder's decision to retain some parablacines in Dendrometrinae was the presence of other genera with partially similar morphology in this subfamily, including related genera that were later transferred to Hapatesinae.[11] Moving forward, the placement of several other genera, such as *Paracrepidomenus* Schwarz, 1906, warrants further investigation.

Distribution

Australia and New Zealand harbor a rich diversity of Parablacini, currently comprising eight genera with twenty-three species[56] (Table 2). However, we suspect that the biodiversity inventory for Australian Parablacini is still far from complete. In addition to the Australian diversity, there is a single parablacine genus, *Sharon*, known from the southern part of the Neotropical region.[26,57]

Table 1. The list of newly sequenced and analyzed genomic data (taxonomic placement proposed in the present study)

Voucher	Species	Subfamily	Tribe	Geographic origin
G19011	*Diplophoenicus* sp.	Morostomatinae		Madagascar
G20007	*Hapatesus tropicus*	Hapatesinae		New Guinea
G21014	*Elatichrosis* sp.	Pityobiinae	Parablacini	Australia
G19006	*Parasaphes* sp.	Pityobiinae	Parablacini	Australia
G21031	*Rousia* sp.	Pityobiinae	Parablacini	Australia
G20012	*Tibionema abdominalis*	Pityobiinae	Tibionemini	Chile
G21037	*Pityobius murrayi*	Pityobiinae	Pityobiini	USA
G21036	*Campyloxenus pyrothorax*	Pityobiinae	Campyloxenini	Chile

Campyloxenini Costa, 1975, **stat. nov.**
Campyloxeninae Costa, 1975: 114.[14]
Type genus: *Campyloxenus* Fairmaire & Germain, 1860.

Remark

Our analyses provide compelling evidence supporting the placement of *Campyloxenus* within the pityobiine clade, where it emerges as a sister to *Tibionema* (Figures 2A and 2E). Based on relationships shown in Figures 2A, 2E, and S3–S7, we propose downranking Campyloxeninae to a tribe within Pityobiinae. Arias-Bohart[26] extensively discussed the morphological distinctiveness of the two constituent genera. *Malalcahuello* exhibits a well-sclerotized cuticle, and *Campyloxenus* displays a more soft-bodied nature resembling fireflies. Adult female light emission was observed by Isai Madríz; reliable information on pupae and larvae is unavailable.

Tibionemini Motyka, Kusy, Arias, Bybee et Bocak, new tribe
urn:lsid:zoobank.org:pub:91027F71-C0E4-4746-A440-DBD9C1894D6A.
= Tibionemini Ulrich, 1988 (*in litt.*),[58] unavailable name.
Type genus. *Tibionema* Solier, 1851.

Diagnosis

Tibionema has a medium-to-large body, flat pronotum, and elytra (Figure 1C), distant longitudinal bulges in the pronotum, and acutely prominent posterior angles of the pronotum (Figure 3C). The prosternum is narrow, and the prosternal process is long and slender (Figure 3F). The lateral edges of the pronotum are complete (Figure 3E). There is a sharp keel between mesocoxae (Figure 3D). Antennae are 11-segmented and serrate; the scapus is parallel-sided, antennomeres 2 and 3 are short and ball like, and antennomeres 1–3 are almost bare and shining (Figure 3E). The abdomen has five visible segments, a short intercoxal process, a straight posterior margin of the penultimate segment, and a triangular, apically rounded last visible abdominal segment. Internal abdominal segments consist of long and narrow penultimate and small ultimate sternites (Figure 3K). *Tibionema* and *Pityobius* differ in the number of antennomeres (twelve in *Pityobius* versus eleven in *Tibionema*); the posterior pronotal angles bent toward the body in *Tibionema* (also in *Hapatesus* and *Oxynopterus*), but not in *Pityobius* (Figure 3A). *Tibionema* has a prominent intercoxal keel in the mesosternum (Figure 3) that has not been observed in other Pityobiinae. *Campyloxenus* differs in much longer antennomere 3. The larva and pupa of *T. abdominalis* were described by Angulo.[59]

Remark

Tibionemini trib. nov. is proposed due to the refuted monophyly of Pityobiinae *sensu* Kundrata et al.[2] and Douglas et al.[3] (Figure 2A, 2E, and 2G). Following the principles of phylogenetic systematics, preserving the subfamily ranks for Parablacinae and Campyloxeninae would necessitate assigning the same rank to *Tibionema*, ultimately increasing the number of subfamilies. The morphological divergence between *Pityobius* and *Tibionema* was recognized. Due to the sparse sampling, *Tibionema* was recovered as a sister to Parablacinae by Kusy et al.[11] and a sister to *Pityobius* by Bi et al.[60] Here, with parablacines and *Campyloxenus* analyzed, the two genera are recovered in distant positions (Figures 2A and 2E).

DISCUSSION

The phylogeny of Gondwanan click beetles

The internal relationships among click beetle subfamilies have been a subject of active debate, with investigations spanning morphological to phylogenomic analyses.[2–9,11,14,18,21,61,62] However, several subfamilies from the Southern continents (Figure 1A) have yet to be included in data-rich molecular analyses. In this study, we examine the relationships of newly sequenced sixty-six orthologs for Morostomatinae, Hapatesinae, and Pityobiinae, including Pityobiini, Parablacini, Campyloxenini, and Tibionemini (see taxonomy for revised ranks; Table 2). This gene-rich dataset represents the best currently available sampling of species-poor Gondwanan click beetles (Table 1).

Our analyses frequently placed *Pityobius* and all Gondwanan groups within a single clade (Figures 2 and S3–S7). Still, the position of Morostomatinae remains uncertain, either as a sister to the hapatesine-pityobiine clade or as a subsequent serial split in the tree, i.e., a sister to the

Table 2. The overview of tribes, genera, diversity, and distribution of Pityobiinae

Tribe/Genus	Type species	Distribution	spp.
Pityobiini Hyslop, 1917			2
Pityobius LeConte, 1853	*Pityobius anguinus* LeConte, 1853	USA: E. & W., Canada: Manitoba	2
Parablacini Kundrata et al., 2016			38
Elatichrosis Hyslop, 1921	*Chrosis exarata* Candèze, 1863	Australia	13
Metablax Candèze, 1869	*Elater acutipennis* White, 1846	New Zealand	5
Ophidius Candèze, 1863	*Ophidius elegans* Candèze, 1863	Australia: NSW, QLD	4
Parablax Schwarz, 1906	*Metablax trisulcatus* Schwarz, 1903	Australia, Tasmania	9
Parasaphes Candèze, 1882	*Parasaphes elegans* Candèze, 1882	Australia: QLD	1
Rousia Calder, 1996	*Rousia dumbrellium* Calder, 1996	Australia: NSW, QLD	1
Sharon Arias-Bohart & Elgueta, 2015	*Asaphes amoenus* Philippi, 1861	Chile	2
Tasmanelater Calder, 1996	*Tasm. pelionensis* Calder, 1996	Tasmania	1
Wynarka Calder, 1986	*Wynarka sylvestre* Calder, 1986	Australia: NSW, VIC, ACT	1
Xuthelater Calder, 1996	*Xuthelater moppiensis* Calder, 1996	Australia: NSW	1
Campyloxenini Costa, 1975			2
Campyloxenus Fairm. & Germ., 1860	*C. pyrothorax* Fairm. & Germ., 1860	Southern Chile, Argentina	1
Malalcahuello Arias-Bohart, 2015	*Mal. ocaresi* Arias-Bohart, 2015	Southern Chile	1
Tibionemini trib. n.			1
Tibionema Solier, 1851	*Tibionema rufiventre* Solier, 1851	Chile	1

NSW – New South Wales, QLD – Queensland, VIC – Victoria, ACT – Australian Capital Territory.

Agrypninae and Dendrometrinae clade (Figures S3–S7). We observed that all focal groups form a clade in 66-gene ML/NT (partitioned and unpartitioned) and ASTRAL (coalescent) analyses at the AA level. Alternatively, we recovered a paraphylum at the ML/NT and ASTRAL/NT analyses of the same dataset (Figures 2 and S3–S7). The four-cluster likelihood mapping (FcLM) analyses show only slightly higher probabilities for the close relationships of Morostomatinae and other Gondwanan groups (Figures 2A–2D). The monophyly of the clade should be reinvestigated in the future with denser sampling and a larger dataset to handle better biological and analytic factors causing pervasive uncertainty in the topologies.[63]

However, the monophyly of Hapatesinae + Pityobiinae *sensu lato* was robustly supported by all genomic analyses (Figures 2 and S3–S6). The only ambiguity in the internal relationships was the relative position of *Pityobius* (Pityobiinae: Pityobiini) and *Hapatesus* (Hapatesinae). Five of the six analyses recovered Hapatesinae as a sister to the redefined Pityobiinae (≥95% in 66-gene ML analyses, Figure 3). While the ASTRAL analysis at the AA level deviates and suggests a switch between *Hapatesus* and *Pityobius*. However, the statistical support is considerably lower (LPP 0.55, Figure S5B). Therefore, we favor Hapatesinae as a sister to the redefined Parablacinae. The congruent relationship between *Hapatesus* and *Pityobius* + *Tibionema* was suggested in the mitogenomic and genomic studies when other taxa were unavailable.[3,11] The morphological distinctiveness of *Hapatesus* justifies the subfamily rank for Hapatesinae.[11]

Based on the preferred topology, we redefine Pityobiinae, which now contains (a) Pityobiini (monogeneric; *Pityobius* was previously part of Pityobiinae[2,3]), (b) Parablacini (nine genera, earlier in Pityobiinae *sensu lato*[9,18] and Parablacinae[2]), (c) Campyloxenini (*Campyloxenus* and *Malalcahuello*, earlier in Agrypninae, later Campyloxeninae[14,26]), and (d) Tibionemini, new tribe (monogeneric, *Tibionema* has been placed in Pityobiinae[3]). We reject the distant position of *Pityobius* and parablacine genera[2,22] and the sister position of *Pityobius* and other Elateridae.[21] Furthermore, we demonstrate that the earlier proposed concept of Pityobiinae consisting of *Tibionema* and *Pityobius* was an artifact of sparse sampling.[3] With the revised taxonomy, the redefined Pityobiinae remains a species-poor subfamily, comprising 13 genera and 43 described species (Table 2).

Further, we expanded and reanalyzed the short-fragment dataset published by earlier authors.[2,22,55,62] Even with additional taxa, the separate positions of *Pityobius* and parablacines are again recovered (Figures 2E and S7). As these lineages represent ancient splits, the information from rRNA genes and mtDNA fragments is likely insufficient for robust analyses. The instability of the backbone is evident from very low statistical support in earlier studies,[2,60] and the present reanalysis of short DNA fragments (Figure S7). While some researchers still follow relationships recovered by short-fragment analyses,[48] we prefer detailed analyses of large multigene datasets.[63] The topologies recovered from such analyses can be tested for the biological and methodological sources of systematic errors. The strictly tested topologies provide a more robust basis for classification than a short-fragment analysis without subsequent tests (Figures 2A and S3–S6).

Zoogeography

Elateridae is found on all continents except Antarctica.[1] The large subfamilies, Elaterinae, Dendrometrinae, and Agrypninae, encompass most described genera and species and exhibit a truly global distribution. In contrast, the lineages studied here are species-poor and mostly confined to small geographic ranges on the continents that formed Gondwana (Figure 1A).

The split of Morostomatinae is estimated at ~136 mya at the time of high connectivity between the Gondwanan continents (Figure 2G). It corresponds with the opening of the South Atlantic at 135 mya.[37] However, Morostomatinae does not occur in continental Africa and India despite the connectivity between these regions and Madagascar during the Cretaceous.

Pityobiini originated ~111 mya. It is the sole modern lineage of the focal clade occurring in the Nearctic region. Yet, its Cretaceous representative *Cretopityobius* has been reported from Burmese amber (Cenomanian).[36] These findings suggest that Pityobiini was once more widespread and persisted only in North America. It is worth noting that Cenomanian Burmese amber is considerably younger than click beetle subfamilies,[24,39] and the fauna includes both Gondwanan and Laurasian taxa. Campyloxenini and Tibionemini originated at ~96 mya, i.e., when South America became isolated from Africa, but earlier than it lost its connection with Antarctica and Australia.[37] The distribution of Parablacini in the southernmost part of South America, New Zealand, and Australia can be attributed to vicariance and Cretaceous connectivity through temperate forests in Antarctica.[64] Their diversification started shortly after the opening of the Tasman Sea, 80 mya. A more extensive sampling would be necessary to delve deeper into this question as South American Parablacini has not been analyzed (Figure 2E).

The modern distribution suggests that the Gondwanan click beetles might face limitations in crossing open seas. For instance, the seas between Australia and southeastern Asia appear to be an impermeable barrier for Australian pityobiine and hapatesine genera, even with the availability of the Wallacea steppingstone connection since the Miocene.[65] Similarly, Morostomatinae has not crossed the Mozambique strait and remains endemic to Madagascar. Thus far, there is no reliable record of the old Gondwanan click beetles from continental Africa. However, Ulrich[58] reported a putative pityobiine larva from Southern Africa. Adult specimens have not yet been collected. Few species in restricted ranges raise macroevolutionary questions regarding diversification and extinction rates. There is no evidence of the dispersal capability of Gondwanan lineages and their ability to undergo rapid speciation. Despite being a very ancient lineage with ample opportunities to colonize new territories during the Cenozoic and Quaternary periods, the Gondwanan lineages still bear the signature of the breakup of the southern continents.

Bioluminescence in click beetles: Exploring the Campyloxenus case

Since the dissolution of the traditional cantharoid clade,[55] our understanding of the origins of bioluminescence in elateriform beetles has progressively advanced.[38,39,41,42,66] Most bioluminescent elateroids are soft bodied (~2,000 spp; fireflies and railworm beetles, Lampyridae, Phengodidae, and Rhagophthalmidae). Jointly with clicking Sinopyrophoridae, they constitute a lampyroid clade *sensu* Kusy et al.[4] It represents an independent origin of bioluminescence, leading to the most extensive radiation of luminescent elateroids.

In contrast, other light-emitting elateroids exhibit pronounced sclerotization and the clicking mechanism (Elateridae, ~100 species), and they are currently placed into three subfamilies. Most are concentrated in Agrypninae, particularly Pyrophorini in current classification, i.e., including Hapsodrilina, Nyctophyxina, and Pyrophorina (the second origin of bioluminescence[7,14,54]). The larva of *Alampoides* Schwarz (Pyrophorinae: Anaissini) is luminescent and might eventually represent an additional, third, origin if distant relationships of Anaissini and Pyrophorini are confirmed.[14,49] Currently, *Coctilelater* Costa is also placed in Anaissini but in a separate position from *Alampoides*.[14] As *C. sanguinicollis* (Candèze) was originally placed in *Pyrophorus*, it should be a bioluminescent species. The genus-level analysis is needed for a detailed investigation of the origins and, eventually, losses of bioluminescence in Agrypninae. To be conservative, we provisionally hypothesize a single origin of bioluminescence in Agrypninae as we do not have sufficiently dense sampling to investigate possible origins and losses of bioluminescence in this group.

Balgus schnusei, currently placed in Thylacosterninae, represents evidence for the third origin of bioluminescence in Elateroidea[50,51] (Figure 2A). The bioluminescence of this taxon was observed by G. L. Tavakilian in Saul, French Guyana.[51] There is no information available on later observations and the larva of *Balgus schnusei*.

The presence of luminous patches and recent observation of the bred specimen by Isai Madríz (personal communication) substantiate the bioluminescence of *Campyloxenus* adults. Isai Madríz bred a *Campyloxenus* larva to the imago and did not observe its luminescence. The larva of *Campyloxenus'* closest relative, at adult stage non-luminescent *Malalcahuello* Arias-Bohart, is unknown. Genomic data analyses unequivocally identify *Campyloxenus* as the fourth well-documented origin of bioluminescence in click beetles (Figures 2A and 2E). We adopt the Elateridae concept introduced by Kusy et al.,[4] which is based on a thorough analysis of 4,200 orthologs and corroborated by multiple tests exploring the sources of signal and conflicts and supporting the monophyly of click beetles. In this sense, we hypothesize four origins of bioluminescence in Elateroidea.

Alternatively, *Sinopyrophorus* will represent the additional, fifth, origin if it is hypothesized as related to Oestodinae and Hemiopinae.[60] Nonetheless, we ardently favor its membership in the lampyroid clade recovered by the phylogenomic analyses.[4] The *Sinopyrophorus* + Oestodinae + Hemiopinae clade asserted by Bi et al.[60] and recently defended by Lawrence et al.[48] is based on analyzing a notably incomplete dataset (completeness ~25%). This dataset was *ad hoc* assembled from newly sequenced *Sinopyrophorus* (mitogenome, rRNA genes) and previously published short DNA fragments.[8,22,55] These data were merged with publicly available mitogenomes of elaterids.[67,68] The statistical support for the (*Oestodes*(*Hemiopus*, *Sinopyrophorus*)) clade was very low (the whole clade BS 13%, and *Hemiopus* + *Sinopyrophorus* BS 41%). The backbone of Elateridae also lacks robust support (the five deepest splits having BS values 15%–49%). The dataset was insufficient for the reliable recovery of deep splits.

An additional report on the bioluminescence referred to *Omalisus* (Omalisinae)[69,] and it was revived by Beutel.[70] The possibility of bioluminescent *Omalisus* was definitively rejected by Burakowski[71] and other authors who frequently collected this species.[72,73]

Undeniably, the bioluminescence in Elateroidea is ancient. Fossil evidence includes a firefly fossil in Cenomanian Burmese amber,[74] while recent dating analyses place the terrestrial luminescence origin in the Early Cretaceous.[39] Our dating analysis suggests the delayed origin of

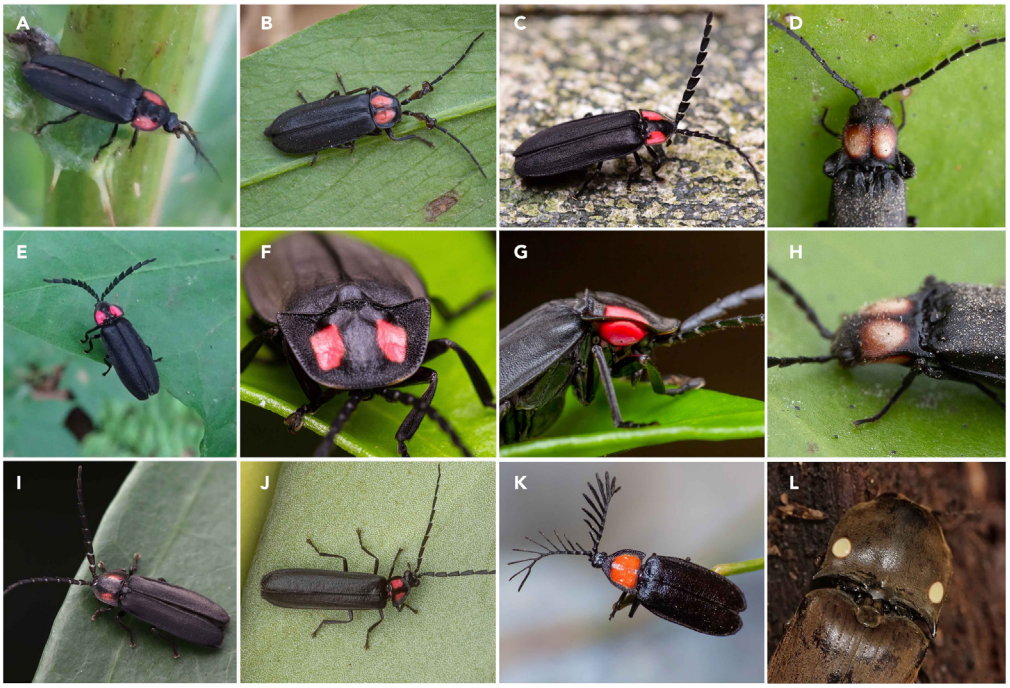

Figure 5. The general appearance of elateroid species

(A) *Pyropyga nigricans* (USA, photo by Erin Moore, USA); (B) *Dysmorphocerus dilaticornis* (Chile, photo by Claudio Maureira, Chile); (C) *Lucidina vitalisi* (China, Zhenjiang, photo by Fan Gao, Nanjing); (D, H) *Campyloxenus pyrothorax*, (Chile, photo by Matías Gargiulo); (E) *Cladodes ater* (Chile, photo by Michael Weyman); (F, G) *Pyratocnema* sp. (photo by Matías Garguilo, Chile); (I) *Lucidina* sp., (China, photo by rosefan2 from inaturalist.org); (J) *Pyractonema* sp. (Chile, photo by Matías Gargiulo); (K) *Vesta cincticollis* (Chile, photo by Claudio Maureira); (L) *Pyrophorus* sp. (Ecuador, photo by M. Motyka).

luminescence in *Campyloxenus*, even without including its sister genus *Malalcahuello* (Figures 2G and S8). Our estimate at 53 mya stands as a maximum age, considering that non-luminescent *Malalcahuello* must have separated from *Campyloxenus* later, as both belong to Campyloxenini. We assume *Campyloxenus* might be somewhat younger than the origin of bioluminescent Pyrophorini (the origin of Pyrophorinae was estimated at 133.7–91.7 mya[39]). It is conceivable that *Balgus schnusei* became bioluminescent even later than *Campyloxenus*, as it is the sole bioluminescent species within its genus. Based on phylogenomic analyses, we can conclude that elateroids' bioluminescence originated independently at least on four occasions and at different times. *Campyloxenus*, as a relatively young taxon, could be a promising model for further studies if new material is available for breeding and *Malalcahuello* is available for analyses. Additional bioluminescence origins and eventual losses can be recovered if Pyrophorinae phylogeny is studied in detail.

The positions, sizes, and shapes of luminous lanterns exhibit substantial variability among click beetles. *Campyloxenus* has only prothoracic luminous lanterns like Pyrophorini: Nyctophyxina (Figures 5D and 5H), and the abdominal spots common in Pyrophorina are absent. Pyrophorini, e.g., *Pyrearinus* Costa and *Nyctophyxis* Costa, and *Campyloxenus* also share the position of lanterns at the posterior pronotal margins instead of lateral ones.[14] *Campyloxenus'* spots differ in diffuse boundaries and larger sizes (Figures 5D and 5H). Their color is orange to pinkish, unlike Pyrophorini (Figure 5L), and somewhat reminds those of *Nyctophyxis ocellatus* (Germar). The large lanterns are visible on the dorsal and ventral sides of the pronotum (Figures 5D and 5H). Our investigation into additional material from regions inhabited by *Campyloxenus* unveils its resemblance to certain Chilean fireflies (Lampyridae: Lampyrinae, Cladodinae, Figure 5E) and soldier beetles (Cantharidae: Dysmorphocerinae; Figure 5B). The diurnal lampyrine fireflies commonly coexist and share leaves with click beetles (Figures 1 and 5). Lampyrine fireflies are unpalatable. They contain steroidal pyrones called lucibufagins, structurally akin to the cardiotoxic bufadienolides found in toads and cardenolides in plants.[43,75] Lucibufagins protect them against spiders, toads, birds, and other predators. Similarly, soldier beetles (Figure 5B) potentially possess a chemical defense[76,77] and often participate in Batesian mimicry rings.[78]

Campyloxenus, like many other small insects, is a potential prey for small birds, spiders, and assassin bugs. Interestingly, this species has a black-colored body and elytra and bright spots on the pronotum, like some other beetles known for their unpalatability (Figure 5). This color pattern is widely distributed and shared by multiple genera and species of similar fireflies and soldier beetles. As unpalatable, phenotypically similar, but unrelated species, they potentially form a Müllerian mimicry ring.[79] Interestingly, the black-red colored unpalatable species are not closely copied by Batesian mimics in Asia and North America, possibly due to the unusual coloration of pronotal patches (Figure 5). The remarkable resemblance between *Campyloxenus* and unpalatable fireflies is an exception. *Campyloxenus* does not occur in masses, and there is no indication that it is unpalatable. Therefore, it should be a Batesian mimic. Further, *Campyloxenus* differs from other click beetles in a weakly sclerotized cuticle akin to fireflies. It might be a coincidence as it is the only true bioluminescent click beetle with weaker cuticle sclerotization.

Alternatively, it might indicate parallel processes leading to the loss of sclerotization in bioluminescent elateroids relying on a warning signal instead of an escape reaction. Other click beetles also readily imitate the sympatrically occurring unpalatable beetles; among them is *Rousia* sp., resembling *Microtrichalus* spp. and *Trichalus* spp. net-winged beetles in Queensland[78] (Figure 4G).

The phenotypic resemblance of *Campyloxenus* and diurnal fireflies (Figure 5) and a shared color signal potentially aiming at diurnal visual predators do not have anything in common with its luminescence. Diurnal lampyrids usually do not emit light or only low-intensity light.[42] Moreover, the diurnal lampyrids emit light of different wavelengths from lanterns positioned in terminal abdominal ventrites.[54,80] Consequently, *Campyloxenus* acquired the ability to produce light without any direct ecological context related to interactions with fireflies in South American ecosystems, where *Campyloxenus* is indigenous (Figure 1). Given the relatively recent emergence of luminescence in *Campyloxenus* (*Malalcahuello* is its non-luminescent sister taxon[26]), it is plausible that some bioluminescent Pyrophorini sympatrically occurred in the area where *Campyloxenus* developed its luminescence although only *Phanophorus* and *Nyctophix* share the range now (see zoogeography section). While the presence of bioluminescent taxa might not be the trigger for *Campyloxenus'* light-emitting capability, it could contribute to the subsequent selection of a more potent signal for protection against potential predators after dusk.[81]

Conclusions

Most phylogenetic studies of beetles have densely sampled faunas of Europe and Northern America, and this was also the case for Elateridae when several Australian, African, and Southern American subfamilies were omitted.[3,23] In this study, we investigated the relationships between Gondwanan subfamilies of click beetles. Given their morphological disparity and ancient origins, it is appropriate to retain earlier described higher-level taxa while redefining Pityobiinae, downranking Parablacinae and Campyloxeninae to tribes within Pityobiinae, and proposing Tibionemini trib. nov. These adjustments aim to uphold accepted family-group taxa while creating a more informative classification that reduces the number of species-poor subfamilies in Elateridae. The vicariance hypothesis following the breakup of Gondwana is the most plausible scenario for the early diversification of these click beetles. The new delimitation of the ancient morostomatine-hapatesine-pityobiine clade offers insights into the group's zoogeography, underscoring the necessity for robust and extensively sampled phylogenies to formulate zoogeographic hypotheses effectively.

Likewise, preceding phylogenetic analyses have still incompletely elucidated the origins of bioluminescence in click beetles. The placement of *Campyloxenus* within a non-luminescent Pityobiinae clade signifies a minimum of the fourth instance of bioluminescence origin within Elateroidea. Our estimation suggests that *Campyloxenus'* luminescent ability is significantly younger than that of the lampyroid clade and Pyrophorini. Additionally, we propose that *Campyloxenus* is a Müllerian mimic of sympatrically occurring unpalatable fireflies and soldier beetles (Figure 5) and could evolve its bioluminescence in the presence of other bioluminescent click beetles.

Limitations of the study

The sampling is limited by the accessibility of the properly fixed material of rare and narrowly endemic species for the genomic analyses. We conservatively count only a single origin of bioluminescence in Agrypninae, as earlier morphology-based phylogenies and classification proposed variable relationships among constituent tribes and subtribes, and we do not have new data. In the future, the robust reconstruction of bioluminescence in Agrypninae should be based on a much denser sampling of the whole subfamily. It is also possible that further bioluminescent taxa will be discovered. Conversely, further observations are needed to confirm the single published report of bioluminescence in *Balgus schnusei*. New ecological data are also needed to investigate the bioluminescence of larvae. Due to a limited fossil record, we depend on a single Pityobiini record from the Burmese amber when considering the possibility that the group was widespread in the Holarctic region. The discussed extinct taxon is morphologically distinct, and further research might question its relationships.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.108440.

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AUTHOR CONTRIBUTIONS

M.M., D.K., E.T.A.-B., S.M.B., and L.B. conceived and designed the study; D.K. and M.M. conducted phylogenetic analyses; L.B. drafted the manuscript; E.T.A.-B. collected the critical taxa and provided field observations; L.B. and E.T.A.-B. analyzed morphology; L.B. and M.M. prepared illustrations; L.B. coordinated the study; L.B., M.M., and D.K. obtained the funding; all authors commented on the drafts and gave the final approval for publication.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Diplophoenicus sp.	Madagascar	G19011
Hapatesus tropicus	New Guinea	G20007
Ophidius sp.	Australia	G21014
Parasaphes elegans	Australia	G19006
Rousia sp.	Australia	G21031
Tibionema abdominalis	Chile	G20012
Pityobius anguinus	USA	G21037
Campyloxenus pyrothorax	Chile	G21036
Chemicals, peptides, and recombinant proteins		
Ethanol	Litolab	Cat# 221430216100
Proteinase K	Thermo Fisher Scientific	Cat# EO0491
AE buffer	QIAGEN	Cat# 67563
Critical commercial assays		
MagAttract HMW DNA extraction kit	QIAGEN	Cat# 67563
Qubit 2.0 Fluorometer	Thermo Fisher Scientific	Cat# Q32850
Deposited data		
Analyzes dataset	Mendeley Data https://doi.org/10.17632/sh7s4jzhmz.1	
Analyzes dataset	Figshare data, V1, https://doi.org/10.6084/m9.figshare.24316531	
Software and algorithms		
Photoshop v.6.0		https://www.adobe.com/products/photoshop.html
Helicon Focus v.8.2.2		https://www.heliconsoft.com/software-downloads/
fastp v.0.21.0	Chen et al. 2018[82]	https://github.com/OpenGene/fastp
FastQC v.0.11.9		https://github.com/s-andrews/FastQC
SPAdes v.3.13.1	Bankevich et al., 2012[83]	https://github.com/ablab/spades
Augustus	Stanke & Waack, 2003[84]	http://augustus.gobics.de/binaries/
BUSCO v.5	Manni et al., 2021[85]	https://busco.ezlab.org/
OrthoDB v.9.1		https://www.orthodb.org/
Orthograph v.0.6.3	Petersen et al., 2017[86]	https://mptrsen.github.io/Orthograph
summarize_orthograph_results.pl	Petersen et al., 2017[86]	https://github.com/mptrsen/Orthograph/tree/master
Geneious v.7.1.9		https://www.geneious.com/download/
TRANSALIGN		http://malde.org/~ketil/biohaskell/transalign/
MAFTT v.7.407	Katoh & Standley, 2013[87]	https://mafft.cbrc.jp/alignment/software/linux.html
AliStat v.1.7		https://github.com/thomaskf/AliStat
SymTest v.2.0.49		https://github.com/ottmi/symtest
IQ-TREE v.2.2.0	Minh et al. 2020[88]	http://www.iqtree.org/
ASTRAL v.5.15.5		https://github.com/smirarab/ASTRAL
BEAST v.1.8.1	Suchard et al., 2018	https://github.com/beast-dev/beast-mcmc
TRACER v.1.7	Rambaut et al., 2018[89]	https://github.com/beast-dev/tracer
TREEANNOTATOR	Suchard et al., 2018	https://github.com/beast-dev/beast-mcmc

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Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Other		
Olympus SZX16 binocular microscope		https://www.olympus-lifescience.com/en/microscopes/stereo/szx16/

RESOURCE AVAILABILITY

Lead contact

Requests for further information should be directed to and will be fulfilled by the lead contact Ladislav Bocak (ladislav.bocak@upol.cz).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Data reported in this paper will be shared by the lead contact upon request.
- Further information and requests for DNA data should be directed to and will be fulfilled by M. Motyka (michal.motyka@upol.cz).

DNA data are available at: Motyka, Michal; Kusy, Dominik; Bohart, Elizabeth; Bybee Seth, Bocak, Ladislav (2023), "Enigmatic Campyloxenus: Shedding Light on the Delayed Origin of Bioluminescence in Ancient Gondwanan Click Beetles," Mendeley Data, V1, https://doi.org/10.17632/sh7s4jzhmz.1 and Figshare data, V1, https://doi.org/10.6084/m9.figshare.24316531. The study contains a taxonomical act and is registered on ZooBank with the Life Science Identifier (LSID) urn:lsid:zoobank.org:pub:91027F71-C0E4-4746-A440-DBD9C1894D6A.

- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

This study did not generate new code.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

The voucher specimens used in the study are deposited in the collection of the Biodiversity and Molecular Evolution, CATRIN, Olomouc, Czech Republic.

METHOD DETAILS

Data collection - Morphology

The photographs of morphological characters were taken by a Canon M6 Mark II camera attached to an Olympus SZX16 binocular microscope. Stacks were assembled using Helicon Focus software and processed in Photoshop 6.0. Vouchers were dry-mounted and deposited in the collections of Biodiversity & Molecular Evolution, CATRIN, Olomouc. Specimens were dissected as in previous studies.[11]

Data collection - Molecular data

We analysed the relationships of predominantly Gondwanan groups Morostomatinae, Hapatesinae, Pityobiinae *sensu* Douglas et al.,[3] Campyloxeninae *sensu* Costa,[14] and Parablacinae *sensu* Kundrata et al.[2] Additional five click beetle subfamilies (Elaterinae, Dendrometrinae, Omalisinae, Agrypninae, and Lissominae, 19 samples) and two subfamilies of Lampyridae (Luciolinae and Lampyrinae, two taxa) were used as outgroups. The list of newly sequenced samples is given in Table 1, and the complete list is in Table S1.

The ethanol-conserved or dry-mounted specimens were used for the DNA isolation with a Qiagen MagAttract HMW DNA extraction kit. DNA was sequenced for 8Gb per sample with Illumina NovaSeq 6000 by Novogene, Inc., Beijing, using (2 × 150 bp) paired-end mode. Raw Illumina reads were filtered with fastp v.0.21.0[82] using -q 28 -u 50 -n 15 -l 50 settings and quality checked with FastQC. The data were processed with SPAdes v.3.13.1,[83] with k-mer sizes of 21, 33, 55, 77 and 99. The resulting contigs were used to train Augustus[84] for species-specific gene models with BUSCO v.5.[85] Predicted models were used for *ab initio* gene predictions. The single-copy ortholog set was collated by searching the OrthoDB v.9.1 database[90] (Table S3). Following Kusy et al. (2018), we carried out Orthograph v.0.6.3 searches[86] with all 95 genes from Zhang et al.[91] against the single-copy ortholog set. Only 66 single-copy orthologs were used for further analyses. Next, we searched new genomic data with Orthograph targeting 66 single-copy orthologs (66NT and 66AA datasets). Terminal stop codons were removed, and internal stop codons at the translational and nucleotide levels were masked using the Perl script summarize_orthograph_results.pl.[86] Additionally, filtered reads were mapped to mitochondrial *cox1*, *rrnL*, and nuclear *LSU*, *SSU* genes using Geneious v.7.1.9. Furthermore, they were merged with earlier published nuclear and mitochondrial fragments[2] (Table S2). The protein-coding genes were aligned using Translation Align in Geneious v.7.1.9, whereas the ribosomal genes were aligned MAFTT v.7.407 using the L-INS-i algorithm.[87] All alignments were visually checked for dubiously aligned regions and outlier sequences.

The proportion of the missing data and pairwise completeness of the 66-genes dataset was computed using AliStat v.1.7. (https://github.com/thomaskf/AliStat). The deviation from stationarity, reversibility, and homogeneity (SRH) were computed using SymTest v.2.0.49 (https://github.com/ottmi/symtest). Heatmaps were generated for NT and AA datasets to visualize the pairwise completeness and deviations from SRH conditions.

We also compiled a four-gene dataset from previously published studies of our laboratory, and we additionally included new taxa listed in Table S2 representing the taxa from the Gondwanan clade (*Pityobius murrayi*, *Tibionema abdominalis*, *Campyloxenus pyrothorax*, *Elatichrosis* sp., *Rousia* sp., *Parasaphes* sp., *Hapatesus tropicus*, and *Diplophoenicus* sp. The dataset contained 185 terminals, including the outgroups. The taxa were chosen to proportionally represent the relative diversity of click beetle lineages and to assemble the dataset with high completeness.

Phylogenetic analyses

The maximum likelihood trees were analysed using IQ-TREE v.2.2.0[88] with partitioned or unpartitioned schemes and the following options: -nt AUTO -m MFP -merit BIC -gmedian -bb 10000 -alrt 10000 -abayes. The gene trees were computed using the same approach as gene-partitioned datasets. The coalescent species tree was estimated using ASTRAL v.5.15.5.[92]

To investigate how the data fit different phylogenetic scenarios of the focal taxa, we tested amino acid (AA) and nucleotide (NT) levels 66-genes datasets using IQ-TREE v.2.2.0[88] with per-site log-likelihoods calculated using -zb 100,000 -zw and -au parameters. Then, we employed the approximately unbiased AU-test,[93] the KH-test (one-sided Kishino–Hasegawa test[94]), the p-SH (p-value of the Shimodaira–Hasegawa test[95]), the p-WKH (p-value of weighted KH test), the p-WSH (p-value of weighted SH test), and c-ELW (Expected Likelihood Weight[96]), and bp-RELL (bootstrap proportion using RELL method[97]). The 66-gene NT dataset was tested to determine whether it supports *Diplophoenicus* as a sister to the Hapatesinae + Pityobiinae clade, and the 66AA dataset supports the (*Diplophoenicus* (Omalisinae (Agrypninae + Dendrometrinae)) clade.

Additionally, we tested the monophyly of the focal clade using four-cluster likelihood mapping[98] in IQ-TREE. We divided the taxa into clusters shown in Figure 2B. All 672 unique quartets were considered.

Divergence dating

We used BEAST v.1.8.1[89] to estimate the time frame of the clade diversification. We pruned the dataset. It contained the focal taxa and *cox1*, *rrnL* mtDNA, and *LSU* rRNA genes (*SSU* rRNA was omitted due to missing data). The dataset was partitioned by genes, with an unlinked site model and the HKY+I+G4 substitution model. We used the universal *cox1* rate (0.0115[99]) as the lognormal relaxed clock. Further, the second analysis used only reported *Cretopityobius pankowskiorum* Otto from Burmese amber to calibrate the split among Pityobiini and other Pityobiinae tribes under the following settings: lognormal prior distribution, initial value 95.0, log(mean) 2.0, log (Stdev) 1.0, and offset 95.0. The resulting node distribution has 102.4 median and 96.43–133.3 95% HDP. Both analyses were set to Speciation: Birth-Death Process. The analysis was run for 100 million generations, with a 10,000 sampling frequency. Tracer v.1.7[89] was used for checking a convergence. The maximum credibility tree was estimated using Treeannotator[89] after discarding the initial 50% of trees as burn-in. Due to topological uncertainty, we also estimated with the same setting the constrained topologies without Morostomatinae, as its position was incongruent with the preferred topology in some analyses.

Palacký University Olomouc
Faculty of Science
Department of Zoology

Studies on neoteny in Coleoptera: phylogenomics, classification, and evolutionary interactions

SUMARY OF PH.D. THESIS / AUTOREFERÁT DIZERTAČNÍ PRÁCE

Mgr. Dominik Kusý

Biology
Zoology

Supervisor: Prof. Ing. Ladislav Bocák, Ph.D.

Olomouc 2024

SUMMARY OF THE PH.D. THESIS

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Study program: ZOO IA18 Zoology

Obhajoba disertační práce se koná dne..... 2024 v ..... hodin v učebně č.....
na Katedře zoologie Přírodovědecké fakulty Univerzity Palackého v Olomouci, 17. listopadu 50, Olomouc. Na stejné adrese se lze také seznámit s disertační prací a posudky.

Abstract

Beetles owe their evolutionary success to metamorphosis. However, their relationships remain controversial, hindering progress in understanding the evolution of different beetle phenotypes. Elateriformia, which includes jewel beetles, pill beetles, fireflies, click beetles, and their relatives, are members of the deepest branch of the "core" Polyphaga (Coleoptera), containing 31 extant families and approximately 43,000 species. These beetles exhibit considerable morphological and ecological diversity. However, their characteristics have led to an unstable classification.

This dissertation investigates the evolution of soft-bodied beetle lineages, focusing on their phylogenetic relationships, temporal dynamics, and the emergence of modified lineages. With eight comprehensive studies, state-of-the-art phylogenomic methods, next-generation sequencing, molecular systematics, and morphology, I aimed to unravel the evolutionary relationships of the studied lineages. The included studies investigated beetle evolution using different data sources such as mitogenomes, nuclear genes, and transcriptomes. I addressed fundamental questions and highlighted the effectiveness of molecular techniques in revealing complex phylogenetic relationships while addressing the possible incongruences and challenges when resolving deep splits.

The primary objective of this study is to clarify the higher phylogeny and phylogenetic placement of enigmatic neotenic soft-bodied lineages within the beetles, especially within Elateriformia. For example, molecular analyses were used to determine the taxonomic affiliations of *Paulusiella*, *Analastesa*, and *Thylodrias*. These analyses challenged conventional classification methods due to the altered morphologies of these species. This highlights the inadequacy of relying solely on morphological characters to accurately infer evolutionary relationships within soft-bodied beetle lineages. Additionally, my research reveals the position of the family Sinopyrophoridae, providing novel insights into the relationships of lineages in elateroid-lampyroid clade and showing that their ancestor was, in fact, a hard-bodied elaterid-like beetle. Moreover, I address the uncertainty surrounding the internal classification of Elateridae, Cantharidae, Lycidae, and Dermestidae by utilizing various molecular datasets.

In addition, my research focuses on the evolutionary basis of life history traits, bioluminescence, and mimicry. Studying the genetic basis and ecological implications of these traits elucidates the adaptive significance of evolutionary innovations and provides a comprehensive picture of beetle diversification through time.

By harnessing the power of molecular techniques and interdisciplinary approaches, I have advanced our understanding of the evolution of soft-bodied beetles and contributed to the broader discourse on biodiversity, adaptation, and evolutionary biology.

Key words: Phylogenomics, evolution, Coleoptera, Elateriformia, Dermestidae, Lycidae, Elateridae, Cantharidae, neoteny, systematics, ontogenetic modifications, molecular dating, bioluminescence, mimicry.

Abstrakt

Brouci vděčí za svůj evoluční úspěch metamorfóze. Nicméně jejich vzájemné vztahy zůstávají kontroverzní, což brzdí pokrok ve výzkumu evoluce jejich rozmanitých fenotypů. Elateriformní brouci, kteří zahrnují skupiny známé jako krasci, světlušky, páteříčci, kovaříci a jejich příbuzné, patřící mezi nejhlubší větve tzv. "core" Polyphaga (Coleoptera), skupinu tvoří 31 recentních čeledí a přibližně 43 000 druhů. Tito brouci vykazují značnou morfologickou a ekologickou diverzitu. Avšak právě jejich ekologická i morfologická rozmanitost vedly k nestabilní klasifikaci.

Tato disertační práce se zabývá evolucí linií měkkotělých brouků s důrazem na jejich fylogenetické vztahy, časovou dynamiku a vznik modifikovaných linií. V osmi komplexních studiích zahrnujících pokročilé fylogenomické metody, sekvenování nové generace, molekulární systematiku a morfologii, jsem se snažil vyřešit evoluční vztahy studovaných skupin. Tyto studie zkoumaly evoluci brouků s využitím různých zdrojů dat, jako jsou mitogenomy, nukleární geny či transkriptomy. Zabýval jsem se základními otázkami příbuznosti a zdůraznil efektivitu molekulárních technik v odhalování složitých fylogenetických vztahů a možných nekonzistencí.

Hlavním cílem této studie je objasnit vyšší fylogenezi a fylogenetické umístění záhadných neotenických linií brouků uvnitř Elateriformia. Molekulární analýzy byly použity k určení taxonomické příslušnosti rodů *Paulusiella*, *Analastesa* a *Thylodrias*. Tyto analýzy vedly k přehodnocení tradičních metod klasifikace kvůli paralelním modifikacím morfologických znaků u těchto druhů. To ukazuje na nedostatečný taxonomický signál morfologických znaků pro přesné určení evolučních vztahů uvnitř linií měkkotělých brouků. Další studie se zabývá pozicí čeledi Sinopyrophoridae. Tato práce poskytuje nové pohledy na vztahy linií v elateroid-lampyroidním kládu a ukazuje, že jejich předek byl ve skutečnosti brouk podobný kovaříkům. Dále jsem se věnoval vyřešením vnitřní klasifikace čeledí Elateridae, Cantharidae, Lycidae a Dermestidae s využitím různých molekulárních datových sad.

Mimo to se mé studie soustředily na evoluci životních strategií, bioluminiscenci a mimikry. A jejich vliv na diverzifikaci a evoluční úspěch studovaných skupin v čase.

Využitím molekulárních technik a interdisciplinárních přístupů jsem snad přispěl k našemu chápání evoluce brouků s „měkkým tělem“ a přispěl k širší diskusi o biodiverzitě, adaptaci a evoluční biologii.

Klíčová slova: Fylogenomika, evoluce, Coleoptera, Elateriformia, Dermestidae, Lycidae, Elateridae, Cantharidae, neotenie, systematika, ontogenetické modifikace, molekularní datování, bioluminescence, mimikry.

Thesis outline

This Ph.D. thesis entitled "**Studies on neoteny in Coleoptera: phylogenomics, classification, and evolutionary interactions**" is composed of 8 published studies representing summarized research made by the author during his Ph.D. study.

The first study (Part I) focuses specifically on the Elateridae family and the placement of enigmatic lineages of modified soft-bodied elateroids, namely *Paulusiella* and *Analastesa*. Both are only known from males, with modified morphology and are unable to click. Here I used mitogenomes and nuclear genes to test their placement in Cebrionini and Elaterinae incertae sedis. *Paulusiella* was recovered as a sister lineage to the subfamily Hemiopinae, and *Analastesa* was found to be one of the serially splitting branches in Cardiophorinae. It has been shown that click beetles affected by ontogenetic changes in many cases converge to similar forms. Therefore, their phylogenetic position cannot be reliably inferred by analyzing morphological characters alone and must be validated by molecular data. This highlights the need for cautious interpretation of morphology in other soft-bodied groups, including fossil taxa described mostly from amber deposits.

In the second study (Part II), the new family Sinopyrophoridae is recovered and detailed phylogenomic analyses of the elateroid-lampyroid clade are performed. In this study, I first begin to explore the sources of conflicting topologies for some of the lineages of Elateriformia that cloud our understanding of the evolution of bioluminescence and neoteny. I also examine in detail the sources of conflicting signals in the data and propose likely solutions. Furthermore, this study shows that the common ancestor of the soft-bodied Lampyridae, Rhagophthalmidae, and Phengodidae was in fact a hard-bodied elaterid like click beetle. And highlight the inability of morphological characters to resolve deep splits in groups with lineages that shifted to soft-bodiness and neoteny from ancestral hard-bodied forms. This study also discusses the general trends and timing of the evolution of bioluminescence in beetles.

The third study (Part III) deals with phylogeny and divergence time estimation of the family Dermestidae. Boundaries between subfamilies were recovered and their origin was dated between the Middle Jurassic and the Upper Cretaceous. Mitogenomics was used to reconstruct their phylogeny and evolution of life history strategies. This family represents another clade with neoteny origins outside of Elateriformia. The position of *Thylodrias contractus* with brachypterous females and flightless males is resolved here. The evolution of Dermestidae represents a new example of ecological shifts already observed in the evolution of beetles: mycetophagy as a feeding habit predisposing a shift to saprophagy. Also discussed are the effect of flightlessness and host specificity as drivers of diversification; a shift

from free-living in crevices to commensalism in the nests of eusocial Hymenoptera; parallel shifts from general saprophagy to keratin feeding; and the increased diversification rate of beetles associated with angiosperms. As part of this study, I have also succeeded in establishing a laboratory breeding colony of *Thylodrias*, which will serve as a distant comparison to neotenic lineages of Elateroidea in further studies of neotenic development.

The fourth study (Part IV) focuses on resolving the phylogeny of the family Cantharidae (Elateroidea) using different types of data and analytical approaches. This group of beetles represents another beetle lineage affected by soft-bodiness. Using a holistic approach and different types of data, relationships between subfamilies and the timing of diversification of lineages is resolved. In addition, characters used in previous morphology-based studies are re-evaluated and the classification and placement of Cretaceous Cantharidae are questioned.

The fifth study (part V) is a large-scale study using Lycidae (Metriorrhynchini), soft-bodied, appropriately colored beetles as a model to propose an effective way how to accelerate research and our understanding of unknown hyperdiverse lineages of tropical insects. Samples were collected from ~700 localities on three continents. The species-rich dataset included ~6500 terminals, and ~1850 putative species delimited at a 5% uncorrected pairwise threshold, of which ~1000 may be unknown to science. The study combines a phylogenomic approach using transcriptomes and genomes to build a phylogenetic backbone that will later be used together with traditional Sanger amplicon sequencing. The phylogenetic position of the *Cautires apterus* male with a modified ontogeny is also discussed. This study was only possible because of the many collaborators who provided the material and most importantly the lifelong efforts of my supervisor. The fact that my supervisor and I were able to collect all the necessary lineages for transcriptome sequencing during a single expedition to New Guinea, which mitigates the problem of large Lycidae genomes, also contributed to the success of this study.

The sixth study (part VI) is a detailed taxonomic work directly related to the previous study **(part V)**. Using a combination of top-down and bottom-up approaches, detailed taxonomic work is conducted for the porrostomine lineage using mtDNA and morphology. The 352 analyzed species were assigned to genera and 8 new genera are described in honor of the local people. Repeated origins of several external morphological characters previously used to delimit genera were identified. Therefore, concordant evidence from the densely sampled mitochondrial phylogenies and male genitalia is preferred. The analyses reveal high phylogenetic diversity and species richness in New Guinea, much lower phylogenetic diversity of the Australian continental fauna, and the limited permeability of Wallacea resulting in a single porrostomine genus in Asia. The study also points to the general acceptance of paraphyletic and polyphyletic taxa in the current classification.

The seventh study (Part VII) deals with the phylogeny of the tribe Lycini (Coleoptera: Lycidae) and shows how sexually dimorphic characters and common aposematic patterns in soft-bodied, aposematically colored, and chemically protected beetles can often mislead morphology-based classification. In this study, a mito-ribosomal dataset was assembled representing ~100 species from across the range. Results show that each specific aposematic pattern occurs in a limited range and that similar body shape and coloration evolved in unrelated sympatric lineages. High intraspecific polymorphism is presumably a result of adaptation of different populations to local mimetic assemblages. Therefore, the delimitation of many phenotypically diverse species should be investigated using molecular data.

The eight study (VIII) is a detailed phylogenomic work showing an independent, delayed origin of bioluminescence in the Elateridae. *Campyloxenus pyrothorax* reveals a separate, recent origin of bioluminescence in elateroids no older than ~ 53 mya. The study focuses on neglected and difficult-to-sample Southern Hemisphere lineages of putative Gondwanan origin. The sampling of many studies is biased toward developed countries and the South Hemisphere endemics are a valuable source of information for true worldwide phylogenies. The possible precipitation of *C. pyrothorax* in assemblages of unique aposematic rings of soft-bodied elateroids is also discussed. This discovery highlights the fourth or fifth origin of bioluminescence in Elateroidea, alongside the lampyroid clade, click beetles Pyrophorini, *Alampoides* and *Coctilelater* in Anaissini (Agrypninae), and *Balgus schnusei* (Thylacosterninae). While phylogenetic findings illuminate the phylogenetic aspects, the complete story awaits further field observations and in-depth genomic analyses of genetic and biochemical pathways used by bioluminescent elateroids.

Preface

Holometaboly is the latest major ontogenetic modification in Arthropoda (Engel & Grimaldi, 2005; Jindra 2019, Misof et al. 2014) and it changed the evolutionary history of animals on Earth. Today, holometabolous insects are the largest group of animals in the number of species, they are ubiquitous and play an important role in all terrestrial ecosystems. Fossil records indicate that the first holometabolan insects appeared in the Carbon and gradually rose to dominance in the Perm (Moran 1994; Nel et al. 2013). At least since the Permian extinction event, they dominate the terrestrial biota and they become indispensable parts of the ecosystems when they become important pollinators of angiosperms in the Cretaceous.

The principal trait, the endogenous development of wings (Medved et al. 2015; Almudi et al. 2020; Ross 2022; Prokop et al. 2023), is phenotypically demonstrated by the transition from the last wingless semaphoront, larva, to a pupa already having wing pads. The pupal stage lasts a substantial time of the transition between the last larval instar, still having the nutritional role and the adult with the predominant reproductive role. Hence, the pupa is a specialized larval instar that is inactive and neither takes food nor reproduces (Truman 2019). The separation of the nutritive and reproductive roles enabled phenotypic divergence opening access to energy sources otherwise unavailable. Therefore, the modification of ontogeny is one of the major macroevolutionary factors leading to novelties determining the success of lineages (Martynov et al. 2022). Developmental plasticity, the ability of an individual to modify its development in response to environmental conditions, might facilitate the evolution of such novel traits. Modifications of serial homologs of legs (wings, gills, mouthparts, antennae) are examples of such a process (Moczek et al. 2011; Fisher et al. 2020; Hu et al. 2019). In insects, the substantial phenotypic and ecological transition between larva and adult is hypothesized to be a key innovation leading to the present dominance of Holometabola, (=Endopterygota) in modern biota (Mayhew 2007). There are 11 recent holometabolan orders, combined having over a million recognized species and representing about 60% of the named animal diversity (Engel 2015).

Prevalently, the holometabolan metamorphosis is a fine-tuned ontogenetic process (Jindra 2019). The Holometabola is characterized by a developmental sequence in which the pupal stage services the transitions between larval/adult semaphoronts and their ecological roles. Measured by the dominance in the modern biota, the advantages of the holometaboly are prevalent. Nevertheless, the pupal stage brings about also disadvantages. Pupa needs time to rebuild the body tissues, is mostly defenseless for a quite long time (only chemical and mechanical protection remains available, e.g., aposematic coloration and setae), exposed to pathogens, and abiotic factors. All organisms pass through ontogenetic phases and metamorphose during their life in some way and there are multiple genes

regulating metamorphosis in insects (Truman & Riddiford, 2002; Jindra 2019). As a successive expression of genes can be modified, some stages may be suppressed, or the ontogenetic development prematurely terminated. The modifications of the temporal synchronization of ontogenetic phases are collectively called heterochrony.

Even though holometaboly is a key factor in the evolutionary success of insects (Rainford et al. 2014; Jindra 2019), some beetle groups especially many lineages of Elateriformia do not pass through full metamorphosis (the last preimaginal instar is active and feeding, adult traits are absent in imago, which differs in most extreme cases from the last larval instar only in sexual organs and a slightly different structure of cuticle) or their transformation from larva to an adult seems to be 'unfinished', i.e., they show incomplete metamorphosis (McMahon et al. 2016; Chafino et al. 2018). Under incomplete metamorphosis, we include sexually mature individuals that show mixed features of the larva, pupa, and adult. As examples, we can list shortened or vestigial appendages (antennae, legs, mouth parts), vestigial to absent elytra and wings (similar to pupal wing pads), lower meso/metathorax length ratio (the trait characteristic for beetle larvae), weakly sclerotized larviform abdomen, weakly sclerotized and simplified female genitalia, and possibly also incomplete sclerotization of the integument. The neoteny should be strictly separated from the ecologically driven loss of wings and the subsequent modification of some structures that have well-known consequences for female fecundity (Tigreros & Davidowitz 2019). Unlike neotenics, as defined by Gould (1977) and others, the groups affected by the ecologically driven loss of wings are not so deeply modified, usually, their modifications are linked to biological associations that make the loss of wings adaptive. I do not include under incomplete metamorphosis winglessness of both sexes that is putatively caused by ecological adaptations (island and high mountain environments) (Waters 2020; Roff 1990). Soft-bodied Elateriformia, i.e., soldier beetles, most net-winged beetles, and fireflies, etc., are traditionally considered completely metamorphosed, but their aberrant phenotypes can be a result of same or similar modifications of metamorphosis as undisputable neotenic forms but affecting only the last phase of the metamorphosis (Kusy et al. 2019). Females always show deeper modifications than conspecific males. Adult traits are progressively expressed from the frontal to posterior parts of the body. Males are seldom obviously modified, but if females are larviform, the males are often miniaturized, have shortened elytra, and can be wingless (Takahashi, et al. 2016; Kusy et al. 2019).

It is widely accepted that genetic and morphological divergence are loosely linked, and that radical phenotypic differences between close relatives can be caused by a relatively simple modification of genetic information that controls metamorphosis (McMahon et al. 2016; Vea et al. 2019). Therefore, the phylogeny can be correctly revealed only with an independent phylogenetic signal. Similar to some parasites, most neotenic taxa were correctly placed in the classification only after molecular evidence was obtained (Kusy et al. 2018; Kusy et al. 2021).

Reconstructing the Tree of Life remains a central goal in biology. Despite the obvious benefits of the large-scale datasets, new challenges appear (Yeates et al. 2016). Investigations based on phylogenomics, which use hundreds to thousands of loci for phylogenetic inquiry, have provided a clearer picture of life's history, but certain branches remain problematic (Steenwyk & King 2024). Therefore, the use of large-scale omics-datasets will not end the presence of conflicting and often incongruent hypotheses but will enable us to evaluate the sources of the inconsistencies and in many cases provide a more complex picture of evolution (Steenwyk & King 2024).

My Ph.D. thesis aims to provide an understanding of the evolution of beetles and Elateriformia through the robust phylogeny, timing, and the reconstruction of the origins of modified (soft-bodied) lineages. I have considered a wide range of information sources, and the studies presented not only resolve some crucial questions about the evolution of beetles and soft-bodied lineages in general, but also demonstrate the power of molecular methods and the need for rigorous evaluation of inferred phylogenies. I wanted to extend the sampling to focus on neotenic lineages to provide critical information about their closest relatives. Therefore, I wanted to estimate the age of heterochronic lineages and their closest relatives, regardless of their morphological divergence. Research in this direction has been minimal, and our current understanding of the diversification rates of the group in question is severely hampered by the lack of such data, which was partially changed by my work.

Conclusion and outlook

My Ph.D. thesis and a set of presented studies relate to the uniting of a single goal: I wanted to resolve the phylogenetic relationships of diverse beetle lineages, especially soft-bodied groups of Elateriformia in particular. To effectively study the genetic and molecular mechanisms underlying diverse phenotypic traits, their role in evolution, and how they shape the origin of diversity. I hope that my research has contributed to the understanding of these phenomena in the soft-bodied lineages with modified ontogenetic development.

Recovery of true phylogeny and disentangling causes of possible incongruence in the phylogeny is essential to effectively investigate the genetic and molecular mechanisms underlying diverse phenotypic traits in biology e.g. evolution of neotenic lineages and their development. It is not surprising that a lot of work remains ahead, especially at the genetic and molecular level. The genetic control of metamorphosis is a hot topic but remains limited to model organisms. However, the nature of the changes that lead to neoteny, and progeny, is not understood. Therefore, it is critical to develop detailed studies of the modification of gene expression and genome structure in neotenic lineages together with gene modification experiments, preferably using several unrelated models, to investigate whether similar heterochronic modifications are controlled by the same or different changes in the genetic machinery. Such studies must be designed with well-understood phylogenetic relationships in mind. **Because nothing in biology makes sense except in the light of evolution.**

Abstracts of Included Studies

Kusy, D., Motyka, M., & Bocak, L. (2023). Ontogenetic modifications produce similar phenotypes in distantly related click beetles (Coleoptera: Elateridae). ***Insect Systematics and Diversity*****, 7(4), 7. (IF = 3.4)**

We analyze the relationships of the click beetles (Elateridae) *Paulusiella* Löbl, 2007, and *Analestesa* Leach, 1824 (=*Cebriognathus* Chobaut, 1899). Both are incapable of jumping, with soft-bodied habitus caused by the incomplete sclerotization of the cuticle during the metamorphosis and unknown females. Their phylogenetic positions have been an uncertain issue. We use mitochondrial genomes and nuclear genes to test their current placement in Cebrionini (=Cebriognathini) and Elaterinae *incertae sedis*, respectively. We recover *Paulusiella* as a sister to *Hemiops* Laporte, 1838 (Hemiopinae) and *Analestesa* as one of the serially splitting branches in Cardiophorinae, both with robust support. Paulusiellini **trib. nov.** is proposed for *Paulusiella* in Hemiopinae due to high morphological disparity. *Analestesa* is transferred to Cardiophorinae, and Cebriognathini Paulus, 1981, an earlier synonym of Elaterinae: Cebrionini, is a synonym of Cardiophorinae Candèze, 1859. The click beetles affected by ontogenetic modifications converge to similar forms. As a result, their phylogenetic position cannot be reliably inferred by morphological analyses and needs to be validated by molecular data. *Paulusiella* and *Analestesa* represent two additional cases of the shift to incomplete sclerotization in elaterids raising the total number to 6. The present transfers of extant taxa between subfamilies call for a cautious interpretation of morphology in other soft-bodied groups, including the taxa described from amber deposits.

Kusy, D., He, J. W., Bybee, S. M., Motyka, M., Bi, W. X., Podsiadlowski, L., Li, X. Y. & Bocak, L. (2021). Phylogenomic relationships of bioluminescent elateroids define the 'lampyroid'clade with clicking Sinopyrophoridae as its earliest member. *Systematic Entomology*, 46(1), 111-123. (IF = 4.841)

Bioluminescence has been hypothesized as aposematic signalling, inter- sexual communication and a predatory strategy, but origins and relationships among bioluminescent beetles have been contentious. We reconstruct the phylogeny of the bioluminescent elateroid beetles (i.e. Elateridae, Lampyridae, Phengodidae and Rhagophthalmidae), analysing genomic data of *Sinopyrophorus* Bi & Li, and in light of our phylogenetic results, we erect Sinopyrophoridae Bi & Li, **stat.n.** as a clicking elaterid-like sister group of the soft-bodied bioluminescent elateroid beetles, that is, Lampyridae, Phengodidae and Rhagophthalmidae. We suggest a single origin of bio- luminescence for these four families, designated as the 'lampyroid clade', and examine the origins of bioluminescence in the terminal lineages of click beetles (Elateridae). The soft-bodied bioluminescent lineages originated from the fully sclerotized elateroids as a derived clade with clicking *Sinopyrophorus* and Elateridae as their serial sister groups. This relationship indicates that the bioluminescent soft-bodied elateroids are modified click beetles. We assume that bioluminescence was not present in the most recent common ancestor of Elateridae and the lampyroid clade and it evolved among this group with some delay, at the latest in the mid-Cretaceous period, presumably in eastern Laurasia. The delimitation and internal structure of the elaterid-lampyroid clade provides a phylogenetic framework for further studies on the genomic variation underlying the evolution of bioluminescence.

Motyka, M., Kusy, D., Háva, J., Jahodářová, E., Bílková, R., Vogler, A. P., & Bocak, L. (2022). Mitogenomic data elucidate the phylogeny and evolution of life strategies in Dermestidae (Coleoptera). *Systematic Entomology*, *47*(1), 82-93. (IF = 4.8)

Dermestidae (Bostrichoidea) exploit diverse food sources including fungal mycelia, but notably they as saprophagous, feeding on decomposing and dried flesh and keratin of animals and plants. Some of them live in spider webs, vertebrate and social insect nests, while others cause damage in human dwellings. Here, we use mitogenomics to reconstruct their phylogeny and evolution of life history strategies. We recovered serial splits of Orphilinae, Thorictinae + Dermestinae, Attageninae, Trinodinae and Megatominae, and we dated the origins of all subfamilies between the Middle Jurassic and Upper Cretaceous. Extant genera started their diversification in the Middle Cretaceous, except for *Dermestes* that originated in the Eocene. Mycetophagy, the likely feeding style of the common ancestor with Endecatomidae, was retained only by Orphilinae. Since the Late Jurassic, most dermestids have been saprophagous with the preference for desiccated tissue. We infer a scenario of feeding preferences from mycetophagy moving to saprophagy, always depending on food with low water content, followed by the shift from cryptic life in crevices and wood, to commensalism with social Hymenoptera, and ultimately feeding on angiosperm pollen as adults. The dependence on spider larders evolved already in the Early Cretaceous, but lineages with this specialized strategy remained species-poor. We date the origin of exploitation of vertebrate carcasses to the Eocene when modern mammalian fauna became dominant. The diversification of Megatominae (62% of known dermestids) and *Attagenus* Latreille (17%) coincides with the radiation of angiosperms.

Motyka, M., Kusy, D., Biffi, G., Geiser, M., Kazantsev, S. V., Bilkova, R., ... & Bocak, L. (2023). Untangling the evolution of soldier beetles (Coleoptera: Cantharidae) and the evaluation of the morphological phylogenetic signal in a soft-bodied elateroid lineage. ***Cladistics, 39*****(6), 548-570. (IF = 3.6)**

This study addresses the long-standing uncertainty about the internal classification of soldier beetles (Elateroidea: Cantharidae). Four datasets were compiled and analysed: 66 genes for 14 terminals, 15 mtDNA genes for 79 terminals, one mtDNA and two rRNA genes for 217 terminals, and barcodes for 576 terminals. Based on congruent topologies, Chauliognathinae is proposed as a sister to the remaining Cantharidae, followed by the redefined Malthininae (including Tytthonyxini), the paraphyletic "dysmorphocerine" lineages (Dysmorphocerinae sensu stricto and Heteromastiginae **subfam. nov.**), and Silinae + Cantharinae as a terminal clade. The present phylogeny supersedes earlier morphology and short-fragment molecular hypotheses that have not converged on a consensus. Few morphological characters corroborate the DNA-based relationships (see the adults and larval keys). However, morphology-based hypotheses have relied on a few informative characters, and no evidence strongly rejects the preferred molecular topology. The interpretation of morphological characters and uncertain polarity resulting from the high phenotypic disparity of Elateroidea are discussed in detail. The dated phylogeny hypothesizes the earliest split within the Cantharidae in the Berriasian stage (Early Cretaceous, ~141 Myr) and the diversification of most extant subfamilies and tribes already in the Late Cretaceous. The most diverse subfamily, Cantharinae, represents a delayed radiation that started during the Eocene climatic optimum, 55.5 Myr. The late origin of Cantharinae questions the classification of Cretaceous Cantharidae as members of Cantharinae. Instead, the results suggest their deeper rooting after separating from dysmorphocerine lineages and before the node between Cantharinae and Silinae.

Motyka, M., Kusy, D., Bocek, M., Bilkova, R., & Bocak, L. (2021). Phylogenomic and mitogenomic data can accelerate inventorying of tropical beetles during the current biodiversity crisis. *Elife*, *10*, e71895. (IF = 8.713)

Conservation efforts must be evidence-based, so rapid and economically feasible methods should be used to quantify diversity and distribution patterns. We have attempted to overcome current impediments to the gathering of biodiversity data by using integrative phylogenomic and three mtDNA fragment analyses. As a model, we sequenced the Metriorrhynchini beetle fauna, sampled from ~700 localities in three continents. The species-rich dataset included ~6500 terminals, ~ 1850 putative species delimited at 5% uncorrected pairwise threshold, possibly ~1000 of them unknown to science. Neither type of data could alone answer our questions on biodiversity and phylogeny. The phylogenomic backbone enabled the integrative delimitation of robustly defined natural genus-group units that will inform future research. Using constrained mtDNA analysis, we identified the spatial structure of species diversity, very high species-level endemism, and a biodiversity hotspot in New Guinea. We suggest that focused field research and subsequent laboratory and bioinformatic workflow steps would substantially accelerate the inventorying of any hyperdiverse tropical group with several thousand species. The outcome would be a scaffold for the incorporation of further data from environmental sequencing and ecological studies. The database of sequences could set a benchmark for the spatiotemporal evaluation of biodiversity, would support evidence - based conservation planning, and would provide a robust framework for systematic, biogeographic, and evolutionary studies.

Kusy, D., Motyka, M., Bilkova, R., & Bocak, L. (2022). How Do Genomic, Mitochondrial, and Morphological Data Contribute to the Linnean Classification of the Porrostomine Net-Winged Beetles (Coleoptera, Lycidae)? *Insect Systematics and Diversity*, *6*(5), 6. (IF = 3.4)

The Lycidae genera have seldom been tested with phylogenetic analyses. Therefore, we assembled genomic data to estimate the phylogenetic backbone of the porrostomines, one of Metriorrhynchina's major clades. Further, mtDNA and morphology were employed to assign 352 analyzed species to genera. We present evidence for the paraphyly of *Metriorrhynchus* and terminal position of *Porrostoma*, revise the generic classification, and describe eight genera: *Maraiakoreus* **gen. nov.**, *Kuarhynchus* **gen. nov.**, *Riedelrhynchus* **gen. nov.**, *Bundikanus* **gen. nov.**, *Yamarhynchus* **gen. nov.**, *Bekorhynchus* **gen. nov.**, *Sundarhynchus* **gen. nov.**, and *Isuarhynchus* **gen. nov.** We synonymize *Stadenus* Waterhouse, 1879, **syn. nov.**, *Metriorrhynchoides* Kleine, 1923, **syn. nov.**, and *Oriomum* Bocak, 1999a, **syn. nov.**, to *Porrostoma* Castelnau, 1838. Next, we propose 75 new combinations and four new species: *Bundikanus styskalai* **sp. nov.**, *Kuarhynchus sisrangensis* s**p. nov.**, *Maraiakoreus argenteus* **sp. nov.**, and *Yamarhynchus sinopassensis* **sp. nov.** We identified repeated origins of several external morphological traits earlier used to delimitate genera. Therefore, we prefer concordant evidence from the densely sampled mitochondrial phylogenies and male genitalia. The analyses identify high phylogenetic diversity and species richness in New Guinea, much lower phylogenetic diversity of the Australian continental fauna, and the limited permeability of the Wallacea that resulted in a single porrostomine genus in Asia. We point to the common acceptance of paraphyletic and polyphyletic taxa in the current classification. As a result, taxonomy has not provided expected support for any state-of-the-art evolutionary and zoogeographic studies. The phylogeny, species inventory, and classification of porrostomines set the basis for future evolutionary and zoogeographical studies.

Kusy, D., Motyka, M., Fusek, L., Li, Y., Bocek, M., Bilkova, R., ... & Bocak, L. (2021). Sexually dimorphic characters and shared aposematic patterns mislead the morphology-based classification of the Lycini (Coleoptera: Lycidae). ***Zoological Journal of the Linnean Society*****,** ***191*****(3), 902-927. (IF = 3.838)**

The Lycini (Elateroidea: Lycidae) contains > 400 species placed in four typologically based genera and numerous subgenera. We assembled a mito-ribosomal dataset representing ~100 species from the whole range and recovered a phylogeny rejecting *Lycus* and *Lycostomus* as polyphyletic assemblages. The male-specific wide elytra and elytral thorns are identified in unrelated *Neolycus* and *Lycus*. The morphological similarity based on sexual dimorphism and aposematic patterns defined terminal clades and misled the genus-rank classification. We delimit *Neolycus*, *Rhyncheros* reinst. name (= *Thoracocalon* **syn. nov**. = *Lyconotus* **syn. nov**.), *Lipernes Lycostomus*, *Haplolycus* and *Lycus*. *Demosis* and six subgenera of *Lycus* are synonymized with *Lycus*. *Celiasis* Laporte, 1840 is kept in the classification as a *nomen dubium* until any specimen is available. The deep lineages are known from the Americas and Asia. Africa was colonized by *Lycus* and *Haplolycus*. Each specific aposematic pattern occurs in a limited range, and the similar body shape and coloration evolved in unrelated sympatrically occurring lineages. High intraspecific polymorphism is putatively a result of the adaptation of various populations to local mimetic assemblages. Therefore, the delimitation of many phenotypically diverse species should be investigated.

Motyka, M., Kusy, D., Arias-Bohart, E. T., Bybee, S. M., & Bocak, L. (2023). Enigmatic *Campyloxenus*: Shedding light on the delayed origin of bioluminescence in ancient Gondwanan click beetles. *Iscience*, *26*(12), 108440. (IF = 5.8)

Gondwanan elaterids, previously thought to be unrelated, include bioluminescent *Campyloxenus* earlier placed in bioluminescent Pyrophorinae. Genomic data suggest close relationships between Gondwanan groups. We maintain Morostomatinae and Hapatesinae and redefine Pityobiinae with Nearctic Pityobiini, Gondwanan Parablacini **stat. nov.**, Campyloxenini **stat. nov.**, and Tibionemini **trib. nov.** Their ancestors putatively underwent differentiation in Gondwana during the Cretaceous separation of southern continents. In contrast with their age, extant groups are species poor. *Campyloxenus* represents a recent origin of bioluminescence, no older than 53 my. Its large pronotal lanterns differ from Pyrophorini and resemble color patches of sympatric beetle co-mimics. This discovery highlights the fourth or fifth origin of bioluminescence in Elateroidea, alongside the lampyroid clade, click beetles Pyrophorini, *Alampoides* and *Coctilelater* in Anaissini (Pyrophorinae), and *Balgus schnusei* (Thylacosterninae). While our phylogenetic findings illuminate the phylogenetic aspects, the complete story awaits further field observations and in-depth genomic analyses of biochemical pathways used by bioluminescent elateroids.

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Curriculum Vitae

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Ph.D. student, Zoology, Palacky University, Olomouc, Faculty of Science (since July 2019)

MSc. Zoology, Palacky University, Olomouc, Faculty of Science (2017–2019)
Bc. Biology and Ecology, Palacky University, Olomouc, Faculty of Science (2014–2017)

Research positions:

Researcher in Laboratory of Biodiversity and Molecular Evolution, CATRIN, Palacky University, Olomouc (since 2020)

Field experience:

2017 – West Papua Province, Indonesia, one month
2018 – Papua New Guinea, Binatang Research Center, Nagada, one month
2019 – Papua New Guinea, Binatang Research Center, Nagada, one month
2023 – Ecuador, one month

Professional Experiences:

November 2019 – May 2020 Research internship at Zoological Research Museum Alexander Koenig (6 months)
Jun 2022 University of Oslo, CEES - Centre for Ecological and Evolutionary Synthesis, Laboratory of Eivind Undheim (1 month)

Selected research projects:

GACR 18-14942S Evolution of aposematic patterns in extensive Muellerian mimicry complexes (2018-2020). Co-investigator.
GACR 22-35327S Elateriform beetles as a model for studying the evolution of neoteny (2022-2024). Co-investigator.

IGA_PřF_2021_026. Phylogenomics of Elateroidea for the reconstruction of ontogenetic modifications. Primary investigator.
Student Grant Project: DSGC-2021-0170. Characterization of putatively neurotoxic venoms of fireflies and click beetles. Primary investigator.

Selected awards:

Price of the rector of University Palacky for the best student publication 2018, 2019

Conferences and Courses:

9th Meeting on Insect Phylogeny in Dresden
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Public outreach

Multiple talks and other activities at Fort of Science https://www.pevnostpoznani.cz/english/

Research interest:

- Evolution of neoteny and bioluminescence - origin of novelties, genetic causes and consequences.
- Molecular phylogenomics of beetles, taxonomy, and molecular systematics.
- Biodiversity genomics and comparative genomics.
- Venom evolution in predatory beetles.
- Phylogenetic conflicts and systematic errors in phylogenies inferred from genome-scale data.

Professional skills:

Bioinformatic analysis; basic programming (shell, python, R, nextflow, snakemake); Laboratory experience (DNA, RNA isolation, PCR, Sanger and Nanopore sequencing); Illumina, PacBio and ONT data handling and processing; Advanced phylogenetic analyses (statistical evaluation of phylogenetic signal, model selection, likelihood tests, etc.); PAML (CODEML) selection tests; Assembling and annotation of mitochondrial and nuclear genomes; genome annotations and comparative genomics methods; transcriptome data assembly and analyses; Analyzing NextRAD population data; Divergence time estimation (Beast, r8s, MCMCtree); processing of Synchrotron Radiation Micro-CT scans; basics of proteomics; Linux, OSX and Windows operating systems; well knowledge about IT hardware; graphics software (Photoshop, Illustrator).

Selected Publications:

*Motyka, M., **Kusy, D**., Arias-Bohart, E. T., Bybee, S. M., & Bocak, L. (2023). Enigmatic *Campyloxenus*: Shedding light on the delayed origin of bioluminescence in ancient Gondwanan click beetles. *Iscience*, 26(12), 108440.

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