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Evoluce a klasifikace tribu Metriorrhynchini (Insecta: Coleoptera: Lycidae)

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Abstrakt:

Lycidae jsou druhově početnou čeledí terestrických brouků (Coleoptera), která obsahuje asi 150 rodů s více než 4000 popsanými druhy po celém světě. Největší diverzita je dokumentována v oblastech tropického deštného lesa. Lycidae jsou významní jak z hlediska počtu druhů, tak evolucí mimetických komplexů nebo neotenních forem. Tato čeleď je v současnosti klasifikována v nadčeledi Elateroidea (Polyphaga: Elateriformia). Tato skupina je charakteristická především měkkým, nekompletně sklerotizovaným tělem a sníženou schopností letu. Všechny druhy jsou schopny produkovat zapáchající, slabě jedovaté látky, které jsou emitovány při vyrušení či podráždění ve formě kapek hemolymfy na krovkách nebo intersegmentálních membránách. Chemická ochrana čeledi Lycidae vede k evoluci pestrého, aposematického zbarvení.

Tato studie je omezena na tribus Metriorrhynchini, který je z hlediska druhové rozmanitosti nejvýznamnější linií čeledi Lycidae. Metriorrhynchini jsou morfologicky definováni kruhovou phallobází samčích kopulačních orgánů, plochou nápadnou nepárovou žlázou v kopulačních orgánech samic, střední areolou kopinatého tvaru na pronotu a většinovou přítomností dalších žeber na pronotu, které vytvářejí postranní areoly. Metriorrhynchini jsou velmi vhodnou modelovou skupinou pro studium vzniku diverzity vzhledem k jejich omezené dispersní schopnosti, uniformní biologii a mimořádně vysokému počtu druhů (přes 1200 druhů ve 41 rodech). Největší počet druhů tribu Metriorrhynchini pochází z kontinentální jihovýchodní Asie, Velkých Sund, Filipín, Moluk, Nové Guinee a severní Austrálie. Další druhy se vyskytují v Subsaharské Africe, na Madagaskaru, na Sri Lance a v Indii. Dosavadní znalosti o zoogeografii této skupiny byly velmi omezené a k dispozici nebyla žádná hypotéza o pravděpodobných oblastech vzniku této skupiny nebo o směrech disperze a jejich datování.

Studie je založena především na molekulárních datech a na jejich základě vytvořených fylogenetických hypotéz. Cílem práce je rekonstrukce ancestrálních území v oblasti

Gondwany a šíření tribu Metriorrhynchini do jihovýchodní Asie, do Afriky a na Madagaskar a jejich srovnání s dosavadními znalostmi o rozpadu Gondwany. Metriorrhynchini se skládají ze dvou linií: afro/orientální a australské. Ancestrální území pro bazální linii celého tribu nelze s jistotou určit vzhledem k topologické nejistotě a tato linie pochází buď z Austrálie, nebo Indie a k jejich rozštěpení došlo v době, kdy se Gondwana začala rozdělovat. Afrotropická fauna a fauna Madagaskaru vznikla disperzí z Indie driftující na sever před 65 až 62 miliony lety. Orientální fauna se diverzifikovala po srážce Indie s Asií a teprve později byla doplněna o linie migrující z australské oblasti, která se dostala do kontaktu s asijským šelfem před asi 25 miliony lety. Orientální fauna obsahuje rody indické i australské linie, přičemž analýzy datování prokázaly starší původ indických rodů.

Dalším cílem této studie je kritická revize vyšší klasifikace všech taxonů tohoto tribu prostřednictvím fylogenetické analýzy založené na kompletní sadě morfologických znaků. Studie rekonstruuje evoluci vybraných morfologických struktur, na kterých byla založena předešlá morfologická klasifikace. Potvrdila jsem několikanásobný vznik podobných struktur žeber na pronotu, zkrácených žeber na krovkách, sekundárních žeber na krovkách u více linií a jejich opakovanou ztrátu a mnohonásobný vznik flabelátních tykadel. Na základě molekulární fylogeneze skupiny, zhodnocení morfologie larev a dospělců jsme navrhli novou klasifikaci, kdy některé taxony jsou jednoznačně podporovány především larválními znaky a jiné znaky adultních stádií. Byly nově ustanoveny subtriby Metriorrhynchina, Metanoeina a Cautirina a subtriby Trichalina a Hemiconderina byly synonymizovány se subtribem Metriorrhynchina.

Klíčová slova: Coleoptera, Lycidae, Metriorrhynchini, Gondwana, fylogeneze, klasifikace, mtDNA, nové subtriby, nové rody, historie šíření

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Abstract:

Lycidae, net-winged beetles, are an abundant family of terrestrial beetles (Coleoptera), which contains about 150 genera with more than formally described 4,000 species worldwide. The greatest diversity is documented in tropical rain forests, while moderate diversity is also known from temperate zones; these beetles do not occur in arid regions. Lycidae are a prominent lineage concerning species richness, evolution mimetic complexes or neotenic modifications of development. This family is currently classified in the superfamily Elateroidea (Polyphaga: Elateriformia). Lycidae is characterized by soft-bodiedness and limited an ability to fly resulting from this trait. As compensation of this limitation they have developed the ability to produce repellent and slightly poisonous compounds, which are released as droplets of hemolymph on the elytra and around inter-segmental joints when the animals are disturbed. Chemical protection of the family Lycidae led to evolution of bright, aposematic coloration patterns.

This study is restricted to the tribe Metriorrhynchini, which are morphologically defined by circular phallobase, flat conspicuous unpaired vaginal gland, pronotum with lanceolate median areola and with carinae which form up to seven areolae in the pronotum. This tribe is the most species rich lineage of Lycidae. Due to their species-richness (over 1200 species in 41 genera), large geographic range, limited ability to disperse and uniform life strategy, Metriorrhynchini can be a suitable model group for studies on diversity, dispersal history and evolution of mimicry. Highest specific and generic diversity of Metriorrhynchini species is known from continental Southeast Asia, the Great Sundas, Philippines, Moluccas, New Guinea and northern Australia. Other species and a few endemic genera, occur in Sub-Saharan Africa, Madagascar, Sri Lanka and India. The previous knowledge of zoogeography of this group has been very limited and no phylogeographic hypothesis has been available for the identification of putative areas of origin or directions of dispersal. We used nuclear and mitochondrial DNA markers to propose phylogenetic and phylogeographic hypotheses.

The principal aim of this thesis was to reconstruct the putative area and time of origin of the tribe Metriorrhynchini and to recover the dispersal routes from the ancestral region around the Indian Ocean rim, e. g., Australia, Southeast Asia, Africa and Madagascar. Here, the phylogenetic hypothesis was considered in the light of the reconstructed break-up of Gondwana. Metriorrhynchini consist of two lineages: the Afro/Oriental and Australian ones. The basal lineage originated from Australia and India, from the time when Gondwana started to break-up. The Afrotropical fauna and fauna of Madagascar came from India drifting to the north about 65 to 62 million years ago. The Oriental fauna diversified from lineages from Indian subcontinent after the collision of India and Asia. Later the Oriental fauna was enriched by migrating lineages from the Australian region about 25 million years ago. Although the Oriental fauna includes the genera from both the Indian and Australian clades, the Indian lineages are more abundant and the analyses showed their older diversification history in the Asian continent.

The secondary aim of this study is the critical revision of classification of all taxa of this tribe. We used the same phylogenetic hypothesis to map the evolution of morphological characters on the tree and we inferred their origin and subsequent modifications. The previous morphological classification was based on the structure of pronotal carinae, elytral costae and the form of antennae. All these characters were mapped with multiple origins of structures and some of them multiple reversals. We hypothesized that evolution of some of these characters is affected by their selective value (the strengthening function of carinae and costae), they originated multiple times when their evolution is correlated with stenoptery (the shortened elytral costa 1) or they evolve when the opposite sex is located using olfactory organs (the flabellate antennae). Using the molecular phylogeny, we evaluated the morphology of larvae and adults; we proposed a new classification, with some taxa defined predominantly with either larval or adult characters. We delimited the subtribes Metriorrhynchina, Metanoeina and Cautirina and we synonymized the subtribes Trichalina and Hemiconderina with the subtribe Metriorrhynchina.

Keywords: Coleoptera, Lycidae, Metriorrhynchini, Gondwana, phylogeny, classification, mtDNA, new subtribes, new genera, dispersal history

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Obsah

1. Úvod

1.1 Klasifikace a biologie čeledi Lycidae

Lycidae jsou jednou z významných čeledí brouků (Coleoptera), jak z hlediska počtu druhů, tak evolucí životních strategií, jako jsou evoluce mimetických komplexů nebo evoluce neotenních forem. Tato čeleď je v současnosti klasifikována v nadčeledi Elateroidea (Polyphaga: Elateriformia). V minulosti však Lycidae tvořili samostatnou nadčeleď Cantharoidea společně s čeleděmi páteříčkovití (Cantharidae), světluškovití (Lampyridae) a několika menšími čeleděmi (Omethidae, Rhagophthalmidae, Phengodidae, Drilidae, Omalisidae a Telegeusidae). Všechny tyto čeledi jsou dnes součástí nadčeledi Elateroidea a jejich společné znaky, které dříve definovaly Cantharoidea, se vyvinuly mnohonásobně (Lawrence & Newton 1995, Bocakova *et al.* 2007).

Lycidae, v české literatuře občas nazývaní dlouhoústcovití nebo zářivkovití, jsou druhově početnou čeledí terestrických brouků, která obsahuje asi 150 rodů s více než 4000 popsanými druhy po celém světě. Podobně jako v jiných skupinách však bude skutečná diverzita pravděpodobně mnohonásobně vyšší (Erwin 1982, Dvorak & Bocak 2007, 2009). Přes téměř kosmopolitní rozšíření je výskyt této skupiny velmi nerovnoměrný. Největší diverzita je dokumentována v oblastech tropického deštného lesa (Kleine 1933, Lawrence 1982, Bocak & Bocakova 1990). Naproti tomu se nevyskytují v aridních oblastech a pouze ve velmi malém počtu druhů v temperátních oblastech s výraznou sezonalitou srážek. Např. pouze 17 druhů je známo ze západní časti palearktické oblasti (Bocak & Bocakova 2008).

Rozšíření této čeledi je důsledkem silné vazby především larev na stinné, celoročně vlhké lesní biotopy. Larvy se vyvíjejí ve dřevě v různém stupni rozkladu, v lesní hrabance nebo v půdě s větším množstvím organického materiálu, v sušších oblastech obvykle v kořenech hluboko v půdě (Bocak & Matsuda 2003). Podmínkou vhodnosti substrátu pro vývoj larev je dostatek tekutin, které obsahují mikrobiální život, v rozkládajícím se materiálu. V dospělosti nepřijímají potravu a pouze některé druhy navštěvují květy a sají nektar. Většina dospělců žije velmi krátce, pravděpodobně v důsledku neschopnosti nahrazovat ztrátu tělesných tekutin. Obě pohlaví jsou ve většině případů v dospělosti okřídlená, jen omezený počet druhů má larviformní samice, u kterých se sexuální zralost projevuje pouze otevřením kopulačních orgánů a změnou struktury kutikuly (Bocak & Bocakova 1990, Wong 1996, Bocakova *et al.* 2007, Levkanicova & Bocak 2009). Lycidae jsou skupinou velmi obtížně klasifikovatelnou pouze na základě morfologických znaků, protože mnoho struktur dosud používaných pro konstrukci fylogeneze a klasifikaci, např. žebra na pronotu nebo krovkách, má pravděpodobně adaptivní hodnotu pro zpevnění struktury slabě sklerotizovaného těla, a proto podléhá rychlé a často paralelní evoluci (Sklenarova *et al.* 2014).

Čeleď Lycidae je charakteristická, podobně jako ostatní tzv. cantharoidní čeledi, především měkkým, nekompletně sklerotizovaným tělem a sníženou schopností letu. Důsledkem této morfologické modifikace je omezená schopnost úniku před predátory. Jako kompenzace tohoto omezení se vyvinula u všech druhů čeledi Lycidae schopnost produkce zapáchajících, slabě jedovatých látek. Při vyrušení či podráždění jsou schopni vylučovat hemolymfu obsahující tyto látky (Moore & Brown 1981; Bocak *et al.* 2008). Chemická ochrana odrazuje jejich predátory od útoku, v tomto případě především pavouky a ptáky (Bocak *et al.* 2008). Kapky hemolymfy se mohou objevovat na okrajích a žebrech krovek, na tykadlech nebo na kloubech mezi femurem a tibií, kde dochází k prasknutí mezisegmentových membrán. Chemická ochrana čeledi Lycidae vede k evoluci pestrého zbarvení, které poskytuje rovněž určitý stupeň ochrany, neboť slouží jako varování pro případné predátory (Alatalo & Mappes 1996). Aposematické zbarvení zahrnuje celou škálu kombinací pestrých barev a černé, především kombinace žlutě/červeně a černě zbarvených částí pronota a krovek (Obr. 3). Aposematicky zbarvené druhy mají tendenci vytvářet agregace, které posilují reakci poučeného predátora a prodlužují dobu před opakováním útoku (Linsley *et al.* 1961).

Lycidae byli v posledních letech použiti jako modelová skupina pro studium evoluce neotenie a studium evoluce aposematických vzorů ve spojení se speciací po rozšíření areálu (Bocak *et al.* 2008, Bocak & Yagi 2010, Malohlava & Bocak 2010). Tyto studie jsou prvními, kdy Lycidae, jako taxonomicky velmi opomíjená skupina, byli použiti pro studium obecných evolučních otázek. Přitom se ukázalo, jak nedostatečné jsou znalosti o evoluci této skupiny, jejich historické biogeografii a jak slabě je podporována současná klasifikace této čeledi.

1.2 Modelová skupina: tribus Metriorrhynchini (Lycidae: Lycinae)

Tato studie je omezena na tribus Metriorrhynchini (Lycidae: Lycinae; Bocak & Bocakova 2008), který je ovšem z hlediska druhové rozmanitosti nejvýznamnější linií čeledi Lycidae. Ačkoliv se jedná o velmi úzce vymezenou část celkové genetické diverzity čeledi Lycidae, Metriorrhynchini jsou velmi vhodnou modelovou skupinou pro studium vzniku diverzity vzhledem k jejich omezené dispersní schopnosti, uniformní biologii a mimořádně vysokému počtu druhů. Kleine (1933) uvádí v katalogu přes 1000 druhů ve 41 rodech a dalších ~200 druhů bylo popsáno později (Bocak 2002). Největší počet druhů tribu Metriorrhynchini pochází z kontinentální jihovýchodní Asie, Velkých Sund, Filipín, Moluk, Nové Guinee a severní Austrálie. Další druhy se vyskytují v Subsaharské Africe, na Madagaskaru, na Sri Lance a v Indii (Bocak 2002, Kazantsev 2007, Kubeček *et al.* 2011). Dosavadní znalosti o zoogeografii této skupiny byly omezeny na deskriptivní delimitace areálů (Bocak 2002, Kazantsev 2012), avšak žádné informace nebyly k dispozici o pravděpodobných oblastech vzniku této skupiny nebo o směrech disperze a jejich datování. Přitom i údaje o rozšíření jednotlivých rodů bylo nutno posuzovat velmi kriticky, protože velká část rodů byla dosud definována pouze na základě typického druhu a pouze velmi vágně byly definovány limity jednotlivých rodů.

2. Přehled dosavadních znalostí o modelové skupině a metodologické přístupy v současné fylogenetické systematice a fylogeografii

2.1 Klasifikace tribu Metriorrhynchini - historie a současný stav poznání

Tropická fauna hmyzu je obecně velmi málo poznaná (Erwin 1982) a znalosti o skupinách bez hospodářského nebo medicinálního významu jsou obvykle omezeny na primární popisy alfa-taxonomické diverzity, rodovou a tribální klasifikaci založenou pouze na *ad hoc* vybraných diagnostických znacích, bez odvození klasifikace od robustní fylogenetické hypotézy. Pouze omezené množství skupin bylo taxonomicky revidováno s použitím současných metod fylogenetické systematiky.

2.2 Druhová diverzita tribu Metriorrhynchini

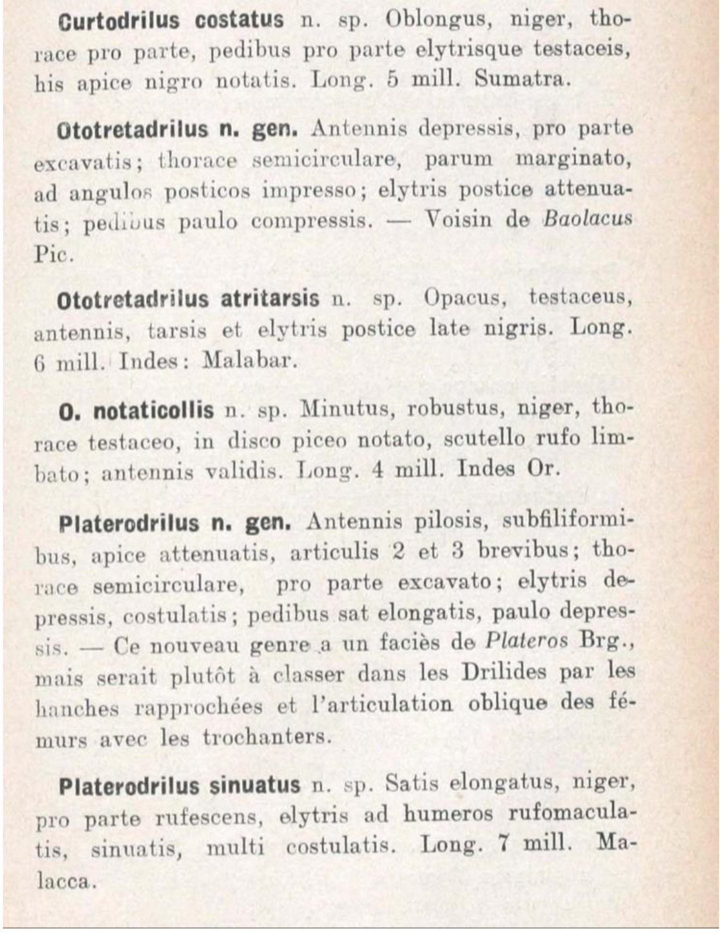
Znalosti druhové diverzity tribu Metriorrhynchini jsou velmi fragmentární a delimitace mnoha druhů a vyšších taxonů není založena na podrobném studiu evoluce, ale výhradně na diagnostických znacích bez jejich evoluční interpretace. Příkladem mohou být práce Maurice Pica z první poloviny 20. století, které neodpovídaly dobré taxonomické praxi ani v době svého vzniku (Obr. 1). Jedná se o podobnou situaci jako ve většině tropických skupin hmyzu, kdy suma našeho poznání vznikla kumulací informací velmi rozdílné kvality. V současnosti známých ~1200 druhů je většinou popsáno pouze na základě zbarvení a vnějších znaků, ve většině případů bez jakýchkoliv doprovodných ilustrací, a formální popisy často neobsahují ani použitelné diagnostické znaky. Vzhledem k této situaci, byla identifikace taxonů zmíněných v této studii prováděna do druhové úrovně pouze ve výjimečných případech, kdy bylo například nutno stanovit typové druhy nově popisovaných rodů nebo popsat nový taxon významný z evolučního pohledu (Bocak *et al.* 2014).

Jednotlivé taxony byly v této studii identifikovány vesměs pouze do úrovně rodů a odhady počtu druhů jsou odvozeny z divergence sekvencí DNA. Přitom je nutno zmínit, že diverzita tropických oblastí je omezeně poznána i z hlediska genetické diverzity a předložená studie je prvním rozsáhlejším datovým souborem v čeledi Lycidae. Obr. 2 ukazuje počty sekvencí DNA řádu Coleoptera uložených v databázi Genbank a pocházejících z jednotlivých center biodiverzity, jak je definoval Myers *et al.* (2000). Přes koncentraci diverzity v těchto centrech, je nejvíce dat k dispozici z Evropy, USA a Japonska.

2.3 Rodová a tribální klasifikace tribu Metriorrhynchini

V tribu Metriorrhynchini (*sensu* Bocak & Bocakova 2008, Sklenarova *et al.* 2014) je v současnosti definováno 49 rodů. První rody byly popsány na začátku 19. století z Austrálie a Wallaceovy linie a bylo v nich dlouho klasifikováno pouze několik druhů. Další rody, dnes tvořící kostru rodové klasifikace, byly popsány na konci 19. století Waterhousem (Waterhouse 1878, 1879). Rodová klasifikace byla dlouho velmi chaotická a limity rodů nebyly zřetelně definovány. Např. rod *Cladophorus* Guérin Méněville, 1830 obsahoval druhy z celého areálu tribu Metriorrhynchini a teprve Bocak (2002) omezil tento rod na jedinou terminální linii australských Metriorrhynchini. Podobně chaotická byla klasifikace australských rodů *Porrostoma* Castelnau, 1838 a *Metriorrhynchus* Geminger & Harold, 1869 (Calder 1998, Bocak 1998). Často byly popisovány monotypické rody velmi nejistého fylogenetického postavení, lišící se pouze unikátní apomorfií, často redukčního charakteru. Kazantsev (2006, 2012) navrhl samostatné rody pro taxony lišící se pouze redukcí žeber na pronotu a krovkách, přestože jejich plasticita byla již dříve prokázána (Bocak 2002).

Klasifikace tribu Metriorrhynchini prodělala v posledních dekádách několik změn. Dříve byla tato skupina klasifikována v samostatných tribech nebo podčeledích (Kleine 1933), později byly tyto taxony spojeny do jediného taxonu Metriorrhynchinae/ini (Bocak & Bocakova 1990, Bocak 2002). Podle předchozích studií byl tribus Metriorrhynchini rozdělen na subtriby: Trichalina Kleine, 1928, Hemiconderina Bocak & Bocakova, 1990 a Metriorrhynchina Kleine, 1926 (Bocak 2002). Klasifikace v jednotlivých obdobích je shrnuta v Tab. 1.



Curtodrilus costatus n. sp. Oblongus, niger, thorace pro parte, pedibus pro parte elytrisque testaceis, his apice nigro notatis. Long. 5 mill. Sumatra.

Ototretadrilus n. gen. Antennis depressis, pro parte excavatis; thorace semicirculare, parum marginato, ad angulos posticos impresso; elytris postice attenuatis; pedibus paulo compressis. — Voisin de *Baolacus* Pic.

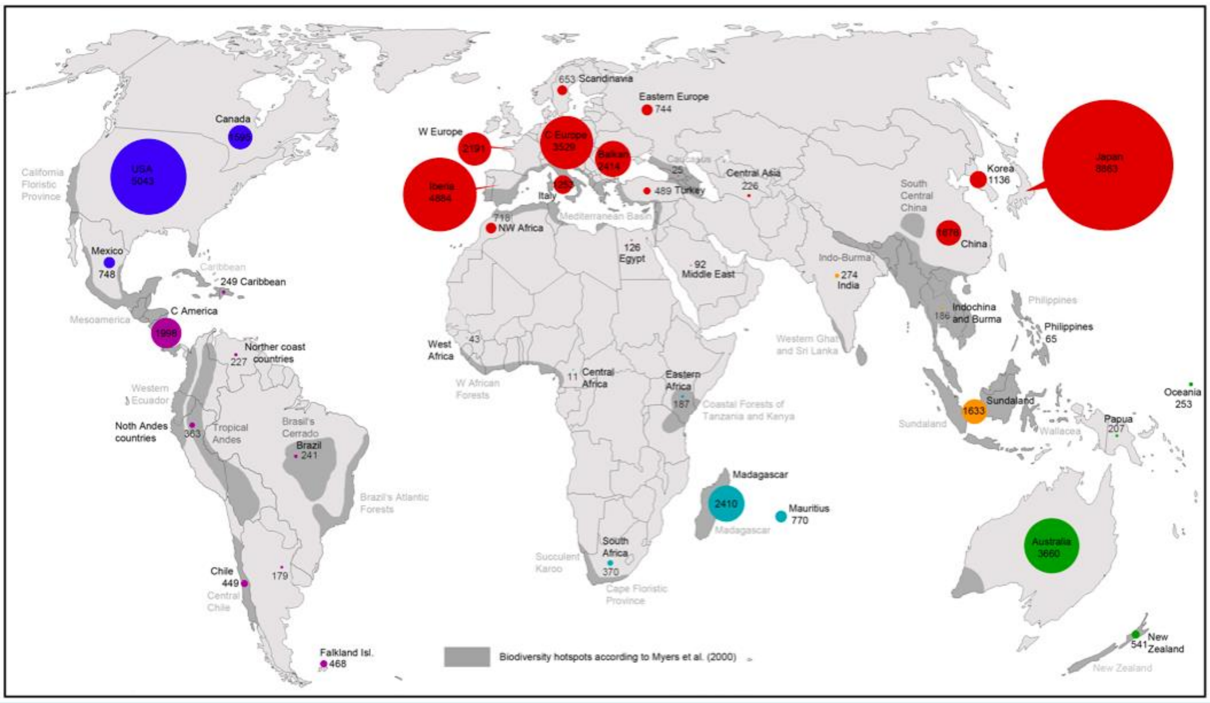
Ototretadrilus atritarsis n. sp. Opacus, testaceus, antennis, tarsis et elytris postice late nigris. Long. 6 mill. Indes : Malabar.

O. notaticollis n. sp. Minutus, robustus, niger, thorace testaceo, in disco piceo notato, scutello rufo limbato; antennis validis. Long. 4 mill. Indes Or.

Platerodrilus n. gen. Antennis pilosis, subfiliformibus, apice attenuatis, articulis 2 et 3 brevibus; thorace semicirculare, pro parte excavato; elytris depressis, costulatis; pedibus sat elongatis, paulo depressis. — Ce nouveau genre a un faciès de *Plateros* Brg., mais serait plutôt à classer dans les Drilides par les hanches rapprochées et l'articulation oblique des fémurs avec les trochanters.

Platerodrilus sinuatus n. sp. Satis elongatus, niger, pro parte rufescens, elytris ad humeros rufomaculatis, sinuatis, multi costulatis. Long. 7 mill. Malacca.

Obr. 1. Historické popisy druhů: typologická taxonomie (Pic, 1921)



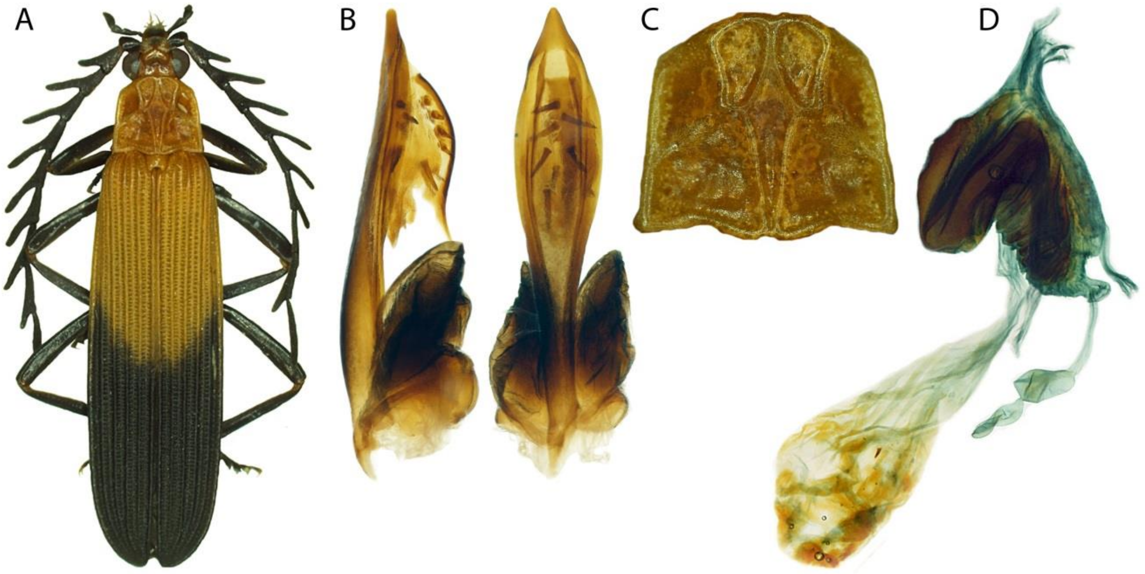
Obr. 2. Počty sekvencí DNA řádu Coleoptera známé z jednotlivých center diverzity definovaných Myersem (Myers *et al.* 2000). Převzato z Bocak *et al.* (2014); © Blackwell Inc.

Tab. 1 Historie klasifikace skupin taxonů dnes klasifikovaných v tribu Metriorrhynchini

Kleine 1933	Bocak & Bocakova 1990 Bocak 2002	Sklenarova *et al.* 2014
Lycidae	Lycidae	Lycidae
Homalisinae		Lycinae
Lycinae	Metriorrhynchinae	Metriorrhynchini
	Conderini	Cautirina
Metriorrhynchina	Metriorrhynchini	Metanoeina
Trichalini	Metriorrhynchina	Metriorrhynchina
Cladophorini	Trichalina	=Dilolycini
Dilolycini	Hemiconderina	=Cladophorini
		=Trichalini
		=Hemiconderini

2.4 Metriorrhynchini: morfologická diverzita

Metriorrhynchini jsou morfologicky definováni kruhovou phallobází samčích kopulačních orgánů, plochou nápadnou nepárovou žlázou v kopulačních orgánech samic, střední areolou kopinatého tvaru na pronotu a většinovou přítomností dalších žeber na pronotu, které vytvářejí postranní areoly (Obr. 3; Bocak 2002). Množství klasifikačních znaků je v čeledi Lycidae omezeno poměrně značnou variabilitou ve tvaru těla vzhledem k jeho slabé sklerotizaci a některé struktury mohou být významně ovlivněny selekčním tlakem, např. zpevňující žebra. Podobně pohlavní orgány jsou často vystaveny silné sexuální selekci a nemůžeme vyloučit rychlý vznik velmi odlišných struktur. Proto preferujeme využití všech dostupných zdrojů informací v souladu s dnešní taxonomickou praxí (Lee *et al.* 2007, Winterton *et al.* 2007).



Obr. 3 Typový rod *Metriorrhynchus* sp.; A – celkový pohled, B – samčí kopulační orgán, C – pronotum se 7 aerolami, D – samičí pohlavní orgán.

2.5 Klasifikace na základě fylogenetické hypotézy: integrace morfologických, molekulárních a zoogeografických dat

Aplikace DNA dat v systematické biologii se bouřlivě rozvíjí od konce 90. let 20. století. Jedná se především o modifikaci klasifikace mnoha skupin na základě molekulárně fylogenetických hypotéz. V počátečním stádiu byl používán pouze jediný molekulární marker, často *cox1* mtDNA nebo 18S rRNA (např. fylogeneze Coleoptera založená na několika desítkách sekvencí *cox1* mtDNA o délce 400 bp, Howland & Hewitt 1995). Postupně byly analýzy založeny na větším počtu taxonů a větším počtu markerů s rozdílnou rychlostí evoluce a poslední práce zabývající se fylogenezí Coleoptera jsou již založeny na tisících taxonů, eventuálně na kompletních mitochondriálních genomech reprezentujících základní linie na úrovni čeledí (Timmermans *et al.* 2010, Bocak *et al.* 2014). Dalším oborem aplikace DNA sekvencí v systematické biologii je použití sekvencí pro identifikaci druhů - barcoding (Hebert *et al.* 2003). Tento ve své podstatě jednoduchý přístup pro zachycení a identifikaci diverzity i mimo taxonomicky prozkoumané oblasti ovšem vyvolal velký odpor pro nejasnosti v postupu stanovení hranic druhů a celkově problematický filozofický základ tohoto přístupu. Právě omezená vazba barcodingu na dosavadní taxonomický výzkum na základě kritického posouzení morfologických znaků vyvolala zásadní nesouhlas některých systematických biologů (Lipscomb *et al.* 2003, Wheeler 2004, Will & Rubinoff 2004). Další aplikací molekulárních dat v systematické biologii je algoritmické stanovení hranic druhů na základě přechodu mezi koalescentní a diverzifikační fází evoluce (GMYC metoda, Pons 2006) nebo identifikace linií jako biologických druhů na základě identifikace diagnostických mutací (DeSalle *et al.* 2005).

Analýza mtDNA poskytuje téměř neomezené množství informací pro robustní identifikaci druhů v taxonomicky opomíjených skupinách a klasifikaci založenou na striktní monofylii definovaných taxonů. To podporuje širokou použitelnost molekulárních dat ve fylogenetické systematice. Vedle možnosti vytvoření molekulární fylogenetické hypotézy pro taxonomickou klasifikaci, poskytuje molekulární fylogeneze informace umožňující hodnocení evoluční historie dané skupiny. Zároveň zpřístupňuje taxonomické informace bez nutnosti hlubších odborných znalostí dané skupiny (Monaghan *et al.* 2006) a představuje nezanedbatelný zdroj informací pro evoluční biologii, populační genetiku a studie celosvětové biologické diverzity. Katalogizace velkého počtu nepopsaných druhů hmyzu může být výrazně urychlena pomocí přístupů založených na analýzách DNA (Vogler & Monaghan 2007).

2.6 Distribuce diverzity tribu Metriorrhynchini

Rozšíření tribu Metriorrhynchini je omezeno na oblasti v minulosti tvořící prakontinent Gondwana a část východní Asie. Areál tak zahrnuje afrotropickou, orientální, australskou oblast a východní část palearktické oblasti. Diverzita Metriorrhynchini je rozložena v rámci celého areálu velmi nerovnoměrně. Většina rodů se vyskytuje v australské oblasti, fauna ostatních oblastí je klasifikována pouze v omezeném počtu rodů, především v rodech *Cautires* a *Xylobanus*.

Australská oblast je druhově i rodově nejbohatší a mnoho morfologicky velmi zřetelně definovaných skupin se vyskytuje pouze v Austrálii a na Nové Guinee. Např. rody *Synchonnus* a *Achras* jsou endemity australského kontinentu a další příbuzné rody jsou známé z Nové Guinee (*Hemiconderis*) a ostrovů východně od Wallaceovy hranice (Sulawesi, Moluky; *Wakarumbia*, *Sulabanus*; Dvorak & Bocak 2007). Morfologicky podobní *Trichalus* a příbuzné rody (dříve hodnoceni jako samostatná podčeleď Trichalina, nyní součástí Metriorrhynchina) jsou další skupinou s australským původem a téměř výlučně se vyskytují v Austrálii a oblasti Wallaceovy linie (pouze rody *Diatrichalus* a *Microtrichalus* se vyskytují i mimo australskou oblast – několik druhů v orientální oblasti a jeden v Yunnanu; Bocak 2002). Největší diverzitu vykazují tropické oblasti australského regionu: např. 40 druhů *Diatrichalus* na Nové Guinee (Bocak 2001), 5 druhů *Diatrichalus* z Filipín, 1 druh z asijského kontinentu a Velkých Sund. Rod *Microtrichalus* se vyskytuje v desítkách druhů v australské oblasti, v několika druzích na Filipínách (Bocak 1998d), ale jeho areál rozšíření zasahuje dále na sever (pouze 1 druh v Yunnanu). *Metriorrhynchus* je další rod v Metriorrhynchina, který má centrum své distribuce v australské oblasti, ale rozšířením zasahuje až na kontinentální Asii. Další endemické linie typické pro oblast Austrálie jsou *Pseudodontocerus, Stadenus, Ditua, Cladophorus, Cautiromimus, Broxylus, Kassemia, Porrostoma* a *Metriorrhynchoides*. Naopak většina druhů rodu *Leptotrichalus* je hlášena z Filipín, několik ze Sulawesi, Malých Sund, Jávy, Sumatry a Bornea a několik málo z asijského kontinentu (Bocak 2000b). Rod *Lobatang,* blízce příbuzný k rodu *Leptotrichalus*, má rovněž většinu svých zástupců v australském regionu, především v Papuánském subregionu, ale velmi malá část z nich překročila Wallaceovu linii a přešla na Filipíny. Rod *Ditua* je zaznamenán především z Nové Guinee a přilehlých ostrovů. Metriorrhynchini tvoří nejbohatší australskou skupinu čeledi Lycidae a ostatní podčeledi a triby jsou v této oblasti zastoupeny pouze několika druhy (Calder 1998).

Faunu Indie reprezentují rody *Cautires*, *Xylobanus* a *Prometaneus* (Bocak & Bocakova 1992). Podobně v afrotropické oblasti jsou známy rody *Cautires* (asi 190 druhů, oblast rozšíření od západní Afriky až po Filipíny a Japonsko; Dudkova & Bocak 2010), *Caenioxylobanus* (2 endemické druhy z Madagaskaru) a tři rody neurčitého postavení popsané Kazantsevem (Kazantsev 2012), které mohou být synonymy rodu *Cautires* (typové exempláře jsou nedostupné pro studium). Celkový počet druhů afrotropické oblasti se pohybuje kolem 200 druhů. Rozšíření rodu *Xylobanus* kopíruje rod *Cautires*, pouze nedosahuje do afrotropické oblasti (Sklenarova *et al.* 2014), a dokázal překročit Wallaceovu linii směrem na východ (Kubecek *et al.* 2010). Metriorrhynchina z východní části orientální oblasti jsou daleko rozmanitější. Ve východní části orientální oblasti se mimo tyto rody vyskytují v omezeném počtu druhů i rody *Metriorrhynchus, Microtrichalus, Diatrichalus* a *Leptotrichalus*, na Filipínách i rody *Cautiromimus* a *Xylometanoues* (Bocak 1998d). Kromě rodů *Cautires* a *Xylobanus*, se zde vyskytuje rod *Metanoeus* typický pro oblast Velkých Sund.

Rodově i druhově je nejchudší Palearktická oblast: *Matsudanoeus* je zde endemický, další rody zasahují do palearktické oblasti pouze částečně z jihu. Rody *Cautires*, *Xylobanus* a *Xylometanoeus* nalezneme v Číně, Japonsku a na ruském Dálném východě. Celkově je počet druhů Metriorrhynchini v tomto regionu velmi nízký a snižuje se s narůstající vzdáleností od orientální oblasti a kontinentálním charakterem klimatu.

2.7 Fylogeografie tribu Metriorrhynchini na základě molekulární fylogeneze

Biogeografie se zabývá studiem distribuce a evoluce organismů v prostoru a čase (Ball 1976). Fylogeografie spojuje geografické informace a údaje o jednotlivých liniích založené na analýzách DNA k odvození evoluční historie moderních taxonů (Avise 2000).

Molekulární datování se stává stále běžnějším nástrojem pro testování historických, biogeografických hypotéz (Smedmark 2010). Současné biogeografické analýzy zkoumající regionální vztahy jsou obvykle založené na molekulárně fylogenetické příbuznosti mezi taxony (Avise 2000, van Welzen *et al.* 2003), poskytují informace o distribučních a speciačních událostech (Wiens & Donoghue 2004). Molekulární fylogenetické metody jsou nezávislým zdrojem informací pro konstrukci fylogenetických hypotéz (Amédégnato *et al.* 2003). Počet kompletních mitochondriálních genomů se stále zvyšuje díky technologickému pokroku, který umožňuje jejich sekvenování v celém rozsahu (Hwang *et al.* 2001, Yamauchi *et al.* 2004). Díky tomu se mohou molekulární data více uplatňovat ve srovnávacích studiích a ve studiu fylogenetických vztahů na úrovni bazálních linií (Gray *et al.* 1999, Nardi *et al.* 2003, Cameron *et al.* 2004, Cameron *et al.* 2006, Cameron *et al.* 2007).

Cílem práce bylo rekonstruovat historii vzniku a šíření tribu Metriorrhynchini po jihovýchodní Asii, Africe a Madagaskaru v závislosti na sekvenci rozpadu Gondwany. Použili jsme fylogenetický přístup k řešení historie disperze a speciace Metriorrhynchini po srážce indickém subkontinentu a Asijské pevninské kry (Hall & Blundell 1996). Výsledky datování vzniku jednotlivých linií na základě molekulárních dat jsme srovnali s dostupnými časovými údaji o pohybu vznikajících kontinentů.

V případě Lycidae chybí fosilní záznamy, a proto byla problematická kalibrace vyprodukovaných normalizovaných stromů. Pro testování konkurenčních hypotéz jsme uvažovali následující kalibrační body:

1) štěpení uvnitř tribu Metriorrhynchini v době před cca 78 miliony lety odvozené z kalibrované fylogeneze celé čeledi (Bocak *et al.* 2008);

2) kalibrační bod byl stanoven na 100 mil., tj. na dobu, kdy ještě existovala Kerguelenská platforma, která potenciálně umožňovala migraci mezi oddělující se Indií a Austrálií;

3) stanovení průměrné evoluční rychlosti mtDNA na 0.0115 substitucí na danou linii za milion let (Brower 1994, aplikováno v rodu *Metriorrhynchus*, Bocak & Yagi 2010). Průměrná rychlost je složena z kombinace rychlostí pomalu a rychle se vyvíjejících genů (Papadopoulou *et al.* 2010).

3. Cíle práce

1. Kriticky revidovat klasifikaci tribu na základě fylogenetické hypotézy založené na studiu morfologické a molekulární diverzity této linie.

2. Vytvořit na základě fylogeneze a současného rozšíření skupiny fylogeografickou hypotézu popisující pravděpodobné oblasti vzniku tribu Metriorrhynchini a datovat významné disperzní události.

4. Materiál a metody: konstrukce fylogenetických stromů, analýza ancestrálních stavů znaků, hodnocení morfologie

Podrobný popis metod je uveden v přiložených publikacích a následující shrnutí obsahuje pouze základní popis použitých metod bez přímé vazby k jednotlivým projektům studie.

4. 1 Materiál

Pro tuto studii byl k dispozici materiál fixovaný pro izolaci DNA zahrnující všechny základní linie tribu Metriorrhynchini. Celkem bylo sekvenováno ~175 druhů. Druhově silně diverzifikované rody byly zastoupeny v analýze maximálně možným počtem druhů. Do analýzy se nepodařilo zařadit větší počet endemických rodů z Nové Guinee.

4.2 Studovaná oblast a její tektonická historie

Studovaná oblast zahrnuje bývalý superkontinent Gondwana, který dal vzniknout současné Africe, Jižní Americe, Antarktidě, Austrálii, Indii a Madagaskaru. Do fylogenetických analýz byly zahrnuty všechny druhově početné rody reprezentující rozšíření tribu Metriorrhynchini a pocházející z pevninské Asie (Indie, Čína, Laos, Malajský poloostrov), ostrovní JV Asie (Velké a Malé Sundy), Japonska, Filipín, Afriky, Madagaskaru, Austrálie a Nové Guinee.

Rozpad Gondwany, následný posun na sever a kolize indického subkontinentu s Asií, představoval nejvýznamnější událost v historii šíření tribu Metriorrhynchini. Počátek kolize mezi Indií a Asií není zcela přesně datován, předpokládá se v období spodního eocénu, cca před 55 až 35 miliony lety (Aitchison *et al.* 2007).

4.3 Molekulární markery, metody laboratorní práce

Studie je založena především na molekulárních datech a na jejich základě vytvořených fylogenetických hypotéz. Cílem současné studie bylo přezkoumat klasifikaci pro všechny taxony spadající do tribu Metriorrhynchini prostřednictvím fylogenetické analýzy založené na kompletní sadě morfologických znaků.

Pro studium tribu Metriorrhynchini jsme použili fylogenetické analýzy založené na sekvencích jaderné DNA (geny 18s, 28s rRNA) a mitochondriální DNA (geny *rrnL, cox1, nad5;* Boore 1999).

Pro izolaci DNA byla použita metathorakální svalovina a zadní pár nohou. Svalová tkáň byla před samotnou izolací vysušena pomocí vakuového koncentrátoru, dále zhomogenizována a inkubována se při 60 - 65ºC, dokud nedošlo k rozložení svaloviny. Vzorky se následně několikrát přečišťují a promíchávají (Vogler *et al.* 1993). U vyčištěného roztoku proběhne ještě měření koncentrace DNA na spektrofotometru (nanodrop ND-1000). Poté se DNA nechá v termocykleru amplifikovat. Po ukončení PCR replikace se provádí elektroforéza jako kontrola úspěšné amplifikace cílového produktu a jeho replikace.

Templát DNA byl použit pro sekvenační reakce, které byly připraveny podle protokolu ABI Applied Biosystems. Každá mikrozkumavka obsahující výsledný produkt Cycle sequencingu byla znovu přečištěna 75µl 95% etanolu a 3µl 3M acetátu sodného a promyta 70% ethanolem. Mikrodestička byla nakonec vysušena ve vakuu. Vyčištěný sekvenační produkt byl rozpuštěn ve formamidu a analyzován na sekvenátoru ABI 3130.

4.4 Alignmenty, fylogenetické analýzy

Chromatogramy byly analyzovány programem Sequencing Analysis (ABI Applied Biosystems, Inc.). Editace sekvencí byla prováděna programem Sequencher 4.10.1 (Gene Codes Corp.). Editované sekvence byly včetně outgroup alignovány následujícími programy: ClustalW 1.83, ClustalX 2.1, T-coffee 8.95, Mafft v. 7, BlastAlign 1.2 a Muscle 3.6. V dalším kroku byly ze sekvencí sestaveny kompletní matice, které byly znovu odkontrolovány v programu Se-Al. K fylogenetické analýze byly použity optimalizační kritéria: metoda maximální parsimonie (MP, program TNT 1.1), metoda maximální pravděpodobnosti (maximum likelihood, ML, program RAxML 7.2.5) a bayesiánská interference (BI, program MrBayes 3.2.1.). Pro kontrolu a vizualizaci výsledných kladogramů a fylogramů byly použity programy Tracer 1.5, FigTree v. 1.3.1 a PAUP*. Za účelem datování vzniku jednotlivých linií jsme použili program Beast 1.6.1. Analýza geografických dat proběhla v programu BayesTraits 2.0. Program RASP 2.1 umožnil mapování rozšíření ancestrálních znaků a rekonstrukci vývoje vybraných morfologických znaků.

4.5 Studium morfologických dat

V rámci této práce byla studována i morfologie sekvenovaných exemplářů. Dokladové exempláře byly po odebrání svalové tkáně pro izolaci DNA preparovány tradičním způsobem a uloženy ve sbírce laboratoře molekulární systematiky na katedře zoologie, PřF UP Olomouc. Vybrané exempláře byly změkčeny v nízko koncentrovaném alkoholu, měkké tkáně obalující kopulační orgány a orgány v hlavové schránce byly odstraněny v horkém roztoku hydroxidu draselném. Jednotlivé části byly odděleny pod preparačním mikroskopem a byla pořízena fotodokumentace, která se stala základem pro vytvoření perových ilustrací, nebo byly fotografie po upravení použity přímo v publikacích.

5. Shrnutí hlavních výsledků studie

Tento přehled shrnuje základní závěry prezentované v této práci. Podrobný popis výsledků a diskuse jsou uvedeny v jednotlivých publikacích.

5.1 Klasifikace tribu Metriorrhynchini

Sklenarova K., Kubecek V. & Bocak L. (2014) Subtribal classification of Metriorrhynchini (Insecta: Coleoptera: Lycidae): an integrative approach using molecular phylogeny and morphology of adults and larvae. Arthropod Systematics and Phylogeny 72 (1): 37-54.

Klasifikace tribu Metriorrhynchini je navržena na základě molekulární fylogeneze skupiny a je podrobně diskutována evoluce vybraných morfologických struktur, na kterých byla založena předešlá morfologická klasifikace. Výsledky ukazují na několikanásobný vznik podobných struktur žeber na pronotu, zkrácených žeber na krovkách, počtu žeber a flabelátních tykadel. Na základě zhodnocení morfologie larev, dospělců a molekulární fylogeneze je navržena nová klasifikace. Byly nově ustanoveny subtriby Metriorrhynchina, Metanoeina a Cautirina a subtriby Trichalina a Hemiconderina byly synonymizovány se subtribem Metriorrhynchina.

Seznam rodových a vyšších taxonů je uveden v Tab. 2

Tab. 2. Seznam rodů včetně synonymie

Metriorrhynchini Kleine, 1926

Type genus: *Metriorrhynchus* Gemminger & Harold, 1869

Metriorrhynchina Kleine, 1926
Type genus: *Metriorrhynchus* Gemminger & Harold, 1869
Achras Waterhouse, 1879
Broxylus Waterhouse, 1878
= *Samanga* Pic, 1921
Cautiromimus Pic, 1926
Cladophorus Guerin Meneville, 1830
= *Odontocerus* Guerin Meneville, 1838
= *Spacekia* Strand, 1936
= *Pseudodontocerus* Pic, 1921
= *Cladophorinus* Kleine, 1926
= *Carathrix* Kleine, 1926
= *Cautiromimus* Pic, 1926
Diatrichalus Kleine, 1926
= *Mimotrichalus* Pic, 1930
Ditua Waterhouse, 1879
Eniclases Waterhouse, 1879
Enylus Waterhouse, 1879
Falsoenylus Pic, 1926

Falsolucidota Pic, 1921
= *Hemiconderis* Kleine, 1926
Flabellotrichalus Pic, 1921
= *Villosotrichalus* Pic, 1921
= *Stereotrichalus* Kleine, 1926
Kassemia Bocak, 1998
Leptotrichalus Kleine, 1925
Lobatang Bocak, 1998
Malacolycus Kleine, 1943
Mangkutanus Kubecek, Dvorak & Bocak, 2011
Marena Kazantsev, 2007
Metriorrhynchoides Kleine, 1926
Metriorrhynchus Gemminger & Harold, 1869
= *Metriorhynchus* Guérin-Méneville, 1838
= *Dilolycus* Kleine, 1926
= *Flabelloporrostoma* Pic, 1923
Mimoxylobanus Pic, 1921
Microtrichalus Pic, 1921
= *Falsoenylus* Pic, 1926
Oriomum Bocak, 1999
Porrostoma Castelnau, 1838
Procautires Kleine, 1925
Pseudodontocerus Pic, 1921
Schizotrichalus Kleine, 1926
Spinotrichalus Kazantsev, 2010
Stadenus Waterhouse, 1879
Stereotrichalus Kleine, 1926
Sulabanus Dvorak & Bocak, 2007
Synchonnus Waterhouse, 1879
Trichalolus Pic, 1923
Trichalus Waterhouse, 1877
Wakarumbia Bocak, 1999
Xantheros Fairmaire, 1877
Xylobanomimus Kleine, 1926
Xylobanomorphus Kleine, 1935

Cautirina Sklenarova *et al.* 2014
Type genus: *Cautires* Waterhouse, 1879
Caenioxylobanus Pic, 1922
Cautires Waterhouse, 1879
= *Bulenides* Waterhouse, 1879
Paracautires Kazantsev, 2012
Prometanoeus Kleine, 1925a
= *Tapromenoeus* Bocak & Bocakova, 1989
Strophicus Waterhouse, 1879
Spartoides Kazantsev, 2012
Tricautires Kazantsev, 2006

Metanoeina Sklenarova *et al.* 2014
Type genus: *Metanoeus* Waterhouse, 1879
Metanoeus Waterhouse, 1879
Xylometanoeus gen. nov
Matsudanoeus gen. nov

5.2 Fylogeografie tribu Metriorrhynchini

Sklenarova K., Chesters D. & Bocak L. (2013) Phylogeography of Poorly Dispersing Net-Winged Beetles: A Role of Drifting India in the Origin of Afrotropical and Oriental Fauna. PLoS ONE 8(6): e67957. doi:10.1371/journal.pone.0067957.

Současné rozložení tribu Metriorrhynchini je výsledkem pohybu kontinentálních fragmentů po rozpadu Gondwany. Metriorrhynchini se skládají ze dvou linií: afro/orientální a australské. Bazální linie pochází z Austrálie a Indie, tedy částí Gondwany definovaných v době separace Austrálie a Indie. Afrotropická fauna a fauna Madagaskaru vznikla disperzí Indie driftující na sever před 65 až 62 miliony lety. Orientální fauna se diverzifikovala po srážce Indie s Asií (rody *Cautires*, *Xylobanus*, *Metanoeus*) a později byla doplněna o linie migrující z australské oblasti, která se dostala do kontaktu s asijským šelfem před asi 25 miliony lety. Orientální fauna obsahuje rody indické i australské linie, přičemž analýzy datování prokázaly starší původ indických rodů.

5.3 Vznik neotenních linií v Metriorrhynchini

Bocak L., Grebennikov V.V. & Sklenarova K. (2014) *Cautires apterus,* a new species and the first record of winless male Lycidae (Coleoptera) discovered in the north Pare Mountains, Tanzania. Annales Zoologici (Warszawa) 64(1): 1-7.

V této studii je popsán nový druh *Cautires apterus* Bocak *et al.* 2014, který je unikátní svou bezkřídlostí, životem v hrabance a faktem, že samice tohoto druhu jsou pravděpodobně larviformní. Jedná se o první potvrzený případ evoluce neotenie v tribu Metriorrhynchini.

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7. Publikované výstupy dizertační práce

(s uvedením impakt faktoru a autorského podílu)

Sklenarova K., Chesters D. & Bocak L. (2013) Phylogeography of Poorly Dispersing Net-Winged Beetles: A Role of Drifting India in the Origin of Afrotropical and Oriental Fauna. PLoS ONE 8(6): e67957, doi:10.1371/journal.pone.0067957 (IF = 3.730; 7/56).

Bocak L., Grebennikov V. V. & Sklenarova K (2014) Cautires apterus, a new species and the first record of wingless male Lycidae (Coleoptera) discovered in the north Pare Mountain, Tanzania. Annales Zoologici 64 (1): 1-7. (IF = 0.660; 114/151).

Sklenarova K., Kubecek V. & Bocak L. (2014) Subtribal classification of Metriorrhynchini (Insecta: Coleoptera: Lycidae) : an integrative approach using molecular phylogeny and morphology of adults and larvae. Arthropod Systematics and Phylogeny 72 (1): 37-54. (IF = 2.318 (Entomologie 9/87).

The declaration of co-author

Article:

Sklenarova K., Chesters D. & Bocak L. (2013) Phylogeography of Poorly Dispersing Net-Winged Beetles: A Role of Drifting India in the Origin of Afrotropical and Oriental Fauna. PLoS ONE 8(6): e67957. doi:10.1371/journal.pone.0067957.

The contributionDouglas Chesters

- devoleped the scripts for bayesian reconstriction of ancestral ranges
- provided an advice for analyses
- commented the manuscript

I confirm with myown signaturethe correctness of information above about myinvolvement in the workon the publication.

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Article:

Sklenarova K., Chesters D. & Bocak L. (2013) Phylogeography of Poorly Dispersing Net-Winged Beetles: A Role of Drifting India in the Origin of Afrotropical and Oriental Fauna. PLoS ONE 8(6): e67957. doi:10.1371/journal.pone.0067957.

The contribution Ladislav Bocak

- collected material in the field
- designed the analyses and experiments
- contributed with information and co-authored the text of the article

I confirm with my own signature the correctness of information above about my involvement in the work on the publication.

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Sklenarova K., Kubecek V. & Bocak L. (2014) Subtribal classification of Metriorrhynchini (Insecta: Coleoptera: Lycidae): an integrative approach using molecular phylogeny and morphology of adults and larvae. Arthropod Systematics and Phylogeny 72.

The contribution Vaclav Kubecek:

- collected part of the material in the field
- contributed with part of dataset and co-authored the text of the article

I confirm with my own signature the correctness of information above about my involvement in the work on the publication.

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Article:

Sklenarova K., Kubecek V. & Bocak L. (2014) Subtribal classification of Metriorrhynchini (Insecta: Coleoptera: Lycidae): an integrative approach using molecular phylogeny and morphology of adults and larvae. Arthropod Systematics and Phylogeny 72 (1): 37-54.

The contribution Ladislav Bocak:

- assembled material for sequencing
- contributed to the design of the analyses
- co-authored the text of the article

I confirm with my own signature the correctness of information above about my involvement in the work on the publication.

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The declaration of co-author

Article:
Bocak L., Grebennikov V.V. & Sklenarova K. (2014) *Cautires apterus*, a new species and the first record of winless male Lycidae (Coleoptera) discovered in the north Pare Mountains, Tanzania. Annales Zoologici (Warszawa) 64(1): 1-7.

The contribution Vasily V. Grebennikov:
- collected material in the field
- contributed with information and co-authored the text of the article

I confirm with my own signature the correctness of information above about my involvement in the work on the publication.

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May 29, 2014

The declaration of co-author

Article:

Bocak L., Grebennikov V.V. & Sklenarova K. (2014) *Cautires apterus*, a new species and the first record of winless male Lycidae (Coleoptera) discovered in the north Pare Mountains, Tanzania. Annales Zoologici (Warszawa) 64(1): 1-7.

The contribution Ladislav Bocak:

- contributed with information and co-authored the text of the article

I confirm with my own signature the correctness of information above about my involvement in the work on the publication.

Autor Signature: Ladislav Bocak

Phylogeography of Poorly Dispersing Net-Winged Beetles: A Role of Drifting India in the Origin of Afrotropical and Oriental Fauna

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Abstract

Ancient dispersal history may be obscured by subsequent dispersal events. Therefore, we intend to investigate the biogeography of metriorrhynchine net-winged beetles, a group characterized by limited dispersal propensity. We used DNA data to construct phylogenies and the BayesTraits and RASP programs to identify putative ancestral areas. Further, we inferred ultrametric trees to estimate the ages of selected nodes. The time frame is inferred from tectonic calibrations and the general mutation rate of the mitochondrial genes. Metriorrhynchini consists of two lineages with Afro/Oriental and Australian distributions. The basal lineages originated in Eastern Gondwana after the split of Australia, India and Madagascar; the Afrotropical and Madagascar Metriorrhynchini separated from the Oriental clades 65 and 62 mya. Several already diversified lineages colonized continental Asia 55–35 mya. A few genera of the Australian clade dispersed to the Oriental region 5–15 mya and reached Eastern India and Southern China. Only *Xylobanus* crossed the Makassar Strait to Sulawesi and does not occur further to the east. The current distribution of Metriorrhynchini is a result of drifting on continental fragments and over-sea dispersal events limited to a few hundreds of kilometers. We conclude that: (1) Afrotropical and Madagascar lineages originated independently from dispersal events during India's drift to the north and the Mozambique Channel completely isolates the respective faunas since then; (2) Oriental fauna is a recently established mixture of the Indian and Australian lineages, with predominance of the older Indian clades; (3) The fauna of islands located north of Australia colonized Sulawesi after collision with the Sundaland margin and the species rich Australian lineages did not reach Western Wallacea or the Philippines. Our results suggest an impact of subtle differences in biological characteristics on biogeographic history of individual lineages, when mostly lowland and flower-visiting lineages were able to disperse across sea channels.

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Introduction

The structure of biotas of the continents that rim the Indian Ocean are the result of the break-up of Gondwana, connectivity between India, Madagascar and Africa, the collision of the Indian subcontinent with Asia, and the formation of the islands of Wallacea [1,2,3,4,5,6]. Studies on dispersal and vicariance history often bring conflicting conclusions depending on the biology of the studied groups as recent dispersals and extinctions can easily obscure older patterns [7,8]. Here, we use poorly dispersing net-winged beetles to study the biotic connectivity among Gondwanan continents and Asia.

The dispersal propensity determines the evolution and distribution of many animal lineages. Some groups are well known as wide dispersers, e.g. diving beetles [9], and are often uninformative in phylogeographic studies addressing ancient zoogeographical patterns. Such studies require widespread and species rich model groups with limited dispersal ability. Further, lineages with an uninterrupted long-term diversification are preferable to avoid confounding impacts of extinctions. The metriorrhynchine beetles are a highly diverse Palaeotropical lineage with ~1400 species and their origin was hypothesized in the Late Cretaceous [10,11]. Low dispersal ability of net-winged beetles is due to strong dependence on rain forest habitats, weak short-distance flight, short adult life span, and the presence of geographically limited aposematic patterns [12,13]. Their diversity and small ranges make Metriorrhynchini a promising model group with a potential to elucidate ancient dispersal histories.

The morphology-based phylogeny of Metriorrhynchini was presented by Bocak [10] and since then information has been accumulating on the diversity of the lineage [14,15]. An apparent trait of the lineage is high morphological diversity in the Australian region, namely in New Guinea and humid areas of Australia where 22 endemic genera occur; some of them, e.g. *Porrostoma* and *Cladophorus*, are represented by hundreds of species [10]. In contrast, only three genera *Cautires*, *Xylobanus*, and *Metanoeus* represent almost complete species-level richness of the Oriental and Afrotropical fauna (altogether ~600 spp., Fig. 1). A few genera occur on both sides of the Wallace line and in all cases the number of species is highly asymmetrical, with the majority of species known from either region (Fig. 1).

The wide distribution of Metriorrhynchini raises the question of how they came to occupy such a range. We expect that drifting on continental fragments beside dispersal played an important role, as their origin is placed well after the breakup of Gondwana [11]. There are several aims of this study: (1) Reconstruct phylogenetic relationships; (2) Identify the areas of origin and present the first worldwide biogeographical analysis of the dispersal routes of Metriorrhynchini in conjunction with plate tectonics [1,5,6]; and (3) Investigate the phylogenetic structure of the hyperdiverse Oriental fauna. The impact of ecological characteristics on biogeographic history is discussed.

Materials and Methods

Sampling, DNA Extraction, PCR Amplification, and Sequencing

Altogether 226 samples of Metriorrhynchini representing ~170 species from all regions from their range were sampled (Fig. 1) with Genbank accession numbers listed in Tab. S1. DNA was extracted using the Wizard SV96 Purification System (Promega Inc.). Extraction yield was measured using a NanoDrop-1000 Spectrophotometer. The PCR settings and cycle sequencing conditions used were as reported by Malohlava & Bocak [16]. Five fragments were sequenced: the 18S rRNA (~1900 base pairs, bp), the D2 region of the 28S rRNA (~630 bp), *rrnL* mtDNA, *tRNA-Leu* with partial *nad1* (~780 bp), *cox1*, *tRNA-Leu* and *cox2* mtDNA (1100 bp), and 1180 bp of *nad5* mtDNA with adjacent *tRNA-Phe*, *tRNA-Glu*, and *tRNA-Ser* (multiple gene fragments are referred as *rrnL*, *cox1*, and *nad5* further). The primers used are listed in Tab. S2. The PCR products were purified using PCRu96 Plates (Millipore Inc.) and sequenced by an ABI 3130 automated sequencer using the Big Dye Sequencing Kit 1.1.

Sequence Handling and Phylogenetic Analyses

Sequences were edited using Sequencher 4.10.1 (Gene Codes Corp.). Protein-coding genes contained few indels and were aligned by ClustalW 1.83 [17]. Length variable loci were separately aligned using four methods: ClustalW 1.83 using penalties 22.5 for gap opening and 0.83 for extension, T-coffee 8.95 [18], Mafft v. 7 [19] and BlastAlign 1.2 [20], all under default parameters, and Muscle 3.6 [21] under the gap opening parameter −600 and gap extension parameter −40. The concatenated supermatrices combined the length variable fragments aligned using various methods and protein coding mtDNA fragments aligned using ClustalW. The alignments were deposited to the Dryad database.

Phylogenies were inferred using Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI) algorithms. The MP analysis was carried out using TNT 1.1 [22]. For ML trees we used RAxML 7.2.5 [23], with separate parameters applied to the 18 partitions (Tab. S3). Confidence was determined with 100 bootstrap replicates utilizing the rapid bootstrap option under the GTRCAT substitution model as given by the AICc criterion in jModelTest 3.7 [24]. Additionally, the dataset was analyzed using MrBayes 3.2.1 [25]. The MCMC was set with independent parameters for 18 partitions under the general time reversible model with a category of invariant sites and gamma distributed rates (GTR+I+G). Four chains were run for 40.10^6 generations, with trees sampled every 1,000 generations. The stationary phase was detected using Tracer 1.5 [26], pre-stationary trees were discarded as the burn-in phase and posterior probabilities determined from the remaining trees.

A likelihood ratio test was used to test the molecular clock hypothesis. Under the null hypothesis L_0, the molecular clock holds, while hypothesis L_1 imposes no clock constraint. The chi-square value is given by $2\log L = 2(\log L_0 - \log L_1)$ where L_0 and L_1 are likelihoods of the tree under the given model, and the p-value is calculated for s-2 degrees of freedom where s is number of terminal branches on the tree [27].

Historical Biogeography Analyses

The absence of the net-winged beetle fossils makes any calibration difficult, and therefore two calibrating points and substitution rate were employed to date splits of interest: (i) The basal split in Metriorrhynchini at ~78 mya inferred from the divergence of Lycini and Calopterini in the dated phylogeny of Lycidae [11]; (ii) Alternatively, we used the arbitrary point 100 mya, when the latest presence of the Kerguelen Plateau [5] could support connection between India and Australia (Fig. 2A); (iii) Finally, the mean substitution rate of mtDNA was fixed to 0.0115 substitutions/lineage/my, which appeared to be satisfactory in *Metriorrhynchus* [13]. The mean rate is a composite rate of rapidly and slowly evolving genes [28].

We estimated the time to the most recent common ancestor for selected clades using a Bayesian approach implemented in Beast 1.6.1 [29]. All analyses were performed using a GTR+I+G model as given by the AICc criterion in jModelTest 3.7 [24], using a relaxed molecular clock and an uncorrelated lognormal model of rate variation among branches. The data were partitioned (Tab. S3), with each partition allowed independent parameters. In all analyses 12.10^7 generations were run and trees sampled every 1,000 generations. Convergence was assessed in Tracer 1.5 [26]. The mtDNA dataset was run four times and results combined.

Using the BI trees, we were interested in the reconstruction of ancestral geographical states at key internal nodes. In total, nine geographic regions were defined: Australia, Sulawesi, the Philippines, Palawan, Madagascar, Africa, the Western Malay Archipelago (Sumatra, Borneo, Java and the Malay Peninsula; referred as Sundas further), continental Asia north of the Kra Isthmus, and an external region for the outgroup, as the sister-group of Metriorrhynchini is unknown. The geographic states at each terminal were input into BayesTraits [30] and the likelihood of each alternative geographic state inferred for nodes of interest. Nodes to be reconstructed were defined on the Bayesian consensus topology. Due to the time consuming nature of defining internal nodes for reconstruction, a Perl script was developed to read newick format trees and prepare the appropriate BayesTraits input commands. The script is made freely available online (https://sourceforge.net/projects/bayestraitswrap/). In order to account for phylogenetic uncertainty, ancestral reconstruction was performed for a number of trees sampled during the stationary phase of the Bayesian search. The likelihoods of each state were calculated where a node of the consensus tree was present in the given sampled tree (BayesTraits command: AddNode), then the average likelihoods calculated over each of 30 sampled trees. We used the MP-based statistical dispersal-vicariance analysis implemented in RASP 2.1 [31] for an alternative ancestral state reconstruction. We randomly selected 1000 Bayesian trees inferred from the Muscle alignment after burning the non-stationary phase; the geographical regions were coded as above.

Results

DNA Sequencing and Estimation of Phylogeny

DNA sequences were obtained for five fragments (Tab. S2). The rRNA fragments varied in length, with four alignment algorithms

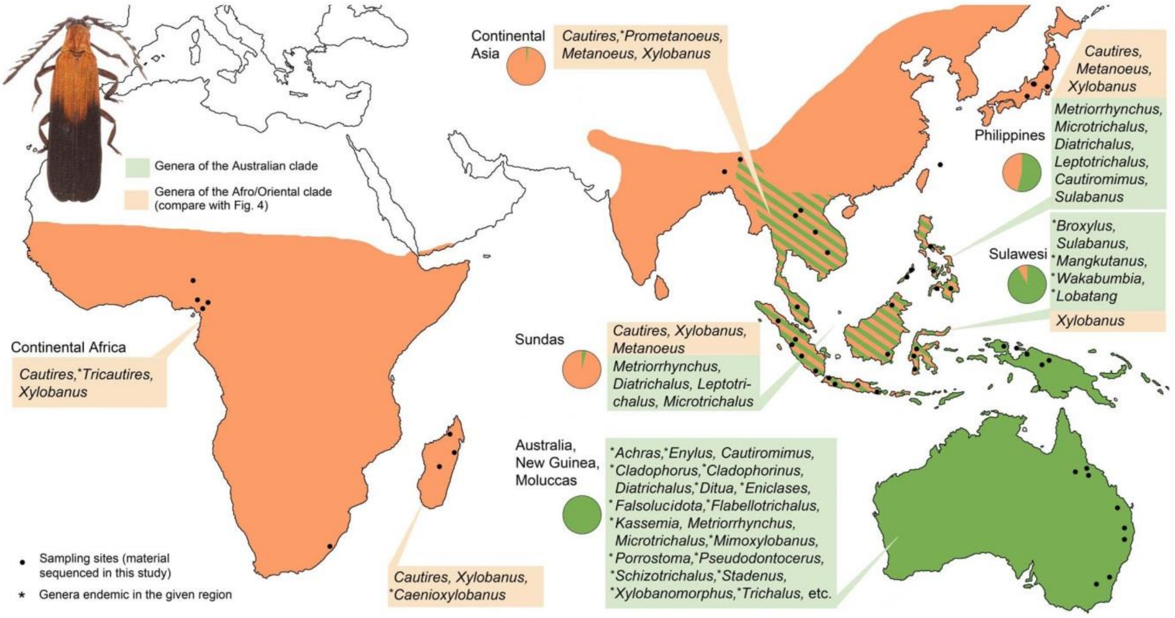

Figure 1. Distribution of Metriorrhynchini. All genera of the Australian clade occurring in the Philippines are also present in Sulawesi.
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producing datasets of 5792–5963 characters. The numbers of characters and their informativeness are given in Tab. S3.

The phylogenetic reconstruction inferred by MP, ML, and BI resulted in similar topologies for the concatenated datasets inferred under the five alignment algorithms (Figs. 3, S1). The trees had fully resolved basal branches, although the arrangement of some clades varied across analyses (Tab. 1).

All trees indicate that Metriorrhynchini represent a monophyletic clade. Three clades were consistently found as basal splits: *Xylobanus*(A)+*Metanoeus*, the Australian clade (*Sulabanus*, *Wakarumbia*, *Porrostoma*, *Metriorrhynchus*, *Microtrichalus*, etc.) and the Afro/Oriental clade (*Xylobanus*(B)+*Cautires*; both genera inferred as a paraphyletic assemblage). The first clade was found as a sister group of either the Australian or Afro/Oriental clade (Tab. 1, Figs. 3–4, Figs. S1–S2). The unconstrained Beast analysis suggested the first topology (Fig. 4). The clade designations refer to their ancestral ranges and are discussed under their respective names further.

Divergence Times and Ancestral Areas

The presence of a molecular clock was tested for using a likelihood ratio test with the ClustalW alignment. The likelihood under a model with no clock constraint was −212138, and −213384 with a clock constraint, giving a likelihood ratio of 1246 (2*(213384–212138)), a significant rejection of the molecular clock at d.f. of 249 and significance cutoff of 0.05.

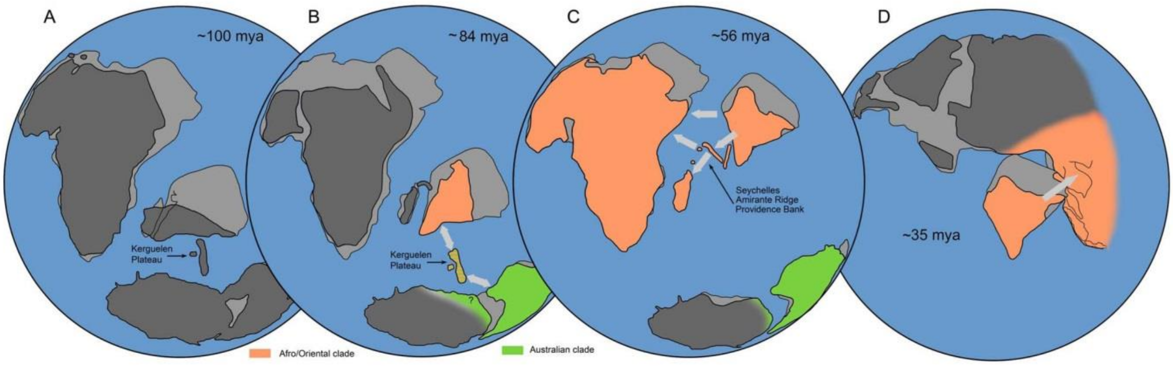

Figure 2. Schematic positions of the Gondwanan continents (A) before the origin of Metriorrhynchini, (B) at the time of the basal split, (C) at the time of dispersal to Africa and Madagascar and (D) at the time of dispersal to continental Asia. Position of continents redrawn from Ali & Aitchison (2008), position of India in Fig. 2D from [47]. Colored areas depict hypothesized ranges of the Afro/Asian and Australian clade; arrows indicate presumed dispersal events.
doi:10.1371/journal.pone.0067957.g002

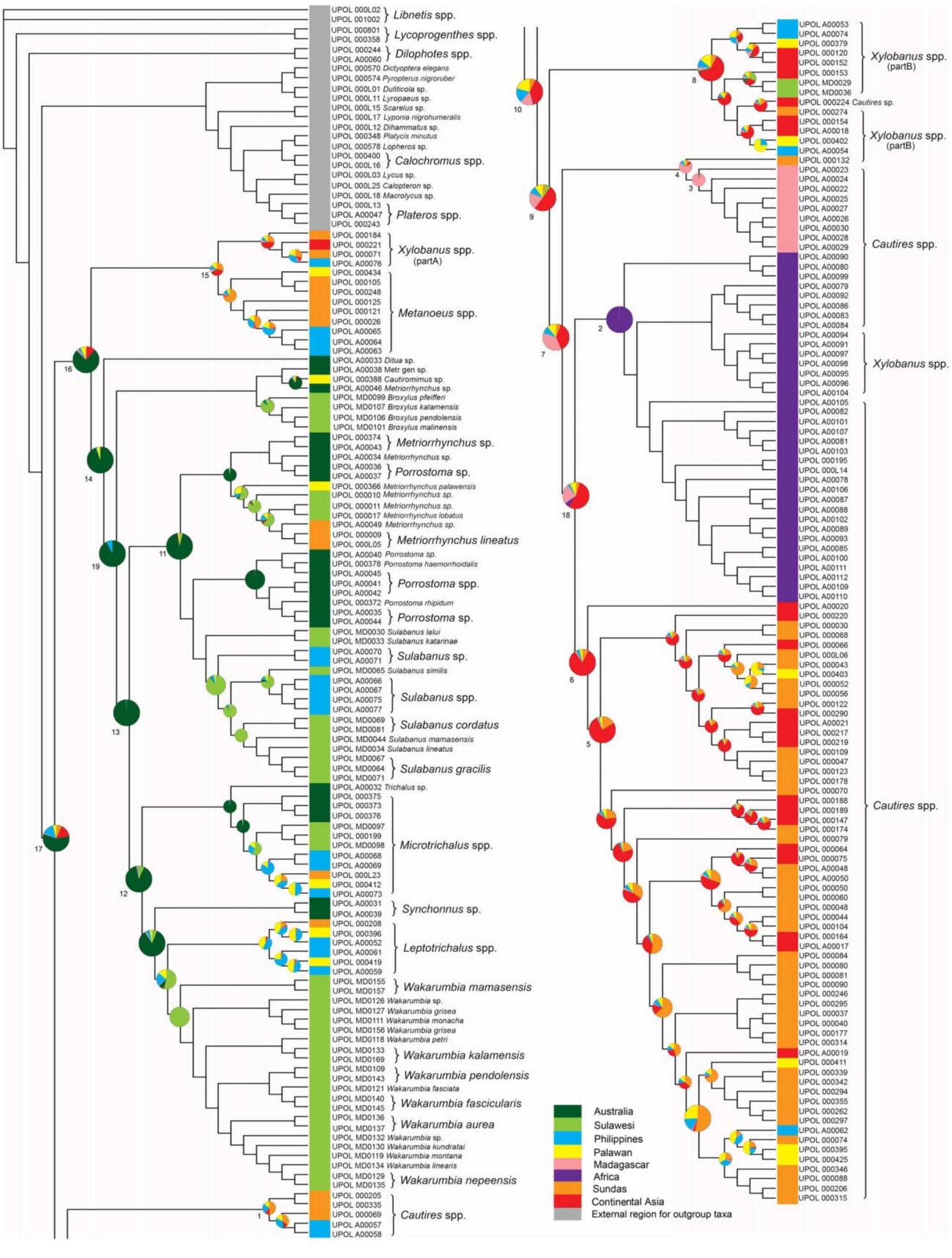

Figure 3. Phylogenetic hypothesis for Metriorrhynchini based on a ML analysis of all available fragments (18S and 28S rRNA, *cox1*, *nad5* and *rrnL* mtDNA). Numbers at the branches designate clades listed in Tab. 3. The charts indicate probabilities of ancestral areas inferred from the BayesTraits analysis.
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Table 1. Nodes recovered by analyses of the datasets produced by five alignment procedures using parsimony (MP), maximum likelihood (ML) and Bayesian (BI) algorithms.

Alignment	**BlastAlign**			**Clustal**			**Muscle**			**Tcoffee**			**Mafft**		
Algorithm	**MP**	**ML**	**BY**	**MP**	**ML**	**BY**	**MP**	**ML**	**BY**	**MP**	**ML**	**BY**	**MP**	**ML**	**BY**
Metriorrhynchini	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Metanoeus(Xylobanus,Cautires)	P	M	M	P	M	M	P	P	P	P	M	M	P	P	M
(*Metanoeus,Xylobanus* partA)	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
(*Xylobanus* partB, *Cautires*)	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
(Australian Metriorrhynchini)	M	–	M	M	–	M	M	M	M	M	M	M	M	M	M
(*Sulab.*(Austr. Metriorh.part)	–	M	–	M	M	–	–	M	M	M	M	M	M	–	–
(*Synch., Leptotr., Wakarumbia)*	M	M	M	–	M	M	–	M	M	–	M	M	M	M	M
(*Porrostoma, Metriorrhynchus*)	–	M	M	M	M	–	–	M	M	–	M	M	P	M	M
(*Trichalus, Microtrichalus*)	M	M	M	M	M	M	–	–	M	–	M	M	M	M	M

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Therefore, speciation events were dated using a relaxed molecular clock as implemented in Beast 1.6.1. Due to uncertainties in molecular dating, we used two tectonic-based calibrations and a general molecular rate as described in the Methods. The normalized tree calibrated by the age of the Metriorrhynchini (~78 mya) sets a time of dispersal to Africa at ~65 mya, and to Madagascar ~62 mya (Fig. 4, Tab. 2). The dispersal events across the Wallace line and to the Philippines are inferred in the congruence with the tectonic history (Fig. 4). The alternative calibrations proposed deeper (when calibrated by the latest presence of the subaerial Kerguelen Plateau) or slightly shallower (calibrated by mutation rate) dating. The complete results are given in Tab. 2.

The program BayesTraits was used to reconstruct the ancestral area states of key nodes in the Metriorrhynchini phylogeny. The analyses gave strong support for the basal split of the Australian and Afro/Oriental lineages (Fig. 3, Tab. 3, S4). High likelihoods were assigned to separate dispersals to Africa and Madagascar. A limited number of lineages crossed the Wallace's line from Sulawesi to Borneo or the Philippines, or the Huxley's line from Asia to the Philippines. The Makassar Strait proved to be an effective barrier to dispersal, as only *Metriorrhynchus* and *Microtrichalus* crossed the line in the westward direction, and *Xylobanus* in the eastward direction. Similar results were inferred from the statistical dispersal-vicariance analysis (Fig. S2).

Discussion

Phylogeny

Here, we propose the first molecular phylogeny of Metriorrhynchini. We confirm their monophyly and basal split into the Afro/Oriental and Australian clade (Figs. 1, 3–4). In contrast to the morphology based study [10], molecular data better resolve the phylogenetic structure of the major clades, with just the position of *Metanoeus* remaining unresolved. Both inferred topologies obtained low support for the position of this clade (Fig. S1). *Xylobanus* and *Cautires* form a monophylum with high support, but both genera were inferred as reciprocically paraphyletic due to multiple origins of the strengthening elytral costae used for the definition of these genera.

Dispersal Propensity

A critical point for further discussion is dispersal propensity of Metriorrhynchini. The earlier studies shoved their limited ability to cross open sea. Bocak & Yagi [13] identified a single dispersal event across the Makassar Strait at the end of mid-Miocene. The strait is now ~115 km wide in the narrowest point, but was less than 100 km wide repeatedly during glacial low stands [32]. Surprisingly, a few metriorrhynchines crossed this strait (Fig. 3) [15]. On the other hand, the ~460 km wide Mozambique Channel was never crossed by any net-winged beetle and the only Lycidae shared between Africa and Madagascar are *Cautires* and *Xylobanus*, which are hypothesized a result of independent dispersal events further (Fig. 3) [33]. This finding is in contrast with frequent dispersal events across the Mozambique Channel in other animal lineages [4]. The low dispersal propensity is additionally supported by very species high turn over between close islands [34] or mountain systems [35,36].

The present differences between faunas of neighboring landmasses enable an estimation of the open-sea distances, which the metriorrhynchines can cross. Therefore, we conservatively accept in further discussion dispersal events across sea channels narrower than 500 kilometers when the geographical proximity lasts over a long time span. Long-distance dispersals across between continents are not considered.

Biogeography and Dating

The analyses indicate that (i) Metriorrhynchini are of eastern Gondwanan origin and the geographical structure of its two principal lineages, the Afro/Oriental and Australian clades, has been preserved until present (Figs. 1, 3); (ii) Rafting on continental fragments played an important role, with dispersal limited to crossing sea channels a few hundreds kilometers in width; and that (iii) The direction of dispersal was most likely from drifting India to Africa, Madagascar, and continental Asia, and from Australia to Asia (Figs. 2, 4). Due to low dispersal propensity, the Metriorrhynchini missed dispersal opportunities commonly exploited by other animals. They are absent from New Zealand in contrast with diversity in Australia [10]. Despite presence in the Far East Metriorrhynchini did not disperse to North America via the Transberingian route [37]. Similarly, the connection between Australia and South America effective until the late Eocene [38] was not traversed by Metriorrhynchini. Concerning the very high diversity of Metriorrhynchini in all parts of their range we suggest that these patterns are not the result of extinctions, but demonstrate deep dispersal history.

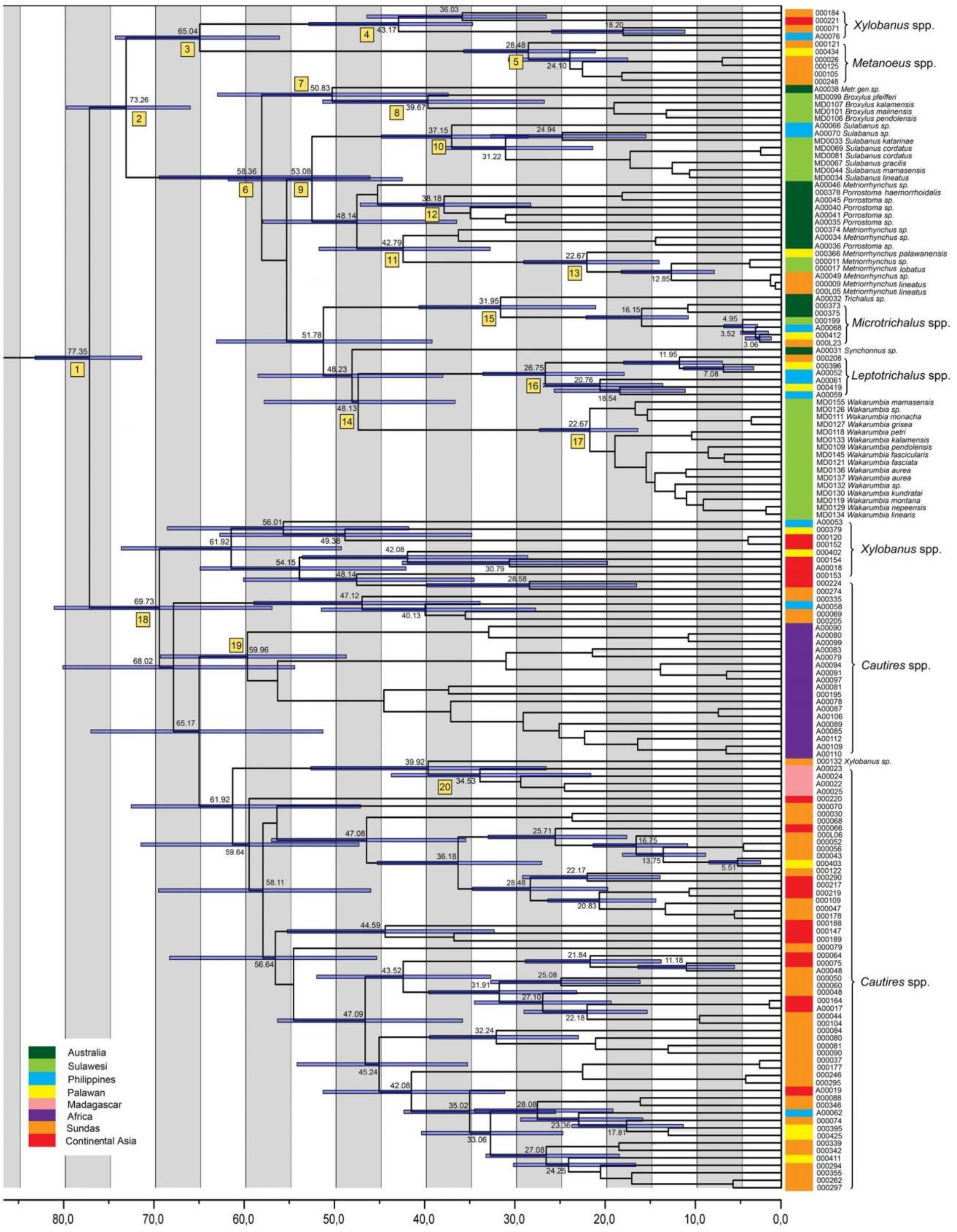

Figure 4. Timing of the Metriorrhynchini radiation. Estimated mean ages of nodes are based on Bayesian analysis of all fragments under the relaxed molecular clock model and the root calibrated at 77.7 mya. The bars depict 95% confidence intervals; the numbers at the branches designate clades listed in Tab. 2.

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Table 2. Estimation of the age of selected nodes inferred from the Bayesian.

Clade number in Fig. 4		Root fixed at 77.7. ±3.02 my		Rate 0.0015% my^{-1}		Root fixed at 100 mya	
	Taxon/node	mean (my)	95% HPD	mean (my)	95% HPD	mean (my)	95% HPD
1	Metriorrhychini (root)	77.35*	71.44–83.22	73.57	63.88–82.81	99.74*	93.86–105.73
2	Austr.(*Xylob.*+*Metan.*)	73.26	66.55–79.97	68.03	61.15–75.32	94.37	86.23–102.93
3	*Xylobanus*(2)+*Metanoeus*	65.04	55.54–75.76	61.92	50.70–72.50	83.34	74.17–92.46
4	*Xylobanus* (clade 1)	43.17	34.70–51.56	41.28	31.06–50.30	55.49	45.12–65.78
5	*Metanoeus*	28.48	21.07–35.71	27.36	20.84–34.14	37.30	28.89–46.57
6	Metriorrhynchini (austr.)	58.36	47.57–67.78	55.90	48.28–62.25	77.37	67.92–86.36
7	*Cautiromimus*+*Broxylus*	50.83	37.50–67.09	49.03	37.65–61.20	65.97	50.83–84.45
8	*Broxylus*	39.67	28.82–51.12	38.73	28.82–47.80	51.95	39.23–68.81
9	*Porrost.*+*Sulab.*+*Metr.*	53.08	42.69–63.70	50.52	43.80–57.34	68.98	59.16–79.19
10	*Sulabanus*	37.15	28.22–45.77	35.78	29.01–42.56	48.25	38.79–58.12
11	*Metriorrhynchus*	42.79	32.74–52.89	40.71	33.25–48.58	55.42	43.87–66.51
12	*Porrostoma*	38.18	28.56–47.46	36.34	28.80–44.05	49.82	39.03–60.14
13	*Metriorrhychus* (Sulaw.)	22.67	15.19–30.46	21.58	15.56–28.09	29.77	21.18–39.12
14	*Leptotrich.*+*Wakarumbia*	48.13	38.41–58.59	45.90	38.61–53.57	62.97	52.60–73.84
15	*Trichalus*	31.95	22.23–41.49	31.11	22.51–40.15	42.12	30.23–54.58
16	*Leptotrichalus*	26.75	19.44–34.38	25.45	19.22–31.75	35.12	26.04–43.78
17	*Wakarumbia*	22.67	17.36–28.38	21.34	17.33–25.88	29.02	23.03–35.20
18	*Xylobanus*+*Cautires*	69.73	59.56–79.40	66.43	60.42–72.73	90.99	83.14–98.60
19	*Cautires* (Afrotropical)	59.96	49.58–69.71	57.11	50.59–63.79	78.06	68.75–87.17
20	*Cautires* (Madagascar)	34.53	24.02–44.99	32.46	24.04–41.45	45.01	33.06–58.23

*Asterisk designates the nodes used for calibration.

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Three approaches were employed to set the sequence of splits inferred from tree topology into a time frame. We used two tectonic events as calibration points: (i) The age of Metriorrhynchini fixed at 78 mya [11] resulted in the dates in Fig. 4, and the inferred time frame is consistent with tectonics of the East Gondwanan region. (ii) The alternative calibration based on the latest presence of the Kerguelen Plateau at 100 mya suggested an unrealistically early dispersal to the Oriental region and Wallacea (Tab. 2) [5]. (iii) The fixed mean substitution rate provided the shallowest estimations amongst the calibrations used, but not substantially different from the preferred dating (Tab. 2). The 'universal' mean clock is prone to error [28] and was applied here to provide another age limit for estimations. Although the inferred dating based on Lycidae phylogeny is congruent with the known sequence of tectonic events resulting in dispersal opportunities, the exact dating remains open for further investigation and we rely herein only on the information inferred from topology, i.e. sequence of events, combined with maximum time limits.

The Early Evolution of Metriorrhynchini: the Split of the Australian and Afro/Asian Clades

Metriorrhynchini is a Palaeotropical clade, which began diversification in Eastern Gondwana (Fig. 2A). The absence of Metriorrhynchini in the Neotropics and the delayed presence in Africa and Madagascar as well as the age inferred from the mutation rate refutes Gondwanan vicariance. Therefore, ancient over-sea dispersal after Gondwanan fragmentation is considered. The basal split between the *Metanoeus*, Australian and Afrotropical/Oriental clade is set in time when Indian was much closer to Australia than the other parts of the present Asia (75–100 mya, Fig. 2A–B). Considering the limited dispersal ability, the over-sea dispersal between Australia and India starting its drift to the north (definitively <1000 km) [39] is preferred to the dispersal event between Australia and Asia (>2500 km, Figs. 2A–B). Deeper dating within confidence intervals or the presence of the remnants of the subaerial Kerguelen Plateau allow assumption of the dispersal at the distance <500 km. The phylogenetic reconstructions suggest origin of Metriorrhynchini either in Australia or India (Figs. 3–4, S1–S2) taken the ambiguity of the phylogenetic inference in consideration. The Australian origin is supported by the higher morphological diversity of the Australian clade (Fig. S1). The second hypothesis is more parsimonious assuming the topology in Fig. S2. The direction of dispersal will have to be based on a more extensive data set.

Late Cretaceous Connectivity between India, Madagascar and Africa

Assuming presence of Metriorrhynchini on the drifting India (Fig. 2), we infer independent Lower Tertiary migrations to Africa (~65 mya) and Madagascar (~62 mya) at the early stage of the differentiation of the Afro/Oriental clade. The topologies and dating of the splits between the Indian and Afrotropical/Madagascar clades refute the vicariance hypothesis, as these Gondwanan fragments lost connectivity 100 mya [4,40], well before the origin of the African and Malagasy clades (Figs. 2, 4). The connectivity of these landmasses between the Maastrichtian and Lower Eocene remains contentious, and an island chain connection [5] or a modified position of India [3], were proposed as alternatives to India's isolation during rifting to the north [2]. The present results support biotic connectivity between India and

Table 3. Reconstructed probabilities of nine geographic areas at each of selected nodes of the Bayesian phylogeny.

Node#	Node code	Defined range:									
		Sundas	**Sulawesi**	**Continental Asia**	**outside**	**Africa**	**Australia**	**Madagascar**	**Philippines**	**Palawan**	**node # in Tab.S4**
1	Cautires1	0.48098	0.00626	0.11454	0	0.00006	0.00757	0.03428	0.21455	0.14177	20
2	Cameroon	0	0.00387	0	0	0.98903	0	0.00481	0.00011	0.00209	55
3	Madagascar1	0.00022	0.00381	0.00284	0	0	0.02506	0.96792	0	0	63
4	Madagascar 2	0.08706	0.02045	0.06322	0	0.00219	0.00962	0.66407	0.09364	0.05975	64
5	Cautires2	0.16606	0.00004	0.76362	0	0	0.00006	0.01782	0.01323	0.03917	124
6	Cautires3	0.07343	0.00026	0.82507	0.00001	0	0.00030	0.02258	0.03902	0.03932	125
7	CautiresMadagasc.	0.05744	0.00032	0.37550	0	0.00303	0.00024	0.36568	0.08801	0.10978	127
8	XylobCautires1	0.05814	0.01505	0.61849	0	0	0.01863	0.04806	0.09906	0.14257	140
9	XylobCautires2	0.02893	0.06566	0.50941	0	0.00004	0.00053	0.20388	0.08926	0.10228	141
10	Cautires4	0.07070	0.00157	0.36993	0	0.01400	0.00132	0.15853	0.17732	0.20662	142
11	MetriorrhSulabanus	0	0.03997	0.00012	0	0	0.93317	0.01446	0.00139	0.01085	184
12	TrichalusWakarumb	0.00008	0.06730	0.00025	0.00001	0.00001	0.90288	0.01954	0.00823	0.00170	225
13	TrichWakSul	0.00053	0.00029	0	0	0.00013	0.99138	0.00074	0.00629	0.00064	226
14	TrichMetr	0.00004	0.00004	0.00002	0.00002	0.00017	0.94138	0.00008	0.01453	0.04373	227
15	MetanoeusXylobanus	0.25720	0.03002	0.33029	0.00026	0.00204	0.03302	0.07744	0.11255	0.15719	239
16	MetanoeusMetr-ini	0.00615	0.00216	0.10669	0	0	0.75762	0.02838	0.03024	0.06877	240
17	Metriorhynchini	0.05665	0.01317	0.15450	0.00007	0.00016	0.57861	0.01155	0.14325	0.04203	241
18	Cautires Cameroon	0.04622	0.00002	0.54908	0	0.05274	0.00086	0.24338	0.03006	0.07766	425
19	Trichalus Broxylus	0	0.00023	0	0	0	0.92463	0.00094	0.07371	0.00039	426

Selected nodes are designated in Fig. 3. For further clades see Tab. S4.
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Africa in this period, across sea channels whose effective width might be lowered by the presence of the islands of the Amirante Ridge (Fig. 2C) [5]. The sampling of Afrotropical metriorrhynchines is limited in the present study and we cannot exclude that further dispersal events from India to Africa may be identified with more comprehensive sampling. Colonization via the migration corridor connecting Asia and Africa through the Arabian Peninsula in the Early Miocene (17–20 mya), which led to faunal and floristic exchanges in other groups [41,42], did not get any support from our data.

Yoder & Novak [4] demonstrated that most of the Malagasy biota is a result of dispersal events originating from the African coast. Metriorrhynchini, although rejected as an old Gondwanan element in Madagascar, suggest a role of India during its rifting to the north as an ancient source of immigrants. The ecological characteristics prevented lycids from the gradual build up of Madagascar biota across the >400 km wide Mozambique Channel and they remained isolated in Madagascar since the colonization of the island.

Origin and Phylogenetic Structure of the Oriental Fauna

Our data imply that Southeast Asian Metriorrhynchini are a phylogenetically heterogeneous assemblage and are formed of two groups: (i) *Cautires*, *Xylobanus*, and *Metanoeus*, which colonized the Oriental Region in Eocene (Fig. 2D, Tab. 2) and (ii), the lineages of the Australian clade (Fig. 5), as recent colonists.

The position of deeply nested lineages which gave an origin to the Madagascar and African fauna supports the India ferry hypothesis as an explanation of the origin of the *Cautires/Xylobanus/Metanoeus* group in the Oriental region, and refutes later, transoceanic dispersal known in some other animals (the Ninety Ridge hypothesis) [42,43]. This pattern is congruent with the role of India in the plant dispersal [44]. The clade was well diversified at the moment of dispersal to Asia and we identified 11 (at 55 mya) to 31 (35 mya) lineages in this clade at the time of the collision between India and Asia (Figs. 2D, 4) [5,45]. The mid Eocene ever-wet rain forest corridor [46] might enable rapid dispersal to east and subsequent diversification (estimated ~800 species at present). Although Indian plants readily colonized Sulawesi [47], Oriental Metriorrhynchini dispersed there much later and in low numbers (Figs. 3–4), probably during episodes of lower sea levels, which enabled dispersal in the opposite direction [13].

The Role of Wallacea in Dispersal of Australian Fauna to the North

Wallacea in the present form is a relatively young configuration of islands and the Australian and Oriental lineages could use this dispersal route since the Australian plate approached Asia 15–20 mya (Fig. 5, Tab. 2) [6]. All dispersal events in the region require the assumption of the over-sea dispersal and the distances to cross might have been a few hundreds kilometers as various islands formed and were eroded in the region [1,6]. Due to rareness of dispersal across such barriers, we still observe substantial differences between the biotas of Wallacea, New Guinea and Northern Australia (Fig. 5). Although effectively used by other organisms [42], these islands provided limited dispersal opportunities for Metriorrhynchini.

Several factors may affect the structure of Metriorrhynchini fauna in Wallacea. The islands at the northern margin of New

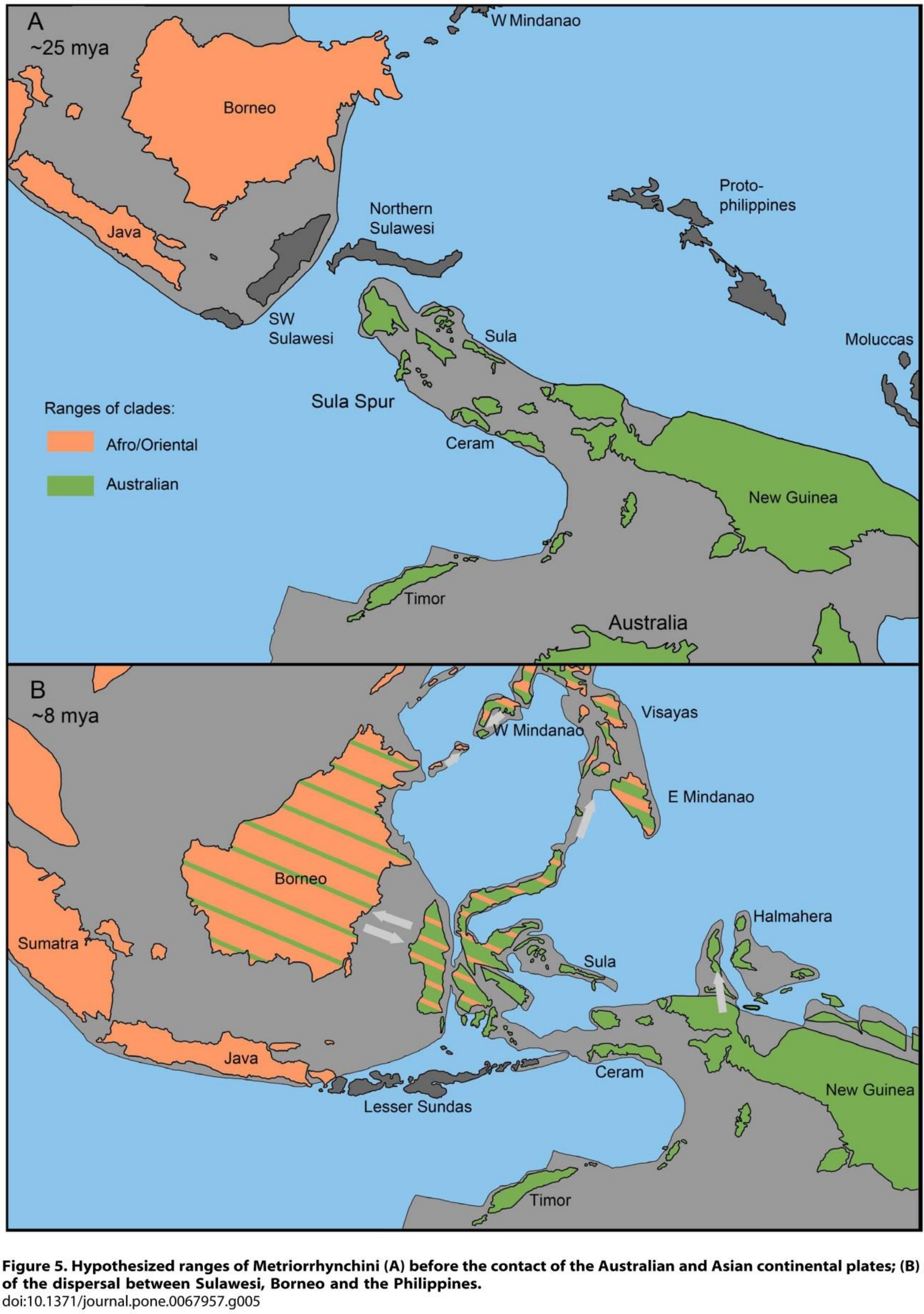

Figure 5. Hypothesized ranges of Metriorrhynchini (A) before the contact of the Australian and Asian continental plates; (B) at time of the dispersal between Sulawesi, Borneo and the Philippines.
doi:10.1371/journal.pone.0067957.g005

Guinea moved westward in the last 20 my (Fig. 5); [6,48] and this might support the dispersal of Australian Metriorrhynchini to west and limit dispersal in the opposite direction. Another important factor are ecological differences: taxa adapted to seasonally semidry habitats and capable of flight outside the rain forest canopy (e.g. flower-visiting *Microtrichalus* and *Metriorrhynchus*) are more widespread. In contrary, the taxa preferring the shaded, moist, under-canopy situations, e.g. *Wakarumbia*, *Sulabanus*, did not disperse across the Makassar Strait. The net-winged beetles exhibit a very conservative life history and even these minor differences in biology have a profound impact on the dispersal success.

Within Wallacea, only the Sulawesi fauna contains representatives of both basal clades (Fig. 1), but the apparent Australian bias in the Sulawesi biota is in contrast with the narrowness of the Makassar Strait (~100 km depending on the sea levels) [31]. Besides isolation by distance it can be supported by the pronounced seasonality of Sulawesi climate. Although almost completely of Australian origin, the Sulawesi fauna consists mainly of genera, which do not occur in Australia and New Guinea, and are either Wallacea endemics (*Wakarumbia*, *Broxylus*, *Mangkutanus*, and *Lobatang*) or occur additionally in the Philippines (*Sulabanus*) (Fig. 1). The origins of these endemic lineages were dated to 23–40 mya (Fig. 5) and we suppose that they might have a long diversification history in the fragmented Sula Spur [6]. We found that several species rich Australian lineages did not reach Sulawesi and the Philippines (e.g. *Porrostoma*, *Cladophorus*), reached only Sulawesi and the Philippines (*Cautiromimus*) and a few colonized additionally continental Asia (*Metriorrhynchus*, *Microtrichalus*). When present in Sulawesi, these genera are species poor [14,15] and we suppose their delayed colonization of Wallacea.

Conclusions

Biological characteristics of the metriorrhynchines have a substantial impact on their evolution. Low dispersal propensity limits Metriorrhynchini to small ranges and even a few hundreds kilometers wide sea channels can limit dispersal between neighboring landmasses. The long-distance dispersal events (>500 km) are absent and repeated expansion of ranges over sea channels were seldom identified. Such immobile organisms provide a distribution pattern that has been historically preserved, and gives a different view on the dispersal history to that of highly mobile animals.

The Indian subcontinent played a central role in the dispersal history of Metriorrhynchini and served as a Noah's Ark, bringing South Gondwanan fauna to Africa (65 mya), Madagascar (62 mya) and Asia (35–55 mya). Australian fauna evolved in complete isolation since the split of the basal lineages until ~20 mya, when several Australian genera dispersed to Asia (Figs. 2–3, 5).

The Metriorrhynchini are represented by ~800 species in humid SE Asia and similarly ~1000 species in New Guinea whose mountain forests have recent origin [44]. Metriorrhynchini are a lineage capable of generating high species level diversity in a very limited space and short time. The Oriental Metriorrhynchini depend on the ever-wet tropical rain forests, and despite geographic proximity only rarely invaded Sulawesi and did not disperse further eastward. In contrast, the Australian flower-visiting or semi-dry condition adapted genera considerably expanded their ranges after crossing sea barriers, and occupy much larger ranges in the Oriental region. These genera occur mostly in dried habitats, including man-disturbed ecosystems.

Supporting Information

Figure S1
(PDF)

Figure S2
(PDF)

Table S1
(PDF)

Table S2
(PDF)

Table S3
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Table S4
(PDF)

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Author Contributions

Conceived and designed the experiments: LB. Performed the experiments: KS. Analyzed the data: KS DC LB. Contributed reagents/materials/analysis tools: LB. Wrote the paper: KS DC LB.

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Supplementary Table S1. Taxa included in the analysis with collecting information, voucher, and GenBank accession numbers.

Supplementary Table S2. Primers and conditions used for PCR amplifications

Supplementary Table S3. The length of DNA fragments, the numbers of informative characters in datasets (gaps as 5th character) and partitions

Supplementary Table S4. Results of the BayesTraits analysis (all analyzed clades)

Supplementary Figure S1. Phylogenetic hypothesis on Metriorrhynchini inferred from the ML analysis.

Supplementary Figure S2. Optimal distributions at each node inferred from the RASP analysis on the set of 1000 Bayesian trees.

Supplementary Table S1. Taxa included in the analysis with collecting information, voucher, and GenBank accession numbers.

Species	Voucher number	Geographic origin	18S	28S	16S	cox1	nad5
Outgroup							
Libnetis sp.	UPOL 001002	Indonesia	DQ181104	DQ181178	DQ181030	DQ181252	DQ181406
Dilophotes sp.	UPOL 000244	Sabah	DQ181066	DQ181140	DQ180992	DQ181214	DQ181368
Plateros sp.	UPOL 000243	Sabah	DQ181065	DQ181139	DQ180991	DQ181213	DQ181367
Platycis minutus	UPOL 000348	Czech Republic	DQ181069	DQ181143	DQ180995	DQ181217	DQ181371
Lycoprogenthes sp.	UPOL 000358	Java	DQ181070	DQ181144	DQ180996	DQ181218	DQ181372
Calochromus sp.	UPOL 000400	Palawan	-	-	-	KC538321	KC538512
Duliticola sp.	UPOL 000L01	Sabah	DQ181037	DQ181111	DQ180963	DQ181185	DQ181339
Libnetis sp.	UPOL 000L02	Sabah	DQ181038	DQ181112	DQ180964	DQ181186	DQ181340
Lycus sp.	UPOL 000L03	South Africa	DQ181039	DQ181113	DQ180965	DQ181187	DQ181341
Calopteron sp.	UPOL 000L25	Ecuador	DQ181053	DQ181127	DQ180979	DQ181201	DQ181355
Lyropaeus sp.	UPOL 000L11	Sabah	DQ181042	DQ181116	DQ180968	DQ181190	DQ181344
Dihammatus sp.	UPOL 000L12	Sabah	DQ181043	DQ181117	DQ180969	DQ181191	DQ181345
Plateros sp.	UPOL 000L13	Sabah	DQ181044	DQ181118	DQ180970	DQ181192	DQ181346
Scarelus sp.	UPOL 000L15	Sabah	DQ181046	DQ181120	KC538782	DQ181194	DQ181348
Calochromus sp.	UPOL 000L16	China	DQ181047	DQ181121	DQ180973	DQ181195	DQ181349
Lyponia nigrohumeralis	UPOL 000L17	China	DQ181048	DQ181122	DQ180974	DQ181196	DQ181350
Macrolycus bocakorum	UPOL 000L18	China	DQ181049	DQ181123	DQ180975	DQ181197	DQ181351
Dictyoptera elegans	UPOL 000570	Japan	DQ181073	DQ181147	DQ180999	DQ181221	DQ181375
Pyropterus nigroruber	UPOL 000574	Japan	DQ181077	DQ181151	DQ181003	DQ181225	DQ181379
Lopheros sp.	UPOL 000578	Japan	DQ181081	DQ181155	DQ181007	DQ181229	DQ181383
Lycoprogenthes sp.	UPOL 000801	Sumatra	DQ181095	DQ181169	DQ181021	DQ181243	DQ181397
Plateros sp.	UPOL A00047	Malaysia	KC538062	KC537854	-	KC538353	KC538544
Dilophotes sp.	UPOL A00060	Philippines	KC538072	KC537863	KC538740	KC538359	KC538552
Ingroup							
Metriorrhynchus lineatus	UPOL 000009	Sumatra	KC538123	KC537913	KC538628	DQ904297	DQ904259
Metriorrhynchus sp.	UPOL 000010	Sulawesi	KC538124	KC537914	-	DQ144659	DQ144685
Metriorrhynchus sp.	UPOL 000011	Sulawesi	KC538125	KC537915	KC538629	DQ144660	DQ144686
Metriorrhynchus lobatus	UPOL 000017	Sulawesi	KC538126	KC537916	KC538630	DQ144662	DQ144688
Metanoeus sp.	UPOL 000026	Borneo	KC538127	KC537917	KC538631	KC538244	KC538436
Cautires sp.	UPOL 000030	Borneo	KC538128	KC537918	KC538632	KC538245	KC538437
Cautires sp.	UPOL 000037	Borneo	KC538129	KC537919	KC538633	KC538246	KC538438
Cautires sp.	UPOL 000040	Borneo	KC538130	KC537920	KC538634	KC538247	KC538439
Cautires sp.	UPOL 000043	Borneo	KC538131	KC537921	KC538635	KC538248	KC538440
Cautires sp.	UPOL 000044	Borneo	KC538132	KC537922	KC538636	KC538249	KC538441
Cautires sp.	UPOL 000047	Sumatra	KC538133	KC537923	KC538637	KC538250	KC538442
Cautires sp.	UPOL 000048	Sumatra	KC538134	KC537924	KC538638	KC538251	KC538443
Cautires sp.	UPOL 000050	Sumatra	KC538135	KC537925	KC538639	KC538252	KC538444
Cautires sp.	UPOL 000052	Sumatra	KC538136	KC537926	KC538640	KC538253	KC538445
Cautires sp.	UPOL 000056	Sumatra	KC538137	KC537927	KC538641	KC538254	KC538446
Cautires sp.	UPOL 000060	Sumatra	KC538138	KC537928	KC538642	KC538255	KC538447
Cautires sp.	UPOL 000064	Laos	KC538139	KC537929	KC538643	KC538256	KC538448
Cautires sp.	UPOL 000066	Laos	KC538140	KC537930	KC538644	KC538257	KC538449
Cautires sp.	UPOL 000068	Borneo	KC538141	KC537931	KC538645	KC538258	KC538450
Cautires sp.	UPOL 000069	Borneo	KC538142	KC537932	KC538646	KC538259	KC538451
Cautires sp.	UPOL 000070	Malaysia	KC538143	KC537933	KC538647	KC538260	KC538452
Xylobanus sp.	UPOL 000071	Borneo	KC538144	KC537934	KC538648	KC538261	KC538453
Cautires sp.	UPOL 000074	Borneo	KC538145	KC537935	KC538649	KC538262	KC538454
Cautires sp.	UPOL 000075	Laos	KC538146	KC537936	-	KC538263	KC538455
Cautires sp.	UPOL 000079	Borneo	KC538147	KC537937	KC538650	KC538264	KC538456
Cautires sp.	UPOL 000080	Borneo	KC538148	KC537938	KC538651	KC538265	KC538457

Cautires sp.	UPOL 000081	Borneo	KC538149	KC537939	KC538652	KC538266	KC538458
Cautires sp.	UPOL 000084	Borneo	KC538150	KC537940	KC538653	KC538267	KC538459
Cautires sp.	UPOL 000088	Malaysia	KC538151	KC537941	KC538654	KC538268	KC538460
Cautires sp.	UPOL 000090	Borneo	KC538152	KC537942	KC538655	KC538269	KC538461
Cautires sp.	UPOL 000104	Borneo	KC538153	KC537943	KC538656	KC538270	KC538462
Metanoeus sp.	UPOL 000105	Borneo	KC538154	KC537944	KC538657	KC538271	KC538463
Cautires sp.	UPOL 000109	Borneo	-	KC537945	KC538658	KC538272	KC538464
Xylobanus sp-	UPOL 000120	Laos	KC538155	KC537946	KC538659	KC538273	KC538465
Metanoeus sp.	UPOL 000121	Sumatra	KC538156	KC537947	KC538660	KC538274	KC538466
Cautires sp.	UPOL 000122	Borneo	KC538157	KC537948	KC538661	KC538275	KC538467
Cautires sp.	UPOL 000123	Java	KC538158	KC537949	-	KC538276	-
Metanoeus sp.	UPOL 000125	Sumatra	KC538159	KC537950	KC538662	KC538277	KC538468
Xylobanus sp.	UPOL 000132	Sumatra	KC538160	KC537951	KC538663	HQ456987	HQ457009
Cautires sp.	UPOL 000147	India	KC538161	KC537952	KC538664	KC538278	KC538470
Xylobanus sp.	UPOL 000152	Laos	KC538162	KC537953	KC538665	KC538279	KC538471
Xylobanus sp.	UPOL 000153	Laos	KC538163	KC537954	KC538666	KC538280	KC538472
Xylobanus sp.	UPOL 000154	Laos	KC538164	KC537955	-	KC538281	-
Cautires sp.	UPOL 000164	Laos	KC538165	KC537956	KC538667	KC538282	KC538473
Cautires sp.	UPOL 000174	Malaysia	KC538166	KC537957	-	KC538283	KC538474
Cautires sp.	UPOL 000177	Malaysia	KC538167	-	KC538668	KC538284	KC538475
Cautires sp.	UPOL 000178	Malaysia	KC538168	KC537958	KC538669	KC538285	KC538476
Xylobanus sp.	UPOL 000184	Borneo	KC538169	KC537959	KC538670	KC538286	KC538477
Cautires sp.	UPOL 000188	Laos	KC538170	KC537960	KC538671	KC538287	KC538478
Cautires sp.	UPOL 000189	Laos	KC538171	KC537961	KC538672	KC538288	KC538479
Cautires sp.	UPOL 000195	South Africa	KC538172	KC537962	KC538673	KC538289	KC538480
Microtrichalus sp.	UPOL 000199	Sulawesi	KC538173	KC537963	KC538674	KC538290	KC538481
Cautires sp.	UPOL 000205	Sumatra	KC538174	KC537964	KC538675	KC538291	KC538482
Cautires sp.	UPOL 000206	Sumatra	KC538175	KC537965	KC538676	KC538292	KC538483
Leptotrichalus sp.	UPOL 000208	Borneo	DQ181064	DQ181138	KC538677	DQ181212	DQ181366
Cautires sp.	UPOL 000217	Japan	KC538176	KC537966	KC538678	KC538293	KC538484
Cautires sp.	UPOL 000219	Japan	KC538177	KC537967	KC538679	KC538294	KC538485
Cautires sp.	UPOL 000220	Japan	KC538178	KC537968	KC538680	KC538295	KC538486
Xylobanus sp.	UPOL 000221	Japan	KC538179	-	KC538681	KC538296	KC538487
Xylobanus sp.	UPOL 000224	Japan	KC538180	KC537969	KC538682	KC538297	KC538488
Cautires sp.	UPOL 000246	Sumatra	KC538181	KC537970	KC538683	KC538298	KC538489
Metanoeus sp.	UPOL 000248	Sumatra	KC538182	KC537971	KC538684	KC538299	KC538490
Xylobanus sp.	UPOL 000262	Borneo	-	KC537972	KC538685	KC538300	KC538491
Xylobanus sp.	UPOL 000274	Borneo	KC538183	KC537973	KC538686	KC538301	KC538492
Cautires sp.	UPOL 000290	Laos	KC538184	KC537974	KC538687	KC538302	KC538493
Cautires sp.	UPOL 000294	Sumatra	KC538185	KC537975	KC538688	KC538303	KC538494
Cautires sp.	UPOL 000295	Sumatra	KC538186	KC537976	KC538689	KC538304	KC538495
Cautires sp.	UPOL 000297	Sumatra	KC538187	KC537977	KC538690	KC538305	KC538496
Cautires sp.	UPOL 000314	Sumatra	KC538188	KC537978	KC538691	KC538306	KC538497
Xylobanus sp.	UPOL 000315	Sumatra	KC538189	KC537979	KC538692	KC538307	KC538498
Cautires sp.	UPOL 000335	Borneo	-	-	KC538693	KC538308	KC538499
Cautires sp.	UPOL 000339	Borneo	KC538190	KC537980	KC538694	KC538309	KC538500
Cautires sp.	UPOL 000342	Borneo	KC538191	KC537981	KC538695	KC538310	KC538501
Cautires sp.	UPOL 000346	Borneo	KC538192	KC537982	KC538696	KC538311	KC538502
Cautires sp.	UPOL 000355	Jawa	-	KC537983	KC538697	KC538312	KC538503
Metriorrh. palawensis	UPOL 000366	Palawan	-	-	KC538698	DQ144665	DQ144691
Porrostoma rhipidum	UPOL 000372	Australia	KC538193	KC537984	KC538699	DQ144678	DQ144702
Microtrichalus sp.	UPOL 000373	Australia	KC538194	KC537985	KC538700	KC538313	KC538504
Metriorrhynchus sp.	UPOL 000374	Australia	KC538195	KC537986	KC538701	KC538314	KC538505
Microtrichalus sp.	UPOL 000375	Australia	KC538196	KC537987	KC538702	KC538315	KC538506
Microtrichalus sp.	UPOL 000376	Australia	KC538197	KC537988	KC538703	KC538316	KC538507
Porrost. haemorrhoidalis	UPOL 000378	Australia	KC538198	KC537989	KC538704	DQ144679	DQ144703
Xylobanus sp.	UPOL 000379	Palawan	KC538199	-	KC538705	KC538317	KC538508
Cautiromimus sp.	UPOL 000388	Palawan	-	-	-	KC538318	KC538509

Cautires sp.	UPOL 000395	Palawan	-	-	KC538706	KC538319	KC538510
Leptotrichalus sp.	UPOL 000396	Palawan	-	-	KC538707	KC538320	KC538511
Xylobanus sp.	UPOL 000402	Palawan	KC538200	-	KC538708	KC538322	KC538513
Cautires sp.	UPOL 000403	Palawan	-	KC537990	KC538709	KC538323	KC538514
Cautires sp.	UPOL 000411	Palawan	KC538201	-	KC538710	KC538324	KC538515
Microtrichalus sp.	UPOL 000412	Palawan	KC538202	KC537991	KC538711	KC538325	KC538516
Leptotrichalus sp.	UPOL 000419	Palawan	KC538203	KC537992	KC538712	KC538326	KC538517
Cautires sp.	UPOL 000425	Palawan	-	-	KC538713	KC538327	KC538518
Metanoeus sp.	UPOL 000434	Palawan	-	-	KC538714	KC538328	KC538519
Cautires sp.	UPOL A00017	Taiwan	-	KC537824	-	-	-
Xylobanus sp.	UPOL A00018	Taiwan	KC538033	KC537825	HQ456946	HQ456964	HQ456988
Cautires sp.	UPOL A00019	Taiwan	KC538034	KC537826	KC538715	KC538329	KC538520
Cautires sp.	UPOL A00020	Taiwan	KC538035	KC537827	-	-	-
Cautires sp.	UPOL A00021	Taiwan	KC538036	KC537828	HQ456947	HQ456965	-
Cautires sp.	UPOL A00022	Madagascar	KC538037	KC537829	KC538716	HQ456966	HQ456986
Cautires sp.	UPOL A00023	Madagascar	KC538038	KC537830	KC538717	KC538330	KC538521
Cautires sp.	UPOL A00024	Madagascar	KC538039	KC537831	KC538718	KC538331	KC538522
Cautires sp.	UPOL A00025	Madagascar	KC538040	KC537832	KC538719	KC538332	KC538523
Cautires sp.	UPOL A00026	Madagascar	KC538041	KC537833	-	KC538333	KC538524
Cautires sp.	UPOL A00027	Madagascar	KC538042	KC537834	-	KC538334	KC538525
Cautires sp.	UPOL A00028	Madagascar	KC538043	KC537835	-	KC538335	-
Cautires sp.	UPOL A00029	Madagascar	KC538044	KC537836	-	KC538336	KC538526
Cautires sp.	UPOL A00030	Madagascar	KC538045	KC537837	KC538720	KC538337	KC538527
Synchonnus sp.	UPOL A00031	Australia	KC538046	KC537838	KC538721	KC538338	KC538528
Trichalus sp.	UPOL A00032	Australia	KC538047	KC537839	KC538722	KC538339	KC538529
Ditua sp.	UPOL A00033	Australia	KC538048	KC537840	KC538723	-	KC538530
Metriorrhynchus sp.	UPOL A00034	Australia	KC538049	KC537841	KC538724	KC538340	KC538531
Porrostoma sp.	UPOL A00035	Australia	KC538050	KC537842	KC538725	KC538341	KC538532
Porrostoma sp.	UPOL A00036	Australia	KC538051	KC537843	KC538726	KC538342	KC538533
Porrostoma sp.	UPOL A00037	Australia	KC538052	KC537844	KC538727	KC538343	KC538534
Metriorrhynchus sp.	UPOL A00038	Australia	KC538053	KC537845	KC538728	KC538344	KC538535
Metriorrhynchus sp.	UPOL A00039	Australia	KC538054	KC537846	KC538729	KC538345	KC538536
Porrostoma sp.	UPOL A00040	Australia	KC538055	KC537847	KC538730	KC538346	KC538537
Porrostoma sp.	UPOL A00041	Australia	KC538056	KC537848	KC538731	KC538347	KC538538
Porrostoma sp.	UPOL A00042	Australia	KC538057	KC537849	-	KC538348	KC538539
Metriorrhynchus sp.	UPOL A00043	Australia	KC538058	KC537850	KC538732	KC538349	KC538540
Porrostoma sp.	UPOL A00044	Australia	KC538059	KC537851	KC538733	KC538350	KC538541
Porrostoma sp.	UPOL A00045	Australia	KC538060	KC537852	KC538734	KC538351	KC538542
Metriorrhynchus sp.	UPOL A00046	Australia	KC538061	KC537853	KC538735	KC538352	KC538543
Cautires sp.	UPOL A00048	Malaysia	KC538063	KC537855	HQ456948	HQ456967	HQ456990
Metriorrhynchus sp.	UPOL A00049	Malaysia	KC538064	KC537856	KC538736	KC538354	KC538545
Cautires sp.	UPOL A00050	Malaysia	KC538065	KC537857	-	-	KC538546
Leptotrichalus sp.	UPOL A00052	Philippines	KC538066	KC537858	HQ456949	HQ456968	HQ456991
Xylobanus sp.	UPOL A00053	Palawan	KC538067	KC537859	KC538737	KC538355	KC538547
Xylobanus sp.	UPOL A00054	Palawan	KC538068	-	KC538738	-	KC538548
Cautires sp.	UPOL A00057	Philippines	KC538069	KC537860	-	KC538356	KC538549
Cautires sp.	UPOL A00058	Philippines	KC538070	KC537861	-	KC538357	KC538550
Leptotrichalus sp.	UPOL A00059	Philippines	KC538071	KC537862	KC538739	KC538358	KC538551
Leptotrichalus sp.	UPOL A00061	Philippines	KC538073	KC537864	KC538741	KC538360	KC538553
Cautires sp.	UPOL A00062	Philippines	KC538074	KC537865	KC538742	KC538361	KC538554
Metanoeus sp.	UPOL A00063	Philippines	KC538075	KC537866	-	KC538362	KC538555
Metanoeus sp.	UPOL A00064	Philippines	KC538076	KC537867	-	KC538363	KC538556
Metanoeus sp.	UPOL A00065	Philippines	KC538077	KC537868	-	KC538364	KC538557
Sulabanus sp.	UPOL A00066	Philippines	KC538078	KC537869	KC538743	KC538365	KC538558
Sulabanus sp.	UPOL A00067	Philippines	KC538079	KC537870	KC538744	KC538366	KC538559
Microtrichalus sp.	UPOL A00068	Philippines	KC538080	KC537871	KC538745	KC538367	KC538560
Microtrichalus sp.	UPOL A00069	Philippines	KC538081	KC537872	KC538746	KC538368	KC538561
Sulabanus sp.	UPOL A00070	Philippines	KC538082	KC537873	KC538747	KC538369	KC538562

Sulabanus sp.	UPOL A00071	Philippines	KC538083	KC537874	KC538748	KC538370	KC538563
Microtrichalus sp.	UPOL A00073	Philippines	KC538084	KC537875	KC538749	KC538371	-
Xylobanus sp.	UPOL A00074	Philippines	KC538085	KC537876	KC538750	-	-
Sulabanus sp.	UPOL A00075	Philippines	KC538086	KC537877	KC538751	KC538372	KC538564
Xylobanus sp.	UPOL 00A076	Philippines	KC538087	KC537878	KC538752	KC538373	KC538565
Sulabanus sp.	UPOL A00077	Philippines	KC538088	KC537879	KC538753	KC538374	KC538566
Cautires sp.	UPOL A00078	Cameroon	KC538089	KC537880	KC538754	KC538375	KC538567
Cautires sp.	UPOL A00079	Cameroon	KC538090	KC537881	KC538755	KC538376	KC538568
Cautires sp.	UPOL A00080	Cameroon	KC538091	KC537882	HQ456950	HQ456969	HQ456992
Cautires sp.	UPOL A00081	Cameroon	KC538093	KC537884	KC538756	KC538377	KC538569
Cautires sp.	UPOL A00082	Cameroon	KC538092	KC537883	KC538757	KC538378	-
Cautires sp.	UPOL A00083	Cameroon	KC538094	KC537885	KC538758	KC538379	KC538570
Cautires sp.	UPOL A00084	Cameroon	KC538095	KC537886	KC538759	KC538380	KC538571
Cautires sp.	UPOL 00A085	Cameroon	KC538096	KC537887	KC538760	KC538381	KC538572
Cautires sp.	UPOL A00086	Cameroon	KC538097	KC537888	KC538761	KC538382	KC538573
Cautires sp.	UPOL A00087	Cameroon	KC538098	KC537889	KC538762	KC538383	KC538574
Cautires sp.	UPOL A00088	Cameroon	KC538099	KC537890	KC538763	KC538384	KC538575
Cautires sp.	UPOL A00089	Cameroon	KC538100	KC537891	KC538764	KC538385	KC538576
Cautires sp.	UPOL A00090	Cameroon	KC538101	KC537892	KC538765	KC538386	KC538577
Xylobanus sp.	UPOL A00091	Cameroon	KC538102	KC537893	-	KC538387	KC538578
Cautires sp.	UPOL A00092	Cameroon	KC538103	KC537894	KC538766	KC538388	KC538579
Cautires sp.	UPOL A00093	Cameroon	KC538104	KC537895	KC538767	KC538389	KC538580
Xylobanus sp.	UPOL A00094	Cameroon	KC538105	KC537896	KC538768	KC538390	KC538581
Xylobanus sp.	UPOL A00095	Cameroon	KC538106	KC537897	KC538769	-	KC538582
Xylobanus sp.	UPOL A00096	Cameroon	KC538107	KC537898	KC538770	-	KC538583
Xylobanus sp.	UPOL A00097	Cameroon	KC538108	KC537899	KC538771	KC538391	KC538584
Xylobanus sp.	UPOL A00098	Cameroon	KC538109	KC537900	KC538772	KC538392	KC538585
Cautires sp.	UPOL A00099	Cameroon	KC538110	KC537901	KC538773	KC538393	KC538586
Cautires sp.	UPOL A00100	Cameroon	KC538111	-	KC538774	KC538394	KC538587
Cautires sp.	UPOL A00101	Cameroon	KC538112	KC537902	-	KC538395	-
Cautires sp.	UPOL A00102	Cameroon	KC538113	KC537903	-	KC538396	KC538588
Cautires sp.	UPOL A00103	Cameroon	KC538114	KC537904	-	KC538397	-
Xylobanus sp.	UPOL A00104	Cameroon	KC538115	KC537905	KC538775	-	KC538589
Cautires sp.	UPOL A00105	Cameroon	KC538116	KC537906	KC538776	-	KC538590
Cautires sp.	UPOL A00106	Cameroon	KC538117	KC537907	KC538777	KC538398	KC538591
Cautires sp.	UPOL A00107	Cameroon	KC538118	KC537908	-	KC538399	-
Cautires sp.	UPOL A00109	Cameroon	KC538119	KC537909	KC538778	KC538400	KC538592
Cautires sp.	UPOL A00110	Cameroon	KC538120	KC537910	KC538779	-	KC538593
Cautires sp.	UPOL A00111	Cameroon	KC538121	KC537911	KC538780	KC538401	KC538594
Cautires sp.	UPOL A00112	Cameroon	KC538122	KC537912	KC538781	KC538402	KC538595
Metriorrhynchus lineatus	UPOL 000L05	Malaysia	DQ181040	DQ181114	DQ180966	DQ181188	DQ181342
Cautires sp.	UPOL 000L06	Malaysia	DQ181041	DQ181115	DQ180967	DQ181189	DQ181343
Cautires sp.	UPOL 000L14	South Africa	DQ181045	DQ181119	DQ180971	DQ181193	DQ181347
Microtrichalus sp.	UPOL 000L23	Malaysia	DQ181052	DQ181126	DQ180978	DQ181200	DQ181354
Xylobanus kundratai	UPOL MD0029	Sulawesi	KC538204	KC537993	-	HQ456972	HQ456994
Sulabanus lalui	UPOL MD0030	Sulawesi	KC538205	KC537994	-	KC538403	KC538596
Sulabanus katarinae	UPOL MD0033	Sulawesi	KC538206	KC537995	KC538783	KC538404	KC538597
Sulabanus lineatus	UPOL MD0034	Sulawesi	KC538207	KC537996	KC538784	KC538405	KC538598
Xylobanus kundratai	UPOL MD0036	Sulawesi	KC538208	KC537997	-	HQ456973	HQ456995
Sulabanus mamasensis	UPOL MD0044	Sulawesi	KC538209	KC537998	KC538785	KC538406	KC538599
Sulabanus gracilis	UPOL MD0064	Sulawesi	KC538210	KC537999	KC538786	KC538407	KC538600
Sulabanus similis	UPOL MD0065	Sulawesi	KC538211	KC538000	-	KC538408	KC538601
Sulabanus gracilis	UPOL MD0067	Sulawesi	KC538212	KC538001	KC538787	KC538409	KC538602
Sulabanus cordatus	UPOL MD0069	Sulawesi	KC538213	KC538002	KC538788	KC538410	KC538603
Sulabanus gracilis	UPOL MD0071	Sulawesi	KC538214	KC538003	KC538789	KC538411	KC538604
Sulabanus cordatus	UPOL MD0081	Sulawesi	KC538215	KC538004	KC538790	KC538412	KC538605
Microtrichalus sp.	UPOL MD0097	Sulawesi	KC538216	KC538005	KC538791	HQ456978	HQ457000
Microtrichalus sp.	UPOL MD0098	Sulawesi	KC538217	KC538006	HQ456956	HQ456979	HQ457001

Broxylus pfeifferi	UPOL MD0099	Sulawesi	KC538218	KC538007	HQ456957	HQ456980	HQ457002
Broxylus malinensis	UPOL MD0101	Sulawesi	KC538219	KC538008	HQ456958	HQ456981	HQ457003
Broxylus pendolensis	UPOL MD0106	Sulawesi	KC538220	KC538009	KC538792	KC538413	KC538606
Broxylus kalamensis	UPOL MD0107	Sulawesi	KC538221	KC538010	KC538793	KC538414	KC538607
Wakarumbia pendolensis	UPOL MD0109	Sulawesi	KC538222	KC538011	KC538794	KC538415	KC538608
Wakarumbia monacha	UPOL MD0111	Sulawesi	KC538223	KC538012	KC538795	KC538416	KC538609
Wakarumbia petri	UPOL MD0118	Sulawesi	KC538224	KC538013	HQ456960	HQ456983	HQ457005
Wakarumbia montana	UPOL MD0119	Sulawesi	KC538225	KC538014	HQ456961	HQ456984	HQ457006
Wakarumbia fasciata	UPOL MD0121	Sulawesi	KC538226	KC538015	KC538796	KC538418	KC538610
Wakarumbia sp.	UPOL MD0126	Sulawesi	KC538227	KC538016	KC538797	KC538419	KC538611
Wakarumbia grisea	UPOL MD0127	Sulawesi	KC538228	KC538017	-	KC538420	KC538612
Wakarumbia nepeensis	UPOL MD0129	Sulawesi	KC538229	KC538018	KC538798	KC538421	KC538613
Wakarumbia kundratai	UPOL MD0130	Sulawesi	KC538230	KC538019	KC538799	KC538422	KC538614
Wakarumbia sp.	UPOL MD0132	Sulawesi	KC538231	KC538020	KC538800	KC538423	KC538615
Wakarumbia kalamensis	UPOL MD0133	Sulawesi	KC538232	KC538021	KC538801	KC538424	KC538616
Wakarumbia linearis	UPOL MD0134	Sulawesi	KC538233	KC538022	KC538802	KC538425	KC538617
Wakarumbia nepeensis	UPOL MD0135	Sulawesi	KC538234	KC538023	KC538803	KC538426	KC538618
Wakarumbia aurea	UPOL MD0136	Sulawesi	KC538235	KC538024	KC538804	KC538427	KC538619
Wakarumbia aurea	UPOL MD0137	Sulawesi	KC538236	KC538025	KC538805	KC538428	KC538620
Wakarumbia fascicularis	UPOL MD0140	Sulawesi	KC538237	KC538026	KC538806	KC538429	KC538621
Wakarumbia pendolensis	UPOL MD0143	Sulawesi	KC538238	KC538027	KC538807	KC538430	KC538622
Wakarumbia fascicularis	UPOL MD0145	Sulawesi	KC538239	KC538028	KC538808	KC538431	KC538623
Wakarumbia mamasensis	UPOL MD0155	Sulawesi	KC538240	KC538029	KC538809	KC538432	KC538624
Wakarumbia grisea	UPOL MD0156	Sulawesi	KC538241	KC538030	KC538810	KC538433	KC538625
Wakarumbia mamasensis	UPOL MD0157	Sulawesi	KC538242	KC538031	KC538811	KC538434	KC538626
Wakarumbia kalamensis	UPOL MD0169	Sulawesi	KC538243	KC538032	KC538812	KC538435	KC538627

Remark. DQ and HQ sequences were published in the previous studies on net-winged beetles (Bocak et al., 2008, Bocak & Yagi 2010; Kubecek et al. 2011). The KC sequences are published here for the first time.

The voucher specimens are deposited in the Laboratory of Molecular Systematics, Department of Zoology, Palacky University, Olomouc, Czech Republic.

Supplementary Table S2. Primers and conditions used for PCR amplifications

Fragment	Code	-mer	Sequence (5’ >> 3’)
18S rRNA	5’	24	GACAACCTGGTTGATCCTGCCAGT
	b5.0	19	TAACCGCAACAACTTTAAT
	ai	22	CCTGAGAAACGGCTACCACATC
	b2.5	20	TCTTTGGCAAATGCTTTCGC
	a1.0	20	GGTGAAATTCTTGGACCGTC
	bi	20	GAGTCTCGTTCGTTATCGGA
	3’I	24	CACCTACGGAAACCTTGTTACGAC
	a2.0	19	ATGGTTGCAAAGCTGAAAC
28S rRNA	ff	20	TTACACACTCCTTAGCGGAT
	dd	19	GGGACCCGTCTTGAAACAC
16S rDNA	16a	20	CGCCTGTTTAACAAAAACAT
	16b	22	CCGGTCTGAACTCAGATCATGT
	ND1A	27	GGTCCCTTACGAATTTGAATATATCCT
cox1 mtDNA	JerM	23	CAACAYYTATTTTGRTTYTTTGG
	Pat	25	TCCATTGCACTAATCTGCCATATTA
	Marilyn	21	TCATAAGTTCAGTATCATTG
	Marcy	27	TARTTCRTATGWTCAATAYCAYTGRTG
nad5 mtDNA	OF1	29	CCTACTCCTGTTTCTGCTTTAGTTCATTC
	R6	29	GAAACGAAAAATCGTATTTAATTTCGACT

Table 3. The length of DNA fragments and the numbers of informative characters in datasets (gaps as 5th character).

Datasets		All data	18S rDNA	28S rDNA	*rrnl-tRNA-nad1*	*coi-tRNAcoii*	*nad5-tRNAs*
# of specimens		249	244	240	227	241	242
Clustal W	# of characters	5792	1913	655	840	1100	1284
	parsimony informative	2233	212	95	416	621	889
BlastAlign	# of characters	5963	1947	668	947	1101	1300
	parsimony informative	2163	205	92	397	621	848
Muscle	# of characters	5827	1921	657	858	1099	1292
	parsimony informative	2220	207	95	411	622	885
T-Coffee	# of characters	5881	1936	669	866	1101	1309
	parsimony informative	2216	208	92	413	621	882
Mafft	# of characters	5822	1909	659	858	1099	1297
	parsimony informative	2243	221	96	418	622	886

Table S4. Results of the BayesTrait analysis (all analyzed clades)

Node	Defined range:								
#	Sundas	Sulawesi	Contin. Asia	outside	Africa	Australia	Madagascar	Philippines	Palawan
18	0,32807	0,02622	0,08885	0	0,00079	0,02615	0,02385	0,32955	0,17649
20	0,48098	0,00626	0,11454	0	0,00006	0,00757	0,03428	0,21455	0,14177
55	0	0,00387	0	0	0,98903	0	0,00481	0,00011	0,00209
63	0,00022	0,00381	0,00284	0	0	0,02506	0,96792	0	0
64	0,08706	0,02045	0,06322	0	0,00219	0,00962	0,66407	0,09364	0,05975
67	0,21414	0,00106	0,00903	0	0	0,00164	0,01456	0,09429	0,66526
68	0,51419	0,00202	0,02937	0	0	0,00268	0,00925	0,10771	0,33477
69	0,77092	0,00086	0,02434	0	0	0,00188	0,00984	0,07595	0,11620
70	0,22706	0,00199	0,55502	0	0,00011	0,00124	0,03854	0,07972	0,09630
76	0,09651	0,00057	0,83519	0	0	0,00009	0,00489	0,02192	0,04084
77	0,19072	0,00199	0,63056	0	0,00040	0,00222	0,04846	0,05009	0,07553
78	0,13407	0	0,78690	0	0,00017	0,00047	0,01920	0,01637	0,04276
79	0,20077	0,00190	0,71169	0	0	0,00264	0,02640	0,03205	0,02453
80	0,17154	0,00314	0,64603	0	0,00015	0,00685	0,05849	0,05146	0,06231
81	0,12858	0,00226	0,67799	0	0	0,00091	0,03355	0,08261	0,07406
82	0,17365	0,00181	0,64440	0	0,00057	0,00189	0,02065	0,08317	0,07384
83	0,07133	0,00078	0,79451	0	0,00021	0,00236	0,05306	0,03991	0,03780
84	0,10204	0,00164	0,77232	0	0	0,01275	0,02762	0,03595	0,04760
86	0,28323	0,00086	0,53106	0	0	0,00166	0,05054	0,06350	0,06909
87	0,09733	0,00013	0,81795	0	0,00025	0,00077	0,02967	0,01744	0,03644
90	0,24255	0,00143	0,55269	0	0	0,00168	0,03407	0,06318	0,10433
91	0,40033	0,00117	0,40022	0	0,00029	0,00092	0,03768	0,06553	0,09384
93	0,64606	0,00456	0,23739	0	0	0,00048	0,01242	0,05356	0,04553
94	0,30633	0,00142	0,50025	0	0,00598	0,00226	0,03225	0,07021	0,08125
103	0,68382	0,00214	0,04240	0	0	0,00723	0,01594	0,09352	0,15494
105	0,22618	0,00243	0,02942	0	0	0,01007	0,00692	0,20295	0,52185
106	0,08102	0,00708	0,01548	0	0	0,00659	0,03948	0,40616	0,44410

110	0,26134	0,00250	0,01838	0	0,00010	0,00250	0,03788	0,33747	0,33982
111	0,53239	0,00112	0,02711	0	0	0,00077	0,01590	0,16576	0,25693
112	0,36663	0,00086	0,34155	0	0	0,00160	0,01535	0,12090	0,15309
118	0,46716	0,00220	0,21820	0	0,00008	0,00280	0,03732	0,12201	0,15021
119	0,62822	0,00404	0,16709	0	0	0,00036	0,01782	0,09419	0,08827
120	0,52113	0,00013	0,37383	0	0	0,00059	0,01297	0,03624	0,05510
121	0,34379	0,00718	0,45107	0,00007	0	0,00313	0,03360	0,07088	0,09028
122	0,20496	0,00075	0,70341	0	0,00020	0,00212	0,01728	0,03938	0,03191
123	0,22740	0,00064	0,62138	0,00001	0,00001	0,00533	0,01681	0,05439	0,07403
124	0,16606	0,00004	0,76362	0	0	0,00006	0,01782	0,01323	0,03917
125	0,07343	0,00026	0,82507	0,00001	0	0,00030	0,02258	0,03902	0,03932
127	0,05744	0,00032	0,37550	0	0,00303	0,00024	0,36568	0,08801	0,10978
130	0,12499	0,00867	0,39791	0,00010	0,00114	0,03012	0,12532	0,12240	0,18934
131	0,11955	0,03122	0,25048	0,00001	0,00049	0,03260	0,06076	0,24317	0,26172
132	0,00044	0,00008	0,00012	0	0	0,00098	0,00131	0,24421	0,75287
134	0,05943	0,01111	0,66261	0,00002	0,00007	0,01264	0,05847	0,08921	0,10644
135	0,14776	0,00407	0,64523	0,00004	0,00011	0,00348	0,04870	0,06977	0,08085
137	0,03404	0,28229	0,32691	0,00016	0,00003	0,10239	0,06841	0,05246	0,13332
139	0,06002	0,01562	0,69908	0,00002	0,00001	0,02758	0,01034	0,06700	0,12033
140	0,05814	0,01505	0,61849	0	0	0,01863	0,04806	0,09906	0,14257
141	0,02893	0,06566	0,50941	0	0,00004	0,00053	0,20388	0,08926	0,10228
142	0,07070	0,00157	0,36993	0	0,01400	0,00132	0,15853	0,17732	0,20662
143	0,00840	0,00219	0,02768	0,00037	0,00022	0,84651	0,01111	0,04060	0,06292
146	0,00129	0,86213	0,00217	0,00008	0,00166	0,06784	0,00586	0,03172	0,02726
153	0,03697	0,59083	0,04589	0,00001	0,00016	0,04965	0,01823	0,13174	0,12652
154	0,00702	0,83416	0,03789	0	0,00027	0,01710	0,00324	0,04239	0,05794
155	0,01464	0,54652	0,00389	0	0	0,04770	0,01044	0,10646	0,27035
160	0,00037	0,00704	0,00047	0,00002	0	0,94944	0,00034	0,02348	0,01883
172	0,00358	0,74455	0,00472	0,00001	0,00063	0,06641	0,00850	0,08855	0,08304
178	0	0,99159	0	0	0	0,00378	0,00023	0,00063	0,00371
179	0,00055	0,89868	0,00242	0	0,00001	0,02639	0,01446	0,03295	0,02454
183	0,00011	0,89517	0,00042	0	0,00001	0,01407	0,00314	0,05800	0,02909
184	0	0,03997	0,00012	0	0	0,93317	0,01446	0,00139	0,01085
206	0	0,99198	0	0	0	0,00178	0,00379	0,001676	0,00072
207	0,01163	0,00291	0,01103	0	0,00003	0,00536	0,01503	0,44108	0,51294
208	0,17242	0,00227	0,02063	0	0,00007	0,00379	0,01377	0,37242	0,41463
209	0,02165	0,01357	0,01630	0,00002	0,00005	0,01228	0,02532	0,42832	0,48250
210	0,01358	0,00608	0,01447	0	0,00006	0,00686	0,00919	0,70429	0,24546
211	0,01821	0,00179	0,04295	0	0,00001	0,00161	0,01503	0,48930	0,43111
212	0,00587	0,53613	0,00795	0,00001	0,00117	0,08395	0,01566	0,20606	0,14320
213	0,00099	0,05712	0,00170	0,00042	0,00019	0,84897	0,01586	0,04423	0,03052
217	0,00063	0,00015	0,00002	0	0	0,00013	0,00321	0,49732	0,49853
218	0,23456	0,00065	0,00454	0	0	0,00046	0,00270	0,37956	0,37752
219	0,01306	0,00012	0,00062	0	0	0,00004	0,00380	0,82981	0,15255
220	0,00020	0,55103	0,00288	0	0,00011	0,00818	0,01045	0,27679	0,15036
223	0	0,00289	0,00019	0	0	0,96642	0,00388	0,01271	0,01386
224	0,00018	0,00168	0,00190	0,00029	0	0,97036	0,00238	0,01971	0,00349
225	0,00008	0,06730	0,00025	0,00001	0,00001	0,90288	0,01954	0,00823	0,00170
226	0,00053	0,00029	0	0	0,00013	0,99138	0,00074	0,00629	0,00064
227	0,00004	0,00003	0,00002	0,00002	0,00017	0,94138	0,00008	0,01453	0,04373
228	0,30559	0,01200	0,06945	0,00002	0,00085	0,00907	0,04290	0,33928	0,22083
230	0,26207	0,00924	0,44753	0,00003	0,00030	0,01237	0,05743	0,09634	0,11468
234	0,22765	0,00795	0,02062	0	0,00001	0,00413	0,01604	0,52249	0,20110
235	0,46743	0,00369	0,04002	0	0,00001	0,00384	0,01294	0,32298	0,14908
236	0,72397	0,00093	0,03026	0	0	0,00054	0,03204	0,12209	0,09017
237	0,76160	0,00073	0,03055	0	0,00036	0,00019	0,00868	0,10000	0,09790
238	0,49536	0,00256	0,03253	0	0,00002	0,00480	0,01057	0,12257	0,33158
239	0,25720	0,03002	0,33029	0,00026	0,00204	0,03302	0,07744	0,11255	0,15719
240	0,00615	0,00216	0,10669	0	0	0,75762	0,02838	0,03024	0,06877
241	0,05665	0,01317	0,15450	0,00007	0,00016	0,57861	0,01155	0,14325	0,04203
425	0,04622	0,00002	0,54908	0	0,05274	0,00086	0,24338	0,03006	0,07766
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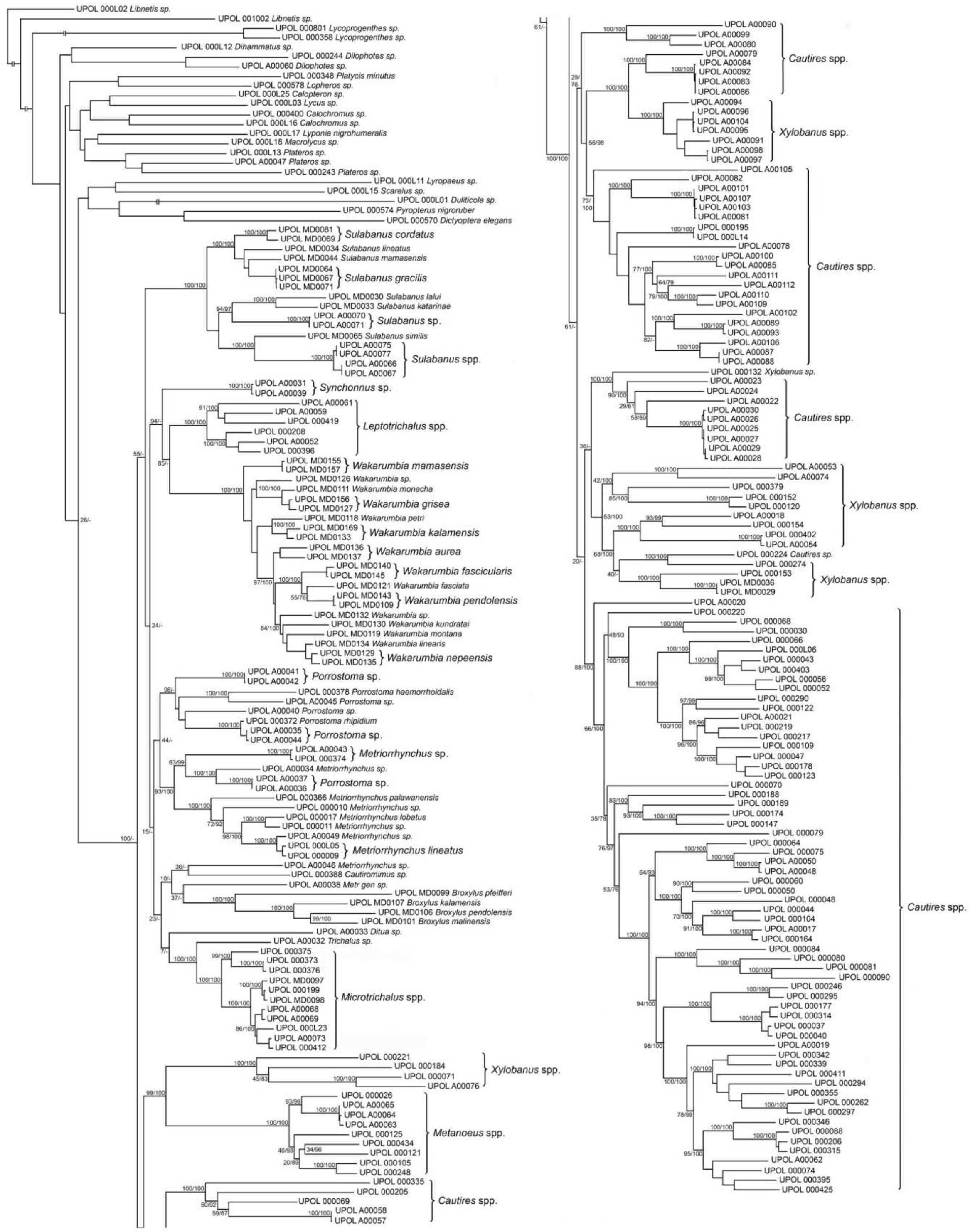
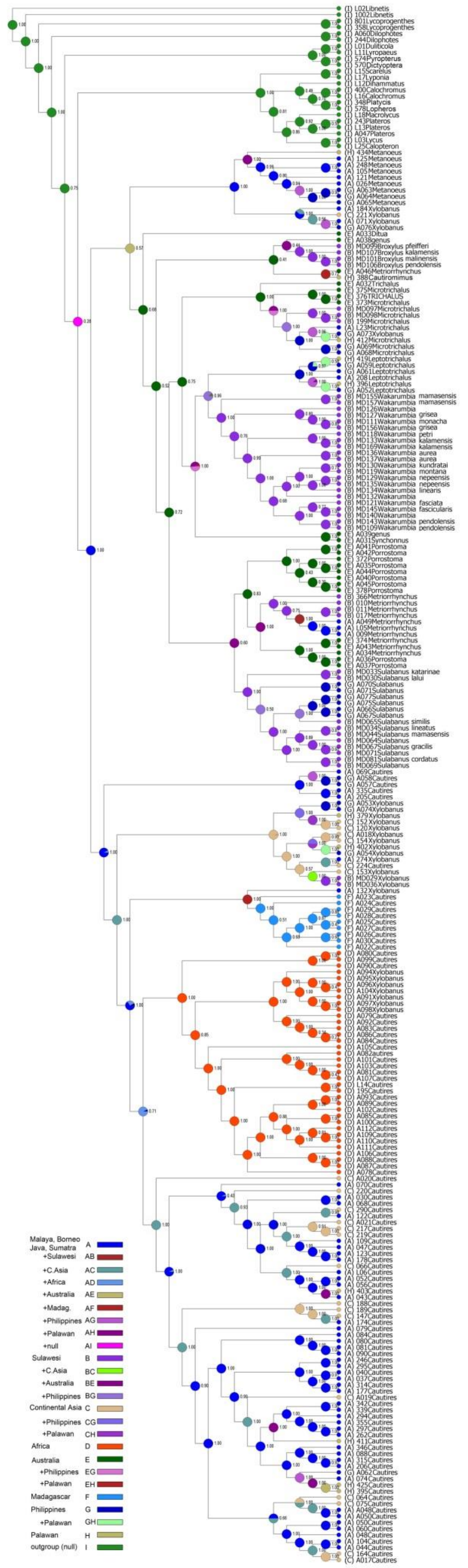
Supplementary Figure S1. Phylogenetic hypothesis on Metriorrhynchini inferred from BlastAlign and ML analysis



Supplementary Figure S2. Optimal distributions at each node inferred from the RASP analysis on the set of 1000 Bayesian trees.



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Subtribal classification of Metriorrhynchini (Insecta: Coleoptera: Lycidae): an integrative approach using molecular phylogeny and morphology of adults and larvae

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Abstract

The classification of Metriorrhynchini, the most diverse lineage of net-winged beetles (Lycidae) containing ~ 1400 species, is revised on the basis of three-marker molecular phylogeny of 175 ingroup taxa, and the adult and larval morphology. The study uses the molecular phylogeny for identification of major lineages and critically considers morphology when adult morphology and sparse information of immature stages alone did not provide enough information for building a robust classification. Reconstruction of the ancestral states of morphological characters on the phylogenetic tree recovered from DNA data presents evidence for multiple origins of the four-costae pattern on the elytra, shortened elytral costa 1, patterns of pronotal areolae and flabellate antennae. As a consequence, revised morphological delineations of the subtribes and genera are proposed: three major lineages are defined as Metriorrhynchina Kleine, 1926, Metanoeina subtrib. nov. and Cautirina subtrib. nov. The subtribes Trichalina Kleine, 1928 and Hemiconderina Bocak & Bocakova, 1990 are synonymized with Metriorrhynchina Kleine, 1926. Metanoeina are studied in detail and three genera are placed in the subtribe: *Metanoeus* Waterhouse, 1879, *Xylometanoeus* gen. nov., and *Matsudanoeus* gen. nov., with *Xylometanoeus japonicus* (Bourgeois, 1902) comb. nov. and *Matsudanoeus yuasai* (Nakane, 1969), comb. nov. as type species, respectively. *Xylobanus basivittatus* Nakane, 1970 is transferred to *Xylometanoeus*. The concepts of genera *Cautires* and *Xylobanus* are based on male and female genitalia. Additionally, the molecular hypothesis is supported by morphology of larvae, when newly proposed Cautirina are characterized by entire tergites in contrast to the longitudinally divided meso- and metathoracic tergites of Metanoeina and Metriorrhynchina. Larval characters support the placement of *Xylometanoeus* in Metanoeina and the close relationships of *Matsudanoeus* and *Metanoeus*. The simultaneous consideration of DNA-based phylogeny and morphology of adults and larvae rejects taxa based on diagnostically usable but strongly homoplastic characters and provides a framework for a robust classification of Metriorrhynchini.

Key words

Metriorrhynchina, Cautirina, Metanoeina, classification, new subtribes, new genera, new synonyms, mtDNA.

1. Introduction

Classification based on phylogenetic relationships is an ultimate goal of systematics, which needs integration of extensive data from various sources (Henning 1966; Wheeler et al. 2013). Here, we revise the subtribal classification of the net-winged beetles of the tribe Metriorrhynchini (Lycidae: Lycinae). This is based on the recently published molecular phylogeny used for an investigation of phylogeography (Sklenarova et al. 2013), the present knowledge on metriorrhynchine larvae (Bocak & Matsuda 2003; Levkanicova & Bocak 2009; Zaitsev, unpublished data) and the morphology of adults (e.g. Bocak 2002; Dudkova & Bocak 2010). Neither larval nor adult morphological data have produced a robust phylogeny alone. The previous studies recovered conflicts in the phylogenetic signal provided by adult morphology and additionally, they were limited by scarcity of information on larvae. The molecular data produced a robust phylogenetic hypothesis, which we compare with morphology to test the traditionally held morphological concepts of the subtribes and genera.

The Metriorrhynchini are an Old World lineage of net-winged beetles with ~1400 valid species-group names (Bocak 2002). The recent studies have confirmed their extraordinary diversity in Southeast Asia, Wallacea, and New Guinea (e.g. Bocak 2000, 2007; Dvorak & Bocak 2009; Weiszenstein & Bocak 2011); 223 species occur in the Afrotropical region (Kazantsev 2012); and 203 species in continental Australia (Calder 1998). Metriorrhynchinae/-ini were given subfamily or tribe rank (Kleine 1926, 1933), and later were merged with Trichalini and Cladophorini in a wider concept of Metriorrhynchinae (Bocak & Bocakova 1990). Recently, this Metriorrhynchinae were downranked to Metriorrhynchini, combined with several other lineages, e.g. Platerodini, Calochromini and Erotini, in the redefined Lycinae (Bocak & Bocakova 2008). The latest concept of Metriorrhynchini recognized subtribes Hemiconderina, Trichalina and Metriorrhynchina. The generic classification was morphology-based and used 72 characters in the mouthparts, thorax and genitalia of both sexes along with the pronotal and elytral structures and shape of antennae (Bocak 2002). Despite the relatively high number of characters and dense sampling, the support for deep relationships in Metriorrhynchini was low. Therefore, we intend to compare the phylogenetic hypothesis inferred from the three-gene dataset (the mtDNA fragments from the dataset by Sklenarova et al. 2013 expanded by critical taxa) with morphological data (Bocak 2002; Bocak & Matsuda 2003; Levkanicova & Bocak 2009) to update the classification of the lineage and to investigate in detail the limits of several species-rich taxa. We demonstrate the power of a densely sampled molecular dataset to produce a topology which corresponds with some morphological traits and strongly rejects the previous approach using strongly homoplastic morphological structures.

2. Material and methods

2.1. Sampling

The dataset of *rrnL* + *tRNA-Leu* + *nad1*, *cox1* + *tRNA-Leu* + *cox2* and *nad5* + *tRNA-Phe* + *tRNA-Glu* + *tRNA-Ser* mitochondrial DNA (further referred as *rrnL*, *cox1* and *nad5*) used for the analysis of Metriorrhynchini by Sklenarova et al. (2013) was expanded by newly produced sequences for *Xylometanoeus* gen. nov. and *Matsudanoeus* gen. nov. (Table 1) and by previously obtained sequences from four unidentified larvae (Levkanicova & Bocak 2009). The list of previously published sequences, geographical origins and accession numbers are given in Table ES1 (Electronic Supplement).

Altogether, 17 genera and about 175 species of Metriorrhynchini were represented by 227 terminals. The number of species is estimated for systematic subsets of the sample where assignment of species names is impossible due to the lack of revisionary taxonomic work (mainly concerning genera *Microtrichalus*, *Leptotrichalus*, *Xylobanus* and *Cautires*). In these parts we consider a clade as a species when terminals show low genetic distance and are morphologically different in some character from their closest relatives. Many other taxa included in the analysis were also identified only to the generic level due to poorly known species level taxonomy. The terminals originate from the whole geographic range of the tribe, but the Oriental region, in particular regarding the genera *Metanoeus* Waterhouse, 1879, *Cautires* Waterhouse, 1879, and *Xylobanus* Waterhouse, 1879 was more densely sampled. In addition, 23 species-level taxa (from 9 genera) representing all lycid subfamilies except Dexorinae and all major tribes of Lycinae were sampled as outgroup taxa. The collections of the Natural History Museums in London, Warsaw and Paris were used for the study of the type material and distributions.

2.2. Laboratory methods

The procedures for DNA extraction, PCR amplification, and sequencing were described in detail by Sklenarova et al. (2013). The morphological part of the study is based on adult and larval semaphoronts. Adult bodies were softened in water, the male genitalia dissected, examined and deposited in glycerol, the female genitalia were treated with hot 10% KOH, dissected and subsequently stained in chlorazol black. Larvae were kept in 70% alcohol and observed without any prior treatment. Illustrations were taken by a digital camera attached to a binocular microscope.

2.3. Phylogenetic analysis

The dataset, without the sequences newly added herein but including 18S and 28S rRNA fragments, was thoroughly analyzed by Sklenarova et al. (2013) and therefore the present analysis is limited to a single alignment procedure (Mafft 7.0; Katoh & Standley 2013) and the maximum likelihood analysis as implemented in RAxML

Table 1. List of newly produced sequences.

Species	**Voucher number**	**Genbank accesion numbers**		
		rrnL	***cox1***	***nad5***
Xylometanoeus basivittatus	UPOL VK0075	KF652135	KF652115	KF652123
	UPOL LB0222	—	—	KF652128
	UPOL LB0223	—	—	KF652129
Xylometanoeus japonicus	UPOL VK0038	—	KF652116	KF652124
	UPOL VK0039	—	KF652117	KF652125
	UPOL VK0090	—	KF652118	KF652126
	UPOL VK0093	—	KF652119	KF652127
Matsudanoeus yuasai	UPOL VK0248	KF652136	—	KF652130
	UPOL VK0249	KF652137	KF652120	KF652131
	UPOL VK0395	—	—	KF652132

7.2.5. (Belshaw & Katzourakis 2005). The methods of the analysis follow those reported in Sklenarova et al. (2013).

2.4. Evolutionary analysis of morphological characters

The ancestral states and the further evolution of selected morphological characters in Metriorrhynchini were reconstructed using the parsimony criterion and the current molecular phylogeny. Four characters were analyzed:

(A) the shape of male antennae (assessed based on antennomere 6): (1) serrate, (2) flabellate (Figs. 11–14). The serrate antennae are similar in shape to the female antennae illustrated in Fig. 15. We consider the antenna as serrate if the apical process is at most 0.5 × the length of the corresponding stem of the antennomere; with a higher value the antenna is scored as flabellate. Although arbitrary, the value 0.5 × lies between commonly encountered types of antenna and values close to it occur in low frequency. Although antennae with greatly lengthened apical processes are commonly designated as pectinate, we do not discriminate between flabellate and pectinate antennae due to continuous variability in the length of processes. The character states are coded as seen on the specimens, e.g. the type-species of *Metriorrhynchus* has flabellate antennae similar to those of *M. doleschali* (Fig. 13), but *Metriorrhynchus* species included in the analysis have serrate male antennae (Fig. 2).

(B) the number of longitudinal elytral costae (assessed for the humeral part of the elytron): (1) four costae, all similar in strength (Figs. 48–49); (2) nine costae, four strong ones, and five weaker ones located between the former and the elytral margins (Figs. 6–9, 47). The strong costae present in both character states are further called primary costae, the weaker ones only present in state 2 are called secondary costae.

(C) the posterior extension of the elytral primary costa 1 (the one closest to the elytral suture) from the elytral base: (1) reaching the apex of the elytron; (2) reaching at most one third of the elytral length.

(D) the pattern of pronotal areolae: the pronotal carinae delimit a maximum of seven areolae; four areolae are present at the anterior margin of the pronotum and they are separated from each other by a midline carina and a pair of fronto-lateral carinae (FLC); a single lanceolate areola is located along midline in the posterior part of the pronotum, bordered laterally by the postero-lateral areolae; the postero-lateral areolae are separated from the fronto-lateral areolae by the postero-lateral carinae (PLC, Figs. 33, 41, 45–46). We distinguish four character states: (1) the complete pattern of seven areolae is present; (2) five areolae are present due to absence of the postero-lateral carinae; (3) three areolae are present due to absence of the lateral carinae (both FLC and PLC) (i.e. slender areola in the middle part of the pronotum and two lateral areolae are present; Figs. 36–38, 42–43); (4) five areolae are present due to absence of the fronto-lateral carinae (FLC) (Fig. 39; *Wakarumbia* type). (5) No areolae are present due to the absence of all carinae, or of all but the frontal part of midline carina. The categorization can be ambiguous as some carinae can be considerably weakened, but they are still present (Fig. 35); or all carinae are inconspicuous, but the pattern of seven areolae is recognizable (*Metanoeus*, Fig. 40). Nevertheless, most cases can be clearly categorized. The *Wakarumbia* type (state 4) is characterized additionally by sharp and straight carinae and differs from superficially similar arrangements found in some outgroup taxa, e.g. some Dictyopterinae or Lycinae: Conderini and Slipinskiini (Bocak & Bocakova 2008).

The above listed characters have been traditionally used in the taxonomy of Lycidae for delineation of genera and tribes, and therefore they were coded for all taxa included in the molecular analysis (Tables 1, ES1; including outgroup taxa), their ancestral states for Metriorrhynchini were reconstructed using the parsimony approach implemented in the Mesquite 2.7.5 software (Maddison & Maddison 2011) and evaluated using the consistency index counted in PAUP 4.8 (Swofford 2002). Additionally, we used the Bayesian estimation as implemented in the BayesTraits 2.0 software for an alternative ancestral state reconstruction of the patterns of pronotal areolae (Fig. ES1). Nodes to be reconstructed were defined on the best ML topology (Fig. 1) and the same distribution of character states at terminals was used as in the MP analysis. The likelihood of each character state was inferred for nodes of interest using the script published by Sklenarova et al. (2013; freely available at https://sourceforge.net/projects/bayestraits-wrap/).

3. Results

3.1. Sequence variation

The aligned and concatenated *rrnL*, *cox1*, and *nad5* mtDNA fragments for 262 terminals formed an alignment of 3143 homologous positions; 1886/1814 characters were parsimony informative including/excluding outgroup taxa. The aligned *rrnL* fragment had 831 positions (407 parsimony informative characters), *cox1* 1099 positions (623 informative), and *nad5* 1213 positions (856 informative).

3.2. Phylogeny and character evolution

The Metriorrhynchini clade was represented in the current analysis as a monophylum with robust support (bootstrap value = BS 99%). The basal splits consisted of the *Metanoeus* clade (*Xylometanoeus* + (*Matsudanoeus* + *Metanoeus*); orange in Fig. 1), *Cautires* clade (*Xylobanus* + *Cautires*; red in Fig. 1) and *Metriorrhynchus* clade (*Metriorrhynchus*, *Trichalus*, *Wakarumbia* and many other genera; green in Fig. 1). The *Cautires* clade and the *Metanoeus* clade were well supported (BS 98 and 91%, respectively), but the *Metriorrhynchus* clade, although recovered by all analyses here and previously (Sklenarova et al. 2013), had low support (BS 17–68%) and formed an unresolved polytomy with the *Metanoeus* clade in the majority consensus recovered from bootstrap trees.

The evolution of the four characters (A)–(D) (see section 2.4.), which have been commonly used for delineation of genera, was optimized under the MP criterion using the tree in Fig. 1. Altogether 7 steps were needed to explain the distribution of flabellate and serrate antennae (CI 0.167, RI 0.944; Fig. 2); 19 steps for the patterns of pronotal areolae (a five-state character, CI 0.158, RI 0.835; Fig. 5); 7 steps for the presence/absence of the secondary costae on the elytra (CI 0.143, RI 0.920; Fig. 3); and 2 steps for the extension of elytral costa 1 (CI 0.5, RI 0.944; Fig. 4). The ancestral character states recovered by parsimony reconstruction in Mesquite are illustrated in Figs. 2–5 and the calculated likelihoods for origins of pronotal patterns are graphically presented in the supplementary Fig. ES1.

Sequences from four metriorrhynchine larvae published by Levkanicova & Bocak (2009) were included in the dataset and these taxa were identified as *Leptotrichalus atricollis* Pic, 1921 (Voucher UPOL ZL2002) and *Sulabanus lalui* Dvorak & Bocak, 2007 (UPOL ZL2010); the sample UPOL ZL2015 was identified as *Cautires* sp. (Metriorrhynchini gen. spec. by Levkanicova & Bocak 2009) and relationships were inferred more precisely for another sample of *Cautires* sp. (UPOL ZL2009) with the current DNA dataset (Fig. 1).

4. Discussion

The older morphology-based classification of metriorrhynchine net-winged beetles recognized several family-group taxa of subfamily or tribe ranks (Kleine 1926, 1933). The comparison of morphology (Bocak & Bocakova 1990) and subsequent morphology-based cladistic analysis by Bocak (2002) suggested monophyly of Metriorrhynchini consisting of Hemiconderina, Trichalina, and Metriorrhynchina, the latter containing also the Oriental and Afrotropical genera *Cautires* and *Xylobanus*.

The current molecular phylogeny (Fig. 1) is incongruent with morphological analyses, with three lineages inferred at the basal split; these are here defined as subtribes of Metriorrhynchini (see Taxonomy section):

(1) The *Cautires* + *Xylobanus* clade (subtribe Cautirina) includes all Afrotropical and most Oriental and East Palearctic Metriorrhynchini.

(2) The *Sulabanus* + *Metriorrhynchus* + *Trichalus* + *Wakarumbia* clade (including all related genera as shown in Fig. 1; subtribe Metriorrhynchina) includes Australian lineages, many of them endemic to Australia and New Guinea, but some dispersed in a low number of species to the Oriental region (e.g. Bocak & Yagi 2010).

(3) The *Xylometanoeus* + *Matsudanoeus* + *Metanoeus* clade (subtribe Metanoeina) includes a limited number of Oriental Metriorrhynchini. The enigmatic position of *Metanoeus* (the only genus of this clade defined prior to the present paper) was discussed by Bocak (2002) when the morphological characters did not provide a clear indication of its relationships to *Metriorrhynchus* or *Cautires*, and even the current molecular dataset does not give a robust support for a sister to the *Metanoeus* + *Xylometanoeus* + *Matsudanoeus* clade. Surprisingly, the Japanese species *Xylobanus basivittatus* and *X. japonicus* and several unidentified *Xylobanus* spp. (their classification in *Xylometanoeus* is discussed in the taxonomical part below) are sister to the clade of *Matsudanoeus yuasai* + *Metanoeus* spp. (Fig. 1). The position of these species compromises monophyly of *Cautires* and *Xylobanus* in the traditional sense (Kleine 1933; Bocak 2002; Kazantsev 2012). Therefore, we defined two new genera in Metanoeina for these species (*Matsudanoeus* and *Xylometanoeus*), studied their morphology in detail and compared it with the morphology of *Cautires* and *Xylobanus* (see Taxonomy section for details).

The subtribe Metriorrhynchina, when Cautirina and Metanoeina are defined and excluded from the subtribe, contains several Australian genera (e.g. *Metriorrhynchus*, *Porrostoma*, *Sulabanus*, Fig. 1). These three genera form a clade with *Trichalus* + *Microtrichalus* (former subtribe Trichalina), *Synchonnus* + *Wakarumbia* (former Hemiconderina) and *Leptotrichalus* (classified in Metriorrhynchina by Bocak 2002) The monophyly of these clades is weakly supported or compromised by presence of *Leptotrichalus* in the clade of *Synchonnus* + *Wakarumbia* (Hemiconderina). Therefore, Metriorrhynchina are redefined and include the genera previously classified in Trichalina and Hemiconderina.

We found that the character states used previously for definition of genera (characters (A)–(D) in section 2.4.) are either plesiomorphies, or apomorphies that evolved several times in distantly related lineages. *Xylobanus* and *Cautires* have been defined by having four and nine elytral costae, respectively (Figs. 3, 47–49). The origin of the nine-costae pattern in the cautirine ancestor was followed either by three independent origins of the four-costae pattern or the four-costae pattern was present at the root of Cautirina and was followed by a single origin of nine-costae pattern and two reversals (Fig. 3). The *Metanoeus* clade contains two branches: *Xylometanoeus* with the four-costae pattern and the *Matsudanoeus* + *Metanoeus* branch with the nine-costae pattern (Fig. 2).

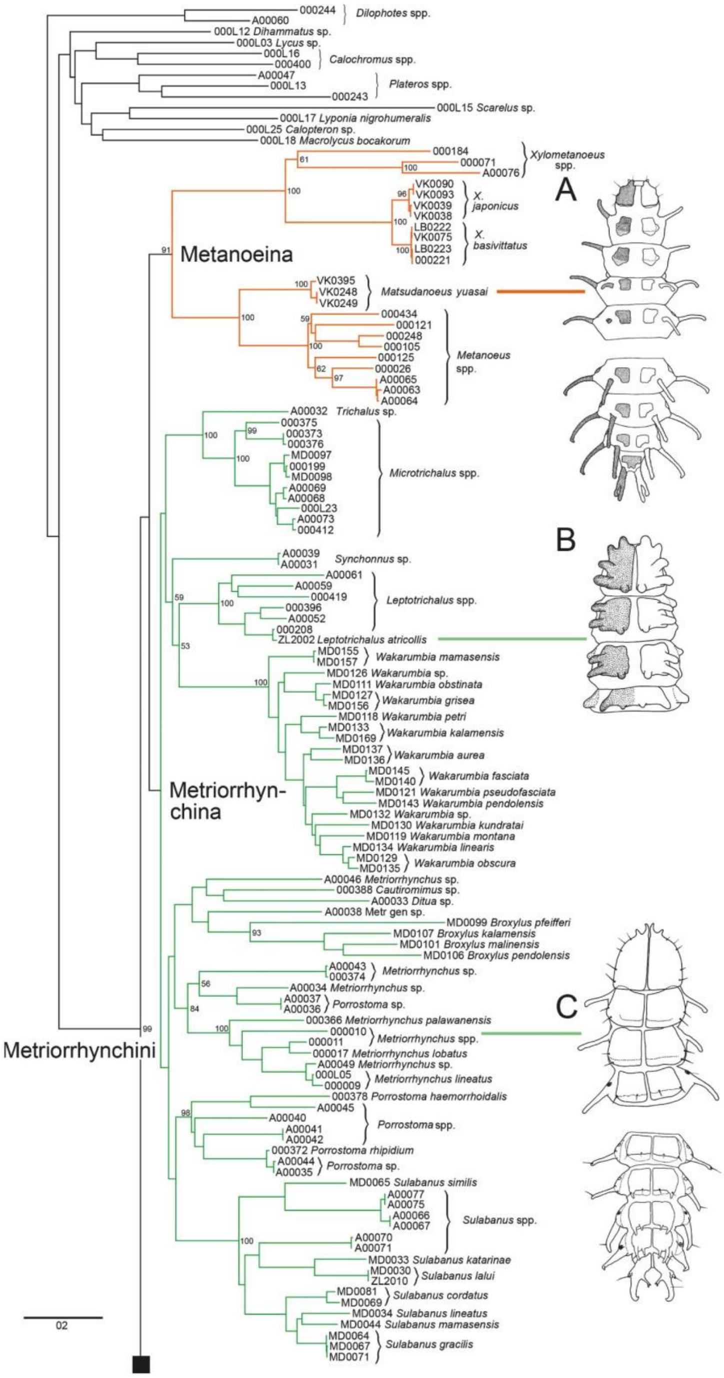
Similarly, the distribution of flabellate antennae suggests multiple origins and our optimization was ambiguous regarding the root of Metriorrhynchini. Therefore, the character should be used with caution in delineation of monophyletic lineages (Fig. 2). We found that *Xylobanus* splits in two sister-clades with flabellate versus serrate antennae; similarly some Australian *Metriorrhynchus*, including the type species, have flabellate antennae but the other, e.g. all species in the Oriental region and Sulawesi, have serrate antennae (Fig. 2). Although the definition of character states "serrate" and "flabellate" by an arbitrary ratio (see Methods) suggests that the changes might be gradual, very few individuals have antennae with a shape of antennomeres close to the given value. We suppose that the flabellate antennae play a role in pheromone communication and therefore, the distribution peaks in serrate and flabellate antennomeres might correspond to the presence/absence of selection for large surface of antennae housing olfactory receptors.

The number of pronotal carinae has been commonly used in systematics of net-winged beetles and the taxa defined based on this character have been accepted since the end of 19th century till recently (e.g. *Bulenides* Waterhouse, 1879, see Dudkova & Bocak 2010). Additionally, new taxa have been based on the number of pronotal areolae recently (e.g. Kazantsev 2012). High likelihood was calculated for the seven-areolae pattern (i.e. for the maximum set of carinae) at the root of Metriorrhynchini (Fig. ES1). Among Lycidae, the seven-areolae pattern only occurs in Metriorrhynchini and it is an autapomorphy of this tribe (while all carinae forming this pattern can also occur in other Lycidae, but not the full set). Subsequently, the various types of reduction of pronotal carinae are recovered in unrelated lineages, and some of them resemble patterns known in other net-winged beetle lineages (Figs. 5, ES1; Dudkova & Bocak 2010). The pattern of absent fronto-lateral carinae (*Wakarumbia* type, state 4; Fig. 39) is similar to those of Dictyopterinae or Conderini and Slipinskiini among Lycinae (Bocak et al. 2008). This pattern of areolae was coded as a single character state also in Conderini (i.e. homology assumption at primary level) in the morphological analysis by Bocak (2002) and as a result Hemiconderini (*Falsolucidota*, *Wakarumbia* and related genera) were recovered as the sister group of all other Metriorrhynchini.

Although with sparse sampling at this moment, we tested the proposal by Bocak (2002) to exclude *Leptotrichalus* from Trichalina and we found that *Leptotrichalus* is closely related to the *Synchonnus* + *Wakarumbia* clade and does not belong to the *Trichalus* + *Microtrichalus* clade (Fig. 1) despite possessing a similarly shortened elytral costa 1 (Fig. 4). Trichalina sensu Bocak (2002) is thus obtained herein as a monophyletic subclade of Metriorrhynchina.

The current analysis provides putative identification for the samples of unknown larvae of Metriorrhynchini reported by Levkanicova & Bocak (2009) and shows the importance of the multi-marker reference database for placement of unidentified samples in the phylogenetic framework as discussed by Bocak et al. (2014). The current sampling enables more precise identification of all four previously unidentified taxa (BS 100%, Fig. 1), although formal identification is unavailable due to the chaotic species-level taxonomy of the group in two cases.

Further, we compared the morphology of larvae of *Matsudanoeus yuasai*, *Metanoeus pendleburyi* (Bocak & Matsuda 2003) and *Xylometanoeus japonicus* (A.A. Zaitsev, unpublished data; Fig. 18) with other Metriorrhynchini. When only morphology was considered, the similarity of larvae of these taxa was noted, but no conclusion on relationships was made (Bocak & Matsuda 2003). The DNA-based association of the Metanoeina (*Metanoeus* + *Xylometanoeus* + *Matsudanoeus* clade) and Metriorrhynchina (Fig. 1) suggests that a midline division of terga is a synapomorphy of these two clades. Similarly, the presence of branched urogomphi supports the relationships of *Matsudanoeus* + *Metanoeus*. Spines at the frontal margin of pronotal hemitergites are ob-


000244
A00060
Dilophotes spp.
000L12 Dihammatus sp.
000L03 Lycus sp.
000L16
000400
Calochromus spp.
A00047
000L13
000243
Plateros spp.
000L15 Scarelus sp.
000L17 Lyponia nigrohumeralis
000L25 Calopteron sp.
000L18 Macrolycus bocakorum
000184
61
000071
100
A00076
Xylometanoeus spp.
100
VK0090
96
VK0093
VK0039
VK0038
X. japonicus
100
LB0222
VK0075
100
LB0223
000221
X. basivittatus
A
91
Metanoeina
100
VK0395
VK0248
VK0249
Matsudanoeus yuasai
100
59
000434
000121
000248
100
000105
000125
62
000026
97
A00065
A00063
A00064
Metanoeus spp.
A00032 Trichalus sp.
100
000375
99
000373
000376
100
MD0097
000199
MD0098
A00069
A00068
000L23
A00073
000412
Microtrichalus spp.
B
A00039
A00031
Synchonnus sp.
A00061
A00059
000419
59
100
000396
A00052
000208
ZL2002 Leptotrichalus atricollis
Leptotrichalus spp.
53
MD0155
MD0157
Wakarumbia mamasensis
100
MD0126 Wakarumbia sp.
MD0111 Wakarumbia obstinata
MD0127
MD0156
Wakarumbia grisea
MD0118 Wakarumbia petri
MD0133
MD0169
Wakarumbia kalamensis
MD0137
MD0136
Wakarumbia aurea
MD0145
MD0140
Wakarumbia fasciata
MD0121 Wakarumbia pseudofasciata
MD0143 Wakarumbia pendolensis
MD0132 Wakarumbia sp.
MD0130 Wakarumbia kundratai
MD0119 Wakarumbia montana
MD0134 Wakarumbia linearis
MD0129
MD0135
Wakarumbia obscura
Metriorrhyn-china
A00046 Metriorrhynchus sp.
000388 Cautiromimus sp.
A00033 Ditua sp.
A00038 Metr gen sp.
MD0099 Broxylus pfeifferi
93
MD0107 Broxylus kalamensis
MD0101 Broxylus malinensis
MD0106 Broxylus pendolensis
A00043
000374
Metriorrhynchus sp.
56
A00034 Metriorrhynchus sp.
A00037
A00036
Porrostoma sp.
84
C
99
100
000366 Metriorrhynchus palawanensis
000010
000011
Metriorrhynchus spp.
000017 Metriorrhynchus lobatus
A00049 Metriorrhynchus sp.
000L05
000009
Metriorrhynchus lineatus
Metriorrhynchini
000378 Porrostoma haemorrhoidalis
98
A00045
A00040
A00041
A00042
Porrostoma spp.
000372 Porrostoma rhipidium
A00044
A00035
Porrostoma sp.
MD0065 Sulabanus similis
A00077
A00075
A00066
A00067
A00070
A00071
Sulabanus spp.
100
MD0033 Sulabanus katarinae
MD0030
ZL2010
Sulabanus lalui
MD0081
MD0069
Sulabanus cordatus
MD0034 Sulabanus lineatus
MD0044 Sulabanus mamasensis
MD0064
MD0067
MD0071
Sulabanus gracilis
02

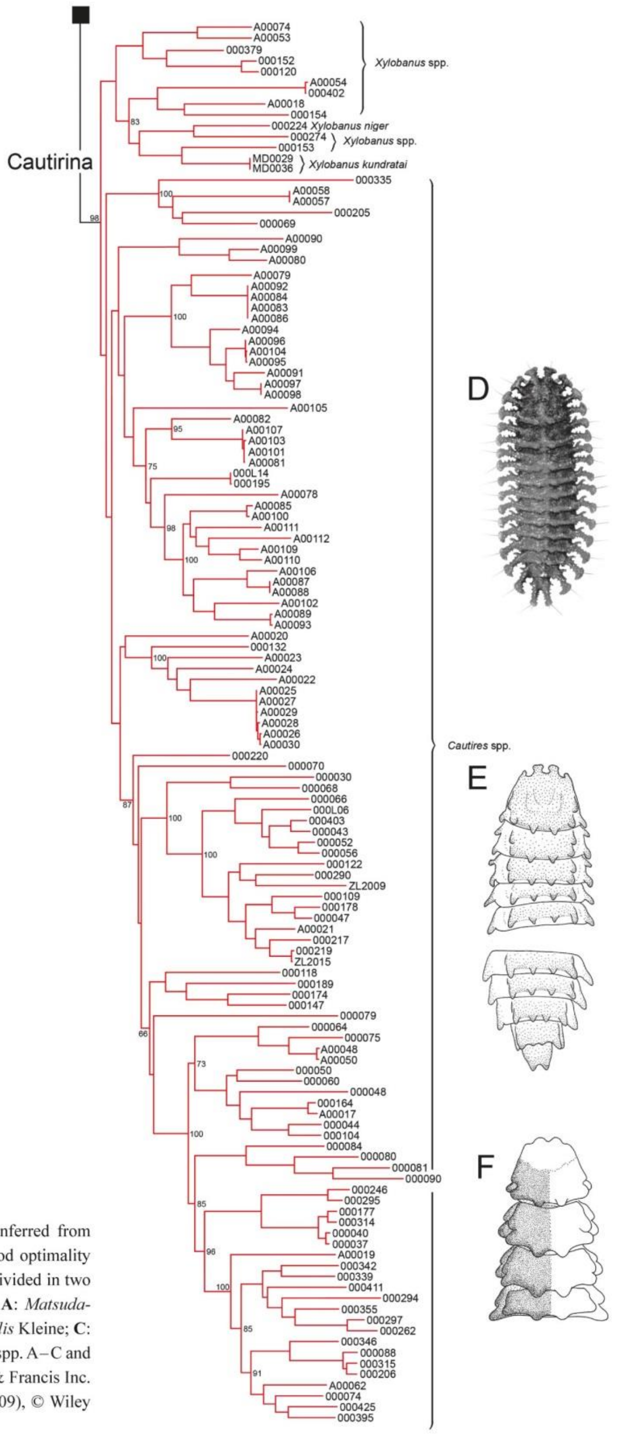
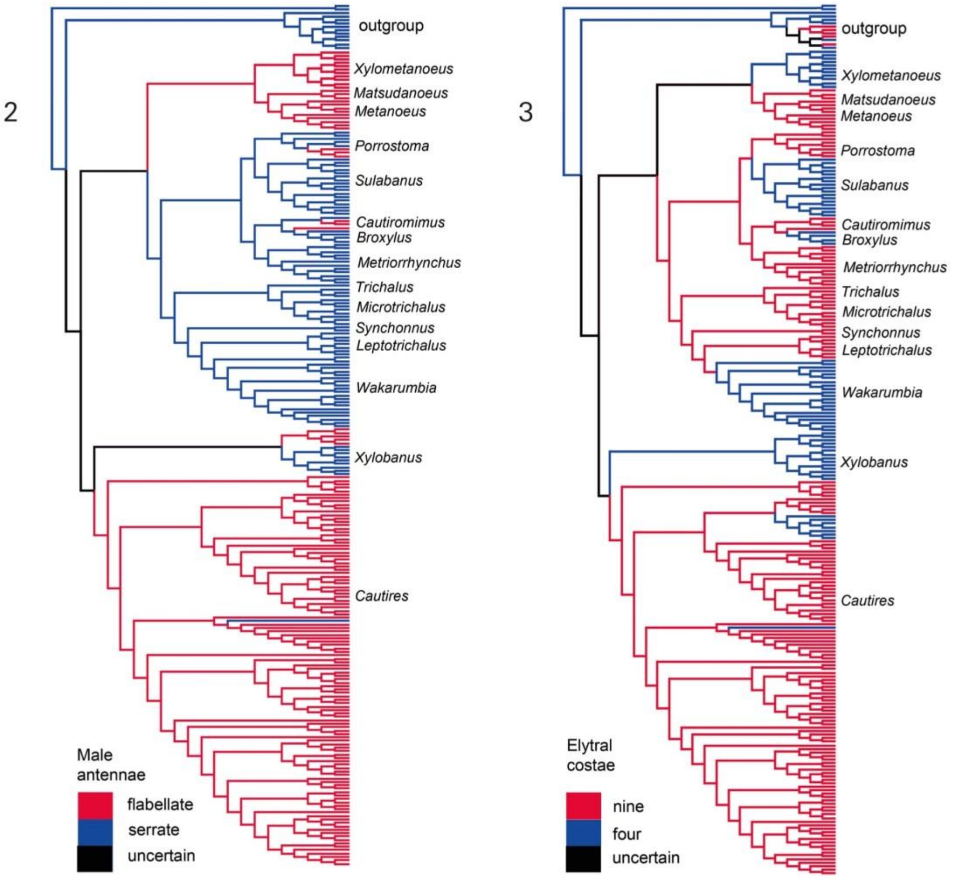


Fig. 1. Phylogenetic tree of Metriorrhynchini inferred from MAFFT alignment using the maximum likelihood optimality criterion; basal part of outgroups omitted. Tree divided in two parts at squares. Larvae, general appearance of **A**: *Matsudanoeus yuasai* (Nakane); **B**: *Leptotrichalus atricollis* Kleine; **C**: *Metriorrhynchus thoracicus* (F.); **D – F**: *Cautires* spp. A – C and E – F from Bocak & Matsuda (2002), © Taylor & Francis Inc. Basingstoke; D from Levkanicova & Bocak (2009), © Wiley & Sons, Inc. Chichester.



Figs. 2–5. Parsimony reconstruction of ancestral character states: **2**: the structure of antennae; **3**: the number of elytral costae; **4**: the length of the elytral costa 1; **5**: the structure of the pronotal areolae.

served in several *Metriorrhynchus* and *Xylometanoeus* and have not been found in any *Xylobanus* or *Cautires*. All these characters support the position of Metanoeina as sister to Metriorrhynchina. The molecular phylogeny supported by some larval and adult morphological characters provides evidence for the newly defined principal lineages.

The consequences of current findings for the taxonomy of Metriorrhynchini are discussed in the taxonomy section below. We show that integration of densely sampled molecular phylogeny with adult and larval morphology provides a strong framework for revised classification with strong explanatory and predictive power. On the other hand, when typologically defined polyphyletic taxa are rejected, and the revised generic and subtribal concepts are defined based on morphology of genitalia or larvae, eventually a combination of several characters must be used for definition of taxa.

5. Taxonomy

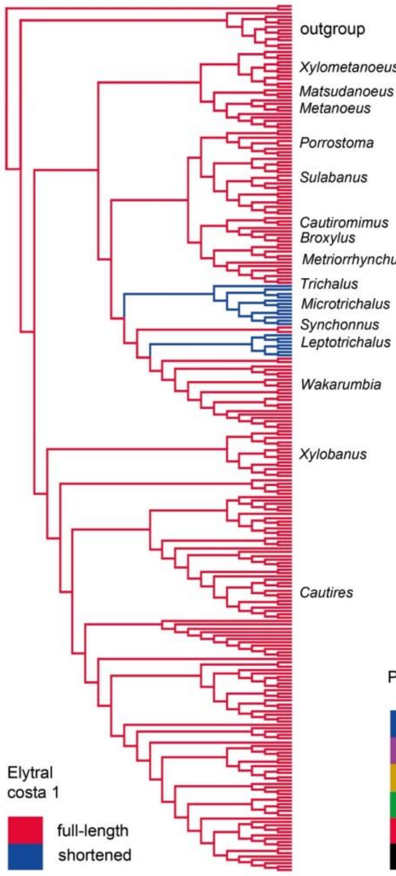
5.1. Tribe Metriorrhynchini Kleine, 1926

Metriorrhynchini Kleine, 1926: 97.

Type genus. *Metriorrhynchus* Gemminger & Harold, 1869.

= Cladophorini Kleine, 1928: 222; Bocak & Bocakova 1990: 641.
Type genus. *Cladophorus* Guérin-Méneville, 1930, pl. 2, fig. 9.
= Dilolycinae Kleine, 1926: 186; Bocak & Bocakova 1990: 641.
= Dilolycini: Kleine, 1933: 84.
Type genus. *Dilolycus* Kleine, 1926: 186.

4



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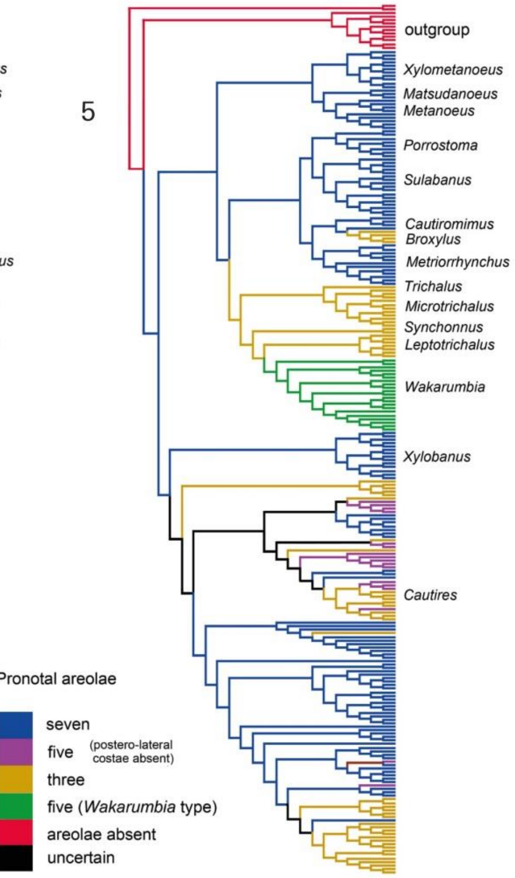
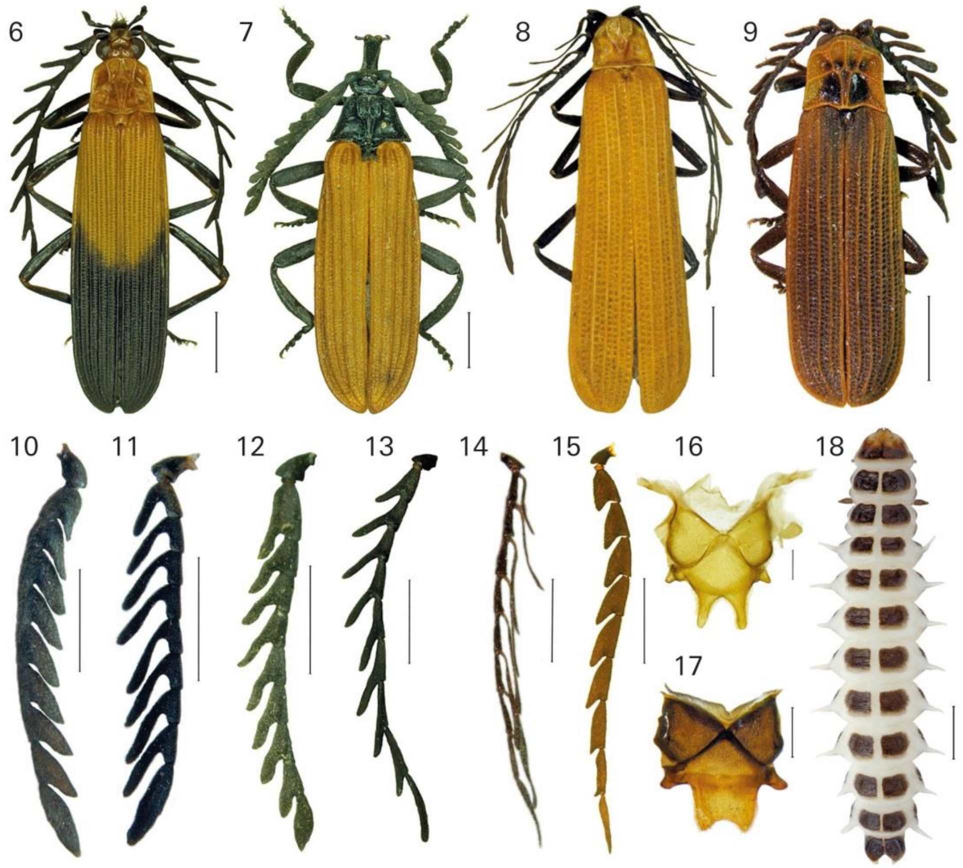


Description. ***Adults***: Body length 2.5–30 mm. Body weakly sclerotized, dorso-ventrally flattened (Figs. 6–9); most species aposematically colored.

Head small (Figs. 6–9), prognathous to hypognathous, partly covered by pronotum; most species without rostrum, flower visiting species rostrate (e.g. *Porrostoma*, *Leptotrichalus*, Figs. 7, 19–21); mouthparts well developed (Figs. 22–32), tiny if head rostrate. Labrum transverse, anterior margin rounded to slightly emarginate (Fig. 26). Mandibles long, slender to robust, shortened in species with rostrum, incisor without teeth (Figs. 23–25). Maxilla with small cardo, stipes plate-like, mala setose. Maxillary palpi 4-segmented. Labium small, without ligula, praementum large, mentum tiny, transverse; labial palpi 3-segmented (Figs. 27–32). Eyes hemispherically prominent. Antennal insertions narrowly separated, antennae 11-segmented, scapus stout, pedicel small, transverse; antennomeres 3–10 serrate to flabellate in males, serrate to shortly flabellate in females (Figs. 10–15).

Pronotum slightly narrower than elytra, flat, with pronotal carinae (Figs. 33–46). Anterior margin convex, anterior angles obtuse, posterior angles projecting. Prosternum transverse (Fig. 44). Mesoscutellum parallel-sided, weakly to deeply emarginate at apex (Figs. 16–17). Metendosternite with simple, robust stalk; arms absent. Elytra subparallel-sided, seldom globular (*Broxylus*); always with longitudinal and regular transverse costae (Figs. 6–9); longitudinal ones in two patterns: (1) four stronger (primary costae) and five weaker ones (secondary costae) (Figs. 6–9, 47, e.g. *Cautires*, *Porrostoma*, *Metriorrhynchus*); (2) only four primary costae present (e.g. *Xylobanus*, *Xylometanoeus*, Figs. 48–49), secondary costae absent; sometimes individual costae reduced in length (*Diatrichalus*, *Leptotrichalus*, etc.). Legs slender, flattened, coxae globular to slightly elongate; trochanters and femora slender; five tarsomeres, tarsomeres 2–4 often with membranous pads. Claws simple (Fig. 84).

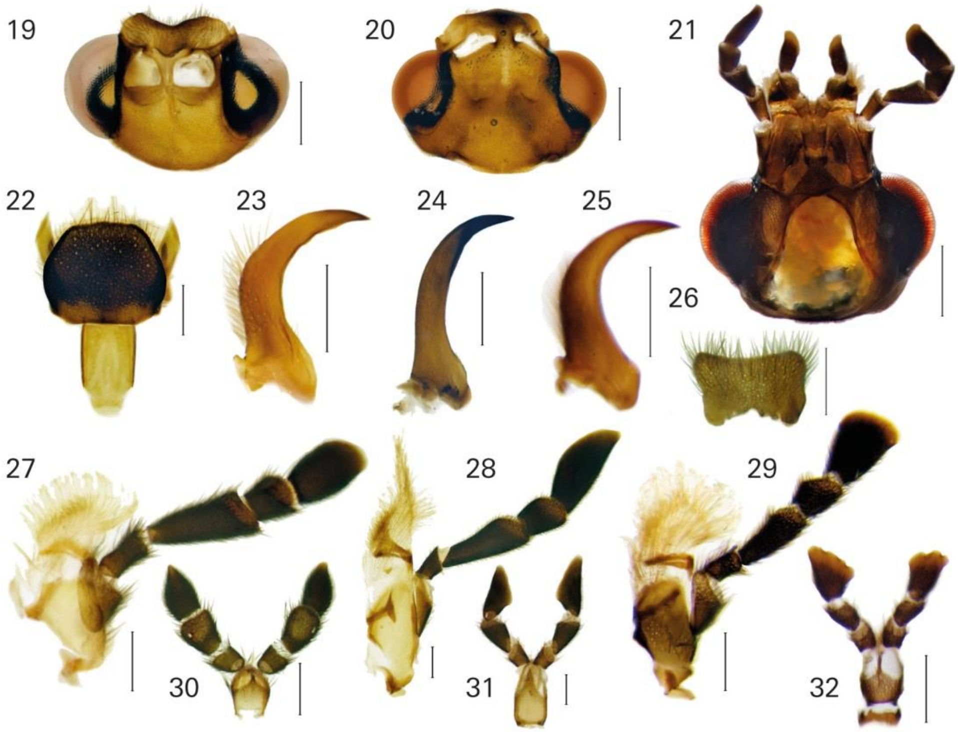


Figs. 6–18. General appearance of **6**: *Metriorrhynchus doleschali* Redtenbacher; **7**: *Porrostoma rhipidium* W. M'Leay; **8**: *Metanoeus bakeri* Kleine; **9**: *Cautires* sp. Antennae of **10**: *Cautires* sp., female; **11**: ditto, male; **12**: *P. rhipidium*, male; **13**: *M. doleschali*, male; **14**: *M. bakeri*, male; **15**: ditto, female. Mesoscutellum of **16**: *M. bakeri*; **17**: *Cautires* sp. Larva, general appearance, of **18**: *Xylometanoeus japonicus*. Scale bars: 2 mm (Figs. 6–15, 18), 0.5 mm (Figs. 16–17).

Abdomen short and much narrower than elytra in most species. Female spiculum gastrale absent. Male genitalia with tubular or partly membranous phallus, circular phallobase, parameres absent (Figs. 50–72). Internal sac regularly with thorns and lamellae (Figs. 50–51, 58–72), seldom completely membranous (*Porrostoma*, Figs. 52–53). Ovipositor with plate-like coxites, either with freely attached rod-like valvifers (Fig. 81) or valvifers reduced (*Metanoeus*, Figs. 73, 79). Styli short, movable (Figs. 73–82). Vagina simple, sac-like, membranous (Figs. 73–82), seldom sclerotized (Fig. 83), with median gland and two lateral accessory glands attached distally. Spermathecal duct short (Figs. 74–75) to very long (*Metanoeus*, Fig. 73). Spermatheca simple, apically bearing y-shaped gland (Figs. 73–83).

Larvae: Only a few genera are known in immature stages (Bocak & Matsuda 2003; Levkanicova & Bocak 2009). The larvae share reduced mala and movable or fixed tergal and pleural processes of variable length. Urogomphi variable in length and shape, long, movable and branched to short and membranous (Figs. 1A,C,D,E, 18).

Diagnosis. The Metriorrhynchini are characterized by several unique characters: Pronotum with carinae usually forming a pattern of four areolae at the anterior margin, single median lanceolate areola in middle and two postero-lateral areolae (unique pattern in Lycidae). However, frequently this set is reduced, in the extreme to a single median areola or only the anterior part of the median carina is present (*Caenioxylobanus*) (Figs. 33–46). Male genitalia with a straight phallus and a circular phallobase, parameres absent (Figs. 50–72). Female genitalia with vagina bearing an unpaired median gland (Figs. 73, 80, 83). Additionally, the Metriorrhynchini are characterized



Figs. 19–32. Head of **19**: *Metanoeus bakeri*; **20**: *Metriorrhynchus doleschali*; **21**: *Cautires* sp. **22**: Labrum and hypopharynx of *M. doleschali*. Mandible of **23**: *M. bakeri*; **24**: *M. doleschali*; **25**: *Cautires* sp. **26**: Labrum of *M. bakeri*. Maxilla of **27**: *M. bakeri*; **28**: *M. doleschali*; **29**: *Cautires* sp. Labium of **30**: *M. bakeri*; **31**: *M. doleschali*; **32**: *Cautires* sp. Scale bars: 0.5 mm (Figs. 19–21), 0.1 mm (Figs. 22–32).

by a pedicel at least four times shorter than antennomere 3 (Figs. 10–15, similar in Lycini and Calopterini) and elytra with longitudinal and transverse costae (similar in Dictyopterini and others, but absent in several lineages such as Lycini, Calochromini etc., Figs. 6–9, 47–49).

Distribution. Afrotropical region including Madagascar, southern part of Arab Peninsula; Oriental region, Australian region including islands of western Pacific (but absent in New Zealand except one introduced species), eastern part of Palearctic region. The dispersal and vicariance history was discussed by Sklenarova et al. (2013).

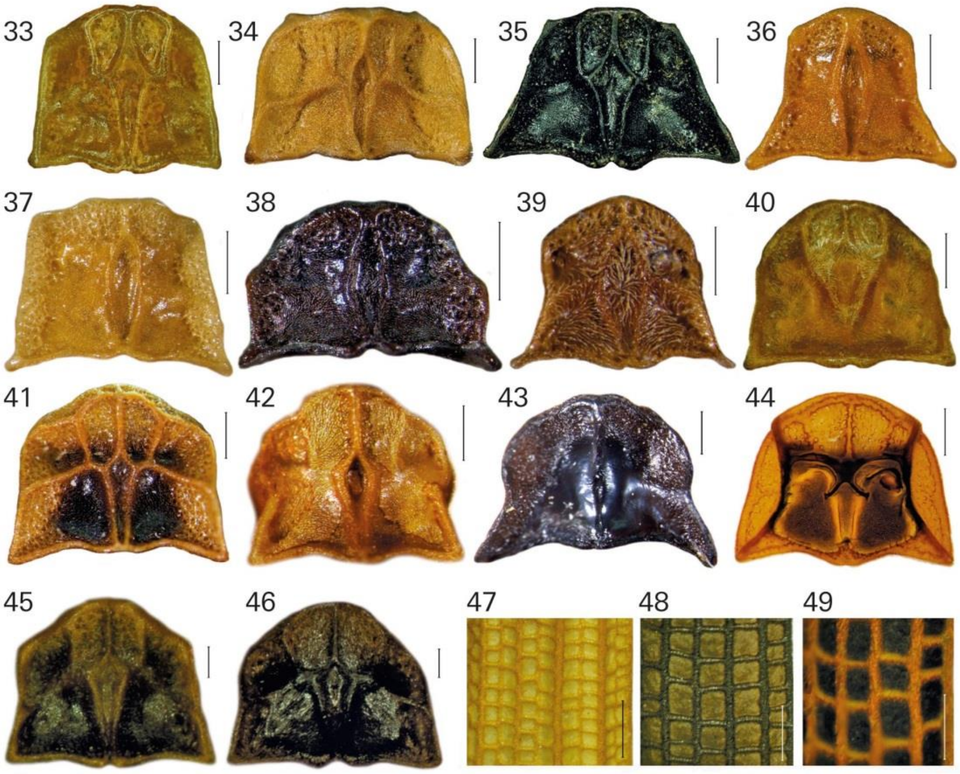
5.2. Subtribe Metriorrhynchina Kleine, 1926

Metriorrhynchini Kleine, 1926: 97.

Type genus. *Metriorrhynchus* Gemminger & Harold, 1869.

= Hemiconderina Bocak & Bocakova, 1990: 645 – **syn. nov.**
Type genus. *Hemiconderis* Kleine, 1926: 162.
= Trichalinae Kleine, 1928: 222 – **syn. nov.**
= Trichalini: Kleine, 1933: 69.
= Trichalina: Bocak & Bocakova, 1990: 646.
Type genus. *Trichalus* Waterhouse, 1877: 82.

Description. ***Adults***: Body length 3–30 mm, most species brightly colored, few uniformly black or metallic blue (*Diatrichalus*, Figs. 6–7, 38). Head with rostrum (*Porrostoma*) or without rostrum (Figs. 6–7, 20), antennae flabellate or serrate in males (Figs. 12–13), sometimes pectinate with lamellae extremely long (*Carathrix*), serrate in females. Mandibles moderately long (Fig. 24), very short when rostrum present; palpomeres variable in shape (Figs. 28, 31). Pronotum usually with seven areolae, sometimes lateral carinae weaker to absent (Figs. 33–39). Mesoscutellum parallel-sided, deeply emarginate at apex. Elytra parallel-sided, seldom globular (*Broxylus*), with four or nine longitudinal costae (Figs. 6–7, 47). Male genitalia with tubular, sometimes apically membranous phallus, internal sac armed with thorns (*Metriorrhynchus*) or membranous (e.g., *Porrostoma*,



Figs. 33–49. Pronotum of **33**: *Metriorrhynchus* sp.; **34**: *Cladophorus* sp.; **35**: *Porrostoma rhipidium*; **36**: *Leptotrichalus* sp.; **37**: *Microtrichalus* sp.; **38**: *Diatrichalus* sp.; **39**: *Wakarumbia* sp.; **40**: *Metanoeus* sp.; **41–44**: *Cautires* spp.; **45**: *Matsudanoeus yuasai*; **46**: *Xylometanoeus japonicus*. Structure of elytral costae of **47**: *Cautires* sp. (Cameroon); **48**: *Xylometanoeus basivittatus*; **49**: *Xylometanoeus* sp. (Borneo). Scale bars: 0.5 mm.

Trichalus, Figs. 50–53). Vagina membranous to heavily sclerotized (some Papuan *Metriorrhynchus*), lateral accessory glands attached directly or via partly sclerotized ducts; spermaduct short to moderately long (Fig. 83).

***Larvae**:* Several genera of Metriorrhynchina are known in immature stages: *Porrostoma* spp. from Australia, *Metriorrhynchus* spp. (the Philippines and Great Sundas), *Leptotrichalus* (Java) and *Sulabanus* (Sulawesi). All known larvae share the longitudinally divided meso- and metathoracic terga and many have the spines at the frontal margin of the pronotum. Urogomphi variable in shape, movable or fixed, seldom absent (e.g. *Metriorrhynchus*; Bocak & Matsuda 2003; Levkanicova & Bocak 2009).

Diagnosis. The Metriorrhynchina consist of morphologically diverse genera, which are difficult to collectively define by a unique feature. Most taxa have seven distinct pronotal areolae. Although these are similar to those of Cautirina, many genera have a slightly different shape of the pronotum and areolae: commonly posterior angles of pronotum are rectangular (Figs. 33–34) or lateral areolae are shallow (Fig. 33). Several genera have areolae modified in a way unknown from other subtribes, e.g. *Leptotrichalus*, *Trichalus*, *Synchonnus* and related genera have a long median areola and the lateral carinae are absent (Figs. 36–38), sometimes patterns of areolae resemble those of Conderini or Dictyopterinae (*Falsolucidota*, *Wakarumbia*, Fig. 39). Additionally, some groups of genera have a shortened primary elytral costa 1 (the genera previously placed in Trichalinae/ini by Kleine 1928, 1933). The male genitalia of Metriorrhynchina are very diverse in the shape of the phallus and sclerotization of the internal sac (Figs. 50–53), but they have never a slender, lanceolate phallus and their internal sac does not have a pair of sclerotized thorns as known in Cautirina (Figs. 66–70). Some Metriorrhynchina have the phallobasal membrane modified in a sclerotized structure (e.g. some *Metriorrhynchus*, Figs. 50–51). Morphologically based identification is possible only using a set of various characters and detailed comparison to other taxa.

List of Metriorrhynchina genera. *Achras* Waterhouse, 1879; *Broxylus* Waterhouse, 1878 (= *Samanga* Pic, 1921); *Cautiromimus* Pic, 1926; *Cladophorus* Guérin-Méneville, 1830 (= *Odontocerus* Guérin-Méneville, 1838; = *Spacekia* Strand, 1936); *Cladophorinus* Kleine, 1926; *Diatrichalus* Kleine, 1926 (= *Mimotrichalus* Pic, 1930); *Ditua* Waterhouse, 1879; *Eniclases* Waterhouse, 1879; *Enylus* Waterhouse, 1879; *Falsolucidota* Pic, 1921 (= *Hemiconderis* Kleine, 1926); *Flabellotrichalus* Pic, 1921 (= *Villosotrichalus* Pic, 1921; = *Stereotrichalus* Kleine, 1926); *Kassemia* Bocak, 1998; *Leptotrichalus* Kleine, 1925; *Lobatang* Bocak, 1998; *Malacolycus* Kleine, 1943; *Mangkutanus* Kubecek, Dvorak & Bocak, 2011; *Marena* Kazantsev, 2007; *Metriorrhynchoides* Kleine, 1926; *Metriorrhynchus* Gemminger & Harold, 1869 (= *Metriorhynchus* Guérin-Méneville, 1838; = *Dilolycus* Kleine, 1926; = *Flabelloporrostoma* Pic, 1923); *Mimoxylobanus* Pic, 1921; *Microtrichalus* Pic, 1921 (= *Falsoenylus* Pic, 1926); *Oriomum* Bocak, 1999; *Porrostoma* Castelnau, 1838; *Procautires* Kleine, 1925; *Pseudodontocerus* Pic, 1921; *Schizotrichalus* Kleine, 1926; *Spinotrichalus* Kazantsev, 2010; *Stadenus* Waterhouse, 1879; *Sulabanus* Dvorak & Bocak, 2007; *Synchonnus* Waterhouse, 1879; *Trichalus* Waterhouse, 1877; *Wakarumbia* Bocak, 1999; *Xylobanomimus* Kleine, 1926; *Xylobanomorphus* Kleine, 1935.

Distribution. Australian region including islands of Western Pacific and eastern part of Oriental region (only *Metriorrhynchus* distributed from southeast Asia to Eastern India and Laos, *Microtrichalus* to southernmost Yunnan, *Leptotrichalus* to Vietnam, *Diatrichalus* recorded from the Philippines, Peninsular Malaysia and the Great Sundas, *Cautiromimus* from the Philippines including Palawan and *Sulabanus* from the Philippines, but not from Palawan). Metriorrhynchina are the only subtribe occurring east of the Wallace line except a few species of *Cautires* and *Xylobanus* on Sulawesi.

Remarks. Most morphological diversity of Metriorrhynchini is known from this subtribe and it resulted in a long list of described genera and definitions of subfamilies and tribes (Kleine 1933; Bocak & Bocakova 1990; Bocak 2002). The previously defined tribes were based on clear, diagnostically highly usable characters, which, however, revealed to be either features evolved multiple times or to define just a restricted terminal branch. The taxa based on these characters are unacceptable in a phylogenetic classification.

The former subtribe Trichalina was recovered as a subordinate branch: the *Trichalus* + *Microtrichalus* clade (Fig. 1); therefore Trichalina Kleine, 1928 is synonymized with Metriorrhynchina Kleine, 1926.

Hemiconderina was described as a subtribe of Metriorrhynchini by Bocak & Bocakova (1990) on the basis of the unique pattern of pronotal carinae, shape of genitalia, and weaker elytral primary costae 1 and 3 (Bocak 2002). The morphology-based analysis recovered these genera as one of principal lineages in Metriorrhynchini due to resemblance of their pronotal areolae pattern to that in the outgroup, but molecular data place them in a terminal position within Metriorrhynchina and suggest an independent origin of the hemiconderine rhomboidal areola. Therefore, we propose to synonymize the subtribe Hemiconderina Bocak & Bocakova, 1990 with Metriorrhynchina Kleine, 1926.

5.3. Subtribe Metanoeina subtrib. nov.

Type genus. *Metanoeus* Waterhouse, 1879.

Description. ***Adults:*** Body length 7–16 mm, most species brightly colored, seldom uniformly black (Fig. 8). Head without rostrum (Fig. 19), antennae flabellate to pectinate in males, serrate in females (Figs. 12–13). Mandibles variable in length (Fig. 23); apical palpomeres slender to pointed (Figs. 27, 30). Pronotum with seven areolae, sometimes carinae obtuse (Figs. 40, 45–46). Mesoscutellum parallel-sided, deeply emarginate at apex. Elytra parallel-sided, with four or nine longitudinal costae (Figs. 48–49). Phallus variable in shape, internal sac armed with thorns and sclerotized lamellae (Figs. 54–55, 58–65). Valvifers vestigial (*Metanoeus*, *Matsudanoeus*; Figs. 73, 79, 80) or slender, branched at base (*Xylometanoeus*; Figs. 74, 77); vagina membranous to partly sclerotized, basal parts of lateral glands often sclerotized (Figs. 73–75, 80), spermaduct very long (*Metanoeus*) or about as long as coxites and valvifers combined (Figs. 74–75, 80).

Larvae: The known larvae share the longitudinally divided meso- and metathoracic terga, the spines at the frontal margin of the pronotum (Bocak & Matsuda 2003). The urogomphi are movable and branched (*Metanoeus*, *Matsudanoeus*, Fig. 1A) or vestigial (*Xylometanoeus*).

The larva of *Xylometanoeus japonicus* (Fig. 18) was illustrated in detail by A.A. Zaitsev on flicker (http://farm4.staticflickr.com/3515/4026462633_96ff6ebb02_z.jpg?zz=1, visited on Aug. 21, 2013).

List of Metanoeina genera. *Metanoeus* Waterhouse, 1879; *Xylometanoeus* gen. nov.; *Matsudanoeus* gen. nov.

Diagnosis. The lineage was inferred as a principal metriorrhynchine lineage by Sklenarova et al. (2013) and a re-analysis here (BS 91%, Fig. 1). The only adult morphological synapomorphy supporting their relationships are the short valvifers and wide, short coxites (Figs. 73–75, 79–80). Further, only a combination of characters can be used for diagnosing Metanoeina: the larvae have longitudinally divided meso- and metathoracic terga (unlike Cautirina but shared with Metriorrhynchina), the pronotal carinae are obtuse and frontal areolae smaller (pronounced in *Metanoeus*, less evident in *Matsudanoeus*, absent in *Xylometanoeus*, Figs. 40, 45–46), the lateral margins of the pronotum are not elevated and the frontal margin is simple; the spermathecal duct is extremely long (*Metanoeus*) to moderately long, bases of ducts to lateral glands of vagina sclerotized in most species (Figs. 73–75, 80). The male genitalia are variable in

Figs. 50–72. Male genitalia, in lateral and dorsal views, of **50–51**: *Metriorrhynchus doleschali*; **52–53**: *Porrostoma rhipidium*; **54–55**: *Metanoeus bakeri*; **56–57**: *Cautires* sp.; **58–59**: *Matsudanoeus yuasai*; **60–61**: *Xylometanoeus japonicus*; **62–63**: *X. basivittatus*; **64–65**: *Xylometanoeus* sp.; **66–67**: *Cautires* sp. (Madagascar, terminal A00030 in Fig. 1); **68–69**: *Cautires* sp. (Cameroon, terminal A00096, previously classified as *Xylobanus*); **70**: *Cautires* sp. (Cameroon, Voucher A00086); **71–72**: *Xylobanus costifer* (Walker). Scale bars: 0.5 mm.

shape (Figs. 54–55, 58–65), but never lanceolate as in *Xylobanus* and *Cautires* (Figs. 56–57, 66–72).

Distribution. Eastern Oriental region (Laos, the Great Sundas, Peninsular Malaysia, Palawan, the Philippines) and eastern Palearctic Region (China, Japan, Russian Far East).

Remark. The analysis supports a deep position of the clade formed by *Metanoeus*, *Matsudanoeus*, and *Xylometanoeus* (Fig. 1). The larval morphology points strongly to relationships of Metanoeina and Metriorrhynchinae by the shared longitudinally divided thoracic terga and presence of thorns at frontal margin of pronotum in *Xylometanoeus* (Fig. 18). Concerning the morphological and molecular disparity of the lineage we propose a new subtribe Metanoeina for these three genera. *Metanoeus* was thoroughly redescribed by Weiszenstein & Bocak (2012) and two new genera are described below.

5.3.1. *Xylometanoeus* gen. nov.

Type species. *Xylobanus japonicus* Bourgeois, 1902.

Description. ***Adults****:* Body length 8–15 mm, dark reddish brown or brightly colored. Head without rostrum; antennae flabellate in males, serrate in females. Mandibles robust; apical palpomeres parallel-sided. Pronotum with seven areolae (Fig. 46). Elytra parallel-sided, with four longitudinal costae (Figs. 48–49). Phallus tubular, short, internal sac with lamellae (Figs. 60–65). Ovipositor short and wide, valvifers branched at base, about as long as coxites, vagina membranous (Figs. 74–75, 77).

Larvae*:* *Xylometanoeus japonicus*, Fig. 18 (previously unpublished information provided by A.A. Zaitsev): Body parallel-sided, 16 mm long, slightly wider in basal part of abdomen, sclerites brown, small, membranes extensive, yellowish-white. Lateral part of epicranium membranous. Eyes small. Mandibles slender, long, slightly curved. Pronotum T1 extensive, with apparent median longitudinal suture, terga T2–T3 and A1–A8 divided in a pair of small hemitergites connected by whitish membrane. Pronotum with small anterior processes; tergites T2 and T3 simple, subquadrate; prosternum small, subtriangular. Sterna T2 and T3 small, less sclerotized. Spiracular plate T2 located in pleural part of mesothorax, small, simple, with spiracular opening in middle of sclerite. Posterior thoracic pleurites present, similar in shape to anterior ones. Abdominal hemi-tergites A1–A8 transverse, weakly sclerotized, each abdominal segment with long, finger-like, lateral membranous process; upper pleurites with spiracular opening at dorsal margin, lower pleurites much smaller. Segment A9 with pair of narrowly divided tergal sclerites and short membranous urogomphi below their apices.

Included species. Due to chaotic species level classification of the Oriental Metriorrhynchini we are not able to identify the Oriental species to the species level (Fig. 1) and only two Japanese species *X. japonicus* and *X. basivittatus* are formally classified in *Xylometanoeus* at present.

Material examined. 1 larva, Russia, South Kurils, Kunashir island, Cape Alekhino, 19. Aug. 2009, in rotten wood of *Acer*. Det. & leg. A.A. Zaitsev, deposited in Zaitsev coll.

Diagnosis. Adult *Xylometanoeus* differ from other Metanoeina in four elytral costae. The larvae of *Xylometanoeus* resemble other Metanoeina in divided meso-, metathoracic terga and prothoracic spines, but they differ from in short, simple urogomphi, undivided pronotal tergum and divided tergum A9 (Fig. 18).

Distribution. *Xylometanoeus* is widely distributed in the Oriental and the eastern part of the Palearctic region. Confirmed records are available from Japan, Laos, Borneo, and the Philippines.

Name derivation. The generic name is derived from the names *Xylobanus* and *Metanoeus* and points to the presence of the *Xylobanus*-like pattern of elytral costae. Gender masculine.

Remark. The species *Xylometanoeus basivittatus* (Nakane, 1970), comb. nov. and *X. japonicus* (Bourgeois, 1902), comb. nov. are transferred from *Xylobanus* Waterhouse, 1879.

5.3.2. *Matsudanoeus* gen. nov.

Type species. *Cautires yuasai* Nakane, 1969.

Description. ***Adults****:* Body length 8–11 mm, dark reddish brown. Head without rostrum; antennae flabellate in males, serrate in females. Mandibles robust; apical palpomeres parallel-sided, obliquely rounded at apex. Pronotum with seven areolae (Fig. 45). Elytra parallel-sided, with nine longitudinal costae. Phallus tubular, short, internal sac with one long and one short thorn (Figs. 58–59). Ovipositor short and wide, valvifers short, almost perpendicular to coxites, vagina short and wide, membranous (Figs. 74–75, 77).

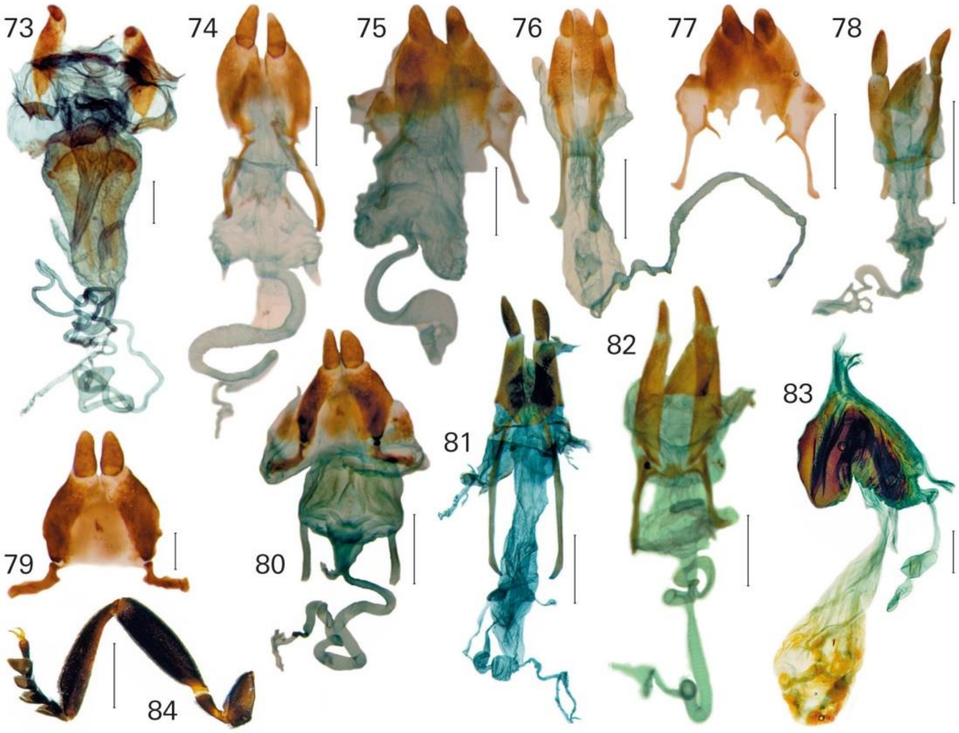
Larvae*:* The larva of *Matsudanoeus yuasai* was described by BOCAK & MATSUDA (2002) as *Cautires yuasai*. All thoracic and abdominal terga A1–A8 are divided in two hemitergites, tergite A9 entire, urogomphi movable, branched (Fig. 1A).

Included species. Only the type-species *Matsudanoeus yuasai* (Nakane, 1969), comb. nov. is classified in the genus. It is transferred here from *Cautires* Waterhouse, 1879.

Diagnosis. *Matsudanoeus* gen. nov. resembles in the general appearance *Cautires*, with which it shares the pattern of pronotal areolae and the presence of secondary costae on the elytra. Unlike most *Cautires*, *Matsudanoeus* has a simple frontal margin of the pronotum. Further, the genus differs in the tubular, well sclerotized phallus and unique thorns of the internal sac (Figs. 58–59). Female genitalia are characteristic in the very short, laterally directed valvifers, which resemble those of *Metanoeus* (Figs. 79–80). Larvae of *M. yuasai* are similar to those of *Metanoeus* and have very long branched urogomphi in contrast to the larvae of *Cautires* (BOCAK & MATSUDA 2003).

Name derivation. The genus is named in honour of Dr. Kiyoshi Matsuda, Takarazuka, a specialist in Lycidae taxonomy. The name merges his family name and "*noeus*", a part of the name *Metanoeus*. Gender masculine.

Distribution. *Matsudanoeus yuasai* is endemic to Japan. Despite a relatively high number of sequenced species, none species has been recorded from the Oriental Region, where *Metanoeus* occur.



Figs. 73–84. Female genitalia of **73**: *Metanoeus bakeri*; **74**: *Xylometanoeus basivittatus*; **75**, **77**: *X. japonicus*; **76**: *Cautires* sp. (Cameroon, terminal A00104 in Fig. 1); **78**: *Xylobanus* sp.; **79–80**: *Matsudanoeus yuasai*; **81**: *Cautires* sp.; **82**: *Xylobanus costifer* (Walker); **83**: *Metriorrhynchus doleschali*. Hind leg of **84**: *Cautires* sp. Scale bars: 0.5 mm.

5.3.3. Key to identification of Metanoeina genera

1 Each elytron with only four longitudinal costae (Figs. 48–49), male genitalia with short, robust phallus (Figs. 60–65); larva with longitudinally divided hemitergites A1–A9 and very short, membranous urogomphi, abdominal segments A1–A8 with a pair of membranous lateral processes (Fig. 18) .... ***Xylometanoeus* gen. nov.**

1' Each elytron with four strong and five weak longitudinal costae; larva with entire, transverse tergum A9 and long, branched and sclerotized urogomphi (Fig. 1A), each abdominal segment A1–A8 with two pairs of movable sclerotized processes (Fig. 1A) **2**

2 Pronotal carinae obtuse, male antennae flabellate, branch of antennomere 3 very slender, basally attached, at least 2× as long as stem of antennomere (Fig. 14), male genitalia with more than two thorns in internal sac (Figs. 54–55); movable processes attached to abdominal terga A1–A8 shorter than width of corresponding tergite .... ***Metanoeus* Waterhouse**

2' Pronotal carinae sharper and more distinct, male antennae flabellate, antennomere 3 with robust branch, which is at most 1.5× as long as stem of antennomere (Fig. 11), male genitalia with internal sac bearing two sclerotized thorns, one of them twice as long as the other (Figs. 58–59); movable processes attached to abdominal terga A1–A8 longer than width of corresponding tergite (Fig. 1) .... ***Matsudanoeus* gen. nov.**

5.4. Subtribe Cautirina subtrib. nov.

Type genus. *Cautires* Waterhouse, 1879.

Description. ***Adults****:* Body length 2.5–21 mm, most species brightly colored, few uniformly black or brown (Fig. 9). Head without rostrum (Figs. 9, 21), antennae flabellate in males, serrate to shortly flabellate in females (Fig. 10). Mandibles moderately long (Fig. 25), palpomeres variable in shape, securiform to pointed at apex (Figs. 29, 32). Pronotum usually with seven areolae, sometimes lateral carinae weaker to absent (Figs. 41–43). Mesoscutellum shallowly emarginate at apex (Fig. 17). Elytra parallel-sided, with four or nine longitudinal costae (Figs. 9, 47). Male genitalia with lanceolate or apically rounded phallus, internal sac armed with two thorns (Figs. 66–72). Valvifers simple, parallel-sided (Fig. 81) seldom fused basally (Fig. 76), short with small sclerite between their bases (*Xylobanus*; Figs. 78, 82); vagina membranous, spermaduct short to moderately long (Figs. 76–77, 81–82).

Larvae: Only several larvae of *Cautires* have been collected and they were described and illustrated by Bocak & Matsuda (2003) and Levkanicova & Bocak (2009). All thoracic terga entire (Fig. 1D,E,F).

Diagnosis. Despite strong support for this clade from molecular data (BS 98%, Fig. 1), the Cautirina are difficult to characterize by the presence of clearly defined morphological characters in the adult stage; only the larvae differ from Metanoeina and Metriorrhynchina in the entire thoracic terga (Fig. 1). Adults share a characteristic shape of the pronotum with elevated lateral margins and seven areolae, but the latter might be reduced to a prominent frontal keel and an obsolete median areola (Figs. 41–44, Dudkova & Bocak 2010). Some Metriorrhynchina have a similar pronotum and then male genitalia must be used for identification. Cautirina have a simple, lanceolate phallus, pointed apically (Figs. 56–57, 66–71) or widely rounded (Figs. 71–72), regularly with a pair of sickle-shaped sclerotized structures in the internal sac or apical lamellae. The Metriorrhynchina and Metanoeina have very variable forms of female genitalia, but never with a similarly slender, finely membranous vagina as found in Cautirina (Figs. 76, 78, 81).

List of Cautirina genera: *Caenioxylobanus* Pic, 1922; *Cautires* Waterhouse, 1879 (= *Bulenides* Waterhouse, 1879); *Paracautires* Kazantsev, 2012; *Prometanoeus* Kleine, 1925 (= *Tapromenoeus* Bocak & Bocakova, 1989); *Spartoides* Kazantsev, 2012; *Tricautires* Kazantsev, 2006; *Xylobanus* Waterhouse, 1879.

Distribution. Afrotropical region including Madagascar and the southernmost part of the Arab Peninsula (Yemen); Oriental region; the eastern part of the Palearctic region (the Himalayas, the eastern slope of the Tibetan Plateau and forest habitats of northeastern China, Japan and Russian Far East), Australian Region: Sulawesi. The ranges of Metriorrhynchina and Cautirina overlap only in a small part of their combined ranges (Sklenarova et al. 2013). A few Cautirina occur in Sulawesi (Kubecek et al. 2011) and none is known east of the Weber's line.

Remarks. The shape of pronotal carinae and elytral costae has been used for definition of genera and higher taxa, both in Cautirina and other Lycidae. The hypothesized phylogeny (Fig. 1) suggests that these structures were often independently modified in unrelated lineages (Figs. 2–5). We suppose that pronotal carinae as well as elytral costae have a strengthening function in the soft-bodied elateroid lineages and are easily modified when the body becomes slender or miniaturized. We hypothesize a multiple origin of the four-costae pattern in Cautirina, which explains the morphological diversity of genitalia in typologically delineated *Xylobanus* as noted by Bocak (2002). The type species, *Xylobanus costifer* (Walker, 1858) was identified as a member of the *Xylobanus* clade in Fig. 1. Other taxa, all with four costae and therefore until now classified as *Xylobanus*, were found in Metanoeina (*X. basivittatus* and *X. japonicus*, Figs. 60–65, herein transferred to *Xylometanoeus*), and in the Afrotropical *Cautires* clade (see distribution of characters in Fig. 3). Further Cautirina species with four-costae pattern are known from Madagascar (e.g. *Caenioxylobanus* Pic, 1922). These species differ from *Xylobanus* in female genitalia and shape of phallus (Figs. 68–72). *Xylobanus* is now defined by the unique shape of the phallus with a rounded apical part, the internal sac bearing lamellae (Figs. 71–72), the female genitalia with valvifers shorter than coxites and a sclerite present between the bases of valvifers (Figs. 78, 82). Males of *Xylobanus* have either serrate or flabellate antennae (Fig. 2). The revised concept of *Cautires* includes taxa with either four or nine elytral costae and most species have flabellate antennae (Figs. 2–3). The male genitalia of *Cautires* have usually a slender, lanceolate phallus (Figs. 56–57, 66–70) and the female genitalia have long slender valvifers without any sclerite between their bases (compare Figs. 81 and 82) or seldom valvifers are short and their bases are connected by a sclerotized bridge (Fig. 76).

Another frequently used character for delineation of genera is the presence of flabellate antennae in males. Similarly to elytral costae or pronotal carinae these evolved frequently in unrelated lineages, e.g. both forms are present in *Xylobanus* and *Cautires* (Fig. 2). The olfactory organs are present on antennae and the expanded surface of antennae might be correlated with more intensive pheromone communication.

Kazantsev (2006, 2012) described three genera: *Tricautires* Kazantsev, 2006, *Paracautires* Kazantsev, 2012 and *Spartoides* Kazantsev, 2012 and based them mostly on the reduction of the number of pronotal areolae and on the shortened elytral costa 3. Additionally, he noted the pointed apical palpomeres in contrast with *Cautires*. The types are deposited in the private collection and unavailable for study (therefore no formal changes are proposed), but we have sequenced a relatively high number of taxa from Cameroon and found that morphologically similar taxa are members of the African *Cautires* clade (Fig. 1) and that this clade has a very diverse shape of apical palpomeres, patterns of pronotal areolae (Fig. 5) and number of elytral costae (Fig. 3). Kazantsev (2012) hypothesized a very old history of Metriorrhynchini in Africa and their restriction to refugia in the African part of the South American-African continent in the Jurassic and Cretaceous, both proposals in deep contrast with the phylogeographic reconstruction by Sklenarova et al. (2013). The supposed ancient origins of African Metriorrhynchini lead him to the proposal of new genera.

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Electronic Supplement Files

at http://www.senckenberg.de/arthropod-systematics ("Contents")

File: SklenarovaEtAl-LycidaeMetriorrhynchini-ASP2014-ElectronicSupplement.pdf. – Table ES1: Previously published sequences included in the analysis with collecting information, voucher, and GenBank accession numbers. – Fig. ES1: Bayesian estimation of the ancestral patterns of pronotal carinae for selected clades.

Sklenarova, K., Kubecek, V., Bocak, L. Subtribal classification of Metriorrhynchini (Coleoptera: Lycidae): an integrative approach using molecular phylogeny and morphology of adults and larvae.

The list of Supplementary Information

Supplementary Tab. S1. Previously published taxa included in the analysis with collecting information, voucher, and GenBank accession numbers.

Supplementary Fig. S1. Bayesian estimation of the ancestral patterns of pronotal carinae for selected clades.

Supplementary Table S1. Previously published taxa included in the analysis with collecting information, voucher, and GenBank accession numbers.

Species	Voucher number	Geographic origin	*rrnL*	*cox1*	*nad5*
Outgroup					
Libnetis sp.	UPOL 001002	Indonesia	DQ181030	DQ181252	DQ181406
Dilophotes sp.	UPOL 000244	Sabah	DQ180992	DQ181214	DQ181368
Plateros sp.	UPOL 000243	Sabah	DQ180991	DQ181213	DQ181367
Platycis minutus	UPOL 000348	Czechia	DQ180995	DQ181217	DQ181371
Lycoprogenthes sp.	UPOL 000358	Java	DQ180996	DQ181218	DQ181372
Calochromus sp.	UPOL 000400	Palawan	-	KC538321	KC538512
Duliticola sp.	UPOL 000L01	Sabah	DQ180963	DQ181185	DQ181339
Libnetis sp.	UPOL 000L02	Sabah	DQ180964	DQ181186	DQ181340
Lycus sp.	UPOL 000L03	S. Africa	DQ180965	DQ181187	DQ181341
Calopteron sp.	UPOL 000L25	Ecuador	DQ180979	DQ181201	DQ181355
Lyropaeus sp.	UPOL 000L11	Sabah	DQ180968	DQ181190	DQ181344
Dihammatus sp.	UPOL 000L12	Sabah	DQ180969	DQ181191	DQ181345
Plateros sp.	UPOL 000L13	Sabah	DQ180970	DQ181192	DQ181346
Scarelus sp.	UPOL 000L15	Sabah	KC538782	DQ181194	DQ181348
Calochromus sp.	UPOL 000L16	China	DQ180973	DQ181195	DQ181349
Lyponia nigrohumeralis	UPOL 000L17	China	DQ180974	DQ181196	DQ181350
Macrolycus bocakorum	UPOL 000L18	China	DQ180975	DQ181197	DQ181351
Dictyoptera elegans	UPOL 000570	Japan	DQ180999	DQ181221	DQ181375
Pyropterus nigroruber	UPOL 000574	Japan	DQ181003	DQ181225	DQ181379
Lopheros sp.	UPOL 000578	Japan	DQ181007	DQ181229	DQ181383
Lycoprogenthes sp.	UPOL 000801	Sumatra	DQ181021	DQ181243	DQ181397
Plateros sp.	UPOL A00047	Malaysia	-	KC538353	KC538544
Dilophotes sp.	UPOL A00060	Philippines	KC538740	KC538359	KC538552
Ingroup					
Metriorrhynchus lineatus	UPOL 000009	Sumatra	KC538628	DQ904297	DQ904259
Metriorrhynchus sp.	UPOL 000010	Sulawesi	-	DQ144659	DQ144685
Metriorrhynchus sp.	UPOL 000011	Sulawesi	KC538629	DQ144660	DQ144686
Metriorrhynchus lobatus	UPOL 000017	Sulawesi	KC538630	DQ144662	DQ144688
Metanoeus sp.	UPOL 000026	Borneo	KC538631	KC538244	KC538436
Cautires sp.	UPOL 000030	Borneo	KC538632	KC538245	KC538437
Cautires sp.	UPOL 000037	Borneo	KC538633	KC538246	KC538438
Cautires sp.	UPOL 000040	Borneo	KC538634	KC538247	KC538439
Cautires sp.	UPOL 000043	Borneo	KC538635	KC538248	KC538440
Cautires sp.	UPOL 000044	Borneo	KC538636	KC538249	KC538441
Cautires sp.	UPOL 000047	Sumatra	KC538637	KC538250	KC538442
Cautires sp.	UPOL 000048	Sumatra	KC538638	KC538251	KC538443
Cautires sp.	UPOL 000050	Sumatra	KC538639	KC538252	KC538444
Cautires sp.	UPOL 000052	Sumatra	KC538640	KC538253	KC538445
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Cautires sp.	UPOL 000066	Laos	KC538644	KC538257	KC538449
Cautires sp.	UPOL 000068	Borneo	KC538645	KC538258	KC538450
Cautires sp.	UPOL 000069	Borneo	KC538646	KC538259	KC538451
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Xylometanoeus sp.	UPOL 000071	Borneo	KC538648	KC538261	KC538453
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Cautires sp.	UPOL 000075	Laos	-	KC538263	KC538455
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Cautires sp.	UPOL 000081	Borneo	KC538652	KC538266	KC538458

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Metanoeus sp.	UPOL 000105	Borneo	KC538657	KC538271	KC538463
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Xylobanus sp.	UPOL 000120	Laos	KC538659	KC538273	KC538465
Metanoeus sp.	UPOL 000121	Sumatra	KC538660	KC538274	KC538466
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Xylobanus sp.	UPOL 000153	Laos	KC538666	KC538280	KC538472
Xylobanus sp.	UPOL 000154	Laos	-	KC538281	-
Cautires sp.	UPOL 000164	Laos	KC538667	KC538282	KC538473
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Xylometanoeus sp.	UPOL 000184	Borneo	KC538670	KC538286	KC538477
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Leptotrichalus sp.	UPOL 000208	Borneo	KC538677	DQ181212	DQ181366
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Xylobanus basivittatus	UPOL 000221	Japan	KC538681	KC538296	KC538487
Xylobanus niger	UPOL 000224	Japan	KC538682	KC538297	KC538488
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Cautires sp.	UPOL 000342	Borneo	KC538695	KC538310	KC538501
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Cautires sp.	UPOL 000355	Jawa	KC538697	KC538312	KC538503
Metriorrh. palawensis	UPOL 000366	Palawan	KC538698	DQ144665	DQ144691
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Metriorrhynchus sp.	UPOL 000374	Australia	KC538701	KC538314	KC538505
Microtrichalus sp.	UPOL 000375	Australia	KC538702	KC538315	KC538506
Microtrichalus sp.	UPOL 000376	Australia	KC538703	KC538316	KC538507
Porrost. haemorrhoidalis	UPOL 000378	Australia	KC538704	DQ144679	DQ144703
Xylobanus sp.	UPOL 000379	Palawan	KC538705	KC538317	KC538508
Cautiromimus sp.	UPOL 000388	Palawan	-	KC538318	KC538509
Cautires sp.	UPOL 000395	Palawan	KC538706	KC538319	KC538510

Leptotrichalus sp.	UPOL 000396	Palawan	KC538707	KC538320	KC538511
Xylobanus sp.	UPOL 000402	Palawan	KC538708	KC538322	KC538513
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Cautires sp.	UPOL A00030	Madagascar	KC538720	KC538337	KC538527
Synchonnus sp.	UPOL A00031	Australia	KC538721	KC538338	KC538528
Trichalus sp.	UPOL A00032	Australia	KC538722	KC538339	KC538529
Ditua sp.	UPOL A00033	Australia	KC538723	-	KC538530
Metriorrhynchus sp.	UPOL A00034	Australia	KC538724	KC538340	KC538531
Porrostoma sp.	UPOL A00035	Australia	KC538725	KC538341	KC538532
Porrostoma sp.	UPOL A00036	Australia	KC538726	KC538342	KC538533
Porrostoma sp.	UPOL A00037	Australia	KC538727	KC538343	KC538534
Metriorrhynchus sp.	UPOL A00038	Australia	KC538728	KC538344	KC538535
Metriorrhynchus sp.	UPOL A00039	Australia	KC538729	KC538345	KC538536
Porrostoma sp.	UPOL A00040	Australia	KC538730	KC538346	KC538537
Porrostoma sp.	UPOL A00041	Australia	KC538731	KC538347	KC538538
Porrostoma sp.	UPOL A00042	Australia	-	KC538348	KC538539
Metriorrhynchus sp.	UPOL A00043	Australia	KC538732	KC538349	KC538540
Porrostoma sp.	UPOL A00044	Australia	KC538733	KC538350	KC538541
Porrostoma sp.	UPOL A00045	Australia	KC538734	KC538351	KC538542
Metriorrhynchus sp.	UPOL A00046	Australia	KC538735	KC538352	KC538543
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Metriorrhynchus sp.	UPOL A00049	Malaysia	KC538736	KC538354	KC538545
Cautires sp.	UPOL A00050	Malaysia	-	-	KC538546
Leptotrichalus sp.	UPOL A00052	Philippines	HQ456949	HQ456968	HQ456991
Xylobanus sp.	UPOL A00053	Palawan	KC538737	KC538355	KC538547
Xylobanus sp.	UPOL A00054	Palawan	KC538738	-	KC538548
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Cautires sp.	UPOL A00058	Philippines	-	KC538357	KC538550
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Leptotrichalus sp.	UPOL A00061	Philippines	KC538741	KC538360	KC538553
Cautires sp.	UPOL A00062	Philippines	KC538742	KC538361	KC538554
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Metanoeus sp.	UPOL A00064	Philippines	-	KC538363	KC538556
Metanoeus sp.	UPOL A00065	Philippines	-	KC538364	KC538557
Sulabanus sp.	UPOL A00066	Philippines	KC538743	KC538365	KC538558
Sulabanus sp.	UPOL A00067	Philippines	KC538744	KC538366	KC538559
Microtrichalus sp.	UPOL A00068	Philippines	KC538745	KC538367	KC538560
Microtrichalus sp.	UPOL A00069	Philippines	KC538746	KC538368	KC538561
Sulabanus sp.	UPOL A00070	Philippines	KC538747	KC538369	KC538562
Sulabanus sp.	UPOL A00071	Philippines	KC538748	KC538370	KC538563
Microtrichalus sp.	UPOL A00073	Philippines	KC538749	KC538371	-
Xylobanus sp.	UPOL A00074	Philippines	KC538750	-	-

Sulabanus sp.	UPOL A00075	Philippines	KC538751	KC538372	KC538564
Xylometanoeus sp.	UPOL 00A076	Philippines	KC538752	KC538373	KC538565
Sulabanus sp.	UPOL A00077	Philippines	KC538753	KC538374	KC538566
Cautires sp.	UPOL A00078	Cameroon	KC538754	KC538375	KC538567
Cautires sp.	UPOL A00079	Cameroon	KC538755	KC538376	KC538568
Cautires sp.	UPOL A00080	Cameroon	HQ456950	HQ456969	HQ456992
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Cautires sp.	UPOL A00082	Cameroon	KC538757	KC538378	-
Cautires sp.	UPOL A00083	Cameroon	KC538758	KC538379	KC538570
Cautires sp.	UPOL A00084	Cameroon	KC538759	KC538380	KC538571
Cautires sp.	UPOL 00A085	Cameroon	KC538760	KC538381	KC538572
Cautires sp.	UPOL A00086	Cameroon	KC538761	KC538382	KC538573
Cautires sp.	UPOL A00087	Cameroon	KC538762	KC538383	KC538574
Cautires sp.	UPOL A00088	Cameroon	KC538763	KC538384	KC538575
Cautires sp.	UPOL A00089	Cameroon	KC538764	KC538385	KC538576
Cautires sp.	UPOL A00090	Cameroon	KC538765	KC538386	KC538577
Cautires sp.	UPOL A00091	Cameroon	-	KC538387	KC538578
Cautires sp.	UPOL A00092	Cameroon	KC538766	KC538388	KC538579
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Cautires sp.	UPOL A00095	Cameroon	KC538769	-	KC538582
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Cautires sp.	UPOL A00098	Cameroon	KC538772	KC538392	KC538585
Cautires sp.	UPOL A00099	Cameroon	KC538773	KC538393	KC538586
Cautires sp.	UPOL A00100	Cameroon	KC538774	KC538394	KC538587
Cautires sp.	UPOL A00101	Cameroon	-	KC538395	-
Cautires sp.	UPOL A00102	Cameroon	-	KC538396	KC538588
Cautires sp.	UPOL A00103	Cameroon	-	KC538397	-
Cautires sp.	UPOL A00104	Cameroon	KC538775	-	KC538589
Cautires sp.	UPOL A00105	Cameroon	KC538776	-	KC538590
Cautires sp.	UPOL A00106	Cameroon	KC538777	KC538398	KC538591
Cautires sp.	UPOL A00107	Cameroon	-	KC538399	-
Cautires sp.	UPOL A00109	Cameroon	KC538778	KC538400	KC538592
Cautires sp.	UPOL A00110	Cameroon	KC538779	-	KC538593
Cautires sp.	UPOL A00111	Cameroon	KC538780	KC538401	KC538594
Cautires sp.	UPOL A00112	Cameroon	KC538781	KC538402	KC538595
Metriorrhynchus lineatus	UPOL 000L05	Malaysia	DQ180966	DQ181188	DQ181342
Cautires sp.	UPOL 000L06	Malaysia	DQ180967	DQ181189	DQ181343
Cautires sp.	UPOL 000L14	South Africa	DQ180971	DQ181193	DQ181347
Microtrichalus sp.	UPOL 000L23	Malaysia	DQ180978	DQ181200	DQ181354
Xylobanus kundratai	UPOL MD0029	Sulawesi	-	HQ456972	HQ456994
Sulabanus lalui	UPOL MD0030	Sulawesi	-	KC538403	KC538596
Sulabanus katarinae	UPOL MD0033	Sulawesi	KC538783	KC538404	KC538597
Sulabanus lineatus	UPOL MD0034	Sulawesi	KC538784	KC538405	KC538598
Xylobanus kundratai	UPOL MD0036	Sulawesi	-	HQ456973	HQ456995
Sulabanus mamasensis	UPOL MD0044	Sulawesi	KC538785	KC538406	KC538599
Sulabanus gracilis	UPOL MD0064	Sulawesi	KC538786	KC538407	KC538600
Sulabanus similis	UPOL MD0065	Sulawesi	-	KC538408	KC538601
Sulabanus gracilis	UPOL MD0067	Sulawesi	KC538787	KC538409	KC538602
Sulabanus cordatus	UPOL MD0069	Sulawesi	KC538788	KC538410	KC538603
Sulabanus gracilis	UPOL MD0071	Sulawesi	KC538789	KC538411	KC538604
Sulabanus cordatus	UPOL MD0081	Sulawesi	KC538790	KC538412	KC538605
Microtrichalus sp.	UPOL MD0097	Sulawesi	KC538791	HQ456978	HQ457000
Microtrichalus sp.	UPOL MD0098	Sulawesi	HQ456956	HQ456979	HQ457001
Broxylus pfeifferi	UPOL MD0099	Sulawesi	HQ456957	HQ456980	HQ457002
Broxylus malinensis	UPOL MD0101	Sulawesi	HQ456958	HQ456981	HQ457003
Broxylus pendolensis	UPOL MD0106	Sulawesi	KC538792	KC538413	KC538606

Broxylus kalamensis	UPOL MD0107	Sulawesi	KC538793	KC538414	KC538607
Wakarumbia obstinata	UPOL MD0111	Sulawesi	KC538795	KC538416	KC538609
Wakarumbia petri	UPOL MD0118	Sulawesi	HQ456960	HQ456983	HQ457005
Wakarumbia montana	UPOL MD0119	Sulawesi	HQ456961	HQ456984	HQ457006
Wak. pseudofasciata	UPOL MD0121	Sulawesi	KC538796	KC538418	KC538610
Wakarumbia sp.	UPOL MD0126	Sulawesi	KC538797	KC538419	KC538611
Wakarumbia grisea	UPOL MD0127	Sulawesi	-	KC538420	KC538612
Wakarumbia obscura	UPOL MD0129	Sulawesi	KC538798	KC538421	KC538613
Wakarumbia kundratai	UPOL MD0130	Sulawesi	KC538799	KC538422	KC538614
Wakarumbia sp.	UPOL MD0132	Sulawesi	KC538800	KC538423	KC538615
Wakarumbia kalamensis	UPOL MD0133	Sulawesi	KC538801	KC538424	KC538616
Wakarumbia linearis	UPOL MD0134	Sulawesi	KC538802	KC538425	KC538617
Wakarumbia obscura	UPOL MD0135	Sulawesi	KC538803	KC538426	KC538618
Wakarumbia aurea	UPOL MD0136	Sulawesi	KC538804	KC538427	KC538619
Wakarumbia aurea	UPOL MD0137	Sulawesi	KC538805	KC538428	KC538620
Wakarumbia fasciata	UPOL MD0140	Sulawesi	KC538806	KC538429	KC538621
Wakarumbia pendolensis	UPOL MD0143	Sulawesi	KC538807	KC538430	KC538622
Wakarumbia fasciata	UPOL MD0145	Sulawesi	KC538808	KC538431	KC538623
Wakarumbia mamasensis	UPOL MD0155	Sulawesi	KC538809	KC538432	KC538624
Wakarumbia grisea	UPOL MD0156	Sulawesi	KC538810	KC538433	KC538625
Wakarumbia mamasensis	UPOL MD0157	Sulawesi	KC538811	KC538434	KC538626
Wakarumbia kalamensis	UPOL MD0169	Sulawesi	KC538812	KC538435	KC538627
Leptotrichalus atricollis	UPOL ZL2002	Jawa	EF143215	EF143230	EF143244
Cautires sp.	UPOL ZL2009	Sumatra	EF143219	EF143234	EF143248
Sulabanus lalui	UPOL ZL2010	Sulawesi	EF143220	EF143235	EF143249
Cautires sp.	UPOL ZL2015	Japan	EF143224	EF143239	-

The voucher specimens are deposited in the Laboratory of Molecular Systematics, Department of Zoology, Palacky University, Olomouc, Czech Republic.

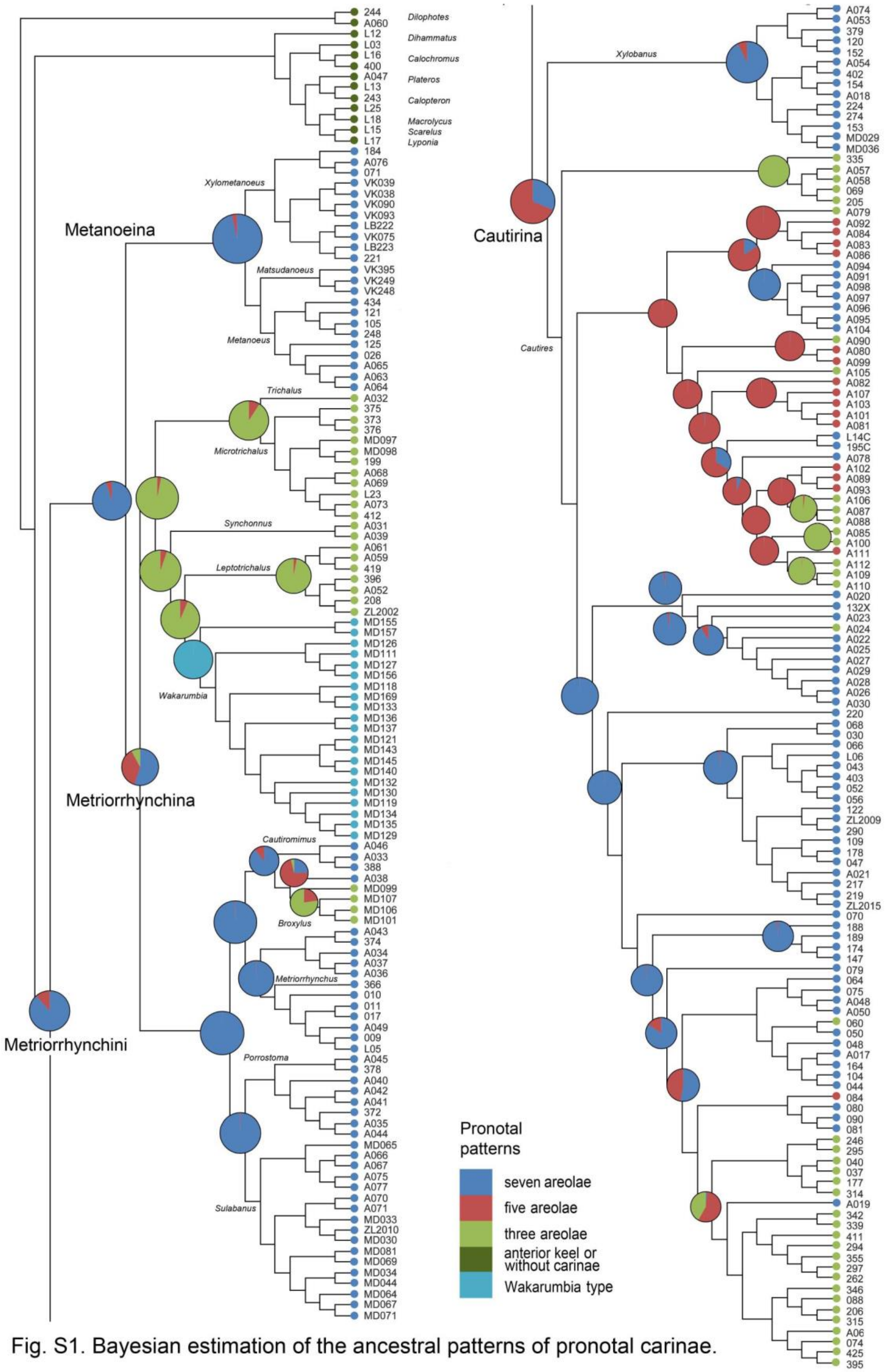

Fig. S1. Bayesian estimation of the ancestral patterns of pronotal carinae.

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CAUTIRES APTERUS, A NEW SPECIES AND THE FIRST RECORD OF WINGLESS MALE LYCIDAE (COLEOPTERA) DISCOVERED IN THE NORTH PARE MOUNTAINS, TANZANIA

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Abstract.— We report the first know case of male hind-wing aptery among the net-winged beetles (Lycidae). Five male specimens of *Cautires apterus* **sp. nov.** were discovered by sifting forest litter in a very small (approximately 300 meters by 50 meters) Kamwala Forest within the North Pare Mountains, Tanzania. Besides being wingless, males of *C. apterus* **sp. nov.** have remarkably small body, shortened elytra and reduced pronotal and elytral costae. Generic assignment of this externally unusual new species is based on the molecular phylogenetic analysis, the structure of the male genitalia and the shape of the pronotum. We discuss biological and evolutionary significance of the discovery and suggest that the winglessness of male lycid beetles correlates with the female neoteny.

⁂

Key words.— male aptery, East Arc Mountains, East Africa, neoteny, Kamwala Forest, Pleistocene refugium.

INTRODUCTION

The net-winged beetles (Lycidae) belong to a group of the elateroid families with incomplete body sclerotization in both sexes and with neotenic modifications in some females (Kundrata and Bocak 2011a). Females in the soft-bodied elateroid lineages often have only vestigial elytra and the hind wings are completely absent, as exemplified in the genera *Omalisus* Geoffrey, 1762 of Omalisidae and *Lamprorhiza* Mulsant, 1862 of Lampyridae (Bocak and Brlik 2008, Branham 2010). Even more dramatic, in some cases mature females are larviform and barely distinguishable externally from the fully grown larvae (Bocak *et al.* 2008). The female winglessness and male brachelytry appear to correlate, since no reduction of male wings has been observed when females are fully winged (Bocak and Bocakova 2008). Two other male morphological characters also appear to correlate with the presence of larviform females: males are much smaller than females and males have reduced hypognathous mouthparts (Bocak and Bocakova 2008). When females do not fly, male flight ability is also limited so males remain in the lowest strata of the forest (personal observation) and are collected in the leaf litter (Kazantsev 1999). Such peculiar life history of these low-dispersing beetles, as

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opposed to the overwhelming majority of other actively flying species, is probably the reason why they are rarely seen and, therefore, relatively understudied.

In this paper we report a remarkable discovery of five male specimens of a new Lycidae beetle obtained by one of us in the East African mountain forests by extensive use of a sifter to sift litter with subsequent specimen extraction by Winkler funnels. These male beetles have markedly shortened elytra and entirely lack hind wings, the latter phenomenon being the first record for males in this family.

MATERIAL AND METHODS

Morphological methods. The species description and diagnosis are based on the male adult semaphoronts, as females are unavailable. Terminal segments of the abdomen were removed and briefly placed in hot 10% KOH to clear them of muscle tissue and fat bodies when male genitalia were studied. Photographs of diagnostic characters and measurements were taken using an Olympus SZX-16 microscope and then traced from the original photographs.

Molecular phylogenetics. The sequences of *rrnL*, *cox1* and *nad5* mtDNA from four paratypes of *C. apterus* sp. nov. were produced using methods as described by Malohlava and Bocak (2010) and combined with a part of the previously published datasets representing all tribes of Lycini and African Metriorrhynchini (Bocak *et al.* 2008, Sklenarova *et al.* 2013). The sequences were submitted to Genbank (Tab. 1). Each fragment was aligned separately using ClustalW 1.83 under default settings and concatenated dataset was analyzed using maximum likelihood in RAxML 7.2.5 and the GTRCAT model (Stamatakis *et al.* 2005). One hundred replicates were produced for bootstrap analysis.

RESULTS

Molecular phylogenetics. Four paratypes of *C. apterus* sp. nov. (Tab. 1) were sequenced for *rrnl*, *cox1* and *nad5* mtDNA fragments. The inferred tree is shown in Fig. 1. The clade represented by *C. apterus* sp. nov. and other African *Cautires* spp. obtained the high support in the bootstrap analysis.

TAXONOMY

Cautires apterus sp. nov.
(Figs 2–8)

Type material. Holotype, male. Tanzania, North Pare Mts., Kamwala For., S3.68565°, E37.63665°, 1844m, 26. Dec. 2012, sift 31, V. Grebennikov leg. (Canadian National Collection of Insect, Arachnids and Nematodes, Ottawa; CNC). Paratypes. 4 males, same data as the holotype (2. spec. in CNC and 2 spec. in Department of Zoology, Palacky University, Olomouc, Czech Republic; LMBC).

Diagnosis. *Cautires apterus* sp. nov. is the only Afrotropical metriorrhynchine net-winged beetle with shortened elytra (Figs 2–3). The very small body measuring only 2.50–3.15 mm distinguishes the species from hundreds other species of African Metriorrhynchini. The new species has an unusually dull brown coloration, small eyes and distinctive male genitalia (Fig. 4).

Description. Male (Fig. 2). Body length 2.50–3.15 mm, pronotal length 0.52 mm, pronotal width 0.55 mm, length of elytra 1.4 mm, minimum frontal eye distance 0.37 mm, maximum eye diameter in lateral view 0.21 mm. Body dark testaceous to brown, lacking any brightly coloration, pronotal margins and elytra testaceous, lighter than thorax and abdomen; antennae with dark colored scapus and pedicel, antennomeres 3–4(6) testaceous, antennomeres 5(7)–9 dark brown, two apical antennomeres light testaceous. Head small, hypognathous, partly covered by pronotum; cranium with prominent frontal part, shortly, densely pubescent. Antennae inserted on tubercles, slender, serrate; scapus robust, pear-like, pedicel small, shorter than wide, antennomeres 3–11 flattened, antennomere 3 triangular, 1.05x longer than antennomere 4 (Fig. 2), subsequent antennomeres becoming gradually more slender and parallel-sided, antennomere 11 parallel-sided, longest. Eyes small, hemispherically prominent, eye

Table 1. The list of sequenced specimens of *C. apterus* sp. nov. (all sequenced specimens are paratypes).

	Gen/Genbank	Accession	Numbers
Voucher #	*rrnL*	*cox1*	*nad5*
UPOL A00652	KF588381	–	KF588386
UPOL A00653	KF588382	–	KF588387
UPOL A00655	–	KF588384	KF588388
UPOL A00656	KF588383	KF588385	KF588389

diameter 0.57 times interocular distance. Mouthparts with long, slender mandibles (Fig. 5); labrum transverse, with widely rounded apex; hypopharynx plate-like, 1.5 times longer than wide; maxillae with setose mala, maxillary palpi 4-segmented, basal palpomere slightly longer than width, ring-like, palpomere 2 twice longer, palpomere 3 robust, as long as wide, palpomere 4 slender, prolonged apically, pointed at tip (Fig. 7). Labium 2-segmented, ligula inconspicuous, setose, palpi 3-segmented, basal palpomere short, palpomere 2 robust, 1.5 times longer than palpomere 1, apical palpomere very slender, pear-like at base, apical two thirds almost parallel-sided, apex pointed (Fig. 6). Pronotum parallel-sided, only slightly wider at basal angles, basal angles well marked and not prominent, lateral margins almost straight, frontal angles obtuse, anterior margin widely rounded; pronotum with single areola attached to posterior margin, frontal and lateral keels absent, surface at anterior margin with irregular punctures (Fig. 8), rest of disc without any microstructure, covered with sparse pubescence. Scutellum bilobed apically, distal processes slender (Fig. 2). Elytra shortened, narrow, separate along midline, each narrowed towards apex; each elytron with 2 robust longitudinal costae and irregular weak transverse costae between them. Internal costa long, almost reaching elytral apex, external costa fused with internal one in apical third, transverse costae inconspicuous in some part of the elytron, elytral costae bearing sparse, erected pubescence (Fig. 3), surface covered with dense microsetae. Hind wings absent. Abdomen large, flat, wide, much longer than elytra, weakly sclerotized. Legs slender, tarsomeres similar in width, slender. Male genitalia with phallus and ring-like phallobase (Fig. 4). Phallus 4.3 times longer than wide in apical third, slightly constricted in middle, widely rounded apically; internal sac with 2 slender sickle-like thorns, parameres absent (Fig. 4).

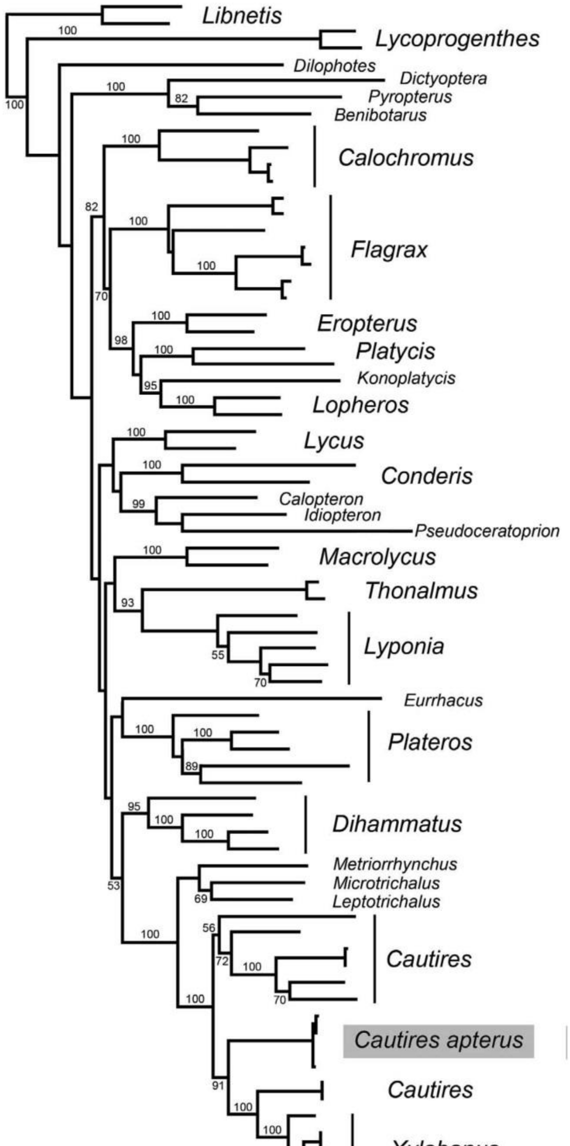


Figure 1. The phylogenetic hypothesis inferred from the maximum likelihood analysis with bootstrap values indication support for recovered relationships.

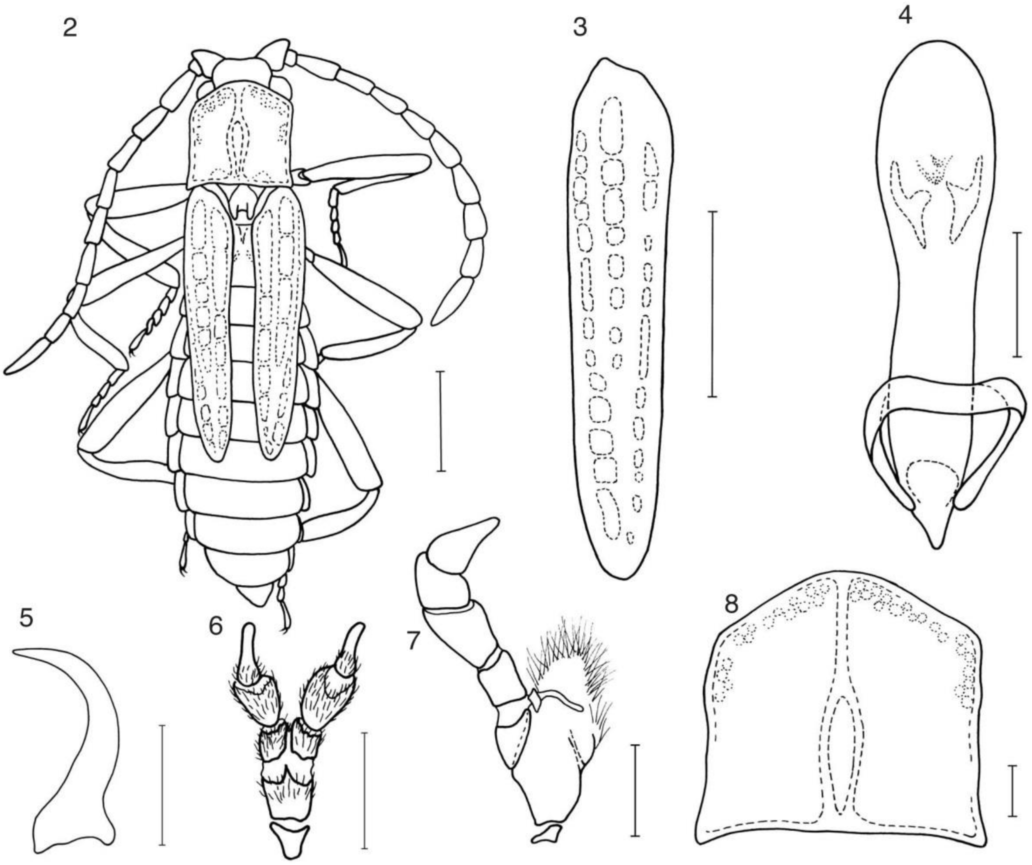
Distribution. This species is known from a single sifting sample of the forest leaf litter taken on approximately 100 square meters in the tiny Kamwala Forest in the North Pare Mountains, Tanzania (Figs 9–11). This is a remarkably small patch of likely original and almost undisturbed moist forest growing on slopes of a minor stream and measuring not more than 300 meters along the stream and some 50 meters across it. A minor trail cuts through the forest. The forest is hidden in the poorly accessible highlands of the otherwise very densely populated North Pare Mountains in northeastern Tanzania and requires at least a kilometre hike from the nearest point accessible by motorbike. Apparently only a few local residents are even aware of its existence. This obscurity, along with some sacred associations, likely contribute to the continuous existence of such a small patch of original forest vegetation. The total volume of this sample with the litter sifted through a 7 mm mesh was 47 individual bags suspended overnight in the Winkler funnels, with each bag containing slightly less than a handful of the sifted substrate. Remarkably, no other male specimens of *C. apterus* sp. nov. were discovered in another similarly-sized sample taken on the same day in Kamwala forest about 100 meters away, nor in about tenfold extensive sampling done in two more North Pare forests: Kindoroko (south of Kamwala Forest) and Minja (north of Kamwala forest); Fig. 10.

Etymology. The specific epithet refers to lack of hind wings in males.

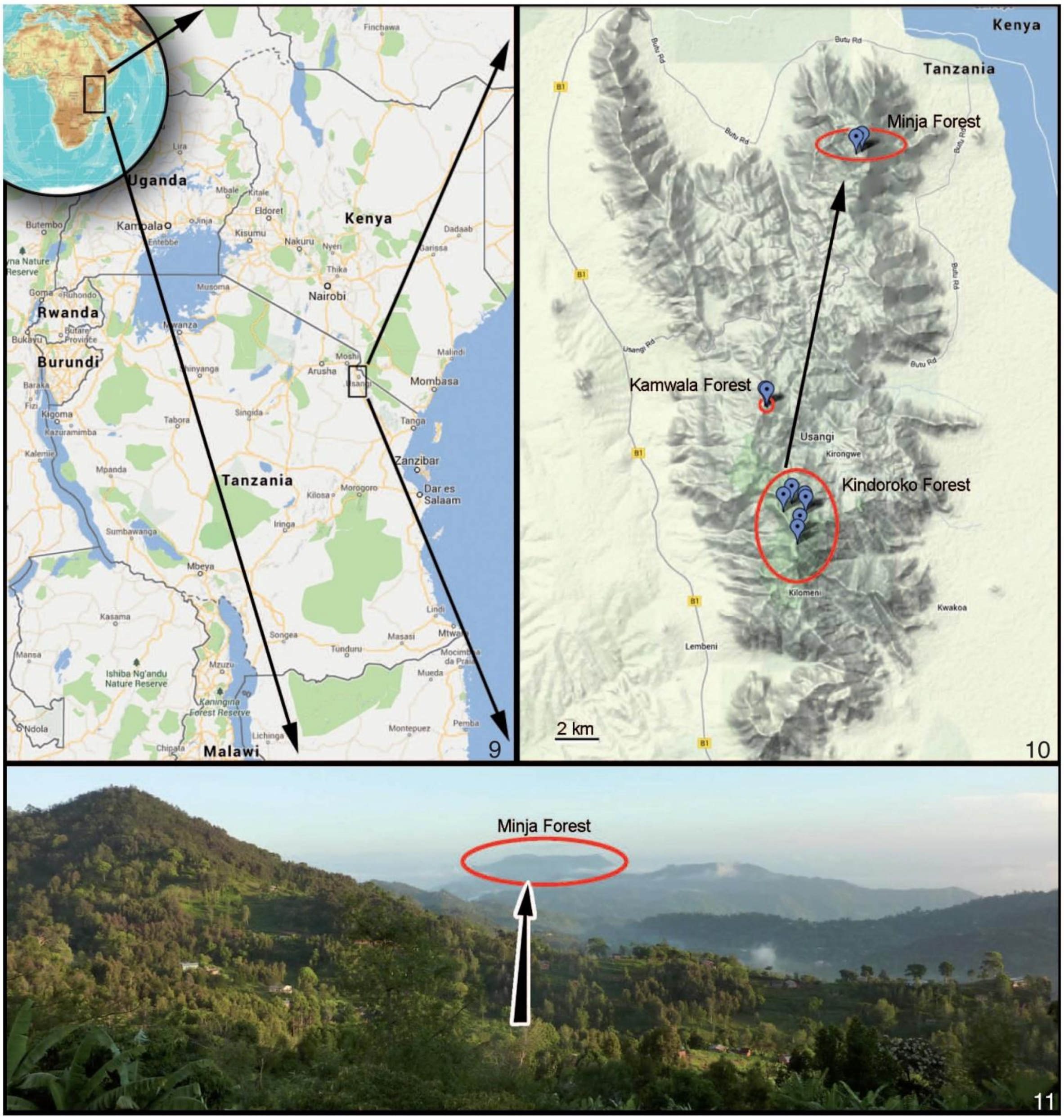
DISCUSSION

Biology and distribution. Adult males of net-winged beetles occur regularly on leaves and usually avoid visiting organic detritus on the soil surface. Larvae and larviform females, on the other hand, develop in the detritus-rich soil or rotten wood (Wong 1996). Collecting all five male specimens of *C. apterus* sp. nov. by sifting suggests that the males of this species remain in the litter, which is unusual for the family. The morphological modifications of *C. apterus* sp. nov., particularly the entire lack of hind wings, suggest limited male dispersal capacity, which in turn would require very stable habitats for continuous species existence. Records of such low-dispersing species normally indicate refugia with uninterrupted presence of stable wet forest habitat during the Pleistocene climate fluctuation (Malohlava and Bocak 2010), such as the North Pare Mountains, a part of the more extensive chain on geologically old East Arc Mountains (Lovett and Wasser 1993). The range of *C. apterus* corresponds with the supposed refugia of East African mountain rain forests and the biodiversity hotspot (Hamilton 1992, Myers *et al*. 2000). Similar range is known for species from the genus *Dexoris* Waterhouse, 1878 (Dexorinae), another Afrotropical net-winged beetle with supposed female neoteny (Bocak and Bocakova 2008).

Classification. The new remarkable species, *C. apterus*, differs markedly in general appearance



Figures 2–8. *Cautires apterus* sp. nov., male: (2) habitus, dorsal view; (3) elytron, dorsal view; (4) male genitalia; (5) mandible; (6) labium; (7) maxilla; (8) pronotum. Scale bar: 0.5 mm (Figs 1–2), 0.1 mm (Figs 3–7).



Figures 9–11. Maps (Figs 9, 10) and landscape image (Fig. 11) showing distribution and approximate habitat of *Cautires apterus* sp. nov. in the North Pare Mountains, Tanzania: (9) East Africa; (10) North Pare Mountains with three sampled forests indicated; (11) view along the longer axis of the North Pare Mountains from the northern end of Kindoroko Forest towards Minja Forest (corresponding in length to the arrow on Fig. 10).

from all Afrotropical net-winged beetles, including those in the genus *Cautires* Waterhouse, 1879. Therefore, we used both molecular and morphological data to infer the relationships of this new taxon. The molecular analysis placed *C. apterus* sp. nov. in the clade of African Metriorrhynchini represented by genera *Cautires* and *Xylobanus*. The results show that the majority of the observed differences correlate with the winglessness and, perhaps, relatively minute body size. Normally *Cautires* and *Xylobanus* males are about

6–17 mm in body length, fully winged and flight capable, with fully developed elytra possessing longitudinal and transverse costae and with seven pronotal areolae (Bocak 2002). The reduction of elytral costae is likely a result of brachelytry. Although two costae of *C. apterus* sp. nov. are more similar to the simple pattern known in the typical *Xylobanus*, we classify the new species in *Cautires* which comprises forms with four and nine costae. Sklenarova *et al.* (2013) showed that *Xylobanus* needs redefinition and that the forms with simple pattern of costae occurring in Africa are not related to *Xylobanus* from South East Asia. We suppose that the winglessness and small body are factors lowering the requirement for rigid body sclerotization. The typical pronotal carinae of *Cautires* form the middle areola, four anterior and two postero-lateral areoles. Although the pronotal carinae might be similarly and occasionally reduced in winged species, the reduction of pronotal keels and elytral costae are more frequently recorded in the small-bodied species such as *C. apterus* sp. nov., as these structures likely have a strengthening function, which is not used when body is miniaturized. The classification of the newly discovered species as a *Cautires* is further based on the shape of male genitalia, which resemble *Cautires* in the following characters: (a.) absence of paramerae, (b.) annuliform phallobase, (c.) internal sac armed with two sickle-like thorns (Fig. 4). *C. apterus* has irregular rough surface in the anterior part of pronotum similarly to *Cautires* and several other Metriorrhynchinae genera such as *Xylobanus* Waterhouse, 1879 (Bocak 2002). The delineation of *Cautires* and *Xylobanus* is complex and needs further study. Most species of *Cautires* are brightly colored and they have usually much larger eyes. The dull coloration and relatively small eyes of *C. apterus* sp. nov. likely result from the cryptic lifestyle of a non-flying forest floor inhabitant.

Correlation of male hind wing aptery and female neoteny. *Cautires apterus* sp. nov. is the first known lycid beetle with hind wings completely lacking. Males of all four recently discovered species of the genus *Alyculus* Kazantsev, 1999 are similar to those of *C. apterus* sp. nov. in having shortened elytra, but the former possess fully developed hind wings and are capable of a weak, slow flight. Although only five specimens of the new species were collected and the females are entirely unknown, their neoteny still can be hypothesized on the basis of repeated correlation between wingless males and larviform females observed in Lycidae and other elateroid beetle families. Lycid males in lineages with neotenic females are regularly smaller similarly to other neotenic lineages (*e.g.* *Platerodrilus* Pic, 1921 and *Lyropaeus* Waterhouse, 1878; see Wong 1996; Palata and Bocak 2012, or *Pseudothilmanus* Pic, 1918; Kundrata and Bocak 2011b). Furthermore, the genus *Alyculus*, the only previously known Lycid beetle with shortened elytra, is also supposed to have neotenic females (Palata and Bocak 2012). The newly described *Cautires* species also resembles other forms with proven neoteny by having a small, slender body and apparently hypognathous mouthparts. All these correlations strongly point to a hypothesis that the females of *C. apterus*, when eventually discovered, will be neotenic.

It should be noted that Lea (1909) described a large-bodied female of *Metriorrhynchus apterus* Lea, 1909 with shortened elytra. The degree of female neotenic modification in this species is much lower and the males are very probably fully winged. The large-bodied neotenic females follow K-strategy and small-bodied males follow r-strategy (Bocak *et al.* 2008). With the larviform females being relatively immobile, the natural selection probably favors the reduction of the flight apparatus of males as well. We observed that males from neotenic lineages, even when capable to flight, remain in the lowest strata of the forest (personal observation on Lycidae: Lyropaeinae in Asia). Therefore, we suppose, that evolution of neoteny in females might also lead to the limited flying ability in males and to the eventual loss of flight capability in some cases, as documented here.

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Evoluce a klasifikace tribu Metriorrhynchini (Insecta: Coleoptera: Lycidae)

AUTOREFERÁT DIZERTAČNÍ PRÁCE

Kateřina Sklenářová

P1527 – Biologie
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Autoreferát dizertační práce

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Název práce: Evoluce a klasifikace tribu Metriorrhynchini (Insecta: Coleoptera: Lycidae)
Školitel: Prof. Ing. Ladislav Bocák, Ph.D.

Obhajoba disertační práce se koná dne .... v .... hod. na učebně č. ………......
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Obsah

Úvod

Lycidae jsou druhově početnou čeledí brouků (Coleoptera) z hlediska evoluce mimetických komplexů nebo evoluce neotenních forem (Bocak *et al.* 2008, Bocak & Yagi 2010). Tato čeleď, v české literatuře občas nazývaná dlouhoústcovití nebo zářivkovití, obsahuje asi 150 rodů s více než 4000 popsanými druhy po celém světě (Kleine 1933, Bocak & Bocakova 1990, Bocak & Bocakova 2008). Největší diverzita je dokumentována v oblastech tropického deštného lesa (Lawrence 1982, Erwin 1982). Naproti tomu se nevyskytují v aridních oblastech a pouze ve velmi malém počtu druhů v temperátních oblastech s výraznou sezonalitou srážek (Bocak & Bocakova 2008). Tato čeleď je v současnosti klasifikována v nadčeledi Elateroidea (Polyphaga: Elateriformia; Lawrence & Newton 1995, Bocakova *et al.* 2007). Lycidae jsou charakterističtí především měkkým, nekompletně sklerotizovaným tělem, sníženou schopností letu a omezenou schopností úniku před predátory. Jako kompenzace tohoto omezení se vyvinula u všech druhů čeledi Lycidae schopnost produkce zapáchajících, slabě jedovatých látek (Moore & Brown 1981). Při vyrušení či podráždění jsou jedinci schopni vylučovat hemolymfu obsahující tyto látky na okrajích a žebrech krovek, na tykadlech nebo na kloubech mezi femurem a tibií, kde dochází k prasknutí mezisegmentových membrán (Bocak *et al.* 2008). Chemická ochrana odrazuje predátory od útoku, rovněž vede k evoluci pestrého aposematického zbarvení (Alatalo & Mappes 1996), které také slouží také varování pro případné predátory (v tomto případě především pavouky a ptáky; Bocak *et al.* 2008). Aposematicky zbarvené druhy mají tendenci vytvářet agregace, které posilují reakci poučeného predátora a prodlužují dobu před opakováním útoku (Linsley *et al.* 1961).

Lycidae byli v posledních letech použiti jako modelová skupina pro studium evoluce neotenie a evoluce aposematických vzorů ve spojení se speciací po rozšíření areálu (Malohlava & Bocak 2010). Tyto studie jsou prvními, kdy Lycidae, jako taxonomicky velmi opomíjená skupina, byli použiti pro studium obecných evolučních otázek. Přitom se ukázalo, jak nedostatečné byly dosavadní znalosti o evoluci této skupiny, jejich historické biogeografii a jak slabě byla podporována současná klasifikace této čeledi. Množství klasifikačních znaků je zde omezeno poměrně značnou variabilitou ve tvaru těla vzhledem k jeho slabé sklerotizaci - některé struktury mohou být významně ovlivněny selekčním tlakem, např. zpevňující žebra. Podobně pohlavní orány jsou často vystaveny silné sexuální selekci a nemůžeme vyloučit rychlý vznik velmi odlišných struktur. Proto preferujeme využití všech dostupných zdrojů informací v souladu s dnešní taxonomickou praxí (Lee *et al.* 2007, Winterton *et al.* 2007).

Tato studie je omezena na tribus Metriorrhynchini, který je z hlediska druhové rozmanitosti nejvýznamnější linií čeledi Lycidae (Lycidae: Lycinae; Bocak & Bocakova 2008). Metriorrhynchini jsou morfologicky definováni kruhovou phallobází samčích kopulačních orgánů, plochou nápadnou nepárovou žlázou v kopulačních orgánech samic, střední areolou kopinatého tvaru na pronotu a většinovou přítomností dalších žeber na pronotu, které vytvářejí postranní areoly. Metriorrhynchini jsou velmi vhodnou modelovou skupinou pro studium vzniku diverzity vzhledem k jejich omezené dispersní schopnosti, uniformní biologii a mimořádně vysokému počtu druhů. Kleine (1933) uvádí v katalogu přes 1000 druhů ve 41 rodech a dalších ~200 druhů bylo popsáno později (Bocak 2002). Největší počet druhů tribu Metriorrhynchini pochází z kontinentální jihovýchodní Asie, Velkých Sund, Filipín, Moluk, Nové Guinee a severní Austrálie (Calder 1998). Další druhy se vyskytují v Subsaharské Africe, na Madagaskaru, na Sri Lance a v Indii (Bocak 2002, Kazantsev 2007, Kazantsev 2012, Kubeček *et al.* 2011).

Do fylogenetických analýz byly zahrnuty všechny základní linie a druhově početné rody reprezentující rozšíření tribu Metriorrhynchini Celkem bylo sekvenováno ~175 druhů pocházejících z pevninské Asie (Indie, Čína, Laos, Malajský poloostrov), z ostrovní JV Asie (Velké a Malé Sundy), Japonska, Filipín, Afriky, Madagaskaru, Austrálie a Nové Guinee. Studovaná oblast zahrnuje bývalý superkontinent Gondwana, který dal vzniknout současné Africe, Jižní Americe, Antarktidě, Austrálii, Indii a Madagaskaru.

Pro izolaci DNA byla použita metathorakální svalovina a zadní pár nohou. Svalová tkáň byla před izolací vysušena ve vakuovém koncentrátoru, homogenizována a inkubována při 60 - 65ºC, dokud nedošlo k rozložení svaloviny. Vzorky se následně několikrát přečišťují a promíchávají (Vogler *et al.* 1993). U vyčištěného roztoku DNA proběhne měření koncentrace DNA na spektrofotometru (nanodrop ND-1000). Poté se DNA nechá v termocykleru amplifikovat. Po ukončení PCR replikace se provádí elektroforéza jako kontrola úspěšné amplifikace cílového produktu a jeho replikace. Sekvenační reakce byly připraveny podle protokolu ABI Applied Biosystems. Vyčištěný sekvenační produkt byl rozpuštěn ve formamidu a analyzován na sekvenátoru ABI 3130. Pro studium tribu Metriorrhynchini jsme použili fylogenetické analýzy založené na sekvencích jaderné DNA (geny 18s, 28s rRNA) a mitochondriální DNA (geny *rrnL*, *cox1*, *nad5*).

Chromatogramy byly analyzovány programem Sequencing Analysis. Editace sekvencí byla prováděna programem Sequencher 4.10.1. Editované sekvence byly včetně outgroup alignovány programy: ClustalW 1.83, ClustalX 2.1, T-coffee 8.95, Mafft v. 7, BlastAlign 1.2 a Muscle 3.6. Ze sekvencí byly sestaveny kompletní matice, které byly znovu kontrolovány v programu Se-Al. K fylogenetické analýze byly použity optimalizační kritéria: metoda maximální parsimonie (MP, program TNT 1.1), metoda maximální pravděpodobnosti (maximum likelihood, ML, program RAxML 7.2.5) a bayesiánská analýza (BA, program MrBayes 3.2.1.). Pro kontrolu a vizualizaci výsledných kladogramů a fylogramů byly použity programy Tracer 1.5, FigTree v. 1.3.1 a PAUP*. Za účelem datování vzniku jednotlivých linií jsme použili program Beast 1.6.1. Analýza geografických dat proběhla v programu BayesTraits 2.0. Program RASP 2.1 umožnil mapování rozšíření ancestrálních znaků a rekonstrukci vývoje vybraných morfologických znaků.

V rámci této práce byla studována také morfologie sekvenovaných exemplářů. Dokladové exempláře byly po odebrání svalové tkáně pro izolaci DNA preparovány tradičním způsobem a uloženy ve sbírce laboratoře molekulární systematiky katedry zoologie, PřF UP v Olomouci. Vybrané exempláře byly změkčeny v nízko koncentrovaném alkoholu, měkké tkáně obalující kopulační orgány a orgány v hlavové schránce byly odstraněny v horkém hydroxidu draselném. Jednotlivé části byly odděleny pod preparačním mikroskopem. Byla pořízena fotodokumentace, fotografie se staly základem pro vytvoření perových ilustrací, nebo byly po upravení použity přímo v publikacích.

Cíle práce

Studie je založena především na molekulárních datech a na jejich základě vytvořených fylogenetických hypotéz.

Cílem práce je rekonstrukce ancestrálních území v oblasti Gondwany a šíření tribu Metriorrhynchini do jihovýchodní Asie, Afriky a na Madagaskar a jejich srovnání s dosavadními znalostmi o rozpadu Gondwany.

Klasifikace tribu Metriorrhynchini prodělala v posledních dekádách několik změn. Dříve byla tato skupina klasifikována v samostatných tribech nebo podčeledích (Kleine 1933), později byly tyto taxony spojeny do jediného taxonu Metriorrhynchinae/ini (Bocak & Bocakova 1990, Bocak 2002). Podle předchozích studií byl tribus Metriorrhynchini rozdělen na subtriby: Trichalina Kleine, 1928, Hemiconderina Bocak & Bocakova, 1990 a Metriorrhynchina Kleine, 1926 (Bocak, 2002). Dalším cílem této studie je proto také kritická revize vyšší klasifikace všech taxonů tohoto tribu prostřednictvím fylogenetické analýzy založené na kompletní sadě morfologických znaků.

Shrnutí hlavních výsledků

Současné rozložení tribu Metriorrhynchini je výsledkem pohybu kontinentálních fragmentů po rozpadu Gondwany. Metriorrhynchini se skládají ze dvou linií: afro/orientální a australské. Ancestrální území pro bazální linii celého tribu pochází buď z Austrálie, nebo Indie, ale nelze jej s jistotou určit, protože k rozštěpení linie došlo v době, kdy se Gondwana začala rozdělovat. Afrotropická fauna a fauna Madagaskaru vznikla disperzí z Indie driftující na sever před 65 až 62 miliony lety. Orientální fauna se diverzifikovala po srážce Indie s Asií a teprve později byla doplněna o linie migrující z australské oblasti, která se dostala do kontaktu s asijským šelfem před asi 25 miliony lety. Orientální fauna obsahuje rody indické i australské linie, přičemž analýzy datování prokázaly starší původ indických rodů.

Klasifikace tribu Metriorrhynchini je navržena na základě molekulární fylogeneze skupiny a je podrobně diskutována evoluce vybraných morfologických struktur, na kterých byla založena předešlá morfologická klasifikace. Výsledky potvrzují několikanásobný vznik podobných struktur žeber na pronotu, zkrácených žeber na krovkách, sekundárních žeber na krovkách u více linií a jejich opakovanou ztrátu a mnohonásobný vznik flabelátních tykadel. Na základě zhodnocení morfologie larev, dospělců a molekulární fylogeneze je navržena nová klasifikace. Byly nově ustanoveny subtriby Metriorrhynchina, Metanoeina a Cautirina a subtriby Trichalina a Hemiconderina byly synonymizovány se subtribem Metriorrhynchina.

V poslední studii je popsán nový druh *Cautires apterus* Bocak *et al.* 2014, který je unikátní svou bezkřídlostí, životem v hrabance a faktem, že samice tohoto druhu jsou pravděpodobně larviformní. Jedná se o první potvrzený případ evoluce neotenie v tribu Metriorrhynchini.

Abstrakty prací

Sklenarova K., Chesters D. & Bocak L. (2013) Phylogeography of Poorly Dispersing Net-Winged Beetles: A Role of Drifting India in the Origin of Afrotropical and Oriental Fauna. PLoS ONE 8(6): e67957. doi:10.1371/journal.pone.0067957.

Ancient dispersal history may be obscured by subsequent dispersal events. Therefore, we intend to investigate the biogeography of metriorrhynchine net-winged beetles, a group characterized by limited dispersal propensity. We used DNA data to construct phylogenies

and the BayesTraits and RASP programs to identify putative ancestral areas. Further, we inferred ultrametric trees to estimate the ages of selected nodes. The time frame is inferred from tectonic calibrations and the general mutation rate of the mitochondrial genes. Metriorrhynchini consists of two lineages with Afro/Oriental and Australian distributions. The basal lineages originated in Eastern Gondwana after the split of Australia, India and Madagascar; the Afrotropical and Madagascar Metriorrhynchini separated from the Oriental clades 65 and 62 mya. Several already diversified lineages colonized continental Asia 55–35 mya. A few genera of the Australian clade dispersed to the Oriental region 5–15 mya and reached Eastern India and Southern China. Only Xylobanus crossed the Makassar Strait to Sulawesi and does not occur further to the east. The current distribution of Metriorrhynchini is a result of drifting on continental fragments and over-sea dispersal events limited to a few hundreds of kilometers. We conclude that: (1) Afrotropical and Madagascar lineages originated independently from dispersal events during India’s drift to the north and the Mozambique Channel completely isolates the respective faunas since then; (2) Oriental fauna is a recently established mixture of the Indian and Australian lineages, with predominance of the older Indian clades; (3) The fauna of islands located north of Australia colonized Sulawesi after collision with the Sundaland margin and the species rich Australian lineages did not reach Western Wallacea or the Philippines. Our results suggest an impact of subtle differences in biological characteristics on biogeographic history of individual lineages, when mostly lowland and flower-visiting lineages were able to disperse across sea channels.

Sklenarova K., Kubecek V. & Bocak L. (2014) Subtribal classification of Metriorrhynchini (Insecta: Coleoptera: Lycidae): an integrative approach using molecular phylogeny and morphology of adults and larvae. Arthropod Systematics and Phylogeny 72 (1): 37-54.

The classification of Metriorrhynchini, the most diverse lineage of net-winged beetles (Lycidae) containing ~ 1400 species, is revised on the basis of three-marker molecular phylogeny of 175 ingroup taxa, and the adult and larval morphology. The study uses the molecular phylogeny for identification of major lineages and critically considers morphology when adult morphology and sparse information of immature stages alone did not provide enough information for building a robust classification. Reconstruction of the ancestral states of morphological characters on the phylogenetic tree recovered from DNA data presents evidence for multiple origins of the four-costae pattern on the elytra, shortened elytral costa 1, patterns of pronotal areolae and flabellate antennae. As a consequence, revised morphological delineations of the subtribes and genera are proposed: three major lineages are defined as Metriorrhynchina Kleine, 1926, Metanoeina subtrib. nov. and Cautirina subtrib. nov. The subtribes Trichalina Kleine, 1928 and Hemiconderina Bocak & Bocakova, 1990 are synonymized with Metriorrhynchina Kleine, 1926. Metanoeina are studied in detail and three genera are placed in the subtribe: *Metanoeus* Waterhouse, 1879, *Xylometanoeus* gen. nov., and *Matsudanoeus* gen. nov., with *Xylometanoeus japonicus* (Bourgeois, 1902) comb. nov. and *Matsudanoeus yuasai* (Nakane, 1969), comb. nov. as type species, respectively. *Xylobanus basivittatus* Nakane, 1970 is transferred to *Xylometanoeus*. The concepts of genera *Cautires* and *Xylobanus* are based on male and female genitalia. Additionally, the molecular hypothesis is supported by morphology of larvae, when newly proposed Cautirina are characterized by entire tergites in contrast to the longitudinally divided mesoand metathoracic tergites of Metanoeina and Metriorrhynchina. Larval characters support the placement of *Xylometanoeus* in Metanoeina and the close relationships of *Matsudanoeus* and *Metanoeus*. The simultaneous consideration of DNA-based phylogeny and morphology of adults and larvae rejects taxa based on diagnostically usable but strongly homoplastic characters and provides a framework for a robust classification of Metriorrhynchini.

Bocak L., Grebennikov V.V. & Sklenarova K. (2014) *Cautires apterus*, a new species and the first record of winless male Lycidae (Coleoptera) discovered in the north Pare Mountains, Tanzania. Annales Zoologici (Warszawa) 64(1): 1-7.

We report the first know case of male hind-wing aptery among the net-winged beetles (Lycidae). Five male specimens of *Cautires apterus* sp. nov. were discovered by sifting forest litter in a very small (approximately 300 meters by 50 meters) Kamwala Forest within the North Pare Mountains, Tanzania. Besides being wingless, males of *C. apterus* sp. nov. have remarkably small body, shortened elytra and reduced pronotal and elytral costae. Generic assignment of this externally unusual new species is based on the molecular phylogenetic analysis, the structure of the male genitalia and the shape of the pronotum. We discuss biological and evolutionary significance of the discovery and suggest that the winglessness of male lycid beetles correlates with the female neoteny.

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Summary

Lycidae, net-winged beetles, are an abundant family of terrestrial beetles (Coleoptera), which contains about 150 genera with more than formally described 4,000 species worldwide. The greatest diversity is documented in tropical rain forests, while moderate diversity is also known from temperate zones; these beetles do not occur in arid regions. Lycidae are a prominent lineage concerning species richness, evolution mimetic complexes or neotenic modifications of development. This family is currently classified in the superfamily Elateroidea (Polyphaga: Elateriformia). Lycidae is characterized by soft-bodiedness and limited an ability to fly resulting from this trait. As compensation of this limitation they have developed the ability to produce repellent and slightly poisonous compounds, which are released as droplets of hemolymph on the elytra and around inter-segmental joints when the animals are disturbed. Chemical protection of the family Lycidae led to evolution of bright, aposematic coloration patterns.

This study is restricted to the tribe Metriorrhynchini, which are morphologically defined by circular phallobase, flat conspicuous unpaired vaginal gland, pronotum with lanceolate median areola and with carinae which form up to seven areolae in the pronotum. This tribe is the most species rich lineage of Lycidae. Due to their species-richness (over 1200 species in 41 genera), large geographic range, limited ability to disperse and uniform life strategy, Metriorrhynchini can be a suitable model group for studies on diversity, dispersal history and evolution of mimicry. Highest specific and generic diversity of Metriorrhynchini species is known from continental Southeast Asia, the Great Sundas, Philippines, Moluccas, New Guinea and northern Australia. Other species and a few endemic genera, occur in Sub-Saharan Africa, Madagascar, Sri Lanka and India. The previous knowledge of zoogeography of this group has been very limited and no phylogeographic hypothesis has been available for the identification of putative areas of origin or directions of dispersal. We used nuclear and mitochondrial DNA markers to propose phylogenetic and phylogeographic hypotheses.

The principal aim of this thesis was to reconstruct the putative area and time of origin of the tribe Metriorrhynchini and to recover the dispersal routes from the ancestral region around the Indian Ocean rim, i.e., Australia, Southeast Asia, Africa and Madagascar. Here, the phylogenetic hypothesis was considered in the light of the reconstructed break-up of Gondwana. Metriorrhynchini consist of two lineages: the Afro/Oriental and Australian ones. The basal lineage originated from Australia and India, from the time when Gondwana started to break-up. The Afrotropical fauna and fauna of Madagascar came from India drifting to the

north about 65 to 62 million years ago. The Oriental fauna diversified from lineages from Indian subcontinent after the collision of India and Asia. Later the Oriental fauna was enriched by migrating lineages from the Australian region about 25 million years ago. Although the Oriental fauna includes the genera from both the Indian and Australian clades, the Indian lineages are more abundant and the analyses showed their older diversification history in the Asian continent.

The secondary aim of this study is the critical revision of classification of all taxa (genus and subtribes) of this tribe. We used the same phylogenetic hypothesis to map the evolution of morphological characters on the tree and we inferred their origin and subsequent modifications. The previous morphological classification was based on the structure of pronotal carinae, elytral costae and the form of antennae. All these characters were mapped with multiple origins of structures and some of them multiple reversals. We hypothesized that evolution of some of these characters is affected by their selective value (the strengthening function of carinae and costae), they originated multiple times when their evolution is correlated with stenoptery (the shortened elytral costa 1) or they evolve when the opposite sex is located using olfactory organs (the flabellate antennae). Using the molecular phylogeny, we evaluated the morphology of larvae and adults; we proposed a new classification, with some taxa defined predominantly with either larval or adult characters. We delimited the subtribes Metriorrhynchina, Metanoeina and Cautirina and we synonymized the subtribes Trichalina and Hemiconderina with the subtribe Metriorrhynchina.

Seznam publikovaných prací
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Sklenarova K., Chesters D. & Bocak L. (2013) Phylogeography of Poorly Dispersing Net-Winged Beetles: A Role of Drifting India in the Origin of Afrotropical and Oriental Fauna. PLoS ONE 8(6): e67957, doi:10.1371/journal.pone.0067957 (IF = 3.730; 7/56).

Sklenarova K., Kubecek V. & Bocak L. (2014) Subtribal classification of Metriorrhynchini (Insecta: Coleoptera: Lycidae) : an integrative approach using molecular phylogeny and morphology of adults and larvae. Arthropod Systematics and Phylogeny 72 (1): 37-54. (IF = 2.318 (Entomologie 9/87).

Bocak L., Grebennikov V. V. & Sklenarova K (2014) *Cautires apterus,* a new species and the first record of wingless male Lycidae (Coleoptera) discovered in the north Pare Mountain, Tanzania. Annales Zoologici 64 (1): 1-7. (IF = 0.660; 114/151).

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Pedagogická činnost
Podíl na výuce: ZOO/OBZO Obecná zoologie
ZOO/SBP Fylogeneze a systém bezobratlých
ZOO/CTP Zoologická cvičení v terénu
BOT/EUBEX Evropská centra biodiverzity (exkurze)
ZOO/KTEN Kurz terénní entomologie
ZOO/BIKZ Biologická klasifikace živočichů
Oponentské posudky bakalářských prací (1x)