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MOLECULAR PHYLOGENY OF THE SUPERFAMILY TENEBRIONOIDEA (COLEOPTERA: CUCUJIFORMIA)

Ph.D. Thesis

P1501 Zoology

Supervisor: Prof. Ing. Ladislav Bocák, Ph.D.

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I undersigned Zuzana Levkaničová declare to have written this Ph.D. thesis alone during 2004-2009 in the Department of Zoology and Anthropology under the supervision of Prof. Ing. Ladislav Bocák, Ph.D. and used the references enclosed in the Ph.D. thesis.

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Abstract: The phylogenetic relationships of the superfamily Tenebrionoidea are investigated here for the first time. The Tenebrionoidea (darkling beetles) is superfamily of speciose rich and complex series Cucujiformia, that is considered as the most derived among the Coloeptera. The Tenebrionoidea itself is very diverse group and contain approximately 30.000 species classified in 30 families. It has been recognized as a relative to the Cucujoidea superfamily, however the position within the Cucujiformia has not been stabilized yet. The intrarelationships of the Tenebrionoidea are also poorly known, since only the studies on either generic or subfamilial level have been published. Here, two nuclear genes SSU and LSU rDNA and two mitochondrial genes rrnL rDNA and cox1 mtDNA of total length approximately 3700 bp were used to reveal the phylogeny of this puzzling group. There were sampled 154 taxa representing 20 families of the superfamily. Both, static and dynamic multiple alignments of combined dataset were performed, followed by the analyses of maximum parsimony and maximum likelihood and bayesian analysis. They confirm the monophyly of the group, proposing its closer relationship to the Lymexyloidea than it has been recognized before. Within the superfamily, four clades of families have been established- tenebrionid, melandryinid, ripiphorid-mordellid-meloid, and scraptiid-pyrochroid. The monophyly of most of families has been confirmed as well, except the families Salpingidae, Pyrochroidae, Anthicidae that have been found paraphyletic and the families Tetratomidae, Melandryidae and Zopheridae found polyphyletic. The paraphyletic status would be changed to monophyletic if certain taxa as Ischaliinae and Eurygeniinae (Anthicidae), Agnathinae (Pyrochroidae), Othniinae (Salpingidae) are excluded out of the family. The polyphyletic families should be revised considering their division in smaller units. There are the high degree of homoplasy and the complexity of the group found as reasons of unsatisfyingly resolved phylogeny of the group. More comprehensive and extensive studies, that would involve both molecular and morphological characters, inclusion of all families as well as of members of the Cucujiformia series and more extensive analyses, will be needed to recognize true relationships within the Tenebrionoidea.

Keywords: Alignment, phylogeny, rDNA, mtDNA, systematics, taxonomy, Tenebrionoidea.

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Contents

1. Introduction.

The Tenebrionoidea, formerly known as Heteromera, is a speciose, morphologically and ecologically heterogenous superfamily of polyphagan beetles. It is placed within the Cucujiformia series. Tenebrionoidea contain approximately 30 000 species classified in 30 families and 71 subfamilies (Lawrence & Newton, 1995). Generally known large families are Tenebrionidae (darkling beetles) and Meloidae (blister beetles). Other species rich families are Anthicidae, Mordellidae, Oedemeridae, Zopheridae and Aderidae, while other families include only one or a few genera.

Traditionally, Tenebrionoidea have been accepted as a lineage within Cucujiformia. The suborder Polyphaga, where they are placed, may have originated ca 270 Mya, the Cucujiformia ca 236 Mya, and the Tenebrionoidea in the Late Triassic according to Hunt *et al.* (2007). The origin of the Meloidae has been determined by the fossil record to an Early Cretaceous period (125–135 Mya), the period of flowering plant radiation (Bologna *et al.*, 2008).

The Heteromera, as a separated section, were for the first time distinguished in the beetle system by P. A. Latreille (1803), the first entomologist, who divided the Coleoptera in supergeneric taxa, based on the tarsal segmentation. Since, Heteromera have been recognized in every classification, though in different positions. Lameere placed it in 1900 in suborder Cantharidiformia; Kolbe, in 1901, found Heteromera in suborder Heterophaga; in 1903 Ganglbauer similarly put it in suborder Polyphaga. All these authors left families Mycetophagidae, Ciidae, Colydiidae either in Clavicornia or Diversicornia section. This trend continued in the beginning of the 20th century. All classifications, including those by Sharp and Muir's (1912) based on the male genital tube, Forbes's (1926) based on the wing venation and wing folding patterns and Poll's (1932) based on the structure of the Malpighian tubules, found a separated superfamily Heteromera. However Sharp and Muir (1912) admitted only few families allied to Tenebrionidae to be a part of the Tenebrionoidea and they placed all remaining families in the Cucujoidea. Böving and Craighead' study of larval types, (Böving & Craighead, 1931), united the Heteromera and Clavicornia in a single superfamily Cucujoidea and they elevated the family Mordellidae to the separated superfamily Mordelloidea and families Meloidae and Rhipiphoridae to the superfamily Meloidea at a coordinated taxon with Cucujoidea. The Peyerimhoff's classification (Peyerimhoff, 1933) merged Heteromera with Cucujoidea, but the cryptonephridial groups were placed in the end

of the system, as the most derived ones. Later, Jeannel and Paulian (1944) published the classification based on structure of the aedeagus and other abdominal features and they established tenebrionoids as the division Heteromeroidea of suborder Heterogastra independently of the division Cucujoidea. They discriminated four sections of Heteromeroidea: Lyttaria, Tenebrionaria, Mordellaria and Oedemeraria.

Crowson's (1955) detailed morphological study of both larvae and adults kept the superfamily Cucujoidea with two recognized sections, Clavicornia and Heteromera. He did not find the differences between them enough substantial to define both of them as superfamilies. According to Crowson, Heteromera arose from primitive Clavicorn types and were the most difficult section to divide into well-characterised families. In this study, he established several new families- Merycidae, Pterogeniidae, elevated several other to family rank: Boridae, Elacatidae, Mycteridae, Inopeplidae and Tetratomidae, the families like Mycetophagidae, Colydiidae, Inopeplidae and Hemipeplidae were transferred from Clavicornia to Heteromera. Although Crowson (1960) mentioned a possibility to establish two or more superfamilies, corresponding with Clavicornia and Heteromera sections, he finally decided to retain a single superfamily because of the unresolved complexity of relationships between families. Crowson (1960) also suggested, that the families Byturidae and Bihpyllidae could be transferred from Clavicornia to Heteromera. Later, Crowson (1966) recognized family Synchroidae and discussed presumable phylogeny of the group. He tentatively proposed a common ancestor of Heteromera, that resembles the family Tetratomidae, both in larval and adult features. As direct descendants were proposed the families Tetratomidae and Mycetophagidae and perhaps Pterogeniidae-Ciidae. The second possible ancestor arose from a tetratomid-like ancestor and might have larval characters like the Zopheridae and adult characters like *Synchroa* and *Stenotrachelus*. From this ancestor might be derived (1) the aderid-anthicid-meloid line, (2) a line leading via Pythidae and Pyrochroidae to Salpingidae, Mycteridae, Boridae and Inopeplidae, (3) a line leading via Synchroid and Zopherid-like forms to Merycidae and Monommidae and Colydiidae and perhaps to true Zopheridae and the Tenebrionid groups of families, and (4) a line leading to Melandryidae and Mordellidae-Rhipiphoridae and including also Scraptiidae. Crowson (1967) moved Prostomidae from Clavicornia to Heteromera. The idea of a more derived Heteromera than primitive Clavicornia section presented Abdullah (1973), who emphasized the heteromeroid aedeagus as a character defining the clade Tenebrionoidea.

Lawrence and Newton (1982) supposed an ancestor, that combines the features of families Tetratomidae and Mycetophagidae and they considered these two families to be the most

primitive ones. Archeocrypticidae, Pterogeniidae and probably Ciidae were supposed to arose directly from this ancestor. It might be followed by a lineage of (1) Tetratomidae, Melandryidae, Mordellidae, Rhipiphoridae, (2) a lineage of Synchroidae, Zopheridae, Prostomidae, Colydiidae, Monommidae, Perimylopidae, Chalcodryidae, Tenebrionidae, (3) a lineage of Oedemeridae, Cephaloidae, Meloidae, (4) a lineage of Pythidae, Pyrochroidae, Pedilidae, Boridae, Mycteridae, Salpingidae, Inopeplidae, Othniidae, (5) and a lineage of Anthicidae, Euglenidae, Scraptiidae, though hesitating with the inclusion of Scraptiidae. In opposition to Crowson's hypothetized tenebrionoids' phylogeny stands an opinion of Mamaev (1973), who has suggested that Heteromera might have arisen polyphyletically and had had a number of the ancestral forms. Iablokoff-Khnzorian (1983) placed the families of Tenebrionoidea within the superfamily Cucujoidea and he divided them on the basis of the structure of male genitalia in four sections- section Hétéromères (Tenebrionidae, Trictenotomidae, Pythidae, Pyrochroidae, Oedemeridae, Cephaloidae, Anthicidae, Aderidae, Meloidae), Colydiomorphes (Rhipiphoridae, Mordellidae, Scraptiidae, Melandryidae, Tetratomidae, Mycetophagidae, Colydiidae), Lathridiomorphes (Lathridiidae, Prostomidae) and Clavicornes. He found classification of the Cucujoidea confused, nevertheless section Hétéromères was considered to be homogeneous and isolated for a long time (Lawrence, Ślipiński and Pakaluk, 1995).

Although Lawrence and Newton (1995) expressed their opinion about a well-limited superfamily Tenebrionoidea, the question about a monophyly of the superfamily has been re-opened by several authors. The monophyly was disputed by Iablokoff-Khnzorian (1983) (see above); Schunger *et al*. (2003) pointed to the absence of autapomorphies inferred from a comprehensive cladistic analysis. Hunt *et al*. (2007) published analyses suggesting polyphyletic Lymexyloidea, that were either nested at the base of Tenebrionoidea forming both together a monophyletic group or found to be closely related to Tenebrionoidea.

On the other hand, Beutel and Friedrich (2005), in their study on larval characters, found Tenebrionoidea monophyletic and well supported as a clade by several larval autapomorphies. As possible synapomorphies, they proposed a posteriorly diverging gula with well developed gular ridges, anteriorly shifted posterior tentorial arms, asymmetric mandibles, the absence or vestigial condition of musculus craniocardinalis and the subdivision of musculus tentoriopharyngalis posterior into several bundles arising from the gular ridges. One potential clade, resulting from their cladistic analysis, suggests the sister-group relationship between Synchroidae and a clade consisting of the salpingid (Pyrochroidae, Salpingidae, Trictenotomidae, Pythidae, Mycteridae, Boridae) and scraptiid (Scraptiidae, Aderidae,

Anthicidae) lineage and Prostomidae. This clade is supported by a distinctly prognathous head and a pad-like maxillary articulating area as synapormophies.

The studies, concerning other cucujiform groups, have not achieved a resolution of the relationships and usually found the Cucujoidea paraphyletic in regards to Tenebrionoidea or Tenebrionoidea and Cleroidea (Hunt *et al.*, 2007; Marvaldi *et al.*, 2009). Buder *et al.* (2008) found as the most basal clades of the cucujoid-tenebrionoid assemblage two cucujoid families, the Silvanidae and Sphindidae, followed by either the monophyletic Ciidae or the Ciidae with the cucujoid Nitidulidae in one monophyletic group. Their study determined families Tenebrionidae, Salpingidae, Zopheridae, Mordellidae, Anthicidae and Tetratomidae plus the cucujoid Monotomidae as the more derived families within the cucujoid-tenebrionoid clade. However, the relationships between them were not resolved except a clade consisting of Tetratomidae, Anthicidae and Monotomidae, that was the only one of tenebrionoids' clade found monophyletic and supported.

The paraphyletic Cucujoidea in respect to the Tenebrionoidea was also suggested by Robertson *et al.* (2004, 2008), in whose analyses of the cerylonid series (2008), a clade of the tenebrionoid taxa, *Bitoma* sp. (Zopheridae), *Cis* sp. (Ciidae) and *Eleodes* sp. (Tenebrionidae), was found in the sister group position to the cerylonid series inside the Cucujoidea. Beutel and Ślipiński (2001) found a weak support for a possible monophyletic group of Cleroidea, Cucujoidea and Tenebrionoidea, with potential synapomorphies as absence of musculus tentoriopraementalis inferior and presence of a short prepharyngeal tube.

The intrarelationships within the superfamily are also not well established (Ślipiński & Lawrence, 1999) and have not yet been seriously studied. Mostly studies dealing with subfamilies, tribes of genera have been published (Bologna & Pinto, 2001, 2002; Bologna *et al.*, 2008; Buder *et al.*, 2008; Burckhardt & Löbl, 1992; Lawrence, 1994 a, b; Lawrence & Pollock, 1994; Nikitsky, 1998; Park & Ahn, 2005; Pollock, 1994, 1995; Schunger *et al.*,

2. Aims of the Ph.D. thesis.

1. Confirmation of the monophyly of the superfamily Tenebrionoidea
2. Recognition and discussion of the relationships within the superfamily
3. Testing of the relationship of the Ripiphoridae to the families Mordellidae, Scraptiidae and Meloidae and the evolution of hypermetaboly within the group

3. Literature review.

3.1 The superfamily Tenebrionoidea Latreille, 1802.

Morphology. The most characteristic feature of the superfamily, that gives an older name to the group, is the heteromerous tarsi, i.e. 5-5-4 tarsomeres in both sexes. Tenebrionoids never have 5-5-5 tarsal formula, sometimes number of tarsomeres may be reduced to 4-4-4, 3-3-3 or 3-4-4 in males. The second significant feature is the tenebrionoid type of male genitalia, whose tegmen is lying either dorsal or ventral, but never completely surrounding the phallus and it forms an incomplete sheath. The characteristic larval features are the mandibles with often asymmetrical molae and without prostheca. Although there are available only few generally valid diagnostic characters, Tenebrionoidea can be distinguished from other beetle lineages as follows:

Adults. Variable in size, 1-80 mm. Eyes often emarginate; antennae usually 11-segmented, variable in shape, seldom clavicorn, antennal insertions often concealed; apically enlarged terminal maxillary palpomeres. Cervical sclerites reduced or absent. Procoxae often conical and projecting, sometimes with long internal extension; protrochantin commonly reduced and concealed; trochanterofemoral joint usually strongly oblique, with femur adjacent coxa (heteromeroid type); empodium indistinctive or absent. Hind wing with maximum 4 veins in medial field. Abdomen with 5 ventrites, 2 or 3 basal connate, without residue of 2^{nd} sternite; 9^{th} segment in male usually reduced to ring-like structure with anterior strut. Aedeagus of incomplete sheath type with tegmen above penis (reversed in some groups) and without anterior strut. Parameres partly or completely fused, sometimes with a pair of articulated processes (Lawrence & Britton, 1991; Lawrence *et al.*, 1999).

Larvae: Elongate and parallel-sided, seldom short and broad; vestiture generally consisting of simple setae. Head with distinct epicranial stem and V-shaped or lyriform frontal arms; frontoclypeal suture absent or distinct; less than 6 stemmata on each side; mandibles asymmetrical and molae often irregularly concave and convex, without prostheca; ventral mouthparts generally retracted; blunt mala. Pretarsal claw mainly bisetose; 9^{th} tergum usually with a pair of fixed urogomphi; 9^{th} sternum sometimes reduced and often with single pair or rows of asperities at base; 10^{th} segment usually transverse; spiracles often annular (Lawrence, 1991).

Bionomics. The members of the superfamily demonstrate various types of diet. Most of them are fungivorous, xylophagous and saprophagous, but there are not missing predators, phytophagous agriculture pests or pests of stored products. A few species are parasitoids.

3.2 The families of the superfamily Tenebrionoidea.

The classification used here follows Lawrence and Newton's (1995) classification (Supplementary material B) with exceptions, that conclude the latest contributions by the other authors. Among these changes are the transfer of subfamilies Hallomeninae and Eustrophinae from family Melandryidae to Tetratomidae (Nikitsky, 1998) and the change of status of families Colydiidae and Monommidae to subfamilies of the family Zopheridae (Ślipiński & Lawrence, 1999).

Mycetophagidae Leach 1815- the hairy fungus beetles.

Morphology. Body oblong to ovate, flattened, pubescent, in small size 1.0-6.5 mm; colour brown to black with yellow or red maculae. Head short, moderately deflexed; antennae with 11 antennomeres, forming an apical loose club; compund eyes relatively large and coarsely faceted; maxillae with separated galeae and laciniae. Pronotum broader than head, sides distinctly margined; tibiae slender, with spurs well developed and serrate, tarsal formula 4-4-4 (females) or 3-4-4 (males) (Young, 2002).

Mycetophagid larvae are elongate, parallel-sided, slightly flattened, up to 8 mmm, usually brown or yellow. Head prognathous, antennae 3-segmented, with segment 2 much longer than 1, mandibles asymmetrical, 4 or 5 stemmata on each side. Legs moderately long; urogomphi simple, slightly upturned (Lawrence, 1991).

Bionomics. Mycetophagidae members are primarily fungivorous, with both larvae and adults feeding on spores or fruiting bodies of various fungi. They can be found associated in fungi-infested leaf litter or wood, most frequently under fungus-grown bark. *Berginus* feeds on pollen and the Chilean genus *Filicivora* on the spores of ferns (Young, 2002).

Classification. The family is distributed worldwide, with approximately 200 species in 18 genera (Young, 2002). Three subfamilies are recognized: Esarcinae (with a single genus *Esarcus* from southern Europe and northern Africa), Bergininae (with a single holoarctic genus *Berginus*) and Mycetophaginae (includes all remaining genera) (Lawrence & Newton, 1995). Mycetophagidae are a well-defined family although it used to be, thanks to the reduced tarsal formula, traditionally placed in Clavicornia (=Cucujoidea). Crowson (1955) moved the

family in the superfamily Tenebrionoidea, finding a basal position together with families Archeocrypticidae, Pterogeniidae, Ciidae and Tetratomidae. Crowson (1966) proposed initial uprise of mycetophagids from a heteromeran ancestral type.

Archeocrypticidae Kaszab 1964- the archaeocryptic beetles.

Morphology. Body hard, elongate-oval to oval; 1.5-3.7 mm in size; brown to black, finely pubescent. Head with distinct frontoclypeal suture; antennae with 11 antennomeres, apical segments forming club; eyes coarsely faceted; mandibles short, bidentate, pubescent prostheca; last maxilar palpomere enlarged. Pronotum as wide as elytra, sides margined; prothoracic intercoxal process extended laterally, procoxal cavities closed, mesocoxal open; legs moderately long, femora and tibiae slender; tarsal formula 5-5-4, rarely 4-4-4, tarsomeres and tarsal claws simple; elytra with fine to coarse rows of punctures. First abdominal sterna connate; aedeagus of the tenebrionoid type, with an unusual sclerotized seminal pump (Young, 2002).

Larvae elongate, parallel-sided, slightly flattened, 2-6mm in length. Head protracted, moderately broad; epicranial stem short, frontal arms lyriform, median endocarina absent; 5 stemmata on each side; antennae 3-segmented, with short, conical sensorium; frontoclypeal suture present; mandibles asymmetrical, bi- or tridentate, with well-developed mola, prostheca absent. Legs well-developed. Tergum A9 with a pair of urogomphi, well separated at base and acute at apex (Lawrence, 1991).

Bionomics. Archeocrypticids are generally found in leaf litter or in other decaying plant material, considered to be saprophagous. Some species feed in the fruiting bodies of Polyporaceae (Lawrence, 1991).

Classification. The family includes approximately 10 genera with 50 species largely pantropically distributed. The family is well defined and it can be easily distinguished from other tenebrionoid families by many adult autapomorphies (Lawrence, 1994a). In the past, archeocrypticids used to be included in the family Tenebrionidae as a tribe until they were elevated by Watt (1974a) to the family level. Archeocrypticids are considered to belong among primitive tenebrionoids and are closer related to Mycetophagidae and Pterogeniidae (Lawrence, 1977; Lawrence, 1991; Lawrence & Newton, 1982). Resemblance of archeocrypticid larvae to the mycetophagid ones is superficial due to their common habitat and feeding preferences (Lawrence, 1991).

Pterogeniidae Crowson 1953.

Morphology. Body oval to oblong, pubescent; 1.5-3.5mm in length. Head globular, without neck; eyes coarsely faceted; 11-segmented, with first segment long, with gradual club; mandibles with broad base, hairy prostheca, extensive mola; apical maxillary palpomere securiform. Prothorax strongly transverse; procoxal cavities open externally; tarsal formula 5-5-4 (Lawrence, 1977). Sexual dimorphism with laterally expanded head (*Pterogenius*) or apically expanded scapes (*Histanocerus*) in males (Burckhardt & Löbl, 1992).

Larvae are elongate, subcylindrical, lightly sclerotized, vestiture of long, simple setae. Head subquadrate, slightly flattened, with a long epicranial stem, flexed to the left, with lyriform frontal arms; 4 or 5 stemmata on each side, antennae 3-segmented, with sensorium as long or longer than 3rd segment; mandibles highly asymmetrical, with large, ridged molae, no prostheca; ventral mouthparts retracted, 3-segmented maxillary palpi, 2-segmented labial palpi. Legs close together. Tergum A9 with a pair of strongly upturned urogomphi, simple or bifurcate (Lawrence, 1991).

Bionomics. Pterogeniids are mycophagous, boring in fruiting bodies of Polyporaceae (Lawrence, 1991).

Classification. The family includes 24 species in five genera, limited to the Indo-Australian region (Burckhardt & Löbl, 1992). Crowson (1955) placed genera *Histanocerus* and *Pterogenius* in Pterogeniidae within heteromerous Cucujoidea. They are considered, together with ciids and archeocrypticiids, to be direct offshoots of a tenebrionoids ancestor (Crowson, 1966; Lawrence & Newton, 1982). The family is believed to belong to an assemblage of the primitive tenebrionoids families, closely related to Archeocrypticidae and both families may have affinities to Ciidae, Tetratomidae and Mycetophagidae (Lawrence, 1977, 1991).

Ciidae Leach in Samouelle1819- the minute tree-fungus beetles.

= Cissides, Cioidae, Orophyidae, Octotemnidae

Morphology. Ovate to elongate, convex to flattened, minute sized body with 0.5-6.0mm, glabrous. Head deflexed, with distinct frontoclypeal suture; antennae 8-10-segmented with the 2- or 3-segmented club that always bears several sensoria; eyes well- developed, prominent. Males may have horns, plates or tubercles on the head and pronotum. Pronotum as wide as the elytra; tarsal formula 4-4-4 or 3-3-3 sometimes, mesocoxae not closed by sterna laterally, elytra without punctate striae. The males with a pubescent fovea in the middle of the first ventrite and the aedeagus with an articulated phallobase to the fused parameres (Thayer & Lawrence, 2002).

Ciid larvae are subcylindrical, parallel-sided, to 7 mm, having a globular hypognathous head and 2- or 3-segmented antennae, with a long sensorium exceeding segment's length. Usually asymmetrical mandibles, mola is usually absent and sometimes replaced by an acute process. Legs short, coxae close together; upturned urogomphi (Lawrence, 1991).

Bionomics. Adults and larvae of Ciidae are internal feeders on fruiting bodies of a variety of Basidiomycetes, but primarily those of Polyporaceae. They are found under bark of logs or in rooting wood. Most of species show a certain degree of host preference (Thayer & Lawrence, 2002).

Classification. There are described about 42 genera with 640 species worldwide (Buder *et al.*, 2008) and, except single species *Sphindocis denticollis* from California belonging to subfamily Sphindociinae, all species belong to subfamily Ciinae with cosmopolitan distribution (Lawrence & Newton, 1995). The subfamily Sphindocinae takes a basal position within the Ciidae (Beutel & Friedrich, 2005). Ciidae were traditionally placed in Bostrichoidea or Cleroidea. Crowson (1955) moved the family in Cucujoidea, section Clavicornia, Crowson (1960) shifted them in the superfamily Tenebrionoidea. Considering both adult and larval characters, the family may be classified in relationships to Mycetophagidae and Tetratomidae (Lawrence, 1991). Lawrence (1977) suggested a possible sister group of Pterogeniidae with family Ciidae, Archeocrypticidae and Piseninae. However, the exact position remains contentious (Thayer & Lawrence, 2002; Buder *et al.*, 2008). They did not find any relationship of Ciidae and Tetratomidae or Mycetophagidae, nevertheless some analyses proposed either the sister group relationship with the cucujoid family Nitidulidae or the basal position of the family within the cucujoid-tenebrionoid assemblage.

Tetratomidae Billberg 1820- the polypore fungus beetles.

Morphology. Oblong to elongate body, convex to somewhat flattened, pubescent and small- 2-17mm, brownish to black colour with reddish markings. Head triangular, antennae with 11 antennomeres either clavate or 3-4 apical antennomeres form a loose club; maxilla reduced; eyes large, obovate. Pronotum broader than head; prothoracic coxae separated by a prosternal process; tarsal formula 5-5-4, tarsomeres not lobed. Male genitalia sometimes inverted (Young & Pollock, 2002).

Tetratomid larvae are elongate and subcylindrical to slightly flattened, lightly sclerotized, 3-17mm long; epicranial suture up to moderately long, frontal arms lyriform or forked, stemmata 5 on each side, antennae 3-segmented, mandibles weakly to strongly asymetrical, mola well developed (*Pisenus*), reduced and tuberculate (*Triphyllia*), replaced by hyaline

processes (Tetratominae) or a membranous lobe (*Penthe*); legs well developed; usually bifid urogomphi (Lawrence, 1991).

Bionomics. Larvae and adults of Tetratomidae feed on the softer fruiting bodies of various Hymenomycetes. Adults feed on the surface, while larvae bore into the tissues. Thus they are commonly found under bark and in fresh or decaying fungal tissues (Lawrence, 1991).

Classification. Tetratomidae are a small family of 13 genera and about 155 species that are distributed almost all over the world except the Australian region. Presently there are recognized five subfamilies: Tetratominae, Piseninae, Penthinae, Hallomeninae and Eustrophinae (Nikitsky, 1998; Young & Pollock, 2002). On the other hand, Lawrence and Newton (1995) omitted Hallomeninae and Eustrophinae keeping them in the family Melandryidae, despite considering the Tetratomidae in their sense paraphyletic. Tradionally, the family was placed in Melandryidae as a subfamily, tribe or several tribes. Sooner, the genera *Tetratoma*, *Penthe* with *Eustrophus* were referred by Böving and Craighead (1931) to the cucujoid family Erotylidae due to their larval adaptations. Crowson (1955) placed them in Tetratomidae, Miyatake (1960) added genus *Pisenus* and Hayashi (1975) genus *Holostrophus*. Tetratomidae and Melandryidae are hard to define as separate lineages. Tetratominae and Eustrophinae show closer relationship to each other than to Melandryidae (Hayashi, 1975) and Eustrophinae are considered to be a link between Melandryidae and Tetratomidae (Crowson, 1966; Viedma, 1971). While the isolated position of Hallomeninae is supported by larval characters (Hayashi, 1972, 1975; de Viedma, 1966, 1971), similarities between Piseninae and Mycetophagidae are obvious (Miyatake, 1960). *Eustrophus* resembles a typical melandryid in imaginal structure (except the simple tibial spurs). *Penthe* and *Eustrophus* cannot be easily associated with *Tetratoma*, while *Mycetoma* is of an intermediate form between *Penthe* and *Eustrophus* (Crowson, 1955). Tetratomids are regarded to be primitive within the Tenebrionoidea as Crowson (1966) illustrated by the proposal of an ancestor of the Tenebrionoidea resembling to the Tetratomidae. Besides the Melandryidae, the family has a strong connection to Mycetophagidae based on both larval and adult characters (Crowson, 1955; Miyatake 1960; Nikitsky, 1998).

Melandryidae Leach 1815- the false darkling beetles.

= Serropalpidae

Morphology. Body varies from narrow, parallel-sided or tapered posteriorly to wide, ovate to subcylindrical, small to large- 2-20mm, coloured brown to black (Pollock, 2002). Head is deflexed, without distinct constriction behind eyes and deeply inserted into the prothorax

(Lawrence & Britton, 1991); eyes at least slightly emarginate; antennae 11-segmented, moniliform to filiform and serrate, with or without 3-5 antennomeres' club, insertions visible; mandibles short, maxillary palpi modified, slightly serrate, the apical palpomere expanded triangular, securiform or cultriform. Elongated first hind tarsomeres, mid and hind tibiae with combs, some species are capable of jumping, distinct hind tibial spurs, tarsal formula 5-5-4 (Pollock, 2002).

Melandryid larvae are elongate, subcylindrical or slightly flattened, usually with slightly sclerotised body, 2.5-30mm. Head prognathous, epicranial suture relatively Y-shaped and long, very short or absent; stemmata 5 on each side or reduced to 2 or 0; antennae 3-segmented; mandibles symmetrical, mola absent or represented by few teeth or tubercles. Legs relatively short and urogomphi minute or absent (Lawrence, 1991).

Bionomics. There are two dominant feeding habits in the family: fungivory (Orchesiini) and xylophagy (the remaining tribes). However, fungi comprise a significant portion of diet even in the xylophagous groups. Adults can be seen active on wood surfaces at night, larvae bore in dead wood or fruiting bodies of fungi (Lawrence, 1991).

Classification. There are known about 24 genera with approximately 430 species, that are widely distributed, with the highest diversity in the tropics (Pollock, 2002). Lawrence and Newton (1995) distinguished four subfamilies, however since than Hallomeninae and Eustrophinae have been transferred in the family Tetratomidae (Nikitsky, 1998), thus only two subfamilies, Osphyinae and Melandryinae, are currently recognized. This classification is also followed by Pollock (2002), who calls for an extensive, phylogenetic study to investigate the placement of the Hallomeninae and Eustrophinae in Tetratomidae and relationships to Melandryidae. Melandryidae are close to Tetratomidae and have affinities in adult structures shared with Mordellidae, Ripiphoridae, Scraptiidae. However, the similarities of anaspidines seem to be convergent (Lawrence, 1991) and the similarities of larvae to Mordellidae as well (Crowson, 1955, 1966). In the past, the family comprised many taxa now placed in various other families - Tetratomidae, Stenotrachelidae, Synchroidae, Pythidae, Pyrochroidae, Scraptiidae. On the basis of several distinct types of larvae mentioned by Lawrence (1991), Pollock (2002) discussed the possible para- or polyphyly of the family. According to Lawrence and Newton (1995), the Melandryinae seem to be monophyletic. The tribal classification appears to be unsuitable (Pollock, 2002).

Mordellidae Latreille 1802- the tumbling flower beetles.

Morphology. Small- 1.5-15mm long, wedge-shaped, humped, laterally compressed body, posteriorly tapered, with a spine-like abdominal process formed by the 7th tergite. Various colour- black, brown, red or yellow; scattered or dense decumbent hairs. Head opistognathous, as wide as thorax, sharply constricted behind eyes; short antennae with 11-antennomeres filiform, in Ctenidiinae pectinate; mandibles short; apical palpomere of maxillary palpi large; eyes lateral, large. Prosternum very short; legs slender, without trochantin, metafemora sometimes enlarged for jumping, metacoxae very large, tibiae and tarsi often with combs of spines, tarsal formula 5-5-4. Pygidium pointed; male genitalia very elongate, parameres often asymmetric and variously modified (Jackman & Lu, 2002).

Mordellid larvae are white, from 3 to 18mm long, very lightly sclerotised, elongate, more or less parallel-sided, subcylindrical. Head globular, long epicranial suture and coincident endocarina; antennae very short, stemmata absent or indistinct, mandibles robust, symmetrical, lacks a mola; thorax sometimes enlarged, legs very short; tergum 9 often with pair of minute urogomphi or median terminal spine (Lawrence, 1991).

Bionomics. Adults are frequent on flowers and feeding on pollen, however there are also known fungivorous species. Mordellid larvae belong to a wood-boring type, they occur primarily in decaying wood and rotten stems of herbaceous plants (*Mordellistena*), few species feed in fungus fruiting bodies (Lawrence, 1991).

Classification. The family consists of about 110 genera and 1500 species distributed all around the world (Jackman & Lu, 2002; Lisberg & Young, 2003). The group is presently divided into two subfamilies: Ctenidiinae containing a single South African species *Ctenidia mordelloides* and Mordellinae including the remaining genera in five tribes (Lawrence & Newton, 1995). Mordellidae, after the separation of Anaspidinae to the family Scraptiidae by Crowson (1955), are a relatively homogenous group. Böving and Craighead (1931) moved Mordellidae from Cucujoidea into the superfamily Mordelloidea and they emphasized the relationship to several melandryid genera. Except Melandryidae, mordellids are closely related to scraptiides and perhaps ripiphorides (Sharp & Muir, 1932; Crowson, 1955, 1966; Lawrence & Newton, 1982).

Ripiphoridae Gemminger and Harold 1870 (1853)- the ripiphorid beetles.

Morphology. Body elongate, wedge shaped, 2.5-14.0mm long, black and orange, red or yellow coloration, glossy integument or pale decumbent hairs. Head hypognathous, deflexed, constricted behind eyes; eyes sometimes very large; antennae either bipectinate or biflabellate

in males or unpectinate in females, 11-segmented; mouthparts sometimes reduced. Pronotum is narrowed behind the head, without lateral margins, however covers scutellum by the extended margin (Evans & Hogue, 2006); elytra smooth, either covering abdomen (*Macrosiagon*) or reduced to scalelike plates (*Ripiphorus*) or completely absent in females (Rhipidiinae) (Falin, 2002). However the Ripiphoridae are a morphologically diverse group so a brief description of individual subfamilies is presented separately.

Species of Pelecotominae and Ptilophorinae are the least specialised, with more or less complete elytra and minimal sexual dimorphism. Male antennae are flabellate. The eyes of Ptilophorinae are almost divided into two parts. The Hemirhipidiinae include small to large beetles with shortened elytra and light antennal dimorphism. The Ripidiinae are the most highly specialised of the ripiphorids, with atrophied mouthparts, large eyes and very short elytra in the male. Female ripidiines are without elytra and are larviform. In the subfamily Ripiphorinae, the elytra are either long and dehiscent, as in *Macrosiagon* and *Metoecus* or short and well separated at the base, as in *Ripiphorus*. The antennae of males are biflabellate and pectinate in females (Lawrence & Britton, 1991; Falin, 2002).

Most of Rhipiphoridae are hypermetamorphic with complex life cycles, thus several larval types may occur in a single species.

1st instar, triungulin type larva is heavily sclerotized, 45-95mm long, shape navicular or crescentic after feeding, vestiture of setae; head without epicranial suture, 4 or 5 stemmata, antennae 2- or 3- segmented, mandibles working vertically; legs slender, elongate, tibiae very long in Rhipidiinae, urogomphi absent (Selander, 1991). 1st instar of *Pelecotoma* is less sclerotized and campodeiform (Švácha, 1985).

Later instars -2nd-6th phase- of Rhipiphorinae ectophagous. Bodies lightly sclerotized, C-shaped, more sparsely covered by setae. Head hypognathous, without epicranial suture, stemmata and labial palpi; antennae and maxillary palpi reduced; mandibles with modified outer surface for cutting, toothed. Thorax and abdomen with conical horns, legs reduced;

2nd phase of Rhipidiinae (endophagous) is apodous, spiracles, antennae, mouthparts absent, with 5 stemmata on each side of head; 3rd phase of Rhipidiinae (endophagous) is pseudoeruciform, without spiracles and with unsegmented antennae and legs; 4th phase-emergent, pseudoeruciform, however with spiracles and with segmented appendages. (Selander, 1991)

Bionomics. Larvae of the primitive subfamilies are free-living predators or ectoparasites of wood-boring beetles larvae. The life cycle was described for *Pelecotoma*: the active first instar finds the host, enters the body and feeds as an endoparasite. After the overwintering,

beetle emerges the host and continues feeding as an ectoparasite attached to the surface of the host body. It undergoes other four instars and the fifth one bores through the wood to prepare the gallery for the adult (Švácha, 1994). The ripiphoride triungulin attaches on flower to an adult wasp (*Macrosiagon*, *Metoecus*) or solitary and semisocial bee (*Ripihorus*) and is carried to a nest, where bores in thorax of hatched host larva (endophagous phase). The ripiphorid larva grows enormously and after approaching maturity of the host larva, the beetle larva emerges and like ectophagous instars feed on the host larva until it is consumed. The Ripidiinae triungulin attaches directly nymphs of cockroaches and only after a short period of externaly feeding, the 2nd instar enters the host. Later, ripidiine larva transfers through the 3rd phase to the 4th instar that emerges and pupates outside of the host (Selander, 1991).

Adult ripiphorides are short living, their feeding habits are unknown (Falin, 2002).

Classification. The family includes 38 genera (Falin, 2002) and 425 species worldwide (Evans & Hogue, 2006). The genera like *Macrosiagon*, *Ripiphorus* (except Australia and Madagascar) and *Trigonodera* (except Europe) are known worldwide. However most species poor genera have restricted distribution, e.g. *Pelecotoma* occurs in North America, Europe, Japan, *Rhipistena* in New Zealand, *Scotoscopus* in Greece, etc. Lawrence and Newton (1995) recognized six subfamilies Pelecotominae, Micholaeminae, Ptilophorinae, Hemirhipidiinae, Rhipidiinae and Rhipiphorinae.

Falin (2002) casts doubt on the monophyly of the family Ripiphoridae based on the absence of a strong synapomorphy that would define them. He emphasizes the need of further work to get better knowledge of the relationships within the family as well as the relationships to other lineages within the Tenebrionoidea. There is hypothesized an early split of the Ripiphorinae off the ancestral lineage, leaving Hemirhipidiinae and Ripidiinae as the most derived sister taxa. Pelecotominae is the most primitive subfamily, but likely non-monophyletic (Falin, 2002). The genera can be arranged from least to morphologicaly the most derived: *Trigonodera*, *Pelecotoma*, *Toposcopus*, *Macrosiagon, Ripiphorus, Pirhidius* (Selander, 1957).

The larval morphology and specific biology, like parasitic habits or hypermetamorphosis, suggest a possible common origin with a family Meloidae. Forbes (1926) proposed a relationship between Ripiphoridae and Meloidae, based on similarities in wing venation. This view corresponds with the Böving and Craighead's (1931) superfamily Meloidea. On the other hand, Crowson (1955), Selander (1957), Bologna & Pinto (2001) and Falin (2002) argue that these characters evolved independently. Falin (2002) expressed support of further studies to understand their relationship.

Considering imaginal characters, the Ripiphoridae are believed to belong to a lineage composed of melandryids, scraptiides and mordellids (Crowson, 1966), or according Lawrence and Newton (1982) to the line with Tetratomidae, Melandryidae, Mordellidae, Scraptiidae, Anthicidae and Aderidae. Ripiphoridae are thought to arise from a common ancestor with the Mordellidae by development of a parasitic mode of life (Selander, 1957; Crowson, 1966; Lawrence & Newton, 1982). Although ripiphorid-mordellid resemblance is obvious (Franciscolo, 1962, 2000) and Crowson (1995) and Falin (2002) regard a sister-group relationship possible, Švácha's work (1994) has questioned it.

To underline the particular features of Ripiphoridae I mention their notional relationship with the order Strepsiptera (Böving & Craighead, 1931; Crowson, 1955, 1960, 1995). However these connections were refuted by several studies based on both morphological and molecular evidences, e.g. Kathirithamby (1989), Whiting *et al.* (1997), Wheeler *et al.* (2001).

Zopheridae Solier 1834- the ironclad beetles, zopherid beetles.

The family Zopheridae currently comprises three groups, that were in the past recognized like individual families. They differ in morphology as well as in bionomy, therefore, they will be presented here separately.

Colydiidae Erichson 1845- the colydiid beetles.

= Adimeridae, Monoedidae, Orthoceridae

Morphology. Elongate, convex to strongly flattened, cylindrical to depressed and parallel-sided body; 1.2-15mm in length; brown to black in coloration; glabrous or variously covered, or modified into scales or bristles. Antenna with 10 or 11 antennomeres, slightly clubbed; highly variable mouthparts. Pronotum with carinate lateral margin, smooth to denticulate; usually open procoxal cavities; elytra entire, costate, carinate, with punctate striae; hind wing may be reduced or absent; closed mesocoxal cavities; tibiae slender, tarsal formula usually 4-4-4 or 3-3-3. Male genitalia tenebrionoid, symmetrical (Ivie, 2002).

Larvae with elongate, parallel-sided, subcylindrical to slightly flattened body; with the length 2-20mm; lightly pigmented. Head protracted; epicranial short to moderately long or absent, frontal arms lyriform or V-shaped; stemmata 5 on each side, arranged in 2 groups; antennae 3-segmented; mandibles symmetrical, usually bidentate, mola either well-developed, tuberculate or reduced. Prothorax sometimes enlarged; legs well developed, 5-segmented; paired, upturned urogomphi, with a pit lying between them (Lawrence, 1991).

Bionomics. Primarily Colydiidae are mycophagous, feeding on decaying plant material or fungi and are associated with rotten logs; however some groups have developed predatory habits and can be found in the galleries of wood boring beetles (Lawrence, 1991).

Classification. The family includes almost 140 genera (Ivie, 2002) and about 1000 species distributed worldwide (Lawrence, 1991).

Colydiidae were a heterogenous assemblage of clavicorn and heteromeran beetles sharing small size, 4-4-4 or 3-3-3 tarsal formula and abruptly clubbed antennae. They were moved from Clavicornia (=Cucujoidea) to Tenebrionoidea by Crowson (1955), based on a type of aedeagus. Many changes have been proposed: Cerylonidae (Crowson, 1955) and Bothrideridae (Lawrence, 1980) were separated from the Colydiidae and both were placed in Cucujoidea. Some misplaced species were recognized and transferred to Tenebrionidae and other families (Lawrence, 1977; Doyen & Lawrence, 1979; Lawrence, 1980; Ivie & Ślipiński, 1990). The reduced tarsi have been found homoplasious (Ślipiński & Lawrence, 1999) and the monophyly of the group still remains contentious (Ślipiński & Lawrence, 1999; Ivie, 2002; Majka *et al.*, 2006). As mentioned above, Colydiidae are currently classified as a subfamily of Zopheridae. The new status was assigned by Ślipiński and Lawrence (1999) on the basis of the phylogenetic analyses of both adult and larval data sets. They were found to be a sister group to Zopherinae clade. Neverthless colydiids were weakly supported without tribe Pycnomerini, that was found to be a member of Zopherinae clade, as was predicted by Lawrence and Newton (1995). The number of the traditional tribes was decreased by synonymizing and uniting many of them in a single tribe Colydiini (Ślipiński & Lawrence, 1999).

Monommatidae *Blanchard 1845*- the monommid beetles.

= Monommidae, Monommatini

Morphology. Compact, ovate, moderately dorsally convex body; 2.3-12mm in size; black in colour and without vestiture. Head prominent; eyes large, almost meeting above; 11-segmented antennae with antennal insertions concealed, 2 or 3 apical antennomeres form flattened club. Pronotum narrowed anteriorly, with distinct lateral margins, punctate surface; procoxal cavities open; procoxae globular, meso- and metacoxae flat, widely separated; tibiae and tarsi slender, tarsal formula 5-5-4; elytra smooth, apically rounded. First ventrite elongate (Ivie, 2002).

Larvae elongate, parallel-sided, slightly flattened, 5-15mm long, lightly pigmented. Head protracted, moderately broad; epicranial stem and median endocarina absent, frontal arms

lyriform; stemmata 5 on each side; antennae 3-segmented; mandibles symmetrical, bidentate, mola reduced, sub-basal, represented by a row of hyaline teeth. Legs short and spinose, 5-segmented, separated; pair of urogomphi between which is a heavily sclerotized pit (Lawrence, 1991).

Bionomics. Monommatins, both adults and larvae feed on a variety of decaying plant material and can be found in soft and decayed stems as well as under bark of rotten logs (Lawrence, 1991).

Classification. There are known 15 genera and about 300 species worldwide, with greater diversity in tropical and subtropical regions (Ivie, 2002; Lawrence, 1991). Although monommatids have long been recognized as an independent tenebrionoid family with a very distinctive body form, they are currently classified as a tribe of Zopheridae. The similarity between them noticed Crowson (1955). Doyen and Lawrence (1979) drew attention to this relationship as well and Lawrence (1994b) suggested that monommids and zopherids form a monophyletic group with the colydiide tribe Pycnomerini. This was supported by the phylogenetic analysis of Ślipiński and Lawrence (1999) which confirmed that exclusion of Monommatidae would make the family Zopheridae paraphyletic. However the intra-group classification is unresolved and needs a revision (Ivie, 2002).

Zopheridae *Solier 1834*- the ironclad beetles.

=Monommatidae, Monommidae, Pycnomerinae

Morphology. Elongate, parallel-sided, flattened to convex; 1.8-34 mm in length; glabrous to covered in setae or scales; smooth or tuberculate or carinae. Head deeply or weakly inserted into prothorax; eyes emarginate, round; antennae with 8-11 segments, with weak to strong antennal club, antennal insertions concealed; maxillary palpi variable. Pronotum with smooth or dentate lateral edges or sometimes absent; procoxae globular, cavities open or closed; tarsi not lobed, tarsal formula 5-5-4 or 4-4-4; elytral punctation seriate; hind wings commonly absent. Ventrites rarely all free; aedeagus sometimes inverted (Ivie, 2002).

Larvae elongate, parallel-sided, subcylindrical or slightly flattened; lightly sclerotised, 5-45mm in length. Head protracted, broad; epicranial stem usually long, or short or absent (*Phelopsis*, *Usechus*, Ulodinae); frontal arms V-shaped or lyriform (*Phelopsis*, *Usechus*, Ulodinae); stemmata 5, 3, 0 on each side; antennae 3-segmented or short; mandibles symmetrical or slightly asymmetrical, robust, sometimes mola reduced; hypostomal rods absent. Legs well developed, sometimes short and spinose; coxae separated; often with rows

or patches of asperities on the tergites; granulate and/or tuberculate 9th tergum with larger urogomphi in *Phelopsis*, *Usechus*, Ulodinae (Lawrence, 1991).

Bionomics. Most Zopherinae are associated with dead, rotten wood, especially attacked by white rot fungi, on which the larvae feed. Adults may be found on surfaces of fungal fruiting bodies (Lawrence, 1991). Adults and larvae of Pycnomerini are associated with rotten plant material (Ivie, 2002).

Classification. The family, in the older sense, counts 26 genera and 125 species worldwide (Lawrence, 1991). Based on larval characters Böving and Craighead (1931) established a new family Zopheridae, excluding the tribes Zopherini and Nosodermini from the family Tenebrionidae. This was confirmed by adult characters and there were added further tenebrionid genera to zopherines (Crowson, 1955; Watt, 1974a; Doyen & Lawrence, 1979), despite doubts about insufficient arguments for establishing an individual family (Triplehorn, 1972). Several times the inclusions and exclusions of subfamily Ulodinae was proposed and rejected (Watt, 1974a; Lawrence, 1991; Lawrence, 1994b). Doyen and Lawrence (1979) drew attention to the relationship of the Zopheridae, Colydiidae and Monommatidae and Lawrence (1994b) suggested that monommids and zopherids form a monophyletic group with the colydiide tribe Pycnomerini. Ślipiński and Lawrence (1999) proposed a monophyletic group consisting of Ulodidae, in a sister-group position to Colydiidae, Zopheridae, Monommatidae and Pycnomerini. The clade comprising Zopheridae, Monommatidae and Pycnomerini was well supported. The family is presently formed by two subfamilies: Colydiinae and Zopherinae, that is now divided into 6 tribes: Usechini, Latometini, Phellopsini, Zopherini plus Monommatini and Pycnomerini.

Ulodidae *Pascoe 1869.*

Morphology. Vestiture of thick hairs or scale-like setae. Antennae with 3-segmented club, with exposed insertions. Procoxae widely separated, procoxal cavities closed; laterally open mesocoxal cavities; tarsal formula in *Meryx* 4-4-4 (Doyen & Lawrence, 1979; Lawrence, 1994b).

Ulodid larvae are diverse with distinct epicranial stem, complete lyriform arms, hypostomal rods and widely separated urogomphi (Doyen & Lawrence, 1979).

Bionomics. Larvae are usually associated with basidiomycete fungi. *Meryx* larvae occur under bark feeding on wood-rotting fungi and both *Ulodes* and *Dipsaconia* feed in the softer fruiting bodies of Polyporaceae and Tricholomataceae (Lawrence, 1994b).

Classification. The family includes 13 genera from southern South America, Australia, New Zealand and New Caledonia. The group used to be placed in the family Zopheridae as a subfamily Ulodinae while a few taxa remained in Tenebrionidae until Lawrence (1994b) united them with Ulodidae.

Perimylopidae *St.George 1839.*

Morphology. Larvae elongate, parallel-sided, in size 7-15mm, with vestiture of simple setae. Head with short epicranial stem, lyriform frontal arms; 5 stemmata on each side; long antennae, with 3rd segment reduced; mandibles with 3 or 4 teeth at the apex, no mola or prostheca, ventral mouthparts retracted. Legs long, widely separated. Tergum A9 with a pair of complex urogomphi (Lawrence, 1991).

Bionomics. Perimylopids are usually collected feeding on plant material under stones and moss and in tufts of tussock grass in very cold environments (Lawrence, 1991).

Classification. The family includes 7 genera occuring in southern Chile, Patagonia and South Georgia and Tasmania (Lawrence & Newton, 1995).

The Perimylopidae were originally proposed for a few tenebrionid genera inhabiting southern Chile and Argentina, however the group was redefined to include the Tasmanian genera *Sirrhas* and *Melytra* (Lawrence, 1994b).

Chalcodryidae *Watt 1974.*

Morphology. Elongate, soft body, 5-18mm in length. Head narrowed behind eyes; antennae 11-segmented, with 3 apical segments enlarged, insertions exposed; mandibles bidentate, mola sclerotised. Procoxal cavities closed, mesocoxal cavities open laterally; legs slender, long, with 5-5-4 tarsal formula (Watt, 1974b).

Larvae elongate, parallel-sided, subcylindrical, lightly pigmented except for dark and heavily sclerotized head, length up to 30mm. Head slightly flattened, tuberculate, with long epicranial stem, V-shaped frontal arms joined anteriorly, without median endocarina; 5 stemmata on each side, short antennae; mandibles bidentate, simple mola, no prostheca; ventral mouthparts retracted. Legs long and slender. Tergum A9 simple, without urogomphi; tergum A10 sclerotized, with a pair of pygopods (Lawrence, 1991).

Bionomics. Adult chalcodryids are usually beaten from moss- or lichen-covered branches in cool, wet forests. Larvae of *Chalcodrya variegata* have been found in refuge galleries in dead twigs or branches, however they feed on lichens or mosses at night (Watt, 1974b).

Classification. The family includes 5 species in 3 genera in New Zealand. Relationships of this group are uncertain. It has several features in common with Zopheridae and Perimylopidae, but it is likely to be close to the base of Tenebrionidae as well (Lawrence, 1991).

Trachelostenidae *Lacordaire 1859.*

Morphology. Elongate, slender body. Head narrowed behind eyes; clypeus angularly depressed at junction with frons; antennae elongate, filiform, concealed insertions; eyes emarginate anteriorly; apical segments of maxillary and labial palpi securiform. Elytra elongate; procoxae projecting, procoxal cavities closed internally and externally; mesocoxal cavities open laterally; metacoxae strongly transverse; tarsal formula 5-5-4. Abdomen with 5 free visible sternites (Watt, 1987).

Classification. The family, represented by 2 species of *Trachelostenus*, is known only from Chile.

The family used to be included in the family Lagriidae or provisionally in the Pythidae (Watt, 1974a) until it was elevated to a familial level by Watt (1987). The relationship to other families is uncertain, but they show some affinities with the primitive Tenebrionidae. The possible sister group relationship with *Leaus tasmanicus* needs to be supported (Lawrence & Newton, 1995).

Tenebrionidae *Latreille 1802*- the darkling beetles.

(including Alleculidae, Blapsidae, Cossyphodidae, Diaperidae, Helopidae, Lagriidae, Nilionidae, Petriidae, Pimeliidae, Rhysopaussidae, Tentyriidae).

Morphology. Hard body, highly variable shape and size (1-80 mm), usually dark; eyes rarely absent, often separated into 2 portions; antennal insertions concealed, 11- or rarely 10-, 9-segmented, variable in shape, apical segment with comound sensilla; mandibles bidentate or tridentate, mandibular mola with or without transverse ridges. Pronotum carinate or extended laterally; procoxal cavities closed externally and open and closed internally, prosternal process convex, at least slightly curved behind the coxae; mesocoxal cavities with or without exposed trochantin, closed laterally; penultimate tarsomere sometimes lobed, tarsal claws sometimes pectinate, tarsal formula 5-5-4, rarely 4-4-4 or 3-3-3; fused elytra in many species, typically with 9 striae, with scutellary striole. Abdomen with intersternal membrane of abdomen exposed, visible sternites 1-3 connate; abdominal paired defensive glands present or

absent (Zolodininae, Pimeliinae, some Lagriinae); aedeagus typically not inverted (Aalbu *et al.*, 2002).

Larvae elongate, cylindrical to slightly flattened, hard-bodied, 5-70mm in length, sometimes short and broad or strongly flattened. Head protracted; epicranial stem long, occasionally short, rarely absent, frontal arms V- or U-shaped, endocarina absent; 5 or fewer or absent stemmata on each side; antennae 3-segmented, with long 2nd and 3rd short segment or reduced, sensorium flattened and dome-like; antennal insertions lateral; frontoclypeal suture distinct; mandibles more or less asymmetrical, short, subtriangular, with 1 to 3 apical teeth, molae well-developed, usually concave, the left tooth often with a projecting premolar lobe or tooth; maxilla with simple, not cleft malar apex. Prothorax slightly larger; legs well-developed, contiguous; tarsungulus sometimes very large, heavily sclerotized, divided into 2 parts; 9th tergite extending onto ventral surface, with apex rounded, triangular bearing an acute median process, pair of urogomphi; single 9th sternum long to very short (Lawrence & Spilman, 1991).

Bionomics. The members of the family Tenebrionidae are primarily saprophagous, feeding on variety of dead plant and animal material. Adults, heavily sclerotized, dark coloured are nocturnal and occur on the ground as wingless ground-dwellers or on the surfaces of logs, tree trunks or they burrow into substrates. Those adults, which are soft-bodied and brightly coloured, occur on foliage or flowers. The ground inhabiting adults live in arid areas and deserts and possess many adaptations in their morphology, physiology and behaviour.

Larvae may be divided in two groups; xylophilous ones specialise on either boring in rotten wood and cambium or occur under bark and in galleries of bark beetles, being mycophagous and predaceous. Geophilous ones live in soil, leaf litter, feeding on roots, rotting vegetation, fungi or are facultative predators.

Several groups live in litoral habitats, in sand dunes or in caves. Some are associated with nests of vertebrates or insects or graze algae, lichens and mosses on bark, rock surfaces and others are pests of crops and stored products (Lawrence & Spilman, 1991).

Classification. The Tenebrionidae are the largest lineage of Tenebrionoidea with approximately 19 000 species in more than 2000 genera worldwide (Aalbu *et al.*, 2002). According to Lawrence and Newton (1995), who have followed Doyen *et al.* (1990), they are classifiied in eight subfamilies: Lagriinae, Phrenapatinae, Zolodininae, Pimeliinae, Tenebrioninae, Alleculinae, Diaperinae, Coelometopinae. However, Aalbu *et al.* (2002) considered Tenebrioninae to be paraphyletic with groups like the Alleculinae, Coelometopinae and Diaperinae falling within them. These authors have distinguished ten

subfamilies with elevating the tribes Bolitophagini, Opatrini and Hypophloeini to a subfamilial level. Recently, Bouchard *et al.* (2005) have recognized ten subfamilies and 96 tribes. Aalbu (2006) generally supported the Bouchard's *et al.* (2005) classification recognizing ten subfamilies and 110 tribes. The classification of Tenebrionidae still remains contentious.

Although some subfamilies, e.g. Nilioninae, Lagriinae, Alleculinae, stood separated from the Tenebrionidae as independent families, they were kept a close relationship with true tenebrionides. On the other hand, the now widely accepted independent families Archeocrypticidae, Chalcodryidae, Perimylopidae, Ulodidae, Zopheridae, Synchroidae, Boridae or subfamily Dacoderinae had been for long time included in the Tenebrionidae. The most primitive branch within the family seems to be represented by lagrioid branch, consisting of Lagriinae and Phrenapatinae. The relationship of two other recognized branches, pimeloid (including Zolodininae and Pimeliinae) and tenebrionid branch (incorporating all remaining subfamilies), has not yet been definitively resolved (Doyen & Tschinkel, 1982; Matthews, 2003).

The phylogenetic relationships of Tenebrionidae are unclear. The potentially related groups include Chalcodryidae, Perimylopidae, Zopheridae, Synchroidae, Cephaloidae and Oedemeridae (Lawrence & Spilman, 1991), but none seem to be very close, due to long independent history of the Tenebrionoidae clade (Watt, 1974a).

Prostomidae *C.G.Thomson 1859*- the jugular-horned beetles.

Morphology. Body elongate, parallel-sided, flattened, yellowish or reddish brown, subglabrous; 5-10mm. Head elongate or very short and broad (*Dryocora*); antennae 11-segmented, 3 apical antennomeres weakly clubbed with pubescence; large, extremely projecting mandibles (*Prostomis*) or expanded laterally and wider than pronotum (*Dryocora*); large, anteriorly projecting genal processes; eyes small; frontoclypeal suture distinct. Lateral pronotal carinae absent; legs slender, coxae small, widely separated; 4-4-4 tarsi; elytra with vertically striped punctures (Young, 2002; Park & Ahn, 2005).

Larvae strongly flattened, lightly pigmented and sclerotized, 8-9mm long, sparse vestiture. Broad head, exserted from prothorax, asymmetrical; frontal arms lyriform, stem short or absent, endocarinae absent; stemmata absent; antennal insertions exposed, antennae elongate, 3-segmented; mandibles heavily sclerotized, asymmetrical, left mandible with prominent molar tooth. Prothorax slightly smaller; legs well developed; abdomen strongly flattened, 9th

segment small, with paired, short and lightly sclerotized urogomphi, 9th sternite with an apical row of asperities (Young, 1991).

Bionomics. Larvae and adults live in heavily deacayed wood. They are frequently found within a mud- or clay-like material between layers of rotting wood (Young, 1991).

Classification. This small family contains only 2 genera and about 27 species (Young, 2002). The genus *Prostomis* occurs worldwide except South America, while *Dryocora* species occur only in New Zealand, Tasmania and Australia.

The genus *Prostomis* was historically treated as a member of the family Cucujidae at a tribal or subfamilial level thanks to their superficial resemblance. Böving (1921) elevated the prostomids to familial level based on larval characters. Adults features, such as wing venation and male genitalia structure, determined its closer affinity with Tenebrionoidea (Wilson, 1930). Crowson (1955) suggested a closer relationship with Inopeplidae and Hemipeplidae or with Colydiid-Mycetophagid group, based on 4-4-4 tarsal formula, but he formally transferred Prostomidae to Tenebrionoidea much later (Crowson, 1967).

Lawrence (1977) suggested, that Prostomidae might be derived from the synchroid-cephaloid-zopherid lineage defined by Crowson (1966). Lawrence and Newton (1982) discussed resemblance of the larval head structure of Prostomidae, Oedemeridae and Cephaloidae, but they preferred relationships of Prostomidae and Colydiidae inferred from the similar type of procoxal cavity and aedeagus. According to Young (1991), the closest relative may be among the Inopeplidae, Salpingidae or Othniidae, all currently united in the widely defined Salpingidae. Schunger *et al.* (2003) confirmed the placement within Tenebrionoidea and the monophyly of the family. There was also suggested a close relationship with Boridae, Mycteridae and Pyrochroidae and the affinities with pythid-pyrochroid lineage were supported.

Synchroidae *Lacordaire 1859*- the synchroa bark beetles.

Morphology. Elongate, tapered, slightly flattened; 7-13mm in length; brownish to black colour, decumbent setae. Head setose; antennae with 11 antennomeres, filiform, insertions concealed under frontal edge near eyes; mandibles strongly curved; maxilla with small lacinia, maxillary palpi with 4 palpomeres, the last one securiform; eyes lateral, large, emarginate. Pronotum slightly broader than head, sides margined, punctate surface; procoxal cavities open externally, closed internally; serrulate tibial spurs; tarsal formula 5-5-4, simple tarsomeres; elytra with confused punctation (Young, 2002).

Larvae elongate, subcylindrical, in length 15-18mm, lightly sclerotized except head and urogomphal apices, vestiture of fine setae, small asperities on dorsum; colour yellowish-white. Head exserted, large; epicranial stem elongate, lyriform frontal arms, without endocarinae; 5 stemmata on each side; antennal insertions fully exposed, antennae 3-segmented; mouthparts retracted; mandibles heavily sclerotized, bidentate, asymmetrical, molar region of right mandible more prominent than the left one; labium free to base of mentum. Thorax elongate with sides subparallel; legs well developed, with spine-like setae. Abdomen subcylindrical; tergite A9 extended ventrally, with single pit between bases of paired, heavily sclerotized urogomphi, urogomphal apices curved upward, 9th sternite with single pair of asperities near margin; segment A10 fused to 9th (Young, 1991).

Bionomics. Both adults and larvae live under bark of deacaying deciduous trees, where they feed on fungi and rotting wood. Adults are nocturnal (Young, 1991).

Classification. The family consists of 2 genera and 8 species, that occur in Indonesia, Japan and North America (Young, 2002).

Originally, *Synchroa* had been placed in Melandryidae, until Böving and Craighead (1931) excluded it on the basis of larval features. The independent Synchroidae were accepted by Crowson (1966), who found its closest relatives among the members of Zopheridae and particularly Stenotrachelidae, based on both larval and adult features. Hayashi (1975) proposed *Synchroa* to be a member of Stenotrachelidae on the basis of similar larval features. Lawrence and Newton (1982) supported the relationship of Synchroidae and Zopheridae, placing them in the lineage with Prostomidae, Colydiidae, Monommidae, Perimylopidae, Chalcodryidae and Tenebrionidae.

Oedemeridae *Latreille 1810*- the false blister beetles, the pollen feeding beetles.

= Ascleridae, Calopodidae, Ditylidae, Nacerdidae, Ganglbaueriidae, Sparedridae, Stenostomatidae

Morphology. Elongate, parallel-sided, soft, slender body; brightly bicoloured, with short, decumbent hairs; small to medium size. Head slightly produced anteriorly; antennal insertions in front of eyes, antennae long, 11-segmented, filiform or serrate; apical maxillary palpomeres enlarged, usually triangular. Pronotum constricted behind, narrower than elytra, without lateral carinae; penultimate tarsal segment lobed beneath, tarsal formula 5-5-4, procoxal cavities open behind; enlarged hind femora in some species; narrow, weakly ribbed elytra, other species with unusual shaped elytra, partially exposing hindwings (Lawrence & Britton, 1991; Vázquez, 2002).

Larvae elongate, parallel-sided, straight or slightly curved ventrally, subcylindrical, slightly pigmented and sclerotized, vestiture scattered, 10-40mm. Head protracted, broad, often asymmetrical; epicranial stem moderately to very long, frontal arms usually V-shaped; stemmata usually absent; antennae well developed, 3-segmented, with reduced 3rd segment; mandibles strongly asymmetrical, bidentate or tridentate, molae large, asymmetrical, transversely ridged. Thorax relatively short, usually with paired patches of asperities on all terga and with asperity-bearing ampullae on some tergites and sternites; legs short; usually urogomphi absent or with a very small pair (Lawrence, 1991).

Bionomics. Adults often occur on flowers feeding on nectar and pollen. Most of larvae feed in dead wood, especially soft and rotten wood, but many Oedemerini are found boring in stems or roots of bushes or herbaceous plants, thus they may be considered to be possible agricultural or horticultural pests. Larvae of *Calopus* have been observed damaging living trees and others occur in driftwood submerged in fresh or salt water (Lawrence, 1991). Adults contain the toxic cantharidin providing them chemical defense (Vázquez, 2002).

Classification. The Oedemeridae are species-rich, they include about 100 genera and 1500 species worldwide (Lawrence, 1991). Lawrence and Newton (1995) have recognized two subfamilies: Calopodinae and Oedemerinae, the latter includes previously independent Nacerdinae. Kriska (2002) regarded the family to be well-defined and monophyletic with strongly supported three subfamilies: Calopodinae, Oedemerinae and Nacerdinae. However Lawrence (2005) described a new subfamily Polypriinae with genera *Dasytomima* and *Polypria* and he confirmed Calopodinae at the subfamilial level and all other oedemerid genera were classified in Oedemerinae.

The closest relatives of oedemerids are thought to be found among the Stenotrachelidae, Synchroidae and Zopheridae (Mamaev, 1973; Hayashi, 1975; Lawrence, 1977; Lawrence, 1991). Lawrence and Newton (1982) proposed a separated lineage with families Oedemeridae, Stenotrachelidae and Meloidae, based on similar larval features between oedemerids and cephaloids Crowson (1955), and the presence of cantharidin in some Oedemeridae and Meloidae.

Stenotrachelidae *C.G.Thomson, 1859*- the false longhorn beetles.

= Cephaloidae

Morphology. Elongate, narrow, convex, soft body, 6-20mm in size, very fine, decumbent setae. Head elongate, diamond or bell-shaped (*Cephaloon*), narrowed behind eyes, constricted behind eyes, forming a neck; antennae slender, 11 antennomeres, with 3-segmented club;

labrum prominent; securiform apical maxillary palpomeres. Pronotum elongate, narrowed anteriorly, lateral pronotal carinae comlete, incomplete or absent (*Cephaloon*); legs long, slender, pro- and mesothoracic trochantins distinct, 5-5-4 tarsi, projecting coxae, tarsal claws simple or pectinate with membranous; elytra narrowed apically. Abdomen with 5 ventrites (Young, 2002).

Elongate larvae, parallel-sided, subcylindrical to slightly flattened, slightly sclerotized, pigmented lightly in *Cephaloon* and *Nematoplus* or more heavily in Stenotrachelinae, vestiture scattered, 10-25mm long. Head protracted, broad, usually asymmetrical; epicranial stem long and frontal arms V-shaped (*Cephaloon*, *Nematoplus*) or epicranial stem absent and frontal arms lyriform (Stenotrachelinae); stemmata 5 or 6 on each side; antennae well-developed, 3-segmented; mandibles strongly asymmetrical, tridentate, molae large and transversely ridged, the left parallel, the right oblique. Legs well-developed; in Stenotrachelinae terga with rows and patches of asperities and tergum 9 granulate or tuberculate, urogomphi larger, upturned, sclerotized at apex; tergum 9 in *Cephaloon* and *Nematoplus* smooth, *Nematoplus* without urogomphi, *Cephaloon* with posteriorly projecting, straight, lightly sclerotized urogomphi (Lawrence, 1991).

Bionomics. Adults are rare and probably short-living. Some have been found in flowers (Arnett, 2000), but Mamaev (1973) proposed that feeding might not be necessary in the genus *Nematoplus*. Larvae feed in rotten wood and those of *Cephaloon* and *Nematoplus* in highly decomposed logs (Lawrence, 1991).

Classification. The Stenotrachelidae is a small family containing 7 genera and about 20 species, distributed in higher latitudes of the Holoarctic region (Lawrence, 1991). Three subfamilies are distinguished: Cephaloinae, Nematoplinae and Stenotrachelinae. The fourth subfamily, Stoliinae, was proposed by Lawrence and Newton (1995).

Stenotrachelus used to be placed in the Melandryidae, however Crowson (1955) drew attention to its resemblance with *Cephaloon*. Subsequently Arnett (1968) transferred the subfamilies Stenotrachelinae and Nematoplinae from the melandryiids and pediliids to the Stenotrachelidae. Mamaev (1973) stressed distinction between *Stenotrachelus* and the Melandryidae and supported Stenotrachelidae and Nematoplidae as independent families. Crowson (1955) emphasized that similarities between *Cephaloon* and meloids exist only in adult stage and proposed a connection to large-bodied forms of Scraptiidae and to melandryid genus *Mikadonius*. Stenotrachelid larvae are similar to zopherid *Phellopsis* and oedemerid Calopodinae (Crowson, 1955; Hayashi, 1975). Hayashi (1975) moved *Stenocephaloon* to Stenotrachelidae, although in previous study (Hayashi, 1963) he treated it as a member of

Melandryidae. Other taxa, potentially close to Stenotrachelidae, are Oedemerinae and bolitophagine Tenebrionidae, to which *Nematoplus* resemble in the larval stage (Mamaev, 1973). The members of the Nematoplinae were also associated with Pyrochroidae or Pedilidae (Young, 2002). Lawrence and Newton (1982) regarded Stenotrachelidae to be a member of an independent lineage with families Oedemeridae and Meloidae, based on similar larval features between oedemerids and cephaloids and unique type of tarsal claws of cephaloids and meloids adults. The common ancestor of Cephaloidae and Meloidae has already been proposed by Abdullah (1965). However Lawrence (1991), as well as Crowson (1955), rejected the connection between Meloidae and Cephaloidae due to the different larval morphology.

Meloidae *Gyllenhal 1810*- the blister beetles.

= Horiidae, Lytiidae, Tetraonycidae

Morphology. Elongate, moderately convex, soft body, heterogeneous shape; 3-30 mm in length; either bicoloured with red or yellow and black or blue, or uniformly coloured, metallic as well; subglabrous or clothed with short, decumbent hairs. Head deflexed, large, strongly constricted behind eyes to form narrow neck; antennae with 11 antennomeres, filiform or moniliform, often modified in male; mandibles more or less curved. Prothorax usually narrower than elytra as well, without lateral carinae; legs long, tarsi slender, 5-5-4, tarsal claws pectinate with a blade-like process beneath each claw, forecoxal cavities open behind, tibiae elongate, variously modified in male; elytra not flat, typically rolled over abdomen. Abdomen soft, last visible sternum of male emarginate to almost completely divided (Nemognathinae); male genitalia with aedeagus elongate, parameres fused only at base or fused entirely (Pinto & Bologna, 2002).

Larval development undergoes hypermetamorphosis with distinctive larval phases.

The triungulin phase is heavily sclerotized, capodeiform or navicular (Nemognathinae); 0.6-4.5mm in length. Head often with a basal transverse ridge; epicranial suture well developed; 1 or 2 stemmata on each side; antennae 3-segmented with long terminal seta on segment 3; mandibles working either horizontally (non-phoretic larvae) or vertically (phoretic larvae), dentate; well developed labial palpi. Thorax with a line of dehiscence; legs slender, elongate; pulvilli absent. The 10^{th} abdominal segment reduced; urogomphi absent, end of abdomen with a pair of large caudal setae except Nemognathinae.

The first grub phase (FG phase) (4-5 instars) with thorax and abdomen membranous, pale, more scarabeiform with growth, 5-25mm in length; numerous body setae. Head becoming

hypognathous with growth; epicranial suture well developed; stemmata replaced by subcuticular black eye spots; antennae with small sensory organ, segment 3 becoming shorter with growth, without a long terminal seta; mandibles massive, without a definite molar area.

The coarctate phase less C-shaped than in late instar FG larva, cuticule very heavily sclerotized, brown, glabrous, with fused segments. Appendages reduced to unsegemented stubs and fused to body.

The second grub phase similar to late-instar FG larva, body setae shorter, head less sclerotized. Legs shorter and thicker (Selander, 1991).

Bionomics. Meloid larvae are predators or parasitoids. Most of Meloinae and Nemognathinae triungulins wait on flowers to infest a bee, that carries them to the nest, where they feed on eggs, larvae and provisions (Selander, 1991). After feeding, the triungulin undergoes ecdysis, becomes scarabaeiform and grows fast. The sixth or seventh instar becomes immobile, the musculature degenerates, respiration is reduced. As coarctate larvae, many species can survive adverse enviromental conditions. The second grub phase follows and pupates. In Nemognathinae, the second grub larva, following pupa and adult are encapsulated within exuvia of previous instar. Many *Epicauta* larvae pupate directly from the first grub phase or fail to diapause in the C phase in response to high temperature. Rarely, a larva pupates directly from the C phase (Selander & Fasulo, 2000). *Epicautina* and *Mylabrina* prey on grasshopper eggs.

Adult meloids are phytophagous, feed on leaves and flowers of several families of plants, few species being serious pests, some specialized adults do not consume any food (Pinto & Bologna, 2002).

The common name, blister beetles, is derived from the presence of cantharidin in meloids' hemolymph, that they exude from leg joints and other body parts when disturbed. The cantharidin is a defensive and probably aggregative terpenoid, that causes blistering of the skin and is highly toxic to mammals. The presence of this substance in Meloidae is connected to prolonged sexual behaviour in the Meloinae (Bologna *et al*., 2008). Some meloids are aposematically coloured.

Classification. The family Meloidae contains almost 3000 species in approximately 125 widely distributed genera (Bologna *et al*., 2008). They were placed in three subfamilies: Eleticinae, Meloinae and Nemognathinae (Selander, 1991b; Lawrence & Newton, 1995). However, Bologna and Pinto (2001) and Bologna *et al*. (2008) list the fourth subfamily Tetraonycinae, that had already been proposed by Bologna (1991). The family is primarily distributed in steppes and other arid regions (Bologna & Pinto, 2002).

The Meloidae used to be associated with the Ripiphoridae on the basis of specialized first larval stage (triungulins) and hypermetamorphosis (Böving & Craighead, 1931), but as mentioned above, under Ripiphoridae, these characters may have evolved independently (Crowson, 1955, 1966; Selander, 1957; Bologna & Pinto, 2001; Falin, 2002). The adult features rather suggest connection with the Anthicidae (Crowson, 1955; Abdullah, 1964; Selander, 1966, 1991) or the Mordellidae-Scraptiidae lineage (Selander, 1991). The sister-group position of the Anthicidae to the Meloidae has not yet been confirmed (Bologna & Pinto, 2001), but they are generally considered to be closely related. A close relationship of meloids to Stenotrachelidae and Oedemeridae has been suggested by Lawrence and Newton (1982) as well as meloids' affinity to Stenotrachelidae by Abdullah (1964).

Eleticinae are supposed to be the most primitive subfamily, based on both larval and adult features, especially possible non-hypermetabolic and non-parasitic larval development. Because of absence of the triungulin, this character can not be taken as distinguishing feature of the family (Bologna & Pinto, 2001). Nemognathinae are basal to all remaining meloids and genus *Tetraonyx* lies in a basal position within a meloine clade (Bologna *et al.*, 2008).

Mycteridae *Blanchard 1845*- the palm and flower beetles, mycterid beetles.

= Hemipeplidae

Morphology. Elongate, convex (*Mycterus*), slightly depressed (*Lacconotus*) or parallel-sided and flattened (*Hemipeplus*); 2.5-9mm in length, vestiture of short setae. Head rostrate (*Mycterus*), narrowed behind eyes (*Hemipeplus*); eyes small, exserted (*Lacconotus*, *Hemipeplus*) or larger, less convex (*Mycterus*), facets coarse to fine; antennae short to moderately long, distal antennomeres extended, exhibiting sexual dimorphism in males (*Mycterus*), insertions not or very slightly concealed by lateral extension of frons; mandibles slightly asymmetrical; distal maxillary palpomere from slightly expanded, securiform, to nearly cultriform. Prothorax subquadrate (*Lacconotus*), campanulate (*Mycterus*), or slightly cordate (*Hemipeplus*); procoxal cavities open, except for *Hemipeplus*, intercoxal process short, not extended between coxae or elongated, extended well between coxae; elytra elongate, parallel-sided to subovate; tarsal formula 5-5-4, penultimate tarsomere expanded laterally. Abdominal ventrites of males (*Lacconotus*, *Mycterus*) with setose patch on V1, V2 or V1-V3 or with protuberance (Pollock, 2002).

Larvae elongate, parallel-sided, strongly flattened, 5-30mm in length; slightly sclerotized except for head and tergum A9. Head protracted, broad, flattened; epicranial stem short or absent, frontal arms lyriform, median endocarina absent except for *Hemipeplus*; stemmata 5

or 2 on each side; antennae 3-segmented; labrum free; mandibles symmetrical, bidentate or tridentate, mola reduced, prostheca absent; ventral mouthparts slightly retracted; labium free to base of mentum. Thorax narrower than abdomen; legs relatively short, widely separated. Abdominal terga and sterna with paired rows of asperities forming incomplete rings; tergum A9 forming an articulated plate, with a pair of urogomphi, sometimes with median process or 2 pits between them; sternum A8 posteriorly excavated and enclosing sternum A9 partly, sternum A9 deeply excavated, forming U-shaped sclerite, which encloses segment A10 (Lawrence, 1991).

Bionomics. Adults are often found on flowers and some mycterids on various palms or grasses. Larvae live under bark or in the leaf axils of monocotyledonous plants. They all appear to be phytophagous (Lawrence, 1991).

Classification. The family includes 30 genera with about 160 species worldwide. Three subfamilies are distinguished, Mycterinae occur in drier areas, Lacconotinae and Hemipeplinae are widely distributed, but most diverse in the tropics (Lawrence, 1991).

The members of the Mycteridae used to be placed into the families Salpingidae, Pythidae and Melandryidae (Mycterinae and Lacconotinae) or Cucujidae (Hemipeplinae) or were recognized as an independent family Hemipeplidae (Arrow, 1930; Crowson, 1955). Crowson and Viedma (1964) proposed the present concept of the family. Mycterids seem to be related to the Boridae and the ‚salpingid' group of families, consisting of Pythidae, Pyrochroidae, Trictenotomidae and Salpingidae (Watt, 1987; Lawrence, 1991; Pollock, 2002). However the relationships among the subfamilies as well as to other families of the Tenebrionoidea remain unclear (Pollock, 2002).

Boridae *C.G.Thomson 1859*- the conifer bark beetles.

Morphology. Elongate, parallel-sided body, convex dorsally, 8-25mm, brown. Head slightly elongate, parallel-sided behind eyes (*Lecontia*) or abruptly narrowed (*Boros*); eyes slightly (*Lecontia*) to moderately (*Boros*) convex; antennae relatively short, 3 apical antennomeres wider, forming club, antennal insertions concealed dorsally; mandibles protrude beyond labrum (*Lecontia*) or not (*Boros*), bidentate; last maxilar palpomere widened, labial palpi similar. Pronotum with lateral carinae; legs slender, coxae elongate, projecting below intercoxal process, coxal cavity broadly open, tibial spurs distinct, tarsal formula 5-5-4, tarsomeres slender, with setose, not lobed or expanded; elytra parallel-sided with broadly rounded apices. Abdomen with 5 ventrites (Pollock, 2002).

Larvae strongly flattened, subparallel, lightly sclerotized, yellowish, scattered setae, 17-23mm (*Boros*) or 38-45mm (*Lecontia*) in length. Head exserted from the prothorax; epicranial suture short, endocarinae absent; 5 stemmata on each side, divided in 2 groups (*Boros*) or absent (*Lecontia*); antennae elongate, 3-segmented, a small conical sensorium; mandibles heavily sclerotized, asymmetrical, left mandible bearing prominent molar tooth, tridentate. Posterior margin of pronotum (*Lecontia*) or anterior margin of mesonotum (*Boros*) with 2 dentiform processes; legs well developed. 9 th tergite heavily sclerotized, hinged; a pair of urogomphi with 2 urogomphal pits between them (Young, 1991).

Bionomics. Adults occur under bark of conifers and in leaf litter. Larvae of *Boros* have been found under loose bark of dead pines (Young, 1991).

Classification. The Boridae is a small family with 3 genera and 6 species (Young, 1991). Two subfamilies are recognized: Borinae with genera *Boros* and *Lecontia* occuring in the Northern Hemisphere and Synercticinae with a single genus *Synercticus* from Australia and New Guinea (Lawrence & Newton, 1995).

The members of the family have been placed in several positions in the classification of Tenebrionoidea. Initially, genus *Boros* used to be associated with Tenebrionidae and later moved either in a position close to Salpingidae and Pythidae or along with the genera *Lecontia* and *Synercticus* in the family Pythidae. However, studies by St.George (1931), Young (1985a), and Lawrence and Pollock (1994) supported an independent family Boridae, with all three genera mentioned above included in it. The Pythidae are believed to be the most closely related family. There are several features of borids in common with lacconotine mycterids or pyrochroids (St.George, 1931; Crowson, 1955; Crowson & Viedma, 1964; Young, 1985a, 1991). Indeed, Boridae are considered to be members of the lineage together with Pythidae, Pyrochroidae, Salpingidae (Inopeplidae, Othniidae included) and Mycteridae (Crowson, 1966; Lawrence & Newton, 1982) or of salpingid group (Watt, 1987; Pollock, 1994). Finally, in the most recent work, Pollock (1994) found a sister group relationship with the family Pyrochroidae.

Trictenotomidae *Blanchard 1845*- the log-boring beetles.

Morphology. Large, 40-80mm in length, cerambycid-like, slightly flattened, with hard integument, shiny or usually coated with thick hairs. Head large, flattened, narrower than prothorax; eyes large, vertical, fine-faceted; antennae long, with 3 apical antennomeres broadened forming a short club; mandibles large, protruding. Prothorax transverse, with lateral carinae; procoxae transverse, divided by a broad prosternal process, front coxal cavities

open from behind and inside, mesocoxae with cavities open laterally; legs long, tarsal formula 5-5-4, tarsal joints cylindrical, thick hairs; elytra taper slightly towards the apices. Aedeagus false-trilobed (Telnov, 2000).

Larvae large, over 100mm, elongate, parallel-sided, slightly flattened, yellowish-white with darker head. Head large, epicranial stem short, frontal arms lyriform, endocarina absent; stemmata absent; antennae long, with 3rd segment reduced; mandibles large, asymmetrical, tridentate, with ridged mola. Thorax short; legs short, widely separated; terga with a series of short, longitudinal ridges forming transverse rows on thorax, longitudinal rows on abdomen; abdominal sterna with short, longitudinal ridges arranged in transverse rows; a pair of posteriorly projecting, up turning urogomphi (Lawrence, 1991).

Bionomics. Trictenotomid larvae live in rotten wood (Lawrence, 1991) or under bark of trees as well as the adults (Telnov, 2000).

Classification. The Trictenotomidae is another small family of Tenebrionoidea that encompasses only two genera *Autocrates* and *Trictenotoma* with 12 species, distributed in Asia (Lawrence, 1991).

The most closely related family is considered to be the family Pythidae (Crowson, 1955; Lawrence, 1991; Beutel & Friedrich, 2005), but they also resemble Boridae in some characters (Watt, 1987). This author found Trictenotomidae as a sister group to a clade comprising of Pilipalpinae, Pythinae, Boridae and Salpingidae in his cladistic analysis. In contrast, Pollock (1994) merged Trictenotomidae in a single clade with Pythidae and Salpingidae, forming a sister group to a Boridae+Pyrochroidae clade. However both authors consider Trictenotomidae to be a part of salpingid group (Trictenotomidae, Salpingidae, Boridae, Pythidae, Pyrochroidae).

Pythidae *Solier 1834*- the dead log beetles.

Morphology. Body elongate, subcylindrical to depressed, dorsum with distinct punctation, 6-22mm in length. Head elongated (*Pytho, Priognathus*) or short, not narrowed posteriorly of eyes; eyes small or large, separated widely from antennal insertions (*Pytho*, *Priognathus*) or emarginate around antennal insertions (*Trimitomerus, Sphalma*); antennae moniliform, with slightly developed club (*Pytho*, *Priognathus*), subfiliform or with elongated antennomeres (*Trimitomerus*); mandibles slender and elongate, mola indistinct or very large (*Sphalma*); terminal palpomere of maxillary palpi securiform (*Pytho*, *Sphalma*) or slightly expanded (*Priognathus*, *Trimitomerus*). Pronotum wider than long, variously convex to flattened, lateral margins smooth (*Pytho, Priognathus*) or with slightly developed carinae (*Trimitomerus*) or

with very distinct border (*Sphalma*), procoxal cavities internally and externally open; elytra elongate, parallel-sided, with scattered punctures or with distinctly raised intervals; mesocoxal cavities laterally open; tarsal formula 5-5-4, tarsi simple. Abdomen with all ventrites free (*Pytho, Priognathus*) or V1, V2 connate (*Trimitomerus* and *Sphalma*) (Pollock, 2002).

Larvae subcylindrical to slightly (*Priognathus, Sphalma*) or strongly flattened (*Pytho*), lightly pigmented and sclerotized, scattered fine setae, 8.5-30mm in length. Head exserted from or slightly retracted within prothorax; epicranial stem short or absent, frontal arms lyriform, endocarinae absent; 5 stemmata on each side, divided in two groups; antennae elongate, 3-segmented; mandibles heavily sclerotized, with 2-3 apical and usually 2 subapical teeth, slightly (*Sphalma*) to conspicuously (*Priognathus, Pytho*) asymmetrical, left mandible with prominent molar tooth, molar region of right mandible slightly more prominent than that of left one. Thorax elongate, anterior margin of metanotum, occasionally mesonotum and abdominal tergites sometimes with 2 posteriorly directed, dentiform processes along meson (*Pytho*); legs well developed, with spine-like setae; between paired, fixed urogomphi usually a single shallow urogomphal pit, urogomphi with secondary branching (*Sphalma*), or spine-like structures (*Priognathus*), or unbranched and possessing smaller spine-like projections (*Pytho*), 9th sternite with a double arch of asperities near anterior margin (Young, 1991).

Bionomics. Both adults and larvae are associated with dead logs, they occur on or under the bark, feeding on decaying wood, either coniferous (*Pytho* spp.) or deciduous (*Sphalma* spp.) trees or on sapwood of conifer logs (*Priognathus* spp.) (Young, 1991).

Classification. The Pythidae, including Pilipalpinae, comprise 15 genera and about 50 species, that are distributed in Holarctic coniferous forests and the temperate parts of the Southern Hemisphere (Young, 1991).

Historically, the family Pythidae used to include some taxa now classified in several families. The broad concept of the family, with inclusion of borids, mycterids, pyrochroids and salpingids, has been proposed by Iablokoff-Khnzorian (1985). However, the concept of the family currently follows Crowson (1967), without genera *Boros* and *Lecontia* (Boridae) (Young, 1985a) and with the addition of *Sphalma* (Young, 1976) and *Anaplopus* (Lawrence, 1987). Repeated transfers of genera between Pythidae and Pyrochroidae were proposed, especially, transfers of the subfamilies Pilipalpinae and Tydessinae (Watt, 1987; Peacock, 1982; Pollock, 1992, 1994). Except Pyrochroidae, the Pythidae are further related with remaining members of salpingid assemblage - Mycteridae, Boridae (Young, 1991) and Trictenotomidae. Based on larval characters, Beutel and Friedrich (2005) suggested the monophyly of the clade Pythidae and Trictenotomidae.

Pyrochroidae *Latreille 1807*- the fire-coloured beetles.

Morphology. Slightly to moderately flattened body, lightly sclerotized, 4-20mm, yellowish to black, often black with red or yellow thorax, dense hairs. Head deflexed, strongly constricted behind the eyes, forming a broad neck; antennae with 11 antennomeres, mostly filiform to pectinate (females) or serrate to plumose (males); eyes emarginate; labrum prominent. Prothorax narrower than elytra, without lateral carinae; long legs, completely open procoxal cavities and laterally open mesocoxal cavities, tarsal formula 5-5-4, tarsi lobed; hind wings rarely reduced or absent (Young, 2002).

Larva with well sclerotized body, 9-35mm in length, lightly pigmented with darker head and 9th tergite. Head exserted, nearly as wide as thorax; epicranial stem short to absent, endocarinae absent; 4 stemmata on each side, divided in 2 groups or absent (*Cononotus*); antennae 3-segmented; mandibles asymmetrical, bidentate to tridentate, molar area of right mandible well developed, left mandible with a prominent molar tooth. Thorax elongate, sides subparallel; legs well developed. Abdomen with 8th tergite more than two times as long as the 7th, 9th tergum hinged, extending ventrally to form the entire terminal segment or urogomphal plate; paired, heavily sclerotized, simple or branched urogomphi with 2 urogomphal pits; 9th sternite with continuous arch of asperities on the anterior margin or 1 (*Pedilus*) or 2 (*Cononotus*) asperities on each anterolateral aspect (Young, 1991).

Bionomics. Larvae are found under loosen bark, within decaying moist wood or in decaying vegetation as well. They consume both decayed wood and fungi tissues, with fungi as a more important part in their diet. Adults are known to occur on flowers, logs (*Pyrochroa*), under stones and decaying vegetation (*Cononotus*) or in flowers and on the leaves (*Pedilus*). Adults appear to be nocturnal (Young, 1991).

Classification. The family consists of approximately 200 species distributed worldwide, with the largest species richness in the temperate regions (Young, 2002). Presently, there are recognized four subfamilies, Tydessinae, Pilipalpinae, Pedilinae and Pyrochroinae, however inclusion of Agnathinae as the fifth subfamily was considered (Lawrence & Newton, 1995).

The concept of the family Pyrochroidae has undergone several changes and the limits remain contentious. Young (1984b) supported placement of *Pedilus* in the Pyrochroidae, as it used to be treated, followed by exclusion of *Ischalia* (to Anthicidae; Young, 1985b). More recently, the subfamilies Tydessinae and Pilipalpinae have been included in Pyrochroidae (Peacock, 1982; Pollock, 1992, 1994, 1995). Pilipalpinae used to be a subfamily of Pythidae until they were elevated to a family status by Nikitsky (1986). The genera *Agnathus* and *Cononotus*

formed Pedilinae on the basis of larval characters, and many autapomorphies of adults (Mamaev, 1976; Doyen, 1979, Young, 2002). The placement of these genera remains unclear, as argued by Pollock (1994), who excluded Agnathinae from pyrochroids. Tydessinae are considered to be the most primitive lineage, coordinated with the clade of Pilipalpinae, Pyrochroinae and Pedilinae (Pollock, 1994, 1995).

The closest relatives may be the families Boridae and Pythidae, Salpingidae and Trictenotomidae (Young, 1991, 2002). The similar connection has been already proposed by Crowson (1966), Lawrence and Newton (1982) and Pollock (1994), however Abdullah (1964) and Watt (1987) rather hypothetised relationship with families Anthicidae, Meloidae and Oedemeridae than with the salpingid group.

Salpingidae *Leach 1815*- the narrow-waisted bark beetles.

= including Aegialitidae, Dacoderidae, Elacatidae, Eurystethidae, Inopeplidae, Othniidae, Tretothoracidae

Morphology. Body elongate to slightly ovate, depressed to subcylindrical, conspicuously waisted, 1.5-7mm in length, punctate, with or without distinct vestiture. Head more or less elongate and rostrate, convex to flattened; eyes absent (*Aglenus*) to large (Othniinae); antennae with 10-11 antennomeres, moniliform to filiform, terminal antennomeres widened (except Inopeplinae) or apical 3-5 antennomeres form club; mandibles short, concealed by labrum. Pronotum narrower than elytra (except *Aglenus* and *Aegialites*) pronotal carinae arcuate; procoxae rounded to projecting, variously separated, procoxal cavities open or closed, protrochantins concealed; elytra elongate, rarely abbreviated (*Inopeplus*), punctures indistinct (*Inopeplus*), scattered or in distinct striae (Salpinginae), vestiture absent to distinct (*Elacatis*); hind wing absent or present; mesocoxae variously separated, mesocoxal cavities closed; tarsal formula 5-5-4 or rarely 4-4-4 (*Aglenus* and *Ocholissa*), tarsi simple, tarsomere with claws longer than any other, in *Aegialites* longer than all tarsomeres together, ventral surface of tarsomeres setose. Abdomen with 5 ventrites, *Aegialites* with first two ventrites immovable (Pollock, 2002).

Larvae slightly to strongly flattened, subparallel, 4-13mm, slightly sclerotized, lightly pigmented, vestiture of scattered, elongate setae. Head exserted from or slightly retracted within prothorax; without epicranial stem, frontal arms lyriform, endocarinae absent or paired endocarinae; stemmata 5, divided in 2 groups or 0 (*Aglenus*) on each side; antennae elongate, 3-segmented, antennal insertions fully exposed; mandibles heavily sclerotized, symmetrical with basal hyaline lobe or asymmetrical (Othniinae, Inopeplinae) with left mandible bearing

prominent molar tooth; maxillary mala undivided. Thorax subparallel-sided; legs well developed, with setae. Abdomen variously flattened to almost cylindrical; 9th tergite sclerotized, with a pair of 2-branched urogomphi; 9th sternite with either 1 or 2 asperities near anterolateral margin, or with double arch of asperities (*Elacatis*) (Young, 1991).

Bionomics. Adults are found in flowers, on leaves, decaying twigs or logs or various decomposing vegetative material. Larvae occur under bark, within decaying logs and decomposing vegetative material. Some were reported to feed on fungi or even prey on scolytids. Aegialitines are found in intertidal zone, where they feed upon algae or small invertebrates (Young, 1991).

Classification. There are known about 45 genera and 300 species distributed worldwide (Pollock, 2002).

According to Lawrence and Newton (1995), the family Salpingidae is nowadays defined in a broad sense, comprising seven subfamilies: Othniinae, Prostominiinae, Agleninae, Inopeplinae, Salpinginae, Aegialitinae and Dacoderinae. The monophyly of the present family Salpingidae is poorly supported (Pollock, 2002, Beutel & Friedrich, 2005).

Crowson (1955) proposed that the Salpingidae are related through *Cononotus* to Mycteridae and even to Anthicidae. Crowson (1955) also established Inopeplidae as a new family. Salpingids have been connected with Pythidae, Pyrochroidae, Mycteridae and Boridae in one lineage (Crowson, 1966; Lawrence & Newton, 1982), which was questioned by Pollock (1994). Watt (1987) defined the Salpingid group as consisting of Pythidae, Pilipalpinae, Trictenotomidae, Boridae and Salpingidae. He supported his concept by the similar type of aedeagus and the structure of mandibles. Within this group, trictenotomids stand in a sister group position to the remaining lineages, which form a single clade. However, the exclusion of Pyrochroidae from this clade, was questioned by Pollock (1994). His analysis has discovered two clades within the salpingid group - first, the unresolved tritomy of Trictenotomidae, Salpingidae and Pythidae and the second formed by Boridae and Pyrochroidae.

Anthicidae *Latreille 1819*- the ant-like flower beetles.

=Notoxidae, Ischaliidae

Morphology. Elongate, soft body, 1.2-6.9mm, with decumbent hairs. Head deflexed, strongly constricted behind eyes forming neck; eyes entire, ovate, emarginate (Eurygeniinae), with short setae; antennae with 11 antennomeres, filiform, serrate, weakly clubbed (*Lagrioida*), subclavate, antennal insertions exposed; mandibles short, strongly curved; variously modified

maxillary palpi. Pronotum widest in anterior third, narrowing in basal half (except Ischaliinae), without lateral carinae (except Ischaliinae), apex with narrow collar or hem, prominent horn projecting over head in Notoxini; procoxal cavities open posteriorly, closed internally, trochantin concealed; mesocoxal cavities separated by mesosternal extension, trochantin evident; metacoxae with short internal hem; legs with slender femora, tibiae and tarsi, tarsal formula 5-5-4, penultimate tarsomeres narrowly lobed beneath, claws simple to appendiculate; elytra entire, with three types of pubescent. Abdomen with 5 visible, free sterna (basal two are fused in Lagrioidinae) (Chandler, 2002).

Larvae elongate, subcylindrical or slightly flattened, lightly sclerotized and pigmented, 3-15mm in length, a few long setae. Head exserted from prothorax; epicranial stem absent (*Pergetus*, Lagrioidinae) or short, frontal arms lyriform, median endocarina absent (Ischaliinae, Lagrioidinae), single (Anthicinae) or paired (*Pergetus*); a pair of stemmata or absent (*Pergetus*) near base of antenna; antennal insertions fully exposed, antennae elongate, 3-segmented with elongate terminal seta; mandibles heavily sclerotized, asymmetrical, molar area of right mandible more prominent than the left one, apices tridentate (*Pergetus*) or bidentate, penicillus or brush of spine-like setae at base of mola. Thorax elongate with longer prothorax; legs well developed, with fine setae; abdomen subcylindrical or slightly flattened, 9^{th} tergite extending ventrally, with a pair of heavily sclerotized, upcurved, fixed urogomphi or absent (Ischaliinae), with or without short secondary branch, 9^{th} sternite small (Young, 1991; Chandler, 2002).

Bionomics. Adults are found within decaying organic debris on the ground or often on flowers and foliage; they are omnivorous, predators, or feed on nectar and pollen. Some species are strongly associated with coastal sand dunes, margins of fresh or salt waters. Many species are attracted to cantharidin, which they seem to accumulate to discourage predators. Larvae are associated with decaying vegetation as well, being omnivorous or mycetophagous. Some feed on eggs or dipteran puparia (Young, 1991).

Classification. The family Anthicidae comprises about 100 genera with over 3000 species worldwide (Chandler, 2002). The family has overcome a large inclusion of several groups in recent period and presently there are recognized ten subfamilies: Eurygeniinae, Lagrioidinae, Afreminae, Macratriinae, Steropinae, Ischaliinae, Copobaeninae, Lemodinae, Tomoderinae and Anthicinae (Lawrence & Newton, 1995). Several of them have been included in other families and higher classification of this family needs revision (Lawrence & Newton, 1995; Chandler, 2002).

Anthicids were associated in one lineage with the Aderidae and Meloidae by Crowson (1966). Lawrence (1977) proposed that the Scraptiidae may be more closely related to anthicids and aderids, on the basis of both larval and adult characters. Indeed, this relationship was concluded by Lawrence and Newton (1982) in the lineage consisting of these three families, despite of a fact that the same authors have questioned the constitution of the family Anthicidae as well as the inclusion of the family Scraptiidae in this lineage. The close relationship between the Anthicidae, Aderidae and Scraptiidae, particularly Anaspidinae, has been confirmed also by Young (1991).

Aderidae *Winkler 1927*- the ant-like leaf beetles.

= Euglenidae, Euglenesidae, Hylophilidae, Xylophilidae

Morphology. Body elongate to oval, convex to slightly flattened, resembling small anthicids, 1-4mm in length; erect or decumbent hairs, dense, sometimes forming pattern; black and red in coloration. Head strongly deflexed, abruptly constricted behind eyes, forming neck, wider than pronotum (except Phytobaenini); larger eyes, weakly to strongly emarginate, with setae between facets; antennae with 11 antennomeres, filiform or thicken gradually towards the apex; small mandibles; securiform maxillary palpi. Pronotum frequently narrowed at apex, base narrower than elytra, lateral margins rounded; procoxal cavities open internally and posteriorly, antepenultimate tarsomere lobed and penultimate reduced, but tarsal formula 5-5-4; elytra entire; males often with pubescent secretory organs on the hind femora. First two abdominal sterna solidly fused (Lawrence & Britton, 1991; Chandler, 2002).

Larvae subcylindrical to flattened, lightly sclerotized and pigmented, vestiture of scattered, long setae. Head prognathous to slightly deflexed; epicranial stem short or absent, frontal arms lyriform, endocarinae absent; stemmata absent, antennal insertions exposed, antennae elongate, 3-segmented, with large sensorium; mandibles heavily sclerotized, asymmetrical, left mandible with prominent molar tooth. Legs well developed, 5-segmented. Paired urogomphi, distally strongly upcurved (Young, 1991).

Bionomics. Our knowledge on biology of aderids is limited. Adults are found on foliage, dead branches, and occasionally in flowers. Larvae occur in rotting wood, leaf litter or nests of other insects and are thought to be saprophagous (Young, 1991; Chandler, 2002).

Classification. The family Aderidae include about 50 genera with more than 1000 species distributed worldwide, however the most species are tropical (Chandler, 2002).

Although Aderidae resemble Anthicidae superficially and are usually placed close to them in classifications (Lawrence & Newton, 1995), they actually differ from anthicids and any other

heteromeran family in several imaginal characters (Crowson, 1955). Their features as a *Byturus*-like metendosternite, lobed antepenultimate and small penultimate tarsal segments, non-heteromeroid trochanters and the internally open front coxal cavities are more clavicorn-like than heteromeran (Crowson, 1955). Also Young (1991) emphasized a number of larval characters distinguishing aderiid larvae from most other ones. Buder's *et al.* (2008) analysis did not indicate relationships between Aderidae and Anthicidae, however has showed association of Aderidae with Sphindidae or with assemblage Coccinelidae, Endomychidae and Lathridiidae, all cucujoid families.

Scraptiidae *Mulsant 1856*- the antlike leaf beetles, false flower beetles, scraptiid beetles.

= Anaspididae

Morphology. Oblong to elongate, parallel-sided or more or less wedge-shaped (Anaspidinae), soft body, 1.3-12mm. Head deflexed, constricted behind eyes, forming a narrow neck; eyes deeply emarginate; long antennae, filiform or enlarged slightly towards the apex; maxillary palpi securiform. Pronotum with lateral carinae, narrower apically; tibial spurs well developed and pubescent, tarsal formula 5-5-4, penultimate tarsal segment lobed beneath (Lawrence & Britton, 1991).

Larvae elongate, subcylindrical, subparallel, lightly sclerotized and pigmented, 3-10mm, with fine setae. Head more or less exserted; epicranial stem short (Anaspidinae) or absent (Scraptiinae), frontal arms lyriform, endocarinae absent; 1 stemmata on each side (Anaspidinae) or absent (Scraptiinae); antennal insertions fully exposed, antennae 3-segmented, 2^{nd} segment with dome-like sensorium, 3^{rd} segment small with an elongate terminal seta; mandibles heavily sclerotized, nearly symmetrical to asymmetrical, molar area well developed, base of mola with brush of spines (Anaspidinae) or absent (Scraptiinae). Thorax elongate, prothorax longer; legs well developed, with fine setae. Abdomen subcylindrical; 9^{th} tergite extended ventrally, with paired, fixed urogomphi (Anaspidinae) or completely dorsal, with large, oblong, dehiscent caudomesal process (Scraptiinae) (Young, 1991).

Bionomics. Adults occur on foliage and flowers, anaspidines on flowers of Apiaceae and Rosaceae near marshes and stream margins. Scraptiines larvae are found under bark, in rotten wood, leaf litter and lichens (Young, 1991).

Classification. The family Scraptiidae is widely distributed and counts about 25 genera and 250 species worldwide (Young, 1991). Two morphologically different subfamilies are defined in Scraptiidae: Scraptiinae and Anaspidinae (Lawrence & Newton, 1995). Crowson (1955)

associated scraptiides with anaspidines and his opinion is widely accepted (Watt, 1987). Based on adult characters, scraptiids were connected with melandryids, and anaspidines with mordellids. The relationships of Scraptiidae to Anthicidae and Aderidae has been proposed by Lawrence (1977) on the basis of larval morphology and his opinion was followed by Watt (1987).

3.3 Molecular markers.

Ribosomal DNA (rDNA) codes ribosomal RNA (rRNA) and is situated in the nucleus of cells. Ribosomal RNA is together with ribonucleoproteins the main component of the ribosomes, that consists of two subunits. The large subunite (LSU) of eukaryotes consists of 5S, 28S and 5.8S rRNA molecules and 34 proteins, the small subunit (SSU) is composed by 18S rRNA and 33 proteins. Except these, cells contain 12S and 16S rRNA (rrnL) molecules in mitochondrias. Eukaryotes generally have many copies of the rRNA genes organized in tandem repeats. The rDNA gene of *Drosophila melanogaster* encodes four individual rRNAs, organized from the 5'end of intergenic spacers /IGS/, external transcribed spacers /ETS/, 18S rRNA (1995 bp), through internal transcribed spacers /ITS 1/, 5.8S rRNA (123 bp), ITS 2a, 2S rRNA (30 bp) and ITS 2 to the 3' end of 28S rRNA (3945 bp) (Tautz *et al.*, 1988).

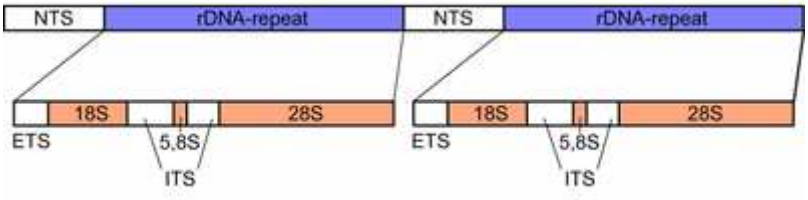


Figure 1: Organization of ribosomal DNA gene. (wikipedia.org)

Genes of rDNA are favoured markers in phylogenetic reconstructions in general (e.g., Caterino *et al.*, 2000; Ridley, 2004), because of their presence in all organisms and in both nucleus and mitochondrias as well. The second reason is the sequences' composition, that consists of conservative regions, that represent 98% of the length, along with variable regions (Smit *et al.*, 2007) and the variability of their secondary structure. The rate of evolution of secondary structure differs and is lineage specific, proposing that specific models would improve phylogenetic resolution (Smit *et al.*, 2007). Nuclear genes evolve more slowly than mitochondrial ones and they are more suitable for investigation of relationships at higher systematic levels (Caterino *et al.*, 2000). 18S rDNA has been supported for resolving the monophyly of insect orders, with the exception of Coleoptera (Kjer, 2004), but it has not been

found to be appropriate for resolving most interordinal relationships, especially at the deeper nodes of the phylogeny (Whiting, 2002). The 28S gene rDNA is faster evolving than the 18S gene (Gomez-Zurita *et al.*, 2007) and have been found useful for resolving relationships among beetle superfamilies and families (Marvaldi *et al.*, 2009).

Mitochondrial DNA (mtDNA) is the DNA from mitochondrias, the organelles responsible for oxidative phosphorylation and electron transport in cells. The mtDNA is a double-stranded circular molecule with 14 000-17 000 base pairs, consisting of 37 genes, from which 13 are protein coding, 22 for tRNA and 2 for rRNA and represents the smallest genome in Metazoa (Cameron, 2007).

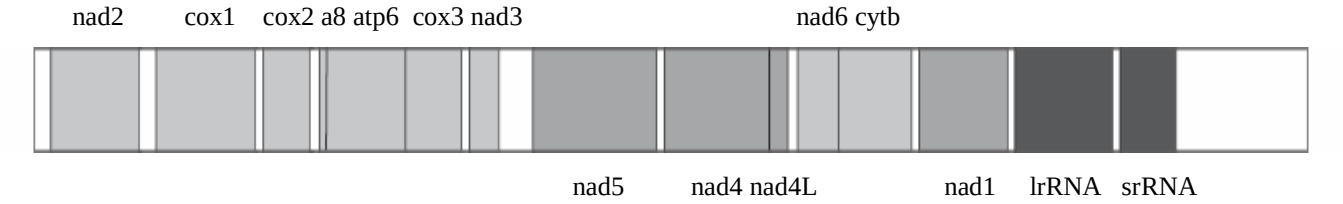


Figure 2: Scheme of mitochondrial DNA (Stewart & Beckenbach, 2005).

Gene COI (cox1) codes for the cytochrome oxidase subunit I, that is a main subunit of the enzyme cytochrome c oxidase, the key enzyme in aerobic metabolism.
Gene 16S rDNA (rrnL) is one of two ribosomal genes in mitochondrial genome. It codes for the small subunit of ribosomes.
Sequences of mitochondrial DNA have been the frequently used characters to infer phylogeny and phylogeography of insects as well as animals in general (Caterino *et al.*, 2000). It is due to their supposed neutral variation, their easy amplification and special features like lack of introns, maternal inheritance, haploidy and absence of recombination events (Zardoya & Meyer, 1996; Orsini *et al.*, 2007).
The mitochondrial DNA has been considered to be a reliable marker in phylogeny of both closely and distantly related taxa (Zardoya & Meyer, 1996; Cameron *et al.*, 2007). Mitochondrial genes are estimated to evolve much faster than nuclear protein-coding genes in insects (Moriyama & Powell, 1997; Monteiro & Pierce, 2001; Lin & Danforth, 2004). However some authors question the usefulness of mtDNA, firstly because mitochondria are frequently influenced by strong selection, that casts doubt on their primarily considered neutral evolution and secondly because they evolve under different evolutionary rules than other genomes (Ballard & Whitlock, 2004).

Since different molecules undergo different evolution, their contribution to the phylogenetic resolution varies. The mitochondrial rRNA genes are useful for resolving phylogenetic problems in the 10-100 million year range, whereas the slowly evolving nuclear rRNA genes are useful in the hundreds of millions of years range (Ridley, 2004). The diverse contributive value of the markers leads to the combination of different types of molecules in the way of collecting as much information as possible. In these days, it is common practice to combine sequences of nuclear and mitochondrial genes, morphological or other relevant data (geographical, ecological, behavioral, etc.) (Sallum *et al.*, 2002; Whitfield *et al.*, 2002; Balke *et al.*, 2005; Bocakova *et al.*, 2007; Hunt *et al.*, 2007; Bocak *et al.*, 2008). Buder *et al.* (2008) has proved the informative value of SSU, cox1 and cox2 genes at the cucujoid-tenebrionoid and familial level.

4. Material and Methods.

4.1 Sampling of taxons.

The data matrix consists of 188 species from which 154 taxa represent the ingroup. The sequences of SSU, LSU, rrnL and cox1 genes for all 154 taxons have been newly sequenced for this study (Supplementary material Table B). The sequences for the representatives of Chrysomeloidea and Curculionoidea superfamilies were provided by the A. P. Vogler's group (BNHM, London).

Twenty of thirty recognized tenebrionoid families (Lawrence & Newton, 1995) were represented in the data set. However we were not successful in the amplification of sequences of genera *Pytho* and *Mycterus*. Eight other missing families in our sampling include mainly species with nearctic distribution, not available for this study. The families recognizing more subfamilies are in the data set represented by taxons ranked in as many subfamilies as possible. The outgroup comprises taxons from all remaining superfamilies of Cucujiformia series and three taxons in the data set are the members of Elateriformia series to include out-Cucujiformia species in the matrix as well. The complete sampling list with the locality of origin, specimens' codenames and GenBank accession number is provided in the Supplementary material Table B.

4.2 Laboratory methods.

All specimens were preserved in 96% alcohol in the field and kept -20°C in the laboratory. The DNA was extracted from the thorax of each individuals using a phenol/chlorophorm protocol as described by Vogler *et al.* (1993).

Two nuclear genes SSU and LSU rDNA and two mitochondrial genes rrnL rDNA and cox1 mtDNA were amplified. The whole SSU rDNA gene was amplified as four overlapping fragments in both directions in the total length about 1900bp. The part of the large subunit nuclear LSU rDNA (670-760bp) and the fragment of the cytochrome oxidase subunit I (723bp) were amplified in both directions as single fragments with the primers '28Sdd' and '28Sff' and 'Jerry' and 'Pat', respectively. The small ribosomal subunit rrnL rDNA (550bp) was amplified using the primers '16Sa' and '16Sb' or in few cases of failed amplification with primer 'ND1-2' (the longer fragment of about 1200bp). Primers' sequences and references are listed in Table 1. The amplifications were carried out using 1U Taq polymerase (Platinum Taq DNA Polymerase, Invitrogen or BioTaq DNA Polymerase, Bioline) with proof-reading

activity in order to mimimize the introduction of artificial mutation, 2mM $MgCl_2$, 50µM each dNTP, 0.2µM each primer and 0.03µg of template in 50µl reaction volume. The typical PCR reactions were performed under the following conditions: 2min at 94°C for initial denaturation; 94°C for 1min, 45°C for 1min, 72°C for 1.5/2min depending on the length of amplifying fragment in 40 cycles; 10min at 72°C for final extension. In the case of unsuccessful amplification of fragment, the higher concentration of template /0.12µg/, Taq polymerase /2.5U/, primers /0.8µM/, dNTPs /300µM/ and $MgCl_2$ /4mM/ were used in the reaction. The fragment of the correct size was separated from the gel if several fragments were amplified. The PCR product was purified using the GeneClean III kit (BIO101Systems QBIOgene). The cycle sequencing reactions were performed using the BigDye Terminator v. 1.1 Cycle Sequencing Kit. The products were purified by alcohol precipitation and the templates were sequenced using ABI3130 Genetic Analyzer (Applied Biosystems). The sequences were edited using Sequencher 4.5 software package (Gene Codes Corporation, Ann Arbor, MI, USA).

Table 1: Primers and its sequences.

Gene	Primer name	Sense	Primer sequence	Reference
SSU	18S 5’	F	5’-GACAACCTGGTTGATCCTGCCAGT- 3’	Shull *et al*. (2001)
	18S b5.0	R	5’-TAACCGCAACAACTTTAAT- 3’	
	18S ai	F	5’-CCTGAGAAACGGCTACCACATC- 3’	
	18S b2.5	R	5’-TCTTTGGCAAATGCTTTCGC- 3’	
	18S a1.0	F	5’-GGTGAAATTCTTGGACCGTC- 3’	
	18S bi	R	5’-GAGTCTCGTTCGTTATCGGA- 3’	
	18S a2.0	F	5’-ATGGTTGCAAAGCTGAAAC- 3’	
	18S 3’I	R	5’-CACCTACGGAAACCTTGTTACGAC- 3’	
LSU	28S ff	F	5’- TTACACACTCCTTAGCGGAT - 3’	Inward (2003)
	28S dd	R	5’-GGGACCCGTCTTGAAACAC- 3’	
rrnL	16S a	F	5’-CGCCTGTTTAACAAAAACAT- 3’	Simon *et al*. (1994)
	16S b	R	5’-CCGGTCTGAACTCAGATCATGT- 3’	
	ND1-2	F	5’-ATCAAAAGGAGCTCGATTAGTTTC- 3’	
cox1	Jerry	F	5’-CAACATTTATTTTGATTTTTTGG- 3’	Simon *et al*. (1994)
	Pat	R	5’-TCCATTGCACTAATCTGCCATATTA- 3’	

4.3 Phylogenetic analyses.

4.3.1 Sequences analyses

Sequences' length variation was counted using MEGA version 3.1 (Kumar *et al.*, 2004). The software DAMBE (Xia & Xie, 2001) was used to calculate the nucleotide frequencies in the sequences.

4.3.2 Multiple alignment

To build an optimal data set, without homoplastic characters, is a crucial step in phylogenetic reconstruction and it is not easy to obtain it with length-variable rDNA sequences. To achieve the maximum, several approaches of aligning of sequences have been performed:

a) a static assignment of homologous sites is produced by progressive multiple alignment using ClustalX version 1.81 (Thompson *et al.*, 1997) in three steps. The process of creating a multiple alignment begins with computing all pairwise alignments, that is followed by constructing a dendrogram. It describes the groupings of the sequences by similarity and it is used as a guide tree for order of sequences' aligning to carry out the final multiple alignment. The alignments were performed under different sets of gap opening and gap extension penalties, including default settings, followed Bocakova *et al.* (2007) as shown in table 2.

Table 2: Alignments settings.

ClustalX	**A**	**B**	**C**	**D**	**E**
Gap opening penalty	5	10	15	15	30
Gap extension penalty					
in pairwise alignment	0.05	0.1	0.15	6.66	6.66
in multiple alignment	0.1	0.2	0.3	6.66	6.66

b) a dynamic homology assignment under direct optimization as well as searching the most parsimonious tree are both implemented in POY 3.0.11 (Wheeler, 1996; Wheeler *et al.*, 2002). The direct optimization performs minimizing of nucleotide changes, including insertion-deletions (indels) in a one-step process; it creates a matrix of costs for substitutions (transitions and transversions) and indels, and simultaneously couples the optimization of the phylogenetic tree. Optimal trees are obtained by rearrangements to the tree topology and correspondences of nucleotide positions to minimize substitutions and length variation in the same time. The analyses were performed on a parallel processing system using a 14 dual-processor (2.8GHz P4. 2GB RAM) cluster at Imperial College London for a maximum of 48h for each run. All tree searches were done under a scheme of equal costs for nucleotide

changes and indels. Tree searches included three sequential stages, following the protocol of Giannini and Simmons (2003) as documented on figure 3. The first step consisted of 40 random sequence addition (RAS) replicates each followed by tree bisection reconnection (TBR) branch swapping. The optimal trees were retained from each independent replicate, followed by up to 10 000 tree fusings (Goloboff, 1999). The second step consisted of several TBR ratchet cycles (Nixon, 1999) performed on the shortest tree from the previous tree fusing and on the shortest and longest tree obtained from each random addition replicate. Finally, the shortest tree from all these analyses was submitted to a TBR search under iterative pass optimization (Wheeler, 2003). The latter run was time consuming, but resulted in a significant reduction of the tree length even when searches were not run to completion.

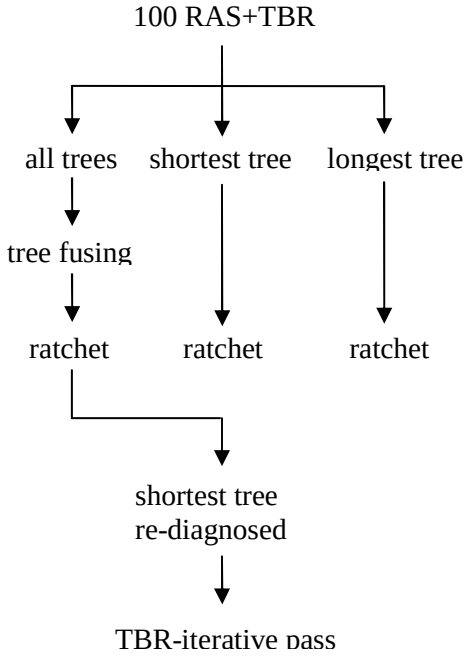


Figure 3: Scheme of tree search protocol by POY according Giannini and Simmons (2003).

c) Last type of alignment employed in this study, is based on the *blastn* algorithm, which determines short non-gapped segments of high similarity between pairs of sequences, implemented in BlastAlign (Belshaw & Katzourakis, 2005). These High-scoring Segment Pairs (HSP) are used as seeds for initiating searches to find longer segments in both directions and can be displayed as “flat query-anchored alignments”. These alignments contain mainly the alignment-conservative regions of the sequences, improving homology assignments. The resulting aligned data matrix was used directly for tree searches.

4.3.3 Tree search methods

4.3.3.1 Maximum Parsimony

Condition of maximum parsimony (MP) assumes the most likely tree with the fewest number of substitutions. The MP analyses were conducted using TNT version 1.0 (Goloboff *et al.*, 2004). The driven search of "New Technology" algorithm was processed simultaneously with implemented sectorial searches, tree ratcheting, tree drifting and tree fusing. The condition to find minimum length tree five times was set for the searches in all analyses. The gaps were treated either as missing or as fifth character state and all characters were considered as unordered and given equal weights. From the shortest trees the consensus 50% majority tree was counted. All trees were rooted by defined outgroup. 100 replications of matrix' resampling with "New Technology Search" were performed and the bootstrap values were assigned to branches, if 50% condition was fulfilled. The trees' characteristics as consistency index (CI), retention index (RI) and rescaled index (RC) were obtained using PAUP v. 4.0b10 (Swofford, 2002).

Due to time-demanding analyses by maximum likelihood approach and bayesian interference, a reduced matrix was prepared. There were included the taxons with all four genes successfully sequenced. The taxons with extremely long insertions were excluded as well as the taxons from numerously represented families. The reduced matrix consists of 110 ingroup taxons and 24 outgroup taxons and these taxons are marked by a star in the sampling list /Supplementary material B/. The ClustalX alignment was applied under the settings as described above, followed by tree search under maximum parsimony condition using software TNT version 1.0. The matrix aligned under default settings (settings "D") was preferably chosen for maximum likelihood and bayesian interference analyses.

4.3.3.2 Bayesian analysis

Bayesian analysis seeks the tree that maximizes the probability of the given data and the model of evolution. It uses Markov Chain Monte Carlo algorithms. The Bayesian interference of phylogeny was performed using a program MrBayes version 3.1.2 at Computational Biology Service Unit (CBSU) from Cornell University. The matrix as well as matrices of individual genes were performed several times in effort of reaching stationary state between the runs. However due to time-limit at the CBSU, the recommended average standard deviation value of the split frequencies (less than 0.01) has not been achieved. The settings for

every bayesian analysis are shown in table 3. As determined by MODELTEST v.3.8, GTR+I+G model was applied.

Table 3: Settings of bayesian analyses.

Bayes	**All**	**SSU**	**LSU**	**rrnL**	**cox1**
Sbst.model (nst)	GTR(6)	GTR(6)	GTR(6)	GTR(6)	GTR(6)
Rate variation	invgamma	invgamma	invgamma	invgamma	invgamma
No.generations	4mil.	2.5mil.	5mil.	5mil.	5mil.
No.runs	2	2	2	2	2
No.chains	4	5	4	4	4
Burn-in	250	8000	250	1000	1000
Std.deviation	0.0498	0.0184	0.0312	0.0430	0.1064

4.3.3.3 Maximum Likelihood

Maximum likelihood (ML) looks for the tree that maximizes the likelihood of observing data given that tree under some model of sequence evolution. Using MODELTEST v.3.8 (Posada & Crandall, 1998), the most appropriate model for DNA substitution of a reduced matrix under both hLRTs and AIC, was determined. The phylogenetic analysis under maximum likelihood (ML) condition with a determined GTR+I+G model and model parameters was performed using PhyML v. 2.4.4 (Guindon & Gascuel, 2003). The settings for every ML analysis are shown in table 4. The branches were evaluated with bootstrap support resulting from 100 resampling' replications. The same steps were applied to individual genes of a reduced matrix as well.

Table 4: Settings of ML analyses.

ML	**All**	**SSU**	**LSU**	**rrnL**	**cox1**
Sbst.model (nst)	GTR(6)	GTR(6)	GTR(6)	GTR(6)	GTR(6)
Proportion invar.sites	0.5490	0.6152	0.6073	0.2071	0.3405
Gamma shape params.	0.5224	0.4140	0.6525	0.4409	0.3980
Type tree improvement	NNI	NNI	NNI	NNI	NNI
Bootstrap no.gens	100	100	100	100	100

4.3.4 Taxonomic Retention Index.

To evalute the trees to the present classification (Lawrence & Newton, 1995), there was created a binary matrix of presence/absence state of characters according the belonging of the taxons to taxonomic level of series, superfamily, family and subfamily (both latter only for taxons of Cucujiformia series). It resulted in the matrix comprising 57 characters for 188 taxons.

The resulting tree of each analysis together with taxonomic matrix was evaluated by counting the retention index for each character of taxonomic matrix (Hunt *et al.*, 2007) using PAUP v. 4.0b10 (Swofford, 2002). To compare the reliability of the type of analysis, the retention index for every taxonomic level- series, superfamily, family and subfamily- was calculated for each analysis.

5. Results.

5.1 Sequences and alignment.

Sequences of all four genes vary in length and nucleotides composition as well. The greatest length variation was found in the SSU gene with absolute difference of 130bp, followed by the LSU (88bp) and the rrnL (28bp) /table 5/.

Table 5: The length of ingroup sequences. Codenames are associated with names in the Sampling list /Supplementary Material B/.

	min. length	max. length	mean
all genes	3674bp (TerEuHo082)	3858bp (SaInIn202)	3697.74bp
SSU	1826bp (MyMyLg146, AdAd197)	1956bp (SaInIn202)	1841.53bp
LSU	628bp (TerEuSy031)	716bp (RhRi087)	640.94bp
rrnL	475bp (RhRi087)	503bp (RhRhMa086)	492.44bp
cox1			723bp

The longest SSU sequences were found in Salpingidae (1958bp and 1943bp; mean 1858.3bp), followed by Riphiphoridae (1923bp; mean 1858bp) and Mordellidae (1894bp; mean 1864.5bp) /figure 4/. Other two families, Oedemeridae and Meloidae, were with a higher mean than the common one. The members of the salpingid subfamily Inopeplinae had the longest insertions at all.

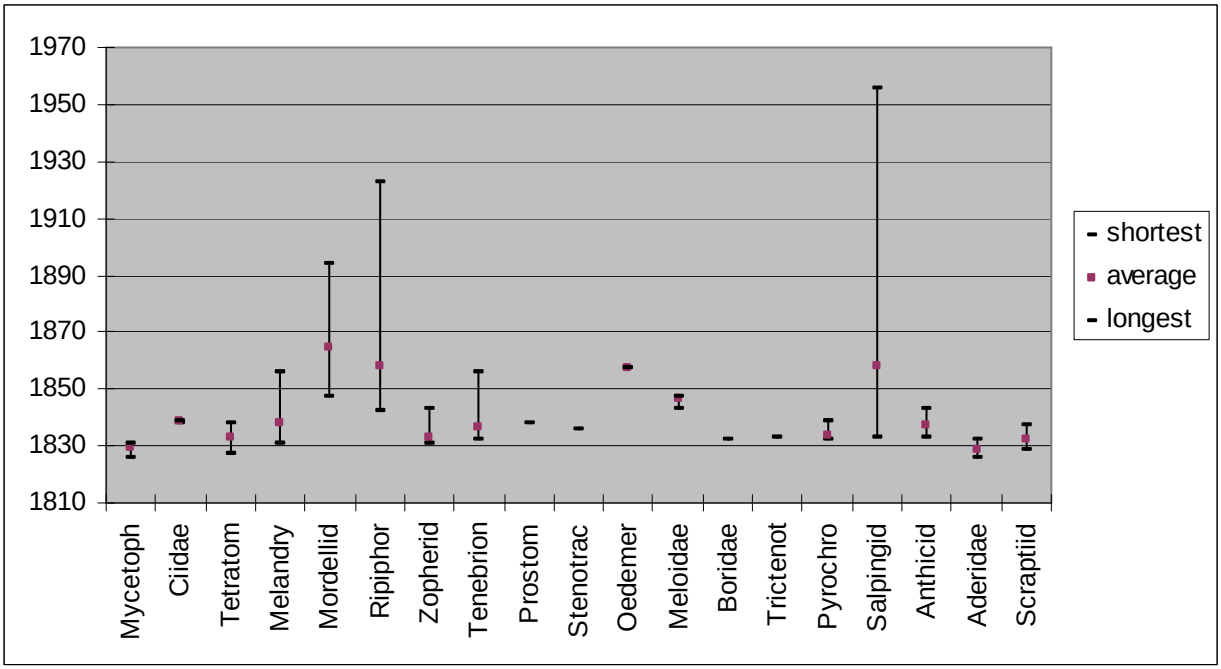


Figure 4: The variation of the SSU gene in families.

In the LSU gene, the longest sequence occured in Ripiphoridae (716bp; mean 657.4bp), Tenebrionidae (695bp; mean 641.9bp) and Prostomidae (693bp) /figure 5/. The six families (Ripiphoridae, Meloidae, Salpingidae, Mordellidae, Oedemeridae, Tenebrionidae) achieved a higher mean than the common one.

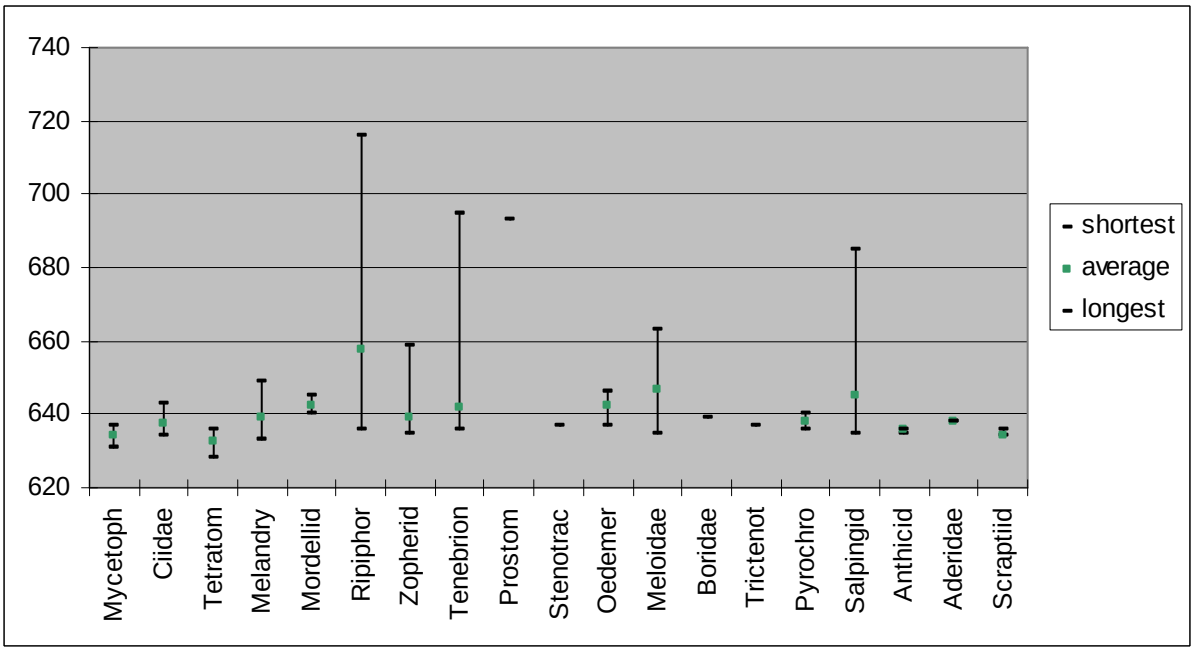


Figure 5: The variation of the LSU sequences in families.

The absolutely longest sequences of the rrnL occured in Ripiphoridae (503bp; mean 490.4bp) and Melandryidae (502bp; mean 493.8bp) /figure 6/.

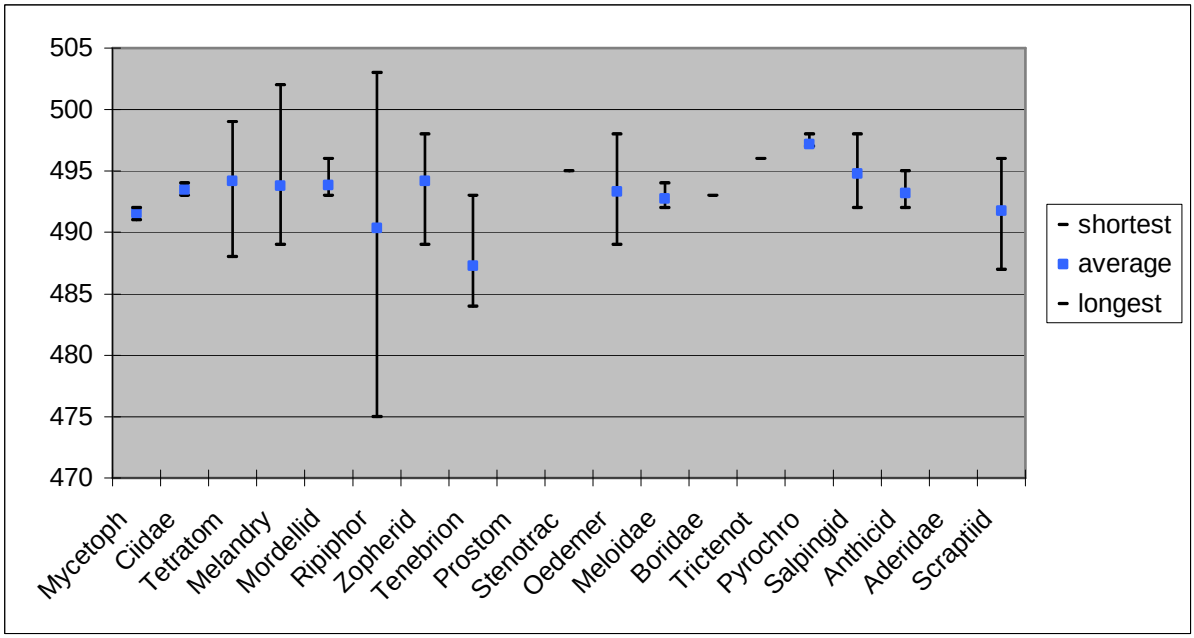


Figure 6: The variation of the rrnL sequences in families.

In all four genes' distance, the greatest variation appeared in the Salpingidae, Ripihoridae, Zopheridae, Tenebrionidae and Melandryidae, based on standard deviation values. However, the highest means achieved Ripiphoridae, Mordellidae, Salpingidae, Oedemeridae and Meloidae /figure 7/. The longest sequences had taxons of Inopeplinae and Ripiphoridae.

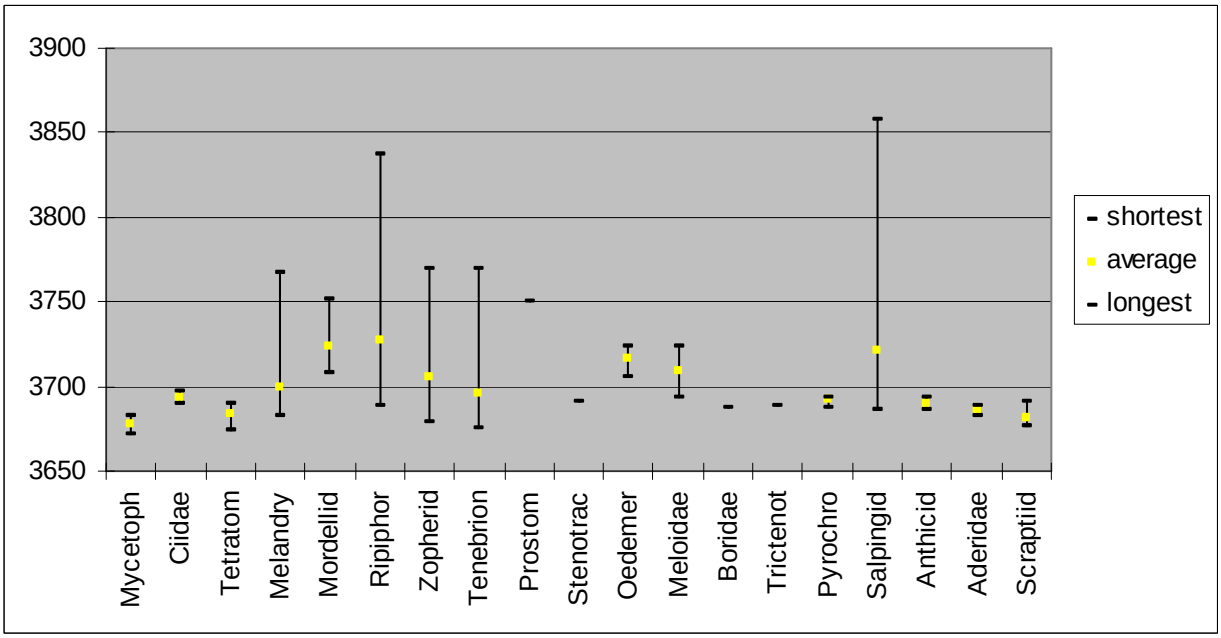


Figure 7: All four genes'sequences in families.

The nucleotides' frequencies are strongly moved toward A-T in both mitochondrial genes, rrnL and cox1, while the nuclear genes, SSU and LSU, contain approximately balanced base composition /table 6; figure 8/. The A-T bias of mitochondrial genes has been already observed in other insect mitochondrial genes as well (e.g. Wetterer *et al.*, 1998; Chippindale *et al.*, 1999; Sallum *et al.*, 2002). The highest content in the SSU gene achieved guanine, in the LSU adenine and guanine, and in the rrnL and cox1 thymine base. As expected, the highest values of A-T content were found on the third coding position of the cox1 gene, with a noticeable increase of adenine in comparison to the second position. The most frequent substitution was the TA transversion, followed by the TC transition.

Table 6: The range of nucleotides' frequencies using software DAMBE (Xia & Xie, 2001). The highest values for a gene or coding position in bold.

A-T	A	C	G	T	A-T
SSU	0.240-0.262	0.216-0.247	**0.258-0.280**	0.233-0.266	0.473-0.528
LSU	**0.247-0.326**	0.175-0.242	0.252-0.315	0.197-0.265	0.443-0.591
rrnL	0.294-0.428	0.070-0.122	0.128-0.209	**0.354-0.444**	0.648-0.872
cox1	0.261-0.371	0.107-0.252	0.129-0.199	**0.277-0.432**	0.538-0.802
1st	**0.261-0.373**	0.100-0.208	0.187-0.299	0.216-0.361	0.477-0.734
2nd	0.170-0.208	0.174-0.249	0.141-0.174	**0.390-0.477**	0.560-0.685
3rd	**0.324-0.560**	0.004-0.324	0.004-0.137	0.183-0.527	0.506-1.087

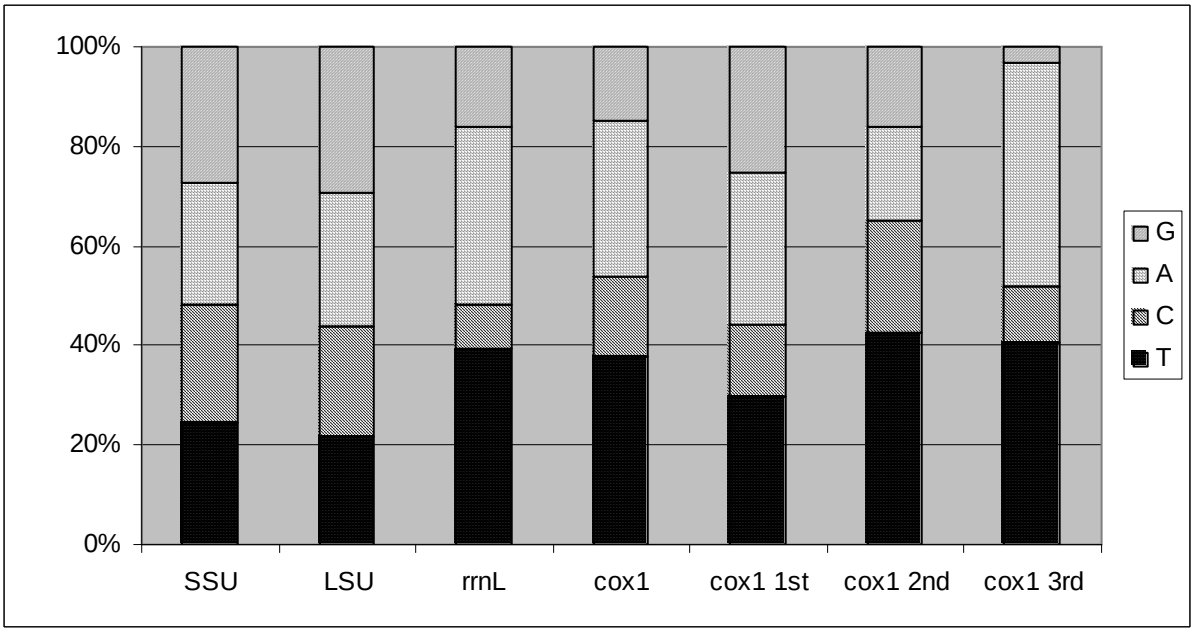


Figure 8: Ratio of nucleotides composition for every gene and every coding position of cox1 gene. Means counted by MEGA 3.1 (Kumar *et al.*, 2004).

As expected, the static alignment performed by ClustalX produced significantly different lengths under different settings of gaps penalties /table 7/, with the longest aligned sequences under the settings "A". In comparison, the alignment performed with *blastn* algorithm (BA), produced aligned genes SSU and LSU with lower number of gaps.

Table 7: Length of alignments under different gap penalties' settings performed by ClustalX (see Material and Methods) and with *blastn* algorithm (BA).

	A	**B**	**C**	**D**	**E**	**BA**
SSU	2053	2043	2021	2005	2009	1994
LSU	785	749	759	727	728	720
rrnL	657	566	536	520	517	601
cox1	838	761	733	724	723	723
total	4333	4119	4049	3976	3977	4038

5.2 Phylogenetic analyses.

5.2.1 Maximum Parsimony analyses.

The parsimony analyses produced different tree topologies, sensitively reacting on both alignment settings and gap state coding (fifth or missing). Despite it, if the gaps were treated as missing, the monophyly of Tenebrionoidea has been supported in all analyses, except Clustal matrix "D".

The shortest tree was produced from the BlastAlign matrix (22838) with CI=0.11, RI=0.39, followed by the Clustal matrix "A" (26813), CI=0.12, RI=0.41 /table 8/. Both these analyses were performed with gaps coded as missing; the shortest tree yielding from analyses with gaps treated as the fifth character was achieved from the Clustal matrix "B" (31426), CI=0.13, RI=0.11. The analysis by direct optimization implemented in POY produced the shortest tree with the length 28696 (data not shown). In contrast, the matrices with gaps treated as the fifth character contained a higher number of informative characters. The most informative characters were found in the Clustal matrix "A" (1813) and achieved higher consistency and retention indexes as well /table 8/. While the highest CI reached the Clustal matrix "A", the highest RI was counted in the Clustal matrix "D".

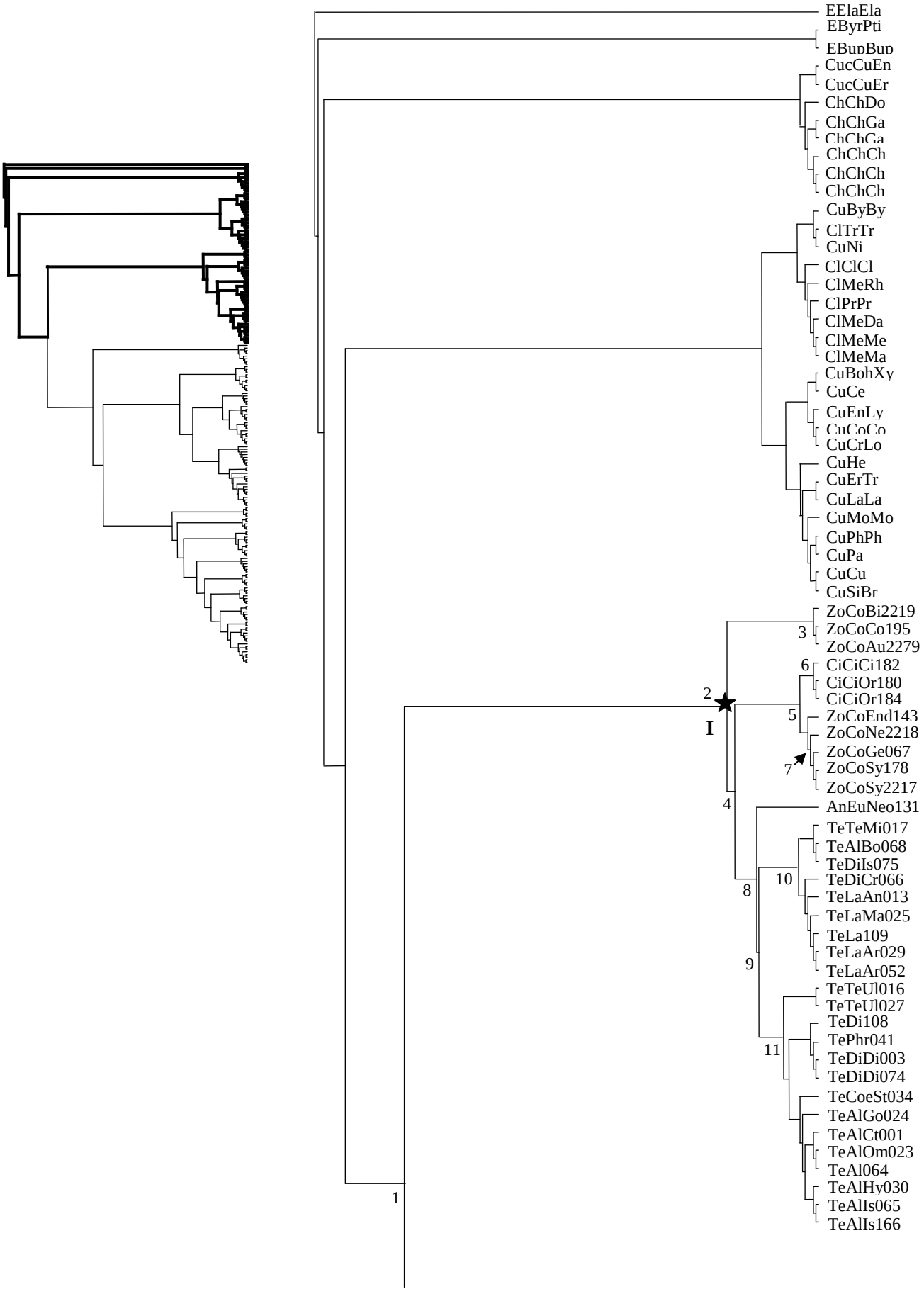
Table 8: Trees characteristics resulting from maximum parsimony analyses of Clustal and BlastAlign aligned matrices. CI, RI, RC calculated from informative characters.

	A		**B**		**C**		**D**		**E**		**BA**	
	5th	**mis**	**5th**	**mis**	**5th**	**mis**	**5th**	**mis**	**5th**	**mis**	**5th**	**mis**
Characters												
total	4333		4119		4049		3976		3977		4038	
constant	2103	2290	2100	2218	2100	2174	2098	2150	2094	2154	1883	2422
variable												
uninform.	417	482	367	415	348	426	317	360	329	366	478	358
inform.	**1813**	1561	1652	1486	1601	1449	1561	1466	1554	1457	1677	1258
Trees												
no.trees	6	10	11	5	3	8	7	3	2	3	1	1
tree length	31677	26813	31426	27810	31480	28187	31504	28540	32108	29102	31476	**22838**
CI	**0.14**	0.12	0.13	0.11	0.12	0.11	0.12	0.11	0.12	0.11	0.11	0.11
RI	0.45	0.41	0.45	0.41	0.45	0.41	**0.47**	0.42	0.46	0.42	0.46	0.39
RC	0.06	0.05	0.06	0.05	0.05	0.05	0.06	0.05	0.05	0.05	0.05	0.04
tax. clades	19	22	19	23	22	25	21	22	18	24	13	25

To assess the credibility of the trees, 41 taxonomical clades, that include the taxonomical units on the level of superfamily, family and subfamily, except one member-taxonomical unit, were diagnosed. The analyses with the gaps' condition as the fifth character recognized less taxonomical clades (from 13 to 22) than those with gaps treated as missing (from 22 to 25) at all. The most taxonomical clades (25) were identified by BlastAlign matrix and Clustal matrix "C" /table 8/. The 21 taxonomical clades were recognized by the POY analysis (data not shown).

Although the stable resolution of the Tenebrionoidea has not been achieved by maximum parsimony, there are four clades within the superfamily, which show more or less stable presence in the consensus trees of all Clustal "missing" matrices. One of the majority consensus trees, resulting tree of Clustal matrix "B", is shown in figure 9. There are visualized the four clades, marked I, II, III, IV and numbered nodes. The clade I, "tenebrionids" clade, consists of the family Tenebrionidae' members, with (matrices "A", "B") or without (matrices "C", "D", "E") members of the subfamily Lagriinae, the Colydiinae' and Ciidae' members and anthicid genus *Neostereopalpus*. The monommids and the genus *Trictenotoma* appear in this clade as well, however only under the "C", "E" settings. The limited family Anthicidae is "floating" in the trees' topology depending on the analysis and it appears in this clade under the "A", "D" settings. The clade II is more constricted and encompasses the Melandryinae' genera *Melandrya*, *Phryganophilus*, *Hypulus*, *Phloiotrya*, *Abdera*, *Microtonus* and *Dircaea* (it misses in the "C" matrix) and the genus *Cephaloon* from the family Stenotrachelidae. The III clade can be divided in two parts, the

family Scraptiidae with melandryid genus *Osphya* and the restricted family Pyrochroidae with salpingid genus *Elacatis* and a taxon from the family Prostomidae in the “A”, “B” matrices. The restricted family Pyrochroidae does not include the genus *Agnathus* and the genus *Tosadendroides* misses only in the “C”, “E” matrices. The clade IV is the largest and comprises all members of five families, Mordellidae, Meloidae, Ripiphoridae, Mycetophagidae and Aderidae; the subfamilies Inopeplinae, Penthinae, Eustrophinae, Hallomeninae, Lagriinae (in matrices “C”, “D”, “E”); melandryid genera *Orchesia*, *Microscapha* and *Anisoxya* and anthicid genus *Ischalia*. In the matrices “B”, “C”, the clade includes the limited family Anthicidae, which misses genera *Ischalia* and *Neostereopalpus* as well. In the trees resulting from the BlastAlign and POY analyses, these four clades are not recognized in the same composition. Only the clades of deeper nodes match with those from Clustal matrices’ trees. This is visible from the table 9, where the numbered nodes from the figure 9 with their presence or absence in each analysis and the bootstrap support are shown.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EBugBug
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CucCuEr
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ClTrTr
CuNi
ClClCl
ClMeRh
ClPrPr
ClMeDa
ClMeMe
ClMeMa
CuBohXy
CuCe
CuEnLy
CuCoCo
CuCrLo
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CuLaLa
CuMoMo
CuPhPh
CuPa
CuCu
CuSiBr
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ZoCoCo195
ZoCoAu2279
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CiCiOr184
ZoCoEnd143
ZoCoNe2218
ZoCoGe067
ZoCoSy178
ZoCoSy2217
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TeDiCr066
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TeLaAr052
TeTeUl016
TeTeUl027
TeDi108
TePhr041
TeDiDi003
TeDiDi074
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TeAlGo024
TeAlCt001
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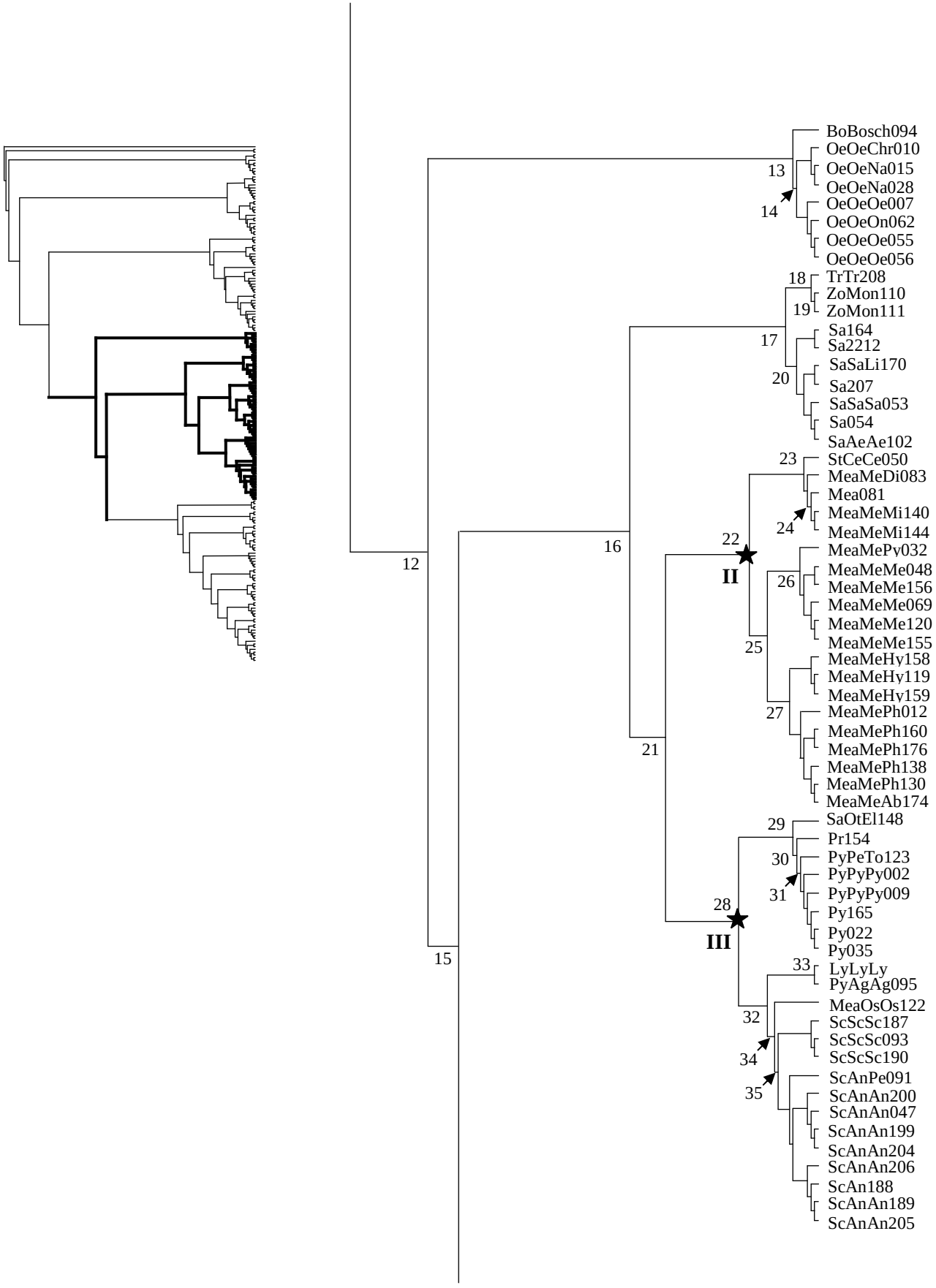
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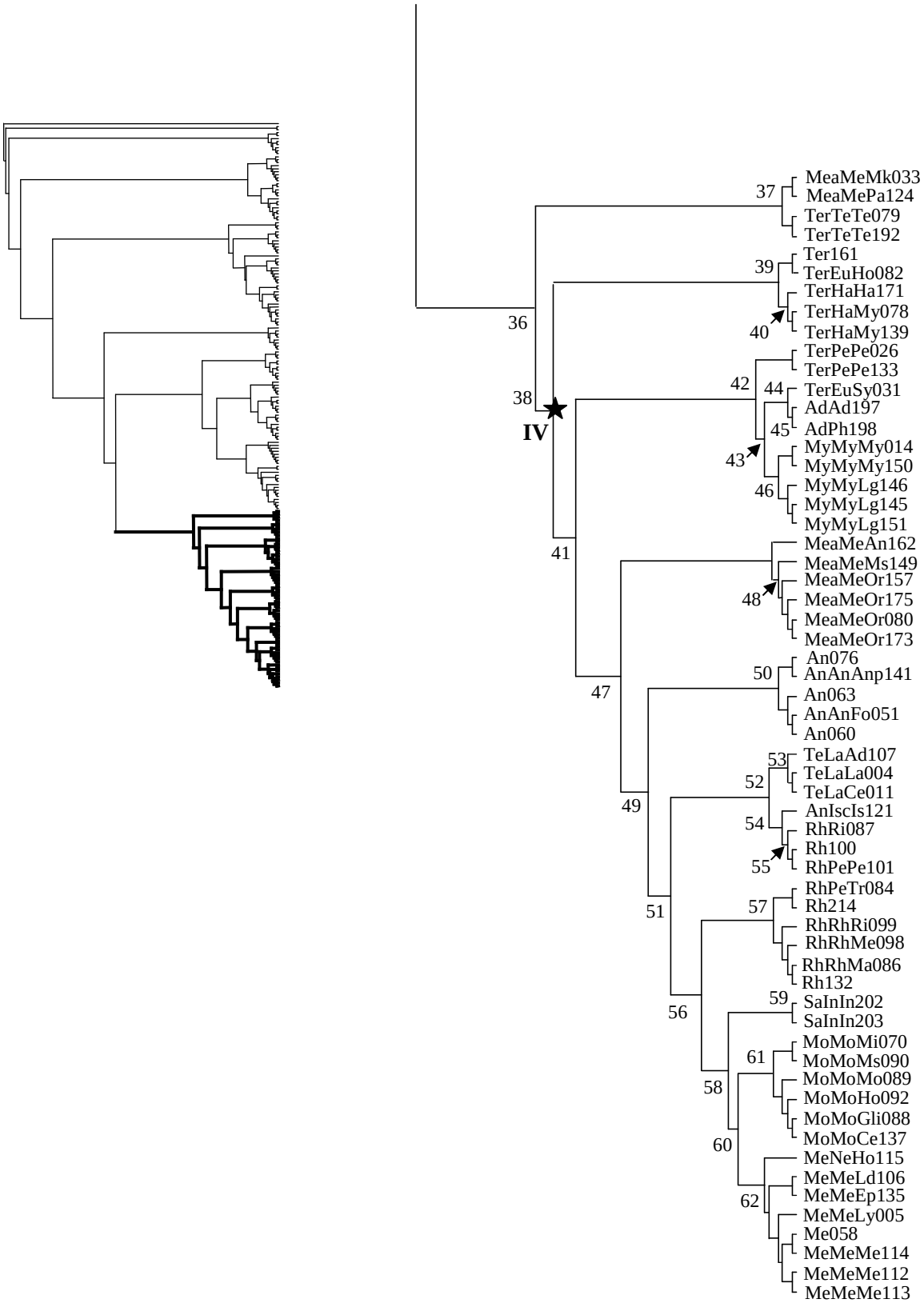


Figure 9: Majority consensus tree from MP analysis Clustal matrix "B", gaps treated as missing. Codenames are associated with names in the Sampling list /Supplementary Material B/. For number of nodes see table 9. Nodes marked by star discussed in the text.

Node	A	B	C	D	E	BA	POY	Node	A	B	C	D	E	BA	POY
1	Y	Y	Y	N	Y	Y	Y	**32**	N	Y	N	N	N	N	N
2	N	Y	N	N	N	N	N	**33**	N	Y	N	N	N	N	N
3	N	79	N	N	64	N	N	**34**	67	62	Y	79	67	N	N
4	N	Y	N	N	N	N	N	**35**	Y	56	Y	N	56	Y	N
5	N	Y	N	N	N	N	N	**36**	N	Y	N	N	N	N	N
6	100	100	100	100	100	100	Y	**37**	61	Y	56	57	Y	Y	N
7	76	55	74	76	51(+Zo143)	Y	N	**38**	N	Y	N	N	N	N	N
8	N	Y	N	N	N	N	N	**39**	100	89	85	90	90	76	N
9	Y	Y	N	N	N	Y	N	**40**	100	100	100	100	100	100	Y
10	N	Y	N	N	N	N	N	**41**	N	Y	N	N	N	N	N
11	N	Y	N	N	N	N	N	**42**	N	Y	Y	Y	N	N	N
12	N	Y	N	N	N	N	N	**43**	N	Y	Y	Y	N	N	N
13	Y	Y	N	N	N	N	N	**44**	N	Y	51	Y	N	N	N
14	100	100	100	100	100	100	Y	**45**	100	97	96	99	96	91	Y
15	N	Y	N	N	N	N	N	**46**	100	92	95	93	90	89	Y
16	N	Y	N	N	N	N	N	**47**	N	Y	N	N	N	N	N
17	Y(-SaAe)	Y	N	N	N	N	N	**48**	69	60	Y	N	Y	66	N
18	Y	Y	Y	N	N	N	N	**49**	N	Y	N	N	N	N	N
19	100	100	100	100	100	100	Y	**50**	99	97	100	99	100	91	Y
20	63(-SaAe)	56	Y	Y	Y	Y	N	**51**	N	Y	N	N	N	N	N
21	N	Y	N	N	N	N	N	**52**	Y	Y	N	N	N	N	N
22	Y	Y	Y(-MeaDi)	N	Y	N	N	**53**	100	100	100	99	97	100	Y
23	86(-MeaDi)	Y	Y(-MeaDi)	Y(-MeaDi)	Y	Y(-MeaDi)	N	**54**	N	Y	62	64	N	N	N
24	100	100	100	100	100	100	Y	**55**	68	70	62	74	Y	N	Y
25	Y	Y	N	Y	Y	N	Y	**56**	N	Y	N	N	Y	N	N
26	99	99	99	100	100	95	Y	**57**	Y	Y	Y	64	67	N	N
27	Y	Y	Y	Y	Y	N	Y	**58**	62	61	61	57	60	Y	N
28	N	Y	N	N	N	N	N	**59**	100	100	100	100	100	100	Y
29	Y	Y	N	Y(-Pr)	N	N	N	**60**	61	Y	Y	54	56	73	Y
30	Y	Y	N	N	N	N	N	**61**	100	100	100	100	100	100	Y
31	70	67	N	Y	N	90	Y	**62**	100	100	100	100	100	100	Y

Table 9: Numbered nodes /figure 9/ with the presence /Y/ or absence /N/ in individual trees and with their boostrap support if >50%.

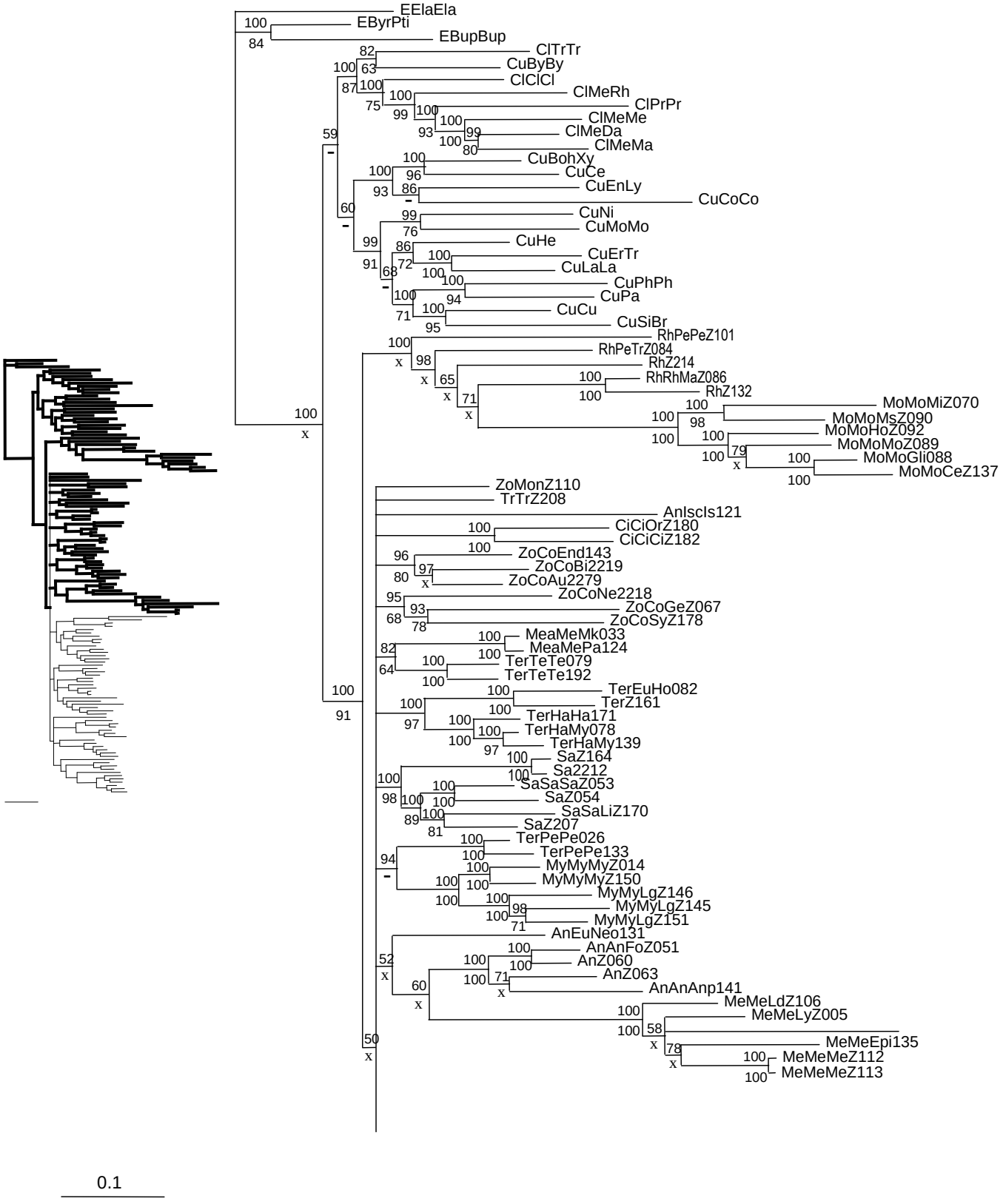
5.2.2 Bayesian analyses

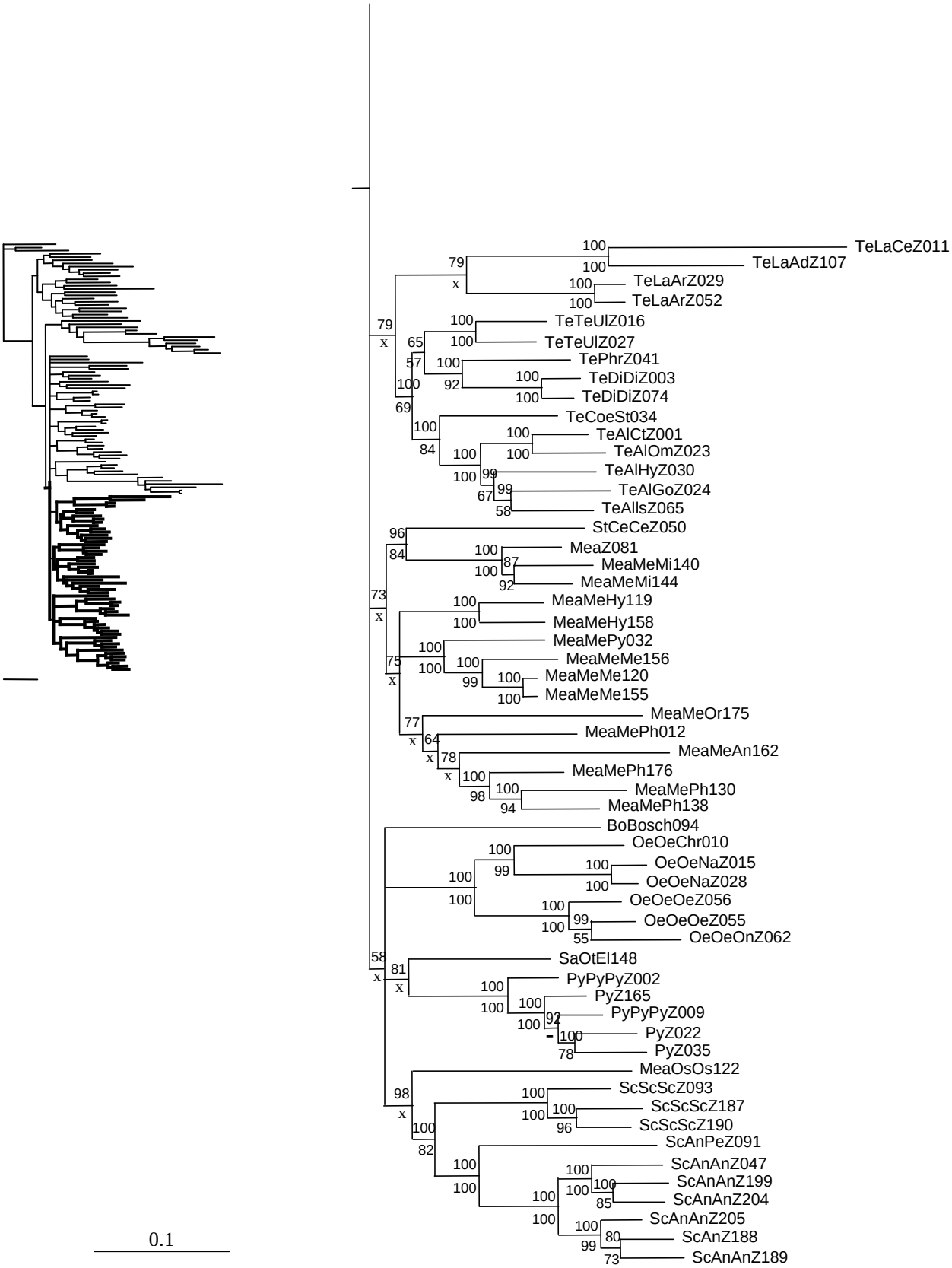
Despite the effort, the bayesian analysis of the full matrix has not been accomplished successfully. There were not more than one million of generations completed, with the average standard deviation value of the split frequencies 0.093. Although the analysis of the reduced matrix (see Material and Methods) got closer to the stationary state /table 3/, the wished result has not been achieved. However its standard deviation value 0.05 represents the best result from all analyses.

The Bayesian interference supported the Cucujiformia and Tenebrionoidea as monophyletic, both with the posterior probability (pp) value 100 /figure 10/. Within the Tenebrionoidea, the clade consisting of families Ripiphoridae and Mordellidae was found in a sister-group relationship to the remaining Tenebrionoidea, though weakly supported (pp=50). Based on this majority consensus tree, only the relationship between Mycetophagidae and Penthinae is supported (pp=94). The relationship between the limited Anthicidae (genera *Ischalia* and *Neostereopalpus* excluded) and Meloidae (pp=60) and a clade consisting of families Boridae, Oedemeridae, Pyrochroidae with salpingid genus *Elacatis* and Scraptiidae with melandryid genus *Osphya* (pp=58) are proposed. The MP clade II, with melandryid' genera and *Cephaloon* was recognized by bayesian analysis as well (pp=73). It is remarkable, that the subfamily Melandryinae would be found monophyletic (pp=75) here, if genera *Mikadonius* and *Paramikadonius* do not miss. These genera create a clade with Tetratominae (pp=82). From the 35 taxonomic clades, as defined in the MP section, 23 were recovered by bayesian analysis.

The analyses of the individual genes by bayesian analysis were performed to test their impact on the phylogeny. Although the genes achieved higher standard deviation value individually than the all genes matrix (see Material and Methods), the resolution of the trees has not been satisfying. The monophyly of Tenebrionoidea was except the cox1 gene supported by all remaning genes: SSU (pp=94), LSU (pp=87) and rrnL (pp=100). The SSU gene identified the highest number (24) of taxonomical clades (see the MP section), followed by the cox1 (18) with LSU (18) genes and rrnL (17).

Figure 10: Consensus tree resulting from the bayesian analysis, with posterior probability values above the branches and boostrap values from maximum likelihood tree under the branches. Codenames are associated with names in the Sampling list /Supplementary Material B/. x = the clade does not exist; - = the clade exists, but with support lower than 50%.




TeLaCeZ011
TeLaAdZ107
TeLaArZ029
TeLaArZ052
TeTeUlZ016
TeTeUlZ027
TePhrZ041
TeDiDiZ003
TeDiDiZ074
TeCoeSt034
TeAlCtZ001
TeAlOmZ023
TeAlHyZ030
TeAlGoZ024
TeAllsZ065
StCeCeZ050
MeaZ081
MeaMeMi140
MeaMeMi144
MeaMeHy119
MeaMeHy158
MeaMePy032
MeaMeMe156
MeaMeMe120
MeaMeMe155
MeaMeOr175
MeaMePh012
MeaMeAn162
MeaMePh176
MeaMePh130
MeaMePh138
BoBosch094
OeOeChr010
OeOeNaZ015
OeOeNaZ028
OeOeOeZ056
OeOeOeZ055
OeOeOnZ062
SaOtEl148
PyPyPyZ002
PyZ165
PyPyPyZ009
PyZ022
PyZ035
MeaOsOs122
ScScScZ093
ScScScZ187
ScScScZ190
ScAnPeZ091
ScAnAnZ047
ScAnAnZ199
ScAnAnZ204
ScAnAnZ205
ScAnZ188
ScAnAnZ189
0.1

5.2.3 Maximum Likelihood analyses

Because of the similar reason as for the bayesian analyses, an unsuccessful completing of analysis due to time-limit, the matrix for ML analyses was reduced (see Material and Methods).

On the resulting ML tree /figure 10/, there were found two monophyletic clades of cucujiformian superfamilies, however not fulfilling monophyletic condition at the series level. Clade consisting of Cleroidea and Cucujoidea superfamilies achieved a low bootstrap value (<50%), in contrast to the Tenebrionoidea that were supported by 91% boostrap value. Within the Tenebrionoidea the "tenebrionids" clade, consisting of families Tenebrionidae (without two Lagriinae taxons; bootstrap value 50%) and Ciidae (100%), subfamily Colydiinae, genus *Trictenotoma* and anthicid genus *Neostereopalpus*, was in a sister-clade position to the remaining tenebrionoids. It is noteworthy, that this clade is consistent with the clade I from the MP analyses. Only the deeper clades achieved the boostrap values higher than 50%. Two other clades corresponding to clades II and IV from the MP analyses were found by ML as well. The families Mordellidae (100%) and Meloidae (100%) were united in a clade with a boostrap support 66%. The monophyletic family Ripiphoridae (<50%) stood to this clade in a sister-group relationship position. These three families formed one clade together, with a boostrap support 58%. The subfamily Melandryinae did not cover up the genera *Orchesia* and *Anisoxya*. There was found one interesting clade more, that consisted of the families Pyrochroidae (100%), Scraptiidae (82%), restricted Anthicidae without genera *Ischalia* and *Neostereopalpus* (100%) and Oedemeridae (100%) and of genera *Boros*, salpingid *Elacatis* and melandryid *Osphya*. From the 35 taxonomic clades, as defined in the MP section, 24 were found.

The individual genes were tested also by ML. Only the genes LSU and rrnL recognized the Tenebrionoidea as monophyletic, though not supported by bootstrap values. Similarly as by baysien analyses, in the tree of SSU gene was found the highest number of taxonomical clades (21), followed by cox1 and LSU genes with 19 resoluted taxonomical clades and rrnL with 18 clades.

To draw a better picture of the resolution, the summary of recovery of the families is presented by testing their monophyly /table 10/ and by taxonomic retention index /table 11/. Seven families have been found monophyletic and if the condition of exclusion few taxa is applied, the families Pyrochroidae, Salpingidae and Anthicidae are found monophyletic as

well. The families Tetratomidae, Melandryidae and Zopheridae were by all types of analyses found polyphyletic. All these findings are supported also by the TRI.

Families	**MP B**	**MP BA**	**Bayes all**	**ML all**	**Subfamilies**	**MP B**	**MP BA**	**Bayes all**	**ML all**
Tenebrionoidea(154/110)	M	M	M	M	Mycetophaginae(5/5)	M	M	M	M
Mycetophagidae (5/5)	M	M	M	M	Ciinae (3/2)	M	M	M	M
Ciidae (3/2)	M	M	M	M	Penthinae (2/2)	M	M	M	M
Tetratomidae (10/9)	Po	Po	Po	Po	Tetratominae (2/2)	M	M	M	M
Melandryidae (28/18)	Po	Po	Po	Po	Eustrophinae (2/1)	Po	Po	-	-
Mordellidae (6/6)	M	M	M	M	Hallomeninae (3/3)	M	M	M	M
Ripiphoridae (9/5)	Po	P	P	M	Melandryinae(26/16)	Po	Po	Po	Po
Zopheridae (10/7)	Po	Po	Po	Po	Mordellinae (6/6)	M	M	M	M
Tenebrionidae (26/15)	Po	M	M	Po	Ripiphorinae (4/2)	M	Po	M	M
Oedemeridae (7/6)	M	M	M	M	Pelecotominae(2/2)	Po	Po	P	M
Meloidae (8/6)	M	M	M	M	Colydiinae (8/6)	P	M	Po	Po
Pyrochroidae (7/5)	Po	Po	M	M	Monommatini (2/1)	M	M	-	-
Py(-PyAgAg095)	M	M	x	x	Alleculinae (8/5)	Po	P	M	M
Salpingidae (10/7)	Po	Po	Po	Po	Diaperinae (5/2)	Po	Po	M	M
Sa(-SaOt148, SaIn)	M	M	M	M	Lagriinae (8/4)	Po	M	M	Po
Anthicidae (7/6)	Po	Po	Po	Po	Tenebrioninae (3/2)	Po	Po	M	M
An(-An121,131)	M	M	M	M	Oedemerinae (7/6)	M	M	M	M
Aderidae (2/-)	M	M	x	x	Meloninae (6/5)	M	M	P	M
Scraptiidae (12/10)	M	M	M	M	Inopeplinae (2/-)	M	M	x	x
					Anaspidinae (9/7)	M	M	M	M
					Scraptiinae (3/3)	M	M	M	M

Table 10: The monophyly of the families and subfamilies by four different types of analyses, MP of BA matrix included. In the parentheses, the number of taxons included in the analysis (full matrix/reduced matrix) is presented. Families Prostomidae, Cephaloidae, Boridae, Trictenotomidae were excluded, because they include only one taxon. M=monophyletic; P=paraphyletic; Po=polyphyletic; x = no taxon of the family/subfamily is present in the matrix; - = only one taxon of the group is present in the matrix.

5.2.4 Taxonomic Retention Index

Series/Families	MP	POY	Bayes	ML	Subfamilies	MP	POY	Bayes	ML
Elateriformia (3/3)	1	1	1	1	Mycetophaginae(5/5)	1	1	1	1
Cucujiformia(185/131)	1	1	1	1	Ciinae (3/2)	1	1	1	1
Lymexyloidea (1/-)	-	-	x	x	Penthinae (2/2)	1	1	1	1
Cleroidea (7/7)	0.833	0.667	0.833	0.833	Tetratominae (2/2)	1	1	1	1
Cucujoidea (15/14)	0.857	0.714	0.923	0.923	Eustrophinae (2/1)	0	0	-	-
Tenebrionoidea(154/110)	0.97	0.939	1	1	Hallomeninae (3/3)	1	1	1	1
Chrysomeloidea (6/-)	1	1	x	x	Melandryinae(26/16)	0.87	0.826	0.923	0.846
Curculionoidea (2/-)	1	1	x	x	Mordellinae (6/6)	1	1	1	1
Mycetophagidae(5/5)	1	1	1	1	Ripiphorinae (4/2)	0.5	0	-	-
Ciidae (3/2)	1	1	1	1	Pelecotominae(2/2)	0	0	0	1
Tetratomidae (10/9)	0.5	0.5	0.6	0.6	Colydiinae (8/6)	0.857	0.857	0.8	0.8
Melandryidae(28/18)	0.833	0.8	0.8	0.75	Monommatini (2/1)	1	1	-	-
Mordellidae (6/6)	1	1	1	1	Alleculinae (8/5)	0.857	0.857	1	1
Ripiphoridae (9/5)	0.875	0.75	0.75	1	Diaperinae (5/2)	0.25	0.25	1	1
Zopheridae (10/7)	0.778	0.778	0.667	0.667	Lagriinae (8/4)	0.857	1	1	0.667
Tenebrionidae(26/15)	0.96	0.92	1	0.929	Tenebrioninae(3/2)	0.5	0.5	1	1
Prostomidae (1/-)	-	-	x	x	Oedemerinae (7/6)	1	1	1	1
Oedemeridae (7/6)	1	1	1	1	Meloninae (6/5)	0.8	0.8	0.75	1
Cephaloidae (1/1)	-	-	-	-	Inopeplinae (2/-)	1	1	x	x
Meloidae (8/6)	1	1	1	1	Anaspidinae (9/7)	1	1	1	1
Boridae (1/1)	-	-	-	-	Scraptiinae (3/3)	1	1	1	1
Trictenotomidae(1/1)	-	-	-	-					
Pyrochroidae (7/5)	0.833	0.833	1	1					
Py(-PyAgAg095)	1	1	x	x					
Salpingidae (10/7)	0.778	0.667	0.833	0.833					
Sa(-SaOt148,SaIn)	1	0.833	1	1					
Anthicidae (7/6)	0.667	0.667	0.6	0.6					
An(-An121,131)	1	1	1	1					
Aderidae (2/-)	1	1	x	x					
Scraptiidae (12/10)	1	0.909	1	1					

Table 11: TRI of the superfamilies, families and subfamilies by four different types of analyses, POY included. In the parentheses, the number of taxons included in the analysis (full matrix/reduced matrix) is presented. 1=Monophyly; x = no taxon of the superfamily/ family/ subfamily is present in the matrix; - = only one taxon of the group is present in the matrix; MP= MP of B matrix.

According the TRI, the best resolution was produced at high levels of classification, as series and superfamilies are, followed by families and subfamilies /figure 11/.

Among all the analyses, the worst perfomance resulted by POY, that reached the lowest values of TRI index at every level of classification /table 12/.

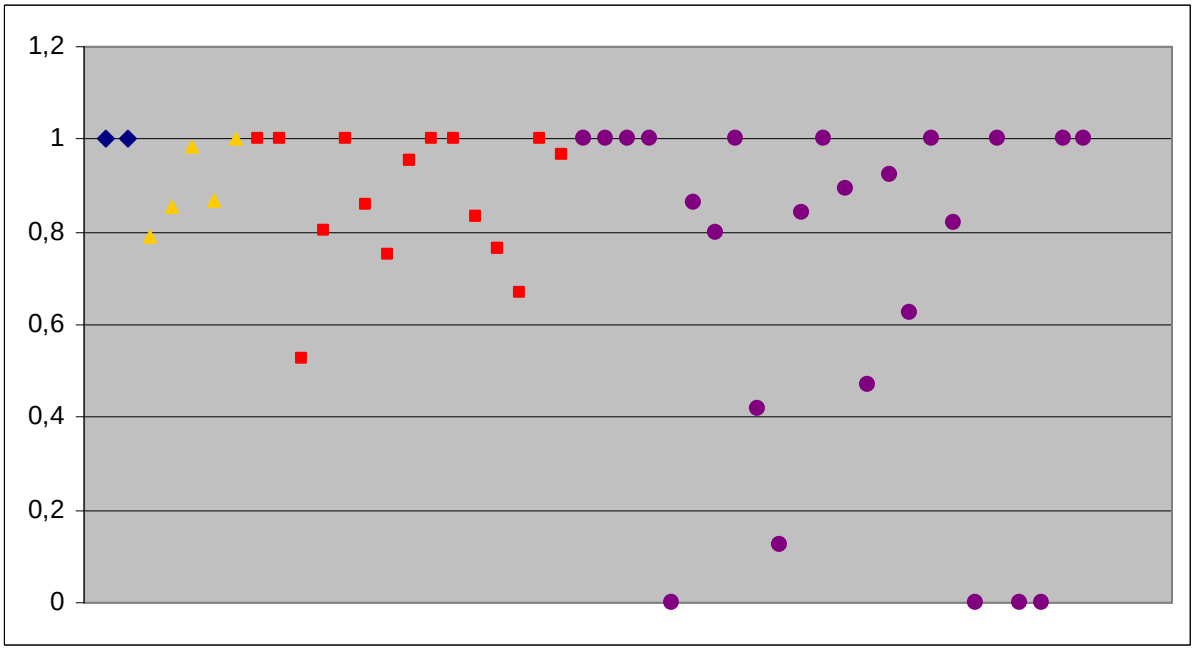


Figure 11: Dispersion of TRI values within each level of classification. It is calculated as a mean of TRI from all the MP analyses with the gap condition as missing, POY, Bayes and ML analyses for every taxonomical unit. Blue symbol = series, yellow symbol = superfamilies, red symbol = families, violet symbol = subfamilies.

	MP A	**MP B**	**MP C**	**MP D**	**MP E**	**POY all**	**Bayes all**	**ML all**
Series	1	1	1	1	1	1	1	1
Superfamily	0.915	0.932	0.932	0.915	0.915	**0.864**	0.952	0.952
Family	0.873	0.873	0.858	0.866	0.873	**0.843**	0.88	0.87
Subfamily	**0.753**	0.767	0.767	0.781	0.781	**0.753**	0.844	0.844

Table 12: The lowest value of TRI among all the trees (except consensus tree) on every level of classification for different types of analyses is presented. The MP analyses are for the gaps treated as missing. The lowest value of TRI for every level of classification is in bold.

6. Discussion.

6.1 Monophyly of the Tenebrionoidea.

The question of the tenebrionoids' monophyly is in dispute. Lawrence and Newton (1995) as well as Beutel and Friedrich (2005) by several larval autapomorphies considered the superfamily Tenebrionoidea to be well-defined and monophyletic, but other authors as Iablokoff-Khnzorian (1983), Schunger *et al*. (2003), because of absence of autapomorphies from a comprehensive cladistic analysis, and Hunt *et al.* (2007) have disclaimed the monophyly of the group. The latter' analyses yielded polyphyletic Lymexyloidea, that were nested at base of the Tenebrionoidea and both together formed a monophyletic group. Our analyses support the monophyly of the superfamily /figure 9, 10; table 9, 10, 11/, except the polyphyly resulted by the MP of Clustal matrix "D". However the Hunt's findings about the Lymexyloidea can not be omitted here because of their inclusion within the Tenebrionoidea by the MP of Clustal matrix "B", POY and the Bayesian analysis of full matrix. The Lymexyloidea are considered either to stand in an isolated position among the Cucujiformia or to be connected with the Cleroidea (adult characters) and Cucujoidea (larval characters) and to stand at base within the Cucujiformia (Young, 2002). The connection between Lymexyloidea and Tenebrionoidea has not been described yet, except of larval parallelism between Mordellidae, Stylopoidea and Lymexyloidea (Crowson, 1960).

6.2 Internal relationships within the Tenebrionoidea.

The intra-classification of the Tenebrionoidea remains unclear (Ślipiński & Lawrence, 1999) and because of complexity of the superfamily, it has not been studied deeply and only the works on generic or familiar level have been published (see Introduction). There are more or less tentatively recognized lineages of families, but relationships between the families and the lineages are not really known. However the range of the superfamily seems to be well established.

By our analyses, the generally believed lineages have not been found. But there are present common clades among analyses, though not fully resolved and unsettled. Four clades are recognized. The tenebrionids clade (I) that consists of the family Tenebrionidae members, with or without members of the subfamily Lagriinae, the Ciidae and the Colydiinae members and anthicid genus *Neostereopalpus*. The Melandryinae clade (II) encompasses the Melandryinae genera and genus *Cephaloon* from the family Stenotrachelidae. The third clade

(III) can be divided in two parts, the family Scraptiidae with the melandryid genus *Osphya* and the restricted family Pyrochroidae with salpingid genus *Elacatis*. The largest clade (IV) comprises all members of five families, Mordellidae, Meloidae, Ripiphoridae, Mycetophagidae and Aderidae; the subfamilies Inopeplinae, Penthinae, Eustrophinae, Lagriinae, Hallomeninae and anthicid genus *Ischalia*. By Bayesian analysis, the Ripiphoridae and Mordellidae stand separately as a sister-group to the remaining families as it was found by Bayesian analyses of Hunt *et al.* (2007).

(I)

Tenebrionidae.

The Tenebrionidae is the largest family within the Tenebrionoidea and as a good example of the superfamily, its subfamilial classification is still not settled. The subfamilies Nilioninae, Lagriinae and Alleculinae used to be separated families. On the other hand many genera previously belonging in the Tenebrionidae are now members of other families. In these days, the discussion about subfamilies is still in process (Aalbu *et al.*, 2002; Bouchard *et al.*, 2005; Aalbu, 2006). The lagrioid branch, consisting of Lagriinae and Phrenapatinae, is the most primitive branch within the family, leaving two other branches, pimeloid (Zolodininae and Pimeliinae) and tenebrionid (remaining subfamilies) unresolved (Doyen & Tschinkel, 1982; Matthews, 2003). The family is monophyletic only by the MP of BA matrix and Bayesian analyses (with pp=79), because the subfamily Lagriinae, either whole or partialy, stands out of remaining tenebrionids. If the Lagriinae are in the common clade with other tenebrionids, they are present in one clade with genera *Crypticus* (Diaperinae) and *Misolampidius* (Tenebrioninae). If they are not included in the Tenebrionidae, their part forms one clade with anthicid genus *Ischalia* and the subfamily Ripidiinae with genus *Pelecotoma*. Within the Tenebrionidae, the subfamily Coelometopinae creates one clade with the Alleculinae, the subfamily Phrenapatinae with the Diaperinae and tenebrionid genus *Uloma*. Subfamilies Diaperinae and Tenebrioninae are polyphyletic here. The similar resolution was found by MP and Bayesian analyses of Hunt *et al.* (2007), where the same classification of subfamilies was described within the Tenebrionidae and the subfamilies Lagriinae and Pimeliinae were standing outside of the family. The family took up together with a part of the Zopheridae an isolated position within the superfamily.

The relationships of the Tenebrionidae to other families are not clear. The possible related groups include Chalcodryidae, Perimylopidae, Zopheridae, Synchroidae, Cephaloidae and Oedemeridae (Lawrence & Spilman, 1991), but none is very close to the Tenebrionidae,

because of their long independent history (Watt, 1974a). In this study, tenebrionids are present together in one clade with the family Ciidae, the zopherid subfamily Colydiinae, Monommatini and genera *Trictenotoma* and *Neostereopalpus*, standing separately from the remaining families. In the MP of BA matrix, Clustal matrix "E" and POY, the family Ciidae stands as a sister-group position to the Tenebrionidae, however without support.

Zopheridae, Trictenotomidae.

The family Zopheridae was originally established by several exclusions of taxons previously placed in the Tenebrionidae (Böving & Craighead, 1931; Crowson, 1955; Watt, 1974a; Doyen & Lawrence, 1979). Only recently, the individual families Colydiidae and Monommatidae were included in the Zopheridae (Ślipiński & Lawrence, 1999), defined as a subfamily Colydiinae and as a tribe Monommatini within a subfamily Zopherinae. The Colydiinae are considered to be unconvincingly monophyletic (Ślipiński & Lawrence, 1999; Ivie, 2002; Majka *et al.*, 2006). In this study, although only the members of the Colydiinae and Monommatini were sampled, they do not form a monophyletic group together in any of analyses. Moreover, the Colydiinae, as an individual group, are monophyletic only in the analysis of the MP of BA matrix, though not supported and they form two separated clades in most of analyses. They are present in the clade comprising the families Tenebrionidae and Ciidae. The Monommatini are found in a common clade with a genus *Trictenotoma*, within the clade of Tenebrionidae, Ciidae and Colydiinae by most analyses, except of the MP of Clustal matrices "A", "B", "D". Bayesian analysis of Hunt *et al.* (2007) found the Monommatini together with a pyrochroid genus *Agnathus* within the clade comprising zopherid subfamilies Usechinae and Zopherinae, that are not sampled here.

The Trictenotomidae are regarded to be a part of salpingid group (Trictenotomidae, Salpingidae, Boridae, Pythidae, Pyrochroidae), either standing in a sister group position to the remaining members (Watt, 1987) or forming one clade with Pythidae and Salpingidae (Pollock, 1994). However, the *Trictenotoma* is present with the Salpingidae in one clade only by the MP of Clustal matrices "A", "B", "C".

Ciidae.

This family is well defined, with one subfamily of a single genus and second one comprising the remaining genera. However its position within the Cucujiformia had not been well settled, until Crowson (1960) shifted it in the superfamily Tenebrionoidea from the Cucujoidae or Cleroidea, where it had been placed. Within the tenebrionoids, it is thought to take up a basal

position and to be related to Mycetophagidae and Tetratomidae (Lawrence, 1991). However the exact position is still uncertain (Thayer & Lawrence, 2002), that underlines Buder *et al.* (2008), whose study has not achieved to settle down the family within the Tenebrionoidea. They did not find any relationship with Tetratomidae or Mycetophagidae, nevertheless some analyses proposed either the sister group relationship with the cucujoid family Nitidulidae or the basal position of the family within the cucujoid-tenebrionoid assemblage. The basal position of Ciidae within the Tenebrionoidea was found also by MP analysis of Hunt *et al.* (2007), but not by Bayesian one, where the family was placed in the clade with the families Anthicidae and Meloidae. Here, as mentioned above, ciids are found in a clade with the Tenebrionidae and Colydiinae, and as a sister-group taxon to the Tenebrionidae in the MP of BA matrix, Clustal matrix "E" and POY.

(II)

Tetratomidae, Melandryidae, Stenotrachelidae.

The families Tetratomidae and Melandryidae are tightly connected. In the past, tetratomids used to be members of Melandryidae and many transfers of subfamilies have occured between them. Traditionally, four subfamilies used to be distinguished in the Melandryidae (Lawrence & Newton, 1995), however Nikitsky (1998) moved the subfamilies Hallomeninae and Eustrophinae in the Tetratomidae, in which three subfamilies had been recognized (Lawrence & Newton, 1995). More recently, Pollock (2002) has followed this transfer as well and he has called for an extensive phylogenetic study to settle down the placement of the Hallomeninae and Eustrophinae in the Tetratomidae. By this study, subfamilies Hallomeninae and Eustrophinae are excluded from Melandryidae. The family Melandryidae, restricted to subfamilies Melandryinae and Osphyinae, is still polyphyletic here. Genus *Osphya* is found as a sister-taxon to the family Scraptiidae (see Scraptiidae). As the family, the subfamily Melandryinae and the tribes Serropalpini and Hypulini can not be defined as monophyletic, thus the discussion about the subfamily has to be hold in a generic level. A clade consisting of genera *Hypulus* (tribe Hypulini) with *Phloeotrya* and *Abdera* (Serropalpini) (node 27 in table 9, figure 9) and a clade of genera *Melandrya* with *Phryganophilus* (Melandryini) (node 26 in table 9, figure 9) form together one monophyletic group (node 25) among all types of analyses. However, this melandryines' clade is not firmly connected to any other taxons. Genera *Orchesia* with *Microscapha* (Orchesiini) as one clade (node 48) are together with melandryines in the analyses of Bayes, MP of BA matrix, Clustal "D" matrix. In remaining analyses, Orchesiini form with *Anisoxya* (Serropalpini) a clade separated from other

melandryines. *Mikadonius* with *Paramikadonius* (Serropalpini) are excluded as well, being united with *Tetratoma* (node 37). *Microtonus* (Hypulini) forms a monophyletic clade with the genus *Cephaloon* in all types of analyses, highly supported by the ML and Bayesian analyses. They occur in the same clade with Melandryini and Serropalpini (node 22) in most of the analyses. The *Cephaloon* by itself used to be treated in a separated family, until other genera of Stenotrachelinae and Nematoplinae have not united them. The resulting *Cephaloon*'s association agrees with Crowson (1955), who noticed its similarities to melandryid *Microtonus*, but disclaims Lawrence and Newton's (1982) placement of Cephaloidae in one lineage with Meloidae and Oedemeridae. Genus *Dircaea* (Serropalpini) stands either with the *Microtonus*-*Cephaloon* clade or separated, without fixing its placement. All these findings agree with Pollock's (2002) opinion of non-monophyly of the family and unsuitable tribal classification.

To discuss the family Tetratomidae it is necessary to discuss its subfamilies apart. As written above, the *Tetratoma* forms the monophyletic clade with melandryines' genera *Mikadonius* and *Paramikadonius* and this clade is present and supported by most of analyses, except POY. The Hallomeninae (node 40) are monophyletic and associated with an eustrophine genus *Holostrophus* and undetermined tetratomid in one common clade (node 39). This highly supported clade appears in most of analyses, except POY, where the Hallomeninae are found in one clade with the *Tetratoma*. The common clade of Hallomeninae and *Holostrophus* was found in MP tree of Hunt *et al.* (2007) as well. The other melandryids and tetratomids were found polyphyletic and unresolved by both MP and Bayesian analyses of these authors. The subfamily Penthinae is monophyletic and stands as a sister-group taxon either to the Mycetophagidae (by analyses of POY, MP of Clustal matrix "E") or to a clade consisting of the Mycetophagidae and Aderidae with an eustrophine genus *Synstrophus* (node 42) (missing in analyses of the MP of BA matrix, Clustal matrix "A"). Although the subfamily Eustrophinae is the only one non-monophyletic tetratomid subfamily, whole family can not be judged as monophyletic one. The above described clades Hallomeninae-*Holostrophus* (except analysis of the MP of BA matrix) and Penthinae-*Synstrophus*-Mycetophagidae are present in one large clade with families Mordellidae, Meloidae and Ripiphoridae, that corresponds to the Melandryid (with Hallomeninae and Eustrophinae being part of it) - Mordellid - Ripiphorid lineage of Crowson (1966) and Lawrence and Newton (1982).

(III)

Scraptiidae.

The scraptiides and anaspidines were united in one family by Crowson (1955) and our study confirms its monophyly. It is confirmed despite the overnumbered Anaspidinae in the sampling and lower bootstrap values in the MP analyses, which in fact supported the family only in two cases (Clustal matrices “B”, “E”). Nevertheless, the bayesian and ML analyses have the family’s monophyly undoubtly affirmed /figure 10/.

Scraptiidae are thought to be relative to Anthicidae, based on larval resemblance (Lawrence, 1977; Watt, 1987), and to be associated through them to Aderidae. However, scraptiides’ relationship to these two families as well as to other ones has not been achieved to fix. By the MP analysis of BA matrix, the common clade of the restricted family Anthicidae with the Scraptiidae has been recovered, but it has not been supported by bootstrap values. Another analysis associating these two families is the ML, where as a scraptiid’ sister-group taxon arose the clade of the Oedemeridae and restricted Anthicidae, however also without boostrap support. Except these two cases, in every analysis, the melandryid genus *Osphya* appears as the sister-taxon /node 34 in table 9, figure 9/, that contributes to recover a higher bootstrap support of the family. The *Osphya* was found to be included in the Scraptiidae also by Hunt *et al.* (2007), either with the Anaspidinae in the bayesian tree or with the *Scraptia* in the MP tree.

Other families comprising the common clade with Scraptiidae are, in most of analyses, the family Pyrochroidae (except the MP analysis of Clustal matrix “D”, BA matrix) and the family Oedemeridae (missing in the MP analyses of Clustal matrices “B”, “C”, “E”). These families with the Prostomidae and genera *Elacatis* and *Boros* create one clade in the bayesian tree (pp=58) /figure 10/. Based on morphology, the connection between the Scraptiidae and Pyrochroidae has not been found, but they were present in one clade in the MP tree of Hunt *et al.* (2007) as well.

Pyrochroidae.

To discuss the family Pyrochroidae, its restriction has to be clarified. The range of the family is not established and only recently the subfamilies Pedilinae (Young, 1984b), Tydessinae and Pilipalpinae (Peacock, 1982; Pollock, 1992, 1994, 1995) were included in. On the contrary, the genera *Ischalia* (Young, 1985b), *Agnathus* and *Cononotus* (Pollock, 1994) were excluded from the Pyrochroidae, although Agnathinae more or less doubtfully (Lawrence & Newton, 1995). Here, the monophyly of the family can be confirmed only under the condition of

exclusion of the genus *Agnathus*, that has not been present within the Pyrochroidae by any of analyses. This could definitely refuse its pyrochroid association, but to bring a light on its position within the Tenebrionoidea has not been succeeded. It has drifted from the common clade with *Boros* and either Salpingidae (MP of the BA matrix, Clustal matrix "E", POY) or Oedemeridae (MP of Clustal matrix "C", Bayesian analysis of full matrix), to the subfamily Colydiinae (ML of full matrix, MP of Clustal matrix "A") or it has been present in common clade with out-tenebrionoid genus *Lymexylon* (MP of Clustal matrix "B"). None of these groupings were confirmed by boostrap support, only by the Bayesian analysis resulting clade found weak support (pp= 54). Bayesian analyses of Hunt *et al.* (2007) placed the *Agnathus* within members of the family Zopheridae. On the other side, the genus *Tosadendroides*, to which the *Agnathus* was considered to be close (Mamaev, 1976; Doyen, 1979), has been found to be the member of the Pyrochroidae by all types of analyses. Because of missing members of the subfamilies Tydessinae and Pilipalpinae, it is the Pedilinae that stands in the outer position to the Pyrochroinae.

There are two different groups regarded as relatives. Generally, it is salpingid group, consisting of families Boridae, Pythidae, Salpingidae and Trictenotomidae, believed to be closest relative (Crowson, 1966; Lawrence, 1977; Lawrence & Newton, 1982; Pollock, 1994; Young, 1991, 2002; Beutel & Friedrich, 2005), however Abdullah (1964) and Watt (1987) have preferred relationship with families Anthicidae, Meloidae and Oedemeridae. In most of analyses, as a sister-group taxon stands either a salpingid genus *Elacatis* alone (except MP of BA matrix) or together with a member of the Prostomidae (MP of Clustal matrices "A", "B"). The possible connection of Pyrochroidae to Othniinae and Oedemeridae was proposed by Young (1991), based on larval similarities. Other connections of the Pyrochroidae with the *Elacatis* are not stable. They are found to be associated in the clade with the family Scraptiidae (except the MP Clustal matrix "D", matrix BA) as well as with the Oedemeridae (MP Clustal matrix "A", bayesian, ML analyses), the genus *Boros* (MP Clustal matrix "A", bayesian, ML analyses) or also with the Anthicidae (ML). The Pyrochroidae with Scraptiidae were found in one clade also by Hunt *et al.* (2007). The relationship of Pyrochroidae to Oedemeridae and Anthicidae, as found by ML, has been suggested by Watt (1987). On the other side, it is only by the MP of Clustal matrix "D", that pyrochroids are in one clade with the supposed relative, the family Salpingidae. The MP of BA matrix finds the Pyrochroidae associated with the subfamilies Colydiinae and Hallomeninae and the genus *Cephaloon*. However, as it is written in the Scraptiidae section, none of these clades are supported by

boostrap values. Only the bayesian tree supported the clade with Scraptiidae, Oedemeridae and *Boros* with posterior probability 54.

Oedemeridae and Boridae.

Despite the family Oedemeridae is large and widely distributed, it is as the family well determined and monophyletic. Its monophyly is supported by all types of analyses here as well, but following Lawrence and Newton (1995), only members of the subfamily Oedemerinae have been sampled. However Kriska (2002) arose the subfamily Nacerdinae as the third subfamily of the Oedemeridae, and its species, *Nacerdes* and *Chrysanthia*, form here a monophyletic group, that is supported by boostrap and posterior probability values (bst=89-98%; pp=99, 100) /figure 10/ and is separated from the remaining Oedemerinae members. But Lawrence (2005) recognized three subfamilies Calopodinae, Oedemerinae, including Nacerdinae, and a new subfamily Polypriinae.

On the other hand, the position of the family within the Tenebrionoidea has not been appointed and only the association either with Stenotrachelidae, Synchroidae and Zopheridae (Mamaev, 1973; Hayashi, 1975; Lawrence, 1977; Lawrence, 1991) or with Stenotrachelidae and Meloidae (Lawrence & Newton, 1982) has been proposed. In this study the family floats between other tenebrionoid families and only the genus *Boros*, either individually or as a sister-taxon to the *Agnathus*, is present in one clade with oedemerids in most of analyses (missing in the MP of BA matrix, Clustal matrix "D", POY). The *Boros*-Oedemeridae connection was found also by Bayesian and MP analyses of Hunt *et al.* (2007) and on the MP tree, the family Salpingidae was present in the same clade in addition to them. Our analyses found this clade only by the MP Clustal matrix "A", "E". In the resulting trees of ML, MP of BA matrix and POY analyses, the Oedemeridae appear in one cluster with the families Anthicidae and Scraptiidae.

Members of family Boridae used to be included in the Pythidae, but since the individual family was established (Young, 1985a; Lawrence & Pollock, 1994), it has been connected also with the families Pyrochroidae, Salpingidae and Mycteridae (Crowson, 1966; Lawrence & Newton, 1982) or with the salpingid group (Watt, 1987; Pollock, 1994). Pollock (1994) found a sister-group relationship with the family Pyrochroidae.

Prostomidae.

Prostomidae used to be placed for a long period in the Cucujoidea until Crowson (1967) moved it to the Tenebrionoidea. The family is connected either with Colydiinae (Lawrence &

Newton, 1982) or with Salpingidae (Young, 1991) and with Boridae, Mycteridae and Pyrochroidae (Schunger *et al.*, 2003). Here, the position of the *Prostomis* has not been found stable. It is connected either with the pyrochroid *Tosadendroides* (MP of Clustal matrices) or with the anthicid *Neostereopalpus* (MP of BA matrix, POY analyses), however none of these clades are supported.

(IV)

Mycetophagidae.

Mycetophagidae are considered to be a well defined family and here its monophyly, though only members of the subfamily Mycetophaginae were sampled, and its placement within the Tenebrionoidea are confirmed. This family is thought to be basal among tenebrionoids with a strong connection to the family Tetratomidae, based on both larval and adult characters (Crowson, 1955; Miyatake 1960; Nikitsky, 1998). As a sister-taxon of mycetophagids acts the tetratomid subfamily Penthinae either alone (analyses of ML, Bayes, POY, MP of Clustal matrix "E") or with an eustrophine genus *Synstrophus*, and the family Aderidae (node 42). The connection of the Mycetophagidae and Penthinae is supported only by Bayesian analysis. In the MP of BA matrix, it is the genus *Synstrophus* with an anthicid genus *Ischalia* in the sister-group position to the Mycetophagidae. However, in all analyses Mycetophagidae are present in one clade with the families Mordellidae, Ripiphoridae and Meloidae. In the MP tree of Hunt *et al.* (2007) a genus *Mycetophagus* was found separated from genera *Litargus* and *Triphyllus*, which were united in one clade with members of the Lymexyloidea, the family Ripiphoridae and subfamily Penthinae. The *Mycetophagus* was a sister-group taxon of the family Ciidae.

Aderidae.

The family Aderidae is small, tropical one and in our sampling list is represented by two genera, that keep monophyletic relationship through all types of analyses. Due to many cucujoid-like characteristics, there have been still doubts of aderid's placement within the Tenebrionoidea. Although Buder *et al.* (2008) found it within cucujoid's families, our analyses show, that its placement within the Tenebrionoidea is right one. This fact is supported also by Bayesian and MP analyses of Hunt *et al.* (2007). They are usually connected with the family Anthicidae, because of their resemblance, but this relationship has not been found here. Our findings associate the Aderidae with the families Mycetophagidae and Tetratomidae, that are considered as primitive ones among tenebrionoids and this fact

would support Crowson (1955), who proposed its possible position among the first of the tenebrionoids' families rather than among derived ones. However, none of the aderids' clades found the boostrap support.

Mordellidae, Meloidae, Ripiphoridae.

The family Mordellidae is considered to be a well defined, with one subfamily that includes most of genera and with one species that stands in an individual subfamily Ctenidiinae. The monophyly of sampled Mordellinae is supported here by all analyses. The family Meloidae consists of four subfamilies (Bologna & Pinto, 2001; Bologna *et al*., 2008), of which we sampled members of Meloinae and Nemognathinae. They hold monophyletic status of the family in this study and the genus *Horia* (Nemognathinae) stands in a basal position to the Meloinae in most of analyses (except bayesian one), as supposed by Bologna *et al.* (2008). The Ripiphoridae is the least known family of these ones and its monophyly is still in doubt as well as its intra- and interrelationships, that need further studying (Falin, 2002). Unfortunately, this study does not answer on ripiphorids' questions as well. The family is monophyletic only by the ML analysis. Within the family, there are two clades recognized. First one consists of Ripidiinae, genus *Pelecotoma* and one undetermined ripiphorid species, second clade encompasses Ripiphorinae, genus *Trigonodera* and another undetermined ripiphorid species. However, only the clade consisting of Ripiphorinae, *Pelecotoma* with the undetermined ripiphorid was supported by all analyses. The high boostrap value (100%) proposes to identify the undertermined species (Rh100) as *Pelecotoma* species. The non-monophyly of the subfamily Pelecotominae, as Falin (2002) suggested, is confirmed here.

Based on adult characters, the families Mordellidae and Ripiphoridae are believed to belong in the same lineage with melandryids and scraptiides (Crowson, 1966) or according Lawrence and Newton (1982) in the line with Tetratomidae, Melandryidae, Mordellidae. The Ripiphoridae are thought to arise from a common ancestor with the Mordellidae by development of a parasitic mode of life (Selander, 1957; Crowson, 1966; Lawrence & Newton, 1982). Although the ripiphorid-mordellid relationship is taken as obvious (Franciscolo, 1962, 2000) or with some reservations possible (Crowson, 1995; Falin, 2002), Švácha (1994) has questioned it, because of missing larval synapomorphies.

The Meloidae is usually associated with the Ripiphoridae, due to similar larval morphology and specific biology, but these characters evolved independently (Crowson, 1955, 1966; Selander, 1957; Bologna & Pinto, 2001; Falin, 2002). Despite disclaim of this relationship, Falin (2002) expressed support of further studying to convincingly stabilize the issue.

The close relationship of the Meloidae to the Anthicidae is rather proposed because of the adult features (Crowson, 1955; Abdullah, 1964; Selander, 1966, 1991), but it has not been confirmed yet (Bologna & Pinto, 2001). Other connections of meloids are suggested to Mordellidae-Scraptiidae (Selander, 1991) or to Stenotrachelidae and Oedemeridae (Abdullah, 1964; Lawrence & Newton, 1982), based on adult morphology. However Lawrence (1991) as well as Crowson (1955) rejected the connection between Meloidae and Stenotrachelidae, thanks to the larval differences.

The MP and ML analyses find mordellids together with Meloidae as a monophyletic group (node 60) and salpingid Inopeplinae in their sister-group's position (node 58), that is supported by boostrap values. The Ripiphoridae-Ripiphorinae, genus *Trigonodera* and one undetermined species are always present with them in the same clade in their sister-group's position to them (node 56). POY has found the same clade except the Inopeplinae. By Baysien analysis of the reduced matrix, Mordellidae and Ripiphoridae are recognized as one highly supported clade, but standing in a sister-group relationship to the remaining Tenebrionoidea. Mordellids with ripiphorids are together with the Lymexyloidea in the sister-group position to the remaining tenebrionoids also by Hunt *et al.* (2007). The Meloidae forms in this analysis one monophyletic group with the family Anthicidae, though weakly supported (pp=52).

Anthicidae.

The family Anthicidae is large and it has included many different groups in the recent past. There are currently recognized ten subfamilies in the family (Lawrence & Newton, 1995), but the classification of this family needs a revision (Lawrence & Newton, 1995; Chandler, 2002).

We can confirm that the family is not monophyletic as presently defined, consistently excluding genera *Ischalia* (subfamily Ischaliinae) by all analyses and *Neostereopalpus* (Eurygeniinae) by most of analyses. Remaining sampled subfamily Anthicinae is highly supported, but its relationship among tenebrionoids has not been solved. Crowson (1966) associated anthicids (included eurygeniines and pedilines) in one line with the families Aderidae and Meloidae, but with regard to both larval and adult characters, there are the Scraptiidae that appear to be more closely related to anthicids and aderids (Lawrence, 1977; Young, 1991). Although Lawrence and Newton (1982) concluded this relationship in one of their lineages, they questioned the composition of the family Anthicidae as well as the inclusion of the family Scraptiidae in this lineage. By the analyses of ML, MP of BA matrix,

Clustal matrix “D” and POY, the subfamily Anthicinae is found in one clade with the Scraptiidae and the Oedemeridae and by the MP of BA matrix, it stands in the position of a sister-group taxon to the Scraptiidae. However, none of these clades are supported. Bayesian analysis proposes a clade comprising Anthicinae and Eurygeniinae with the Meloidae, though with a low support (pp=52; pp=60 without Eurygeniinae). Hunt *et al.* (2007) also found the anthicid-meloid association, either with Eurygeniinae (Baysien analysis) or without (MP). By the remaining MP analyses, the subfamily does not keep any stable position. The *Ischalia* is, by the MP Clustal matrices analyses, found as a sister taxon to a ripiphorid clade consisting of *Pelecotoma* and Ripidiinae. Analyses of the MP of BA matrix and POY connect *Ischalia* with the eustrophine genus *Synstrophus*. The subfamily Eurygeniinae used to be included in the Lagriidae (Tenebrionidae), Ischaliinae (Anthicidae) and Lemodinae (Pyrochroidae). The eurygeniid genus *Neostereopalpus* is present in the clade with the Tenebrionidae, Ciidae and Colydiinae by most of analyses, except baysien one.

Salpingidae.

Salpingidae is another family that presently includes previous members of other families. It comprises seven subfamilies (Lawrence & Newton, 1995) and its broad range (Pollock, 2002) as well as heterogenous larval morphology (Beutel & Friedrich, 2005) question the monophyly of the family. Indeed, the family can be considered monophyletic only under the condition of exclusion genus *Elacatis* (subfamily Othniinae) and the subfamily Inopeplinae. The exclusion of the Inopeplinae and its association with the mordellid-meloid clade might be caused due to a long-branch attraction, that has not been investigated here. This subfamily was, by Baysien analysis of full matrix, as well as by Baysien and MP analyses of Hunt *et al.* (2007), found included with the subfamilies Salpinginae and Aegialitinae in one common clade. The genus *Elacatis* (Othniinae) stands as a sister-group taxon to the restricted Pyrochroidae either alone (except MP of BA matrix) or together a member of the Prostomidae (MP of Clustal matrices “A”, “B”). Based on larval similarities, the possible connection of the Pyrochroidae to Othniinae and Oedemeridae was proposed by Young (1991).

The Salpingidae have been connected with Pythidae, Pyrochroidae, Mycteridae and Boridae in one lineage (Lawrence, 1977; Lawrence & Newton, 1982), but Pollock (1994) considered this lingeage to be unstable. His analysis supported two clades within the salpingid group- first one, unresolved tritomy of Trictenotomidae, Salpingidae and Pythidae and second one consisting of Boridae and Pyrochroidae. Unfortunately, the Salpingidae has not achieved a stable position within Tenebrionoidea, only its most frequent association in one common

clade with genera *Boros* and *Agnathus* and Monomminae with *Trictenotoma* is noteworthy. MP analysis of Hunt *et al.* (2007) produced a common clade of the Salpingidae and Oedemeridae.

6.3 The evolution of hypermetamorphosis.

Hypermetamorphosis is a complete metamorphosis with several larval types in different instars, that distinctly contrast. The early larval stage, triungulin is well-sclerotized, active, determined for finding host; the later stages are parasitic, immobile or later ones are non-feeding and falling in diapause. Various insect groups exhibit hypermetaboly: the beetle families Meloidae and Ripiphoridae, the families Mantispidae (Neuroptera), the Acroceridae (Diptera), the Eucharitidae (Hymenoptera) and the order Strepsiptera.

Although the direct relationship between Mordellidae-Ripiphoridae-Meloidae is not obvious, they have been through other links, as mordellids-ripiphorids (Selander, 1957; Crowson, 1966, 1995; Lawrence & Newton, 1982; Franciscolo, 1962, 2000; Falin, 2002), meloids-ripiphorids (Crowson, 1955, 1966; Selander, 1957; Bologna & Pinto, 2001; Falin, 2002), meloids to mordellid-scraptiides (Selander, 1991), connected together. Although our analyses produced one monophyletic clade with all these families included, there were found the Ripiphoridae as the sister-group taxon to the monophyletic group of the Meloidae and Mordellidae. The supposed monophyletic groups Mordellidae-Ripiphoridae or Meloidae-Ripiphoridae have not been found.

Within the Tenebrionoidea, the hypermetamorphosis appears in the Meloidae and Ripiphoridae, and according our results, these two families are present in one clade with the Mordellidae. Although there might be a possibility of one arising of the hypermetamorphosis within the ripiphorid-mordellid-meloid clade and its secondary lost in the Mordellidae, it is denied by the absence of the triungulin type of larva in the primitive subfamilies Pelecotominae (Ripiphoridae) and Eleticinae (Meloidae) and probable absence of other larval types at all in the Eleticinae (Pinto *et al.*, 1996). It also proposes that the hypermetamorphosis can not be considered as a common feature of these two families and it has had to be evolved two times, as an independently developed character. This is underlined also by the fact, that the triungulins and later instars of both families evidently differ (Selander, 1991; Beutel & Friedrich, 2005).

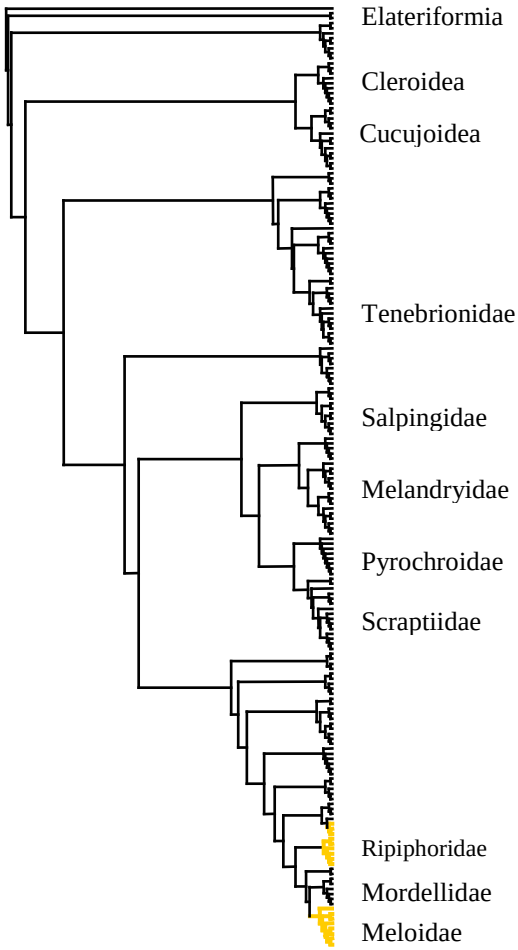


Figure 12: Hypermetamorphosis within the Tenebrionoidea highlighted in yellow on the MP tree of Clustal matrix “B”.

7. Conclusion.

This Ph.D. thesis represents the first phylogenetic analysis of the superfamily Tenebrionoidea. The monophyly of the superfamily as well as the monophyly of the families Oedemeridae, Ciidae, Meloidae, Mycetophagidae, Mordellidae, Scraptiidae and Aderidae has been confirmed. Remaining families as defined by Lawrence and Newton (1995) have been found either paraphyletic (Anthicidae, Pyrochroidae and Salpingidae) or polyphyletic (Zopheridae, Tetratomidae and Melandryidae). The Anthicidae, Pyrochroidae and Salpingidae would be considered monophyletic if the families' limits were changed by exclusion of the subfamilies Ischaliinae and Eurygeniinae from the Anthicidae, the Agnathinae from the Pyrochroidae, the Othniinae from the Salpingidae. The families Tenebrionidae and Ripiphoridae were found either mono- or paraphyletic and this issue has not been succeeded to stabilize.

Four clades have been found within the Tenebrionoidea. The tenebrionid clade consists of the families Tenebrionidae, Ciidae and the subfamily Colydiinae. The melandryinae clade, as named, comprises the members of the subfamily Melandryinae and the genus *Cephaloon*, from the family Stenotrachelidae. In the third clade could be recognized two groups, the family Scraptiidae with the melandryid genus *Osphya* and the restricted family Pyrochroidae with salpingid genus *Elacatis*. The largest clade contains all members of five families, Mordellidae, Meloidae, Ripiphoridae, Mycetophagidae and Aderidae and members of the subfamilies Inopeplinae, Penthinae, Eustrophinae, Lagriinae, Hallomeninae and the anthicid genus *Ischalia*. However, better knowledge of relationships between them has not been achieved.

The high degree of homoplasy, the complexity of the group, lack of information and high variation of morphological characters within families are presented as the main reasons of the unsatisfying situation of the group's classification (Beutel & Friedrich, 2005; Buder *et al.*, 2008). Further research, that would involve both molecular and morphological characters, inclusion of members of all families and of the Cucujiformia series as well as more extensive analyses, will be needed to recognize natural relationships within the Tenebrionoidea.

8. Souhrn.

V této práci jsou zkoumány fylogenetické vztahy nadčeledi Tenebrionoidea. Tenebrionoidea (potemníkovití) je jednou z nadčeledí druhovo bohaté a složité série Cucujiformia, která je považovaná za nejodvozenější sérii v rámci Coleoptera. Samotné Tenebrionoidea jsou velmi různorodou skupinou a obsahují přibližně 30 000 druhů klasifikovaných v 30 čeledích. Jako jejich nejbližší příbuzná nadčeleď je považovaná nadčeleď Cucujoidea, avšak postavení Tenebrionoidea v rámci Cucujiformia nebylo ještě potvrzeno. Vztahy mezi čeleděmi v rámci Tenebrionoidea nejsou známé, protože byly publikovány jenom práce na úrovni rodů nebo podčeledí. V naši práci byly použity sekvence 2 nukleárních genů SSU a LSU rDNA a 2 mitochondriálních genů rrnL rDNA a cox1 mtDNA v celkové délce přibližně 3700 bp pro 154 taxonů reprezentujících 20 čeledí. Pro rozpoznání fylogeneze skupiny byly použity statický i dynamický alignment, následované analýzami maximální parsimonie, maximální pravděpodobnosti a bayesiánskou analýzou. Monofylie nadčeledi byla potvrzena, a byl navržen její vztah k nadčeledi Lymexyloidea, bližší jak se předtím uvádělo. V rámci nadčeledi byly rozpoznány 4 klády- skupina čeledi Tenebrionidae, podčeledi Melandryinae, skupina čeledí Ripiphoridae-Mordellidae-Meloidae a skupina Scraptiidae-Pyrochroidae. Monofylie většiny čeledí byla potvrzena, jenom čeledi Salpingidae, Pyrochroidae a Anthicidae byly parafyletické a čeledi Tetratomidae, Melandryidae a Zopheridae byly určeny jako polyfyletické. Kdyby byli podčeledi Ischaliinae a Eurygeniinae (čeleď Anthicidae), Agnathinae (čeleď Pyrochroidae) a Othniinae (čeleď Salpingidae) vyčleněny, tak by tyhle čeledi taky splňovali podmínku monofyletičnosti. Polyfyletické čeledi by měli být zrevidovány a mělo by být zváženo jejich rozdělení do menších jednotek. Jako hlavní důvody neuspokojivě rozřešené fylogeneze skupiny bych uvedla vysoký stupeň homoplazie a celkovou složitost skupiny. Na rozpoznání pravdivých vztahů nadčeledi Tenebrionoidea bude potřebná více komplexní a rozsáhlejší studie, která by zahrnovala jednak molekulární i morfologické znaky, jednak zástupce všech čeledí a všech nadčeledí série Cucujiformia v rámci rozsáhlých analýz.

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10. Abbreviations.

AIC	Akaike information criterion
BA	alignment by *blastn* algorithm
BNHM	British Natural History Museum, London
CBSU	Computational Biology Service Unit, Cornell University
CI	consistency index
COI	cytochrome oxidase subunit I
DAMBE	Data Analysis and Molecular Biology and Evolution
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
ETS	external transcribed spacers
F	primer of forward sense
hLRTs	Hierarchical likelihod ratio tests
IGS	intergenic spacers
ITS	internal transcribed spacers
LSU	large subunit ribosome, 28S rDNA gene
MEGA	Molecular Evolutionary Genetics Analysis
$MgCl_2$	magnesium chloride
ML	maximum likelihood
MP	maximum parsimony
mtDNA	mitochondrial DNA
nst	number of substitutions
PCR	polymerase chain reaction
pp	posterior probability value
R	primer of reverse sense
RAS	random sequence addition replicates
RC	rescaled index
rDNA	ribosomal DNA
RI	retention index
RNA	ribonucleic acid
rrnL	16S ribosomal molecule in mitochondrias
SSU	small subunit ribosome, 18S rDNA gene
Taq	Thermus aquaticus polymerase

TBR	tree bisection reconnection branch swapping
TNT	Tree analysis using New Technology software

11. List of figures.

12. List of tables.

13. References.

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Supplementary material.

A. Classification.

Order COLEOPTERA

Suborder ARCHOSTEMATA

Suborder MYXOPHAGA

Suborder ADEPHAGA

Suborder POLYPHAGA

Series STAPHYLINIFORMIA
Superfamily HYDROPHILOIDEA
Superfamily STAPHYLINOIDEA

Series SCARABAEIFORMIA
Superfamily SCARABAEOIDEA

Series ELATERIFORMIA
Superfamily SCIRTOIDEA
Superfamily DASCILLOIDEA
Superfamily BUPRESTOIDEA
Superfamily BYRRHOIDEA
Superfamily ELATEROIDEA

Series BOSTRICHIFORMIA
Superfamily DERODONTOIDEA
Superfamily BOSTRICHOIDEA

Series CUCUJIFORMIA
Superfamily LYMEXYLOIDEA
Superfamily CLEROIDEA
Superfamily CUCUJOIDEA
Superfamily TENEBRIONOIDEA
MYCETOPHAGIDAE
- Esarcinae
- Mycetophaginae
- Bergininae

ARCHEOCRYPTICIDAE
PTEROGENIIDAE
CIIDAE (Cisidae, Cioidae)
- Sphindociinae
- Ciinae

TETRATOMIDAE
- Piseninae
- Tetratominae
- Penthinae
- Hallomeninae
- Eustrophinae

MELANDRYIDAE (Serropalpidae)
- Melandryinae
- Osphyinae

MORDELLIDAE
- Ctenidiinae
- Mordellinae

RIPIPHORIDAE (Rhipiphoridae)
- Pelecotominae
- Micholaeminae
- Ptilophorinae
- Hemirhipidiinae
- Ripidiinae
- Ripiphorinae

ZOPHERIDAE (Colydiidae, Monommatidae)
- Colydiinae
- Zopherinae

ULODIDAE
PERIMYLOPIDAE
CHALCODRYIDAE
TRACHELOSTENIDAE
TENEBRIONIDAE (Alleculidae, Lagriidae, Nilionidae, Petriidae, Rhysopaussidae, Tentyriidae)
- Lagriinae
- Nilioninae
- Phrenapatinae
- Zolodininae
- Cossyphodinae
- Pimeliinae
- Tenebrioninae
- Alleculinae
- Diaperinae
- Stenochiinae

PROSTOMIDAE
SYNCHROIDAE
OEDEMERIDAE
- Polypriinae
- Calopodinae
- Oedemerinae

STENOTRACHELIDAE (Cephaloidae)
- Stenotrachelinae
- Cephaloinae
- Nematoplinae
- Stoliinae

MELOIDAE
- Eleticinae
- Meloinae
- Tetraonycinae
- Nemognathinae

MYCTERIDAE (Hemipeplidae)
- Mycterinae
- Eurypinae
- Hemipeplinae

BORIDAE
- Borinae

Synercticinae
TRICTENOTOMIDAE
PYTHIDAE
PYROCHROIDAE (Pedilidae, Pilipalpidae)
Tydessinae
Pilipalpinae
Pedilinae
Pyrochroinae
Agnathinae
SALPINGIDAE (Aegialitidae, Dacoderidae, Elacatidae, Eurystethidae, Inopeplidae, Othniidae, Tretothoracidae)
Othniinae
Prostominiinae
Agleninae
Inopeplinae
Aegialitinae
Salpinginae
Dacoderinae
ANTHICIDAE (Ischaliidae)
Lagrioidinae
Afreminae
Ischaliinae
Eurygeniinae
Macratriinae
Steropinae
Copobaeninae
Lemodinae
Tomoderinae
Anthicinae
ADERIDAE (Euglenidae, Hylophilidae, Xylophilidae)
SCRAPTIIDAE (Anaspididae)
Scraptiinae
Anaspidinae

Superfamily CHRYSOMELOIDEA

Superfamily CURCULIONOIDEA

B. Sampling list.

Superfamily	Family	Subfamily	Species	CodeName	Locality	GenBank Accession Numbers			
						18S	28S	16S	COI
Elateroidea	Elateridae	Denticollinae	Denticollis linearis*	EElaEla	Czech Republic	DQ100498	DQ198741	DQ198651	DQ198573
Byrrhoidea	Ptilodactylidae	Cladotominae	Paralichas pectinatus*	EByrPti	Japan	DQ100486	DQ198722	DQ198633	DQ198556
Buprestoidea	Buprestidae	Buprestinae	Anthaxia hungarica*	EBupBup	France	DQ100484	DQ198702	DQ198623	DQ198545
Lymexiloidea	Lymexilonidae		Lymexylon navale	LyLyLy		AY748185	missing seq	DQ202588	DQ221992
Cleroidea	Melyridae	Danaceinae	Danacea nigritarsis*	ClMeDa	Czech Republic	no	no	EF508035	EF508048
		Rhadalinae	Aplocnemus perforatus*	ClMeRh	Morocco	EF209702	no	EF508037	EF508050
		Melyrinae	Falsomelyris granulata*	ClMeMe	Morocco	EF209700	no	EF508038	EF508051
		Malachiinae	Carphurus sp.*	ClMeMa	Malaysia	EF209731	no	EF508040	EF508053
	Trogossitidae	Trogossitinae	Trogossita japonica*	ClTrTr	Japan	EF209679	no	EF508041	EF508054
	Cleridae	Clerinae	Clerus mutillarius*	ClClCl	Slovakia	EF209691	no	EF508043	EF508056
	Prionoceridae	Prionocerinae	Idgia sp.*	ClPrPr	Indonesia	EF209685	FJ903952	EF490157	EF490187
Cucujoidea	Bothrideridae	Xylariophilinae	Xylariophilus sp.*	CuBohXy	Slovakia	EF209827	FJ903953	EF490158	EF490188
	Nitidulidae		Nitidulidae gen.sp.*	CuNi	Malaysia	EF210012	FJ903954	FJ903788	FJ904081
	Byturidae	Byturinae	Byturus aestivus*	CuByBy	Czech Republic	EF209816	no	no	no
	Phalacridae	Phalacrinae	Stilbus testaceus*	CuPhPh		no	no	no	no
	Erotylidae	Tritominae	Cyrtomorphus sp.*	CuErTr		no	no	no	no
	Endomychidae	Lycoperdininae	Mycetina sp.*	CuEnLy	Indonesia	EF209845	no	no	no
	Cucujidae		Cucujus mniszechi*	CuCu	Japan	EF209775	no	no	no
	Silvanidae	Brontinae	Dendrophagus sp.*	CuSiBr	Japan	EF209768	no	no	no
	Passandridae		Hectathrum sp.*	CuPa	Indonesia	EF209773	no	no	no
	Helotidae		Helota gemmata*	CuHe	Japan	EF209758	no	no	no
	Languriidae	Langurinae	Tetraphala aenea*	CuLaLa	Indonesia	EF209803	no	no	no
	Coccinelidae	Coccinelidae	Psyllobium vingintiduopunctatum*	CuCoCo	Czech Republic	EF209854	no	no	no
	Monotommidae	Monotominae	Monotoma sp.*	CuMoMo	Czech Republic	EF209756	no	no	no
	Cerolynidae		Philotermus sp.*	CuCe	Japan	EF209834	no	no	no
	Cryptophagidae	Loberinae	Loberus sp.	CuCrLo		no	no	no	no
Chrysomeloidea	Chrysomelidae	Chrysomelinae	Chrysolina hyperici	ChChCh		AY748121	missing seq	AF097090	DQ222025
			Gonioctena olivacea	ChChCh	United Kingdom	AJ622061	missing seq	AJ841310	AY904888
			Calligrapha multipunctata	ChChCh		AJ841419	missing seq	AJ841303	AM283119
		Donaciinae	Donacia vulgaris	ChChDo	Russia	AY748122	missing seq	AY232579	AY232522
		Galerucinae	Pyrrhalta viburni	ChChGa	Germany	AJ841497	missing seq	AJ841378	AM283212
			Diabrotica undecimpunctata	ChChGa	USA	AJ781618	missing seq	AJ781555	AM283202
Curculionoidea	Curculionidae	Entiminae	Diaprepes abbreviatus	CucCuEn		AY157729	missing seq	CN475651	DN200219
		Erirhininae	Tanysphyrus lemnae	CucCuEr		AJ850023	missing seq	missing seq	DQ155948

Tenebrionoidea	Mycetophagidae	Mycetophaginae	Litargus sp.*	MyMyLg145	Malaysia	EF209880	FJ903902	EF490145	EF490173
			Litargus sp.*	MyMyLg146	Indonesia	EF209881	FJ903903	FJ903751	FJ904036
			Mycetophagus atomarius*	MyMyMy150	Czech Republic	EF209882	FJ903906	FJ903753	FJ904038
			Litargus connexus*	MyMyLg151	Czech Republic	EF209883	FJ903907	FJ903754	FJ904039
			Mycetophagus quadripustulatus*	MyMyMy014	SlovakRepublic	EF209884	FJ903813	EF490159	FJ903965
	Ciidae	Ciinae	Orthocis pygmaeus*	CiCiOr180	Slovak Republic	EF209885	FJ903926	EF490136	EF490164
			Cis boleti*	CiCiCi182	Czech Republic	EF209886	FJ903927	FJ903769	FJ904059
			Orthocis festivus	CiCiOr184	Slovak Republic	EF209888	FJ903928	missing seq	FJ904060
	Tetratomidae	Penthinae	Penthe japana*	TerPePe026	Japan	FJ903789	FJ903821	FJ903694	FJ903972
		Eustrophinae	Synstrophus macrophtalmus	TerEuSy031	Japan	EF209901	FJ903826	missing seq	FJ903977
		Hallomeninae	Mycetoma suturale*	TerHaMy078	Czech Republic	EF209903	FJ903855	FJ903719	FJ904002
		Tetratominae	Tetratoma fungorum*	TerTeTe079	Czech Republic	FJ903794	FJ903856	FJ903720	FJ904003
		Eustrophinae	Holostrophus orientalis*	TerEuHo082	Japan	EF209905	FJ903858	FJ903723	FJ904006
		Penthinae	Penthe sp.*	TerPePe133	Indonesia	EF209891	FJ903893	FJ903743	FJ904028
		Hallomeninae	Mycetoma sp.*	TerHaMy139	Japan	EF209909	FJ903897	FJ903747	FJ904032
			Tetratomidae gen.sp.*	Ter161	Japan	EF209893	FJ903915	FJ903758	FJ904047
		Hallomeninae	Hallomenus binotatus*	TerHaHa171	Slovak Republic	EF209917	FJ903921	FJ903764	FJ904053
		Tetratominae	Tetratoma ancora*	TerTeTe192	Slovak Republic	EF209890	FJ903933	FJ903774	FJ904065
	Melandryidae	Melandryinae	Phloiotrya bellicosa*	MeaMePh012	Japan	EF209900	FJ903811	FJ903688	FJ903963
			Phryganophilus ruficollis*	MeaMePy032	Japan	EF209902	FJ903827	FJ903699	FJ903978
			Mikadonius gracilis*	MeaMeMk033	Japan	FJ903791	FJ903828	FJ903700	FJ903979
			Melandrya modesta	MeaMeMe048	Japan	FJ903792	FJ903833	missing seq	FJ903982
			Melandrya pictipennis	MeaMeMe069	Japan	missing seq	FJ903850	FJ903716	FJ903998
			Orchesia imitans	MeaMeOr080	Japan	EF209904	missing seq	FJ903721	FJ904004
			Melandryidae gen.sp.*	Mea081	Japan	FJ903795	FJ903857	FJ903722	FJ904005
		Melandryinae	Dircea sp.	MeaMeDi083	Czech Republic	missing seq	missing seq	FJ903724	FJ904007
			Hypulus cingulatus*	MeaMeHy119	Japan	EF209906	FJ903884	EF490138	EF490166
			Melandrya sp.*	MeaMeMe120	Japan	FJ903797	FJ903885	FJ903739	FJ904024
		Osphyinae	Osphya orientalis*	MeaOsOs122	Japan	EF209898	FJ903887	EF490139	EF490167
		Melandryinae	Paramikadonius crepuscula*	MeaMePa124	Japan	EF209895	FJ903889	FJ903740	FJ904025
			Phloiotrya planiuscula*	MeaMePh130	Japan	EF209907	FJ903890	FJ903741	FJ904026
			Phloiotrya flavitarsis*	MeaMePh138	Japan	EF209908	FJ903896	FJ903746	FJ904031
			Microtonus sp.*	MeaMeMi140	Malaysia	EF209910	FJ903898	FJ903748	FJ904033
			Microtonus dimidiatus*	MeaMeMi144	Japan	EF209896	FJ903901	EF490137	EF490165
			Microscapha sp.	MeaMeMs149	Malaysia	EF209911	FJ903905	FJ903752	FJ904037
			Melandrya dubia*	MeaMeMe155	Slovak Republic	EF209899	FJ903909	FJ903755	FJ904041
			Melandrya barbata*	MeaMeMe156	Slovak Republic	EF209897	FJ903910	FJ903756	FJ904042
			Orchesia micans	MeaMeOr157	Czech Republic	EF209912	FJ903911	missing seq	FJ904043
			Hypulus quercinus*	MeaMeHy158	Czech Republic	EF209913	FJ903912	FJ903757	FJ904044

		Hypulus acutangulus	MeaMeHy159	Japan	EF209914	FJ903913	missing seq	FJ904045
		Phloiotrya obscura	MeaMePh160	Japan	FJ903799	FJ903914	missing seq	FJ904046
	Melandryinae	Anisoxya fuscula *	MeaMeAn162	Czech Republic	EF209915	FJ903916	FJ903759	FJ904048
		Orchesia undulata	MeaMeOr173	Slovak Republic	EF209918	FJ903922	missing seq	FJ904054
		Abdera quadrifasciata	MeaMeAb174	Slovak Republic	EF209919	missing seq	FJ903765	FJ904055
		Orchesia minor*	MeaMeOr175	Slovak Republic	EF209920	FJ903923	FJ903766	FJ904056
		Phloiotrya rufipes*	MeaMePh176	Slovak Republic	FJ903800	FJ903924	FJ903767	FJ904057
Mordellidae	Mordellinae	Mordellista brevicauda*	MoMoMi070	Slovak Republic	EF209926	FJ903851	FJ903717	FJ903999
		Glipa ishigakiana*	MoMoGli088	Japan	EF209921	FJ903862	EF490143	EF490171
		Mordella brachyura*	MoMoMo089	Slovak Republic	EF209922	FJ903863	EF490144	EF490172
		Mordellistena neuwaldeggiana*	MoMoMs090	Slovak Republic	EF209923	FJ903864	FJ903727	FJ904010
		Hoshihanomia perlata*	MoMoHo092	Japan	EF209925	FJ903866	FJ903729	FJ904012
		Cephaloglipa angustatissima*	MoMoCe137	Indonesia	EF209927	FJ903895	FJ903745	FJ904030
Ripiphoridae	Pelecotominae	Trigonodera lokejii*	RhPeTr084	Japan	EF209932	FJ903859	FJ903725	FJ904008
	Ripihorinae	Macrosiagon cyaniveste*	RhRhMa086	Japan	EF209933	FJ903860	FJ903726	FJ904009
	Ripidiinae	Ripidiinae gen.sp.	RhRi087	Malaysia	EF209934	FJ903861	EF490148	EF490177
	Ripiphorinae	Metoecus paradoxus	RhRhMe098	Czech Republic	EF209928	missing seq	FJ903730	FJ904014
	Ripiphorinae	Ripiphorus flaviventris	RhRhRi099	Costa Rica	EF209929	FJ903870	missing seq	FJ904015
		Ripiphoridae gen.sp.	Rh100	Greece	EF209930	FJ903871	FJ903731	missing seq
	Pelecotominae	Pelecotoma fennica*	RhPePe101	Czech Republic	EF209931	FJ903872	EF490147	EF490176
		Ripiphoridae gen.sp.*	Rh132	Indonesia	EF209935	FJ903892	FJ903742	FJ904027
		Ripiphoridae gen.sp.*	Rh214	Zambia	EF209936	FJ903946	FJ903782	FJ904075
Zopheridae	Colydiinae	Gempylodes lewisi*	ZoCoGe067	Japan	EF209938	FJ903848	EF490156	EF490186
		Endophloeus serratus*	ZoCoEnd143	Japan	EF209939	FJ903900	FJ903750	FJ904035
		Synchita humeralis*	ZoCoSy178	Slovak Republic	EF209940	FJ903925	FJ903768	FJ904058
		Colydium elongatum	ZoCoCo195	Czech Republic	missing seq	FJ903934	EF490160	EF490189
		Synchita sp.	ZoCoSy2217	Japan	EF209941	FJ903948	FJ903784	FJ904077
		Neotrichus serraticollis*	ZoCoNe2218	Japan	EF209942	FJ903949	FJ903785	FJ904078
		Bitoma siccana*	ZoCoBi2219	Japan	EF209943	FJ903950	FJ903786	FJ904079
		Aulonium trisulcum*	ZoCoAu2279	Czech Republic	EF209944	FJ903951	FJ903787	FJ904080
	Zopherinae	Monommatini gen.sp.*	ZoMon110		EF209937	FJ903878	EF490142	EF490170
	Zopherinae	Monommatini gen.sp.	ZoMon111		missing seq	FJ903879	FJ903735	FJ904020
Tenebrionidae	Alleculinae	Cteniopus sulphureus*	TeAlCt001	Slovak Republic	EF209948	FJ903802	FJ903682	FJ903955
	Diaperinae	Diaperis boleti*	TeDiDi003	Slovak Republic	EF209945	FJ903804	FJ903684	FJ903957
	Lagriinae	Lagria hirta	TeLaLa004	Czech Republic	EF209949	FJ903805	missing seq	FJ903958
	Lagriinae	Cerogria bryanti*	TeLaCe011	Indonesia	EF209951	FJ903810	FJ903687	FJ903962
	Lagriinae	Anisistyra rugipennis	TeLaAn013	Japan	EF209954	FJ903812	FJ903689	FJ903964
	Tenebrioninae	Uloma sp. *	TeTeUl016	Japan	EF209955	FJ903815	FJ903690	FJ903966
	Tenebrioninae	Misolampidius sp.	TeTeMi017	Japan	EF209956	FJ903816	missing seq	FJ903967

	Alleculinae	Omophlus rugosicollis*	TeAlOm023	Slovak Republic	EF209957	FJ903818	FJ903692	FJ903969
	Alleculinae	Gonodera luperus*	TeAlGo024	Czech Republic	EF209958	FJ903819	FJ903693	FJ903970
	Lagriinae	Macrolagria robusticeps	TeLaMa025	Japan	EF209959	FJ903820	missing seq	FJ903971
	Tenebrioninae	Uloma sp. *	TeTeUl027	Japan	EF209960	FJ903822	FJ903695	FJ903973
	Lagriinae	Arthromacra amamiana*	TeLaAr029	Japan	EF209971	FJ903824	FJ903697	FJ903975
	Alleculinae	Hymenalia sp.*	TeAlHy030	Japan	EF209961	FJ903825	FJ903698	FJ903976
	Coelometopinae	Strongylium sp.*	TeCoeSt034	Indonesia	EF209962	FJ903829	EF490152	EF490182
	Phrenapatinae	Phrenapatinae gen.sp.*	TePhr041	Czech Republic	EF209947	FJ903831	EF490154	EF490184
	Lagriinae	Arthromacra decora*	TeLaAr052	Japan	FJ903793	FJ903836	FJ903704	FJ903985
	Alleculinae	Alleculinae gen.sp.	TeAl064	Slovak Republic	EF209964	FJ903845	FJ903713	FJ903994
	Alleculinae	Isomira antennata*	TeAlIs065	Slovak Republic	EF209965	FJ903846	FJ903714	FJ903995
	Diaperinae	Crypticus quiquilius	TeDiCr066	Czech Republic	EF209966	FJ903847	missing seq	FJ903996
	Alleculinae	Borboresthes sp.	TeAlBo068	Japan	missing seq	FJ903849	FJ903715	FJ903997
	Diaperinae	Diaperis lewisi*	TeDiDi074	Japan	EF209946	FJ903852	EF490153	EF490183
		Ischnodactylus sp.	TeDiIs075	Japan	missing seq	FJ903853	FJ903718	FJ904000
	Lagriinae	Adynata brevicollis*	TeLaAd107		EF209952	FJ903875	FJ903733	FJ904017
	Diaperinae	Diaperinae gen.sp.	TeDi108		EF209995	FJ903876	FJ903734	FJ904018
	Lagriinae	Lagriini gen.sp.	TeLa109		EF209953	FJ903877	missing seq	FJ904019
	Alleculinae	Isomira sp.	TeAlIs166	Morocco	EF209969	FJ903919	FJ903762	FJ904051
Prostomidae		Prostomidae gen.sp.	Pr154	Malaysia	EF210011	FJ903908	missing seq	FJ904040
Oedemeridae	Oedemerinae	Oedemera virescens	OeOeOe007	Slovak Republic	EF209972	FJ903807	missing seq	FJ903959
		Chrysanthia viridissima*	OeOeChr010	Slovak Republic	EF209973	FJ903809	FJ903686	FJ903961
		Nacerdes hilleri*	OeOeNa015	Japan	EF209974	FJ903814	EF490146	EF490174
		Nacerdes umenoi*	OeOeNa028	Japan	FJ903790	FJ903823	FJ903696	FJ903974
		Oedemera podagrariae*	OeOeOe055	Slovak Republic	EF209976	FJ903839	FJ903707	FJ903988
		Oedemera femorata*	OeOeOe056	Slovak Republic	EF209977	FJ903840	FJ903708	FJ903989
		Oncomerella venosa*	OeOeOn062	Japan	EF209975	FJ903843	FJ903711	FJ903992
Stenotrachelidae	Cephaloinae	Cephaloon pallens*	StCeCe050	Japan	EF209980	FJ903834	EF490135	FJ903983
Meloidae	Meloinae	Lytta vesicatoria*	MeMeLy005	Czech Republic	EF209985	FJ903806	EF490140	EF490168
		Meloidae gen.sp.	Me058		EF209986	FJ903841	FJ903709	FJ903990
	Meloinae	Lydomorphus bifoveiceps*	MeMeLd106		EF209987	FJ903874	FJ903732	FJ904016
		Meloe uralensis*	MeMeMe112	Czech Republic	EF209981	FJ903880	FJ903736	FJ904021
		Meloe decorus*	MeMeMe113	Czech Republic	EF209982	FJ903881	FJ903737	FJ904022
		Meloe proscarabaeus	MeMeMe114	Czech Republic	EF209983	FJ903882	FJ903738	FJ904023
	Nemognathinae	Horia roepkei*	MeNeHo115	Malaysia	EF209984	FJ903883	EF490141	EF490169
	Meloinae	Epicauta sp.*	MeMeEp135	Malaysia	EF209988	FJ903894	FJ903744	FJ904029
Boridae	Borinae	Boros schneideri*	BoBosch094	Slovak Republic	EF209989	FJ903868	EF490134	EF490163
Trictenotomidae		Trictenotoma sp.*	TrTr208	Malaysia	EF209990	FJ903945	EF490155	EF490185
Pyrochroidae	Pyrochroinae	Pyrochroa coccinea*	PyPyPy002	Slovak Republic	EF209991	FJ903803	FJ903683	FJ903956

		Pyrochroa sp.*	PyPyPy009	Japan	EF209992	FJ903808	FJ903685	FJ903960
		Pyrochroidae gen.sp.*	Py022	Japan	EF209993	FJ903817	FJ903691	FJ903968
		Pyrochroidae gen.sp.*	Py035	Japan	EF209994	FJ903830	FJ903701	FJ903980
	Agnathinae	Agnathus decoratus	PyAgAg095	Czech Republic	EF209998	FJ903869	missing seq	FJ904013
	Pedilinae	Tosadendroides okamatoi	PyPeTo123	Japan	EF209996	FJ903888	missing seq	EF490175
		Pyrochroidae gen.sp.*	Py165	Czech Republic	EF209997	FJ903918	FJ903761	FJ904050
Salpingidae	Salpinginae	Salpingus sp.*	SaSaSa053	Czech Republic	EF210008	FJ903837	FJ903705	FJ903986
		Salpingidae gen.sp.*	Sa054	Czech Republic	EF210009	FJ903838	FJ903706	FJ903987
	Aegialitinae	Aegialites raikokensis	SaAeAe102	Russia	EF210001	FJ903873	missing seq	EF490178
	Othniinae	Elacatis sp.*	SaOtEla148	Malaysia	EF210002	FJ903904	EF490149	EF490179
		Salpingidae gen.sp.*	Sa164	Indonesia	EF210006	FJ903917	FJ903760	FJ904049
	Salpinginae	Lissodema sp.*	SaSaLi170	Malaysia	EF210003	FJ903920	FJ903763	FJ904052
	Inopeplinae	Inopeplus sp.	SaInIn202	Malaysia	EF209999	FJ903939	EF490150	EF490180
	Inopeplinae	Inopeplus sp.	SaInIn203	Malaysia	EF210000	FJ903940	FJ903777	FJ904070
		Salpingidae gen.sp.*	Sa207	Malaysia	EF210004	FJ903944	FJ903781	FJ904074
		Salpingidae gen.sp.*	Sa2212	Indonesia	FJ903801	FJ903947	FJ903783	FJ904076
Anthicidae	Anthicinae	Formicomus pedestris*	AnAnFo051	Slovak Republic	EF210016	FJ903835	FJ903703	FJ903984
		Anthicidae gen.sp.*	An060	Indonesia	EF210013	FJ903842	FJ903710	FJ903991
		Anthicidae gen.sp.*	An063	Slovak Republic	EF210015	FJ903844	FJ903712	FJ903993
		Anthicidae gen.sp.	An076	Japan	EF210014	FJ903854	missing seq	FJ904001
	Ischaliinae	Ischalia sp.*	AnIscIs121	Malaysia	EF210017	FJ903886	EF490132	EF490161
	Eurygeniinae	Neostereopalpus niponicus*	AnEuNeo131	Japan	EF210018	FJ903891	EF490133	EF490162
	Anthicinae	Anthicomorphus suturalis*	AnAnAnp141	Japan	EF210019	FJ903899	FJ903749	FJ904034
Aderidae		Aderus sp.	AdAd197	Malaysia	EF210021	FJ903935	missing seq	FJ904066
		Phytobaenus amabilis	AdPh198	Czech Republic	EF210022	FJ903936	missing seq	FJ904067
Scraptiidae	Anaspidinae	Anaspis rufulabris*	ScAnAn047	Czech Republic	EF210025	FJ903832	FJ903702	FJ903981
		Pentaria badia*	ScAnPe091	Slovak Republic	EF209924	FJ903865	FJ903728	FJ904011
	Scraptiinae	Scraptia sp.*	ScScSc093	Japan	EF210026	FJ903867	EF490151	EF490181
		Scraptia sp.*	ScScSc187	Indonesia	EF210023	FJ903929	FJ903770	FJ904061
	Anaspidinae	Anaspidinae gen.sp.*	ScAn188	Japan	EF210027	FJ903930	FJ903771	FJ904062
		Anaspis hayashii*	ScAnAn189	Japan	EF210028	FJ903931	FJ903772	FJ904063
	Scraptiinae	Scraptia sp.*	ScScSc190	Indonesia	EF210029	FJ903932	FJ903773	FJ904064
	Anaspidinae	Anaspis thoracica*	ScAnAn199	Czech Republic	EF210030	FJ903937	FJ903775	FJ904068
		Anaspis frontalis	ScAnAn200	Czech Republic	EF210031	FJ903938	FJ903776	FJ904069
		Anaspis lurida*	ScAnAn204	Morocco	EF210032	FJ903941	FJ903778	FJ904071
		Anaspis trifasciata*	ScAnAn205	Morocco	EF210033	FJ903942	FJ903779	FJ904072
		Anaspis pulicaria	ScAnAn206	Morocco	EF210034	FJ903943	FJ903780	FJ904073

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Identification of net-winged beetle larvae (Coleoptera: Lycidae) using three mtDNA fragments: a comparison of their utility

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Abstract. We investigated the effectiveness of short mitochondrial DNA fragments for the identification of lycid larvae. The rrnL, cox1 and nad5 mtDNA sequences from 17 specimens of immature stages of Lycidae and Lampyridae were combined with a previously published dataset of homologous fragments representing all major lineages of Lycidae and outgroups. Their relationships were analysed under parsimony criteria. We demonstrate that high-density profiles are necessary for accurate identification of unknown samples to generic and tribal levels and that a multilocus approach is critical for obtaining reliable results. Although widely used, the cox1 mtDNA fragment showed the worst performance for identification at genus level when the query species was not present in the library. Stronger support for deeper branches came from rrnL mtDNA. The neotenic female larvae and male adult stages of Platerodrilus sp. and Macrolibnetis depressus Pic, 1938 were associated by mtDNA fragments. Based on the present identification, larvae of Dictyopterini (Dictyopterini gen. sp., Dictyoptera aurora Herbst, 1784), Sulabanus sp., Leptotrichalus sp. (Metriorrhynchini) and Macrolibnetis depressus Pic, 1938 (Platerodrilini) are described for the first time. Further species of Platycis Thomson, 1859, Plateros Bourgeois, 1979, Macrolycus Waterhouse, 1878, Cautires Waterhouse, 1879 and Lyponia Waterhouse, 1878 are identified by morphology and molecular markers. The data on larval morphology and their usefulness for classification are discussed.

Introduction

The size and scope of phylogenetic analyses using molecular data on Coleoptera has increased steadily in recent years, leading to a general improvement in our understanding of phylogenetic relationships among beetle lineages (Hunt et al., 2007). Nevertheless, the importance of morphological data cannot be underestimated and we should not cease the study of morphology as a source of phylogenetic information (Lipscomb et al., 2003; Will et al., 2005; Wheeler, 2008). The availability of molecular data brought about an opportunity to identify immature stages without rearing to the adult stage (e.g. Miller et al., 2005; Caterino & Tishechkin, 2006; Scheffer et al., 2006; Ahrens et al., 2007). In this way, we can improve the robustness of phylogenies, and enable further studies on life histories and the morphological evolution of poorly known lineages.

Here, we focus on net-winged beetles (Elateroidea: Lycidae), which represent one of many beetle groups with unsatisfactorily known larval morphology and biology. The Lycidae is an extensive lineage, with over 4000 described species of which only 2% are known in a larval stage (Miller, 2002; Bocak & Matsuda, 2003; Bocak & Bocakova, 2008). The main reason for such limited knowledge is the biology of the group. The highest diversity occurs in humid tropical regions, where systematic and long-term field research is scarce. In addition, the larval stages may take up to several years and their growth is very slow. Lycids feed on liquids with a high content of microscopic organisms and it is difficult to maintain rotten wood or soil and the associated microbial life in the laboratory for long time (Bocak & Matsuda, 2003). Failure to breed lycids has been reported by

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several authors (Wong, 1996, 1998; Bocak & Matsuda, 2003; B. Burakowski, personal communication). Our experiments in breeding were successful only if larvae were collected in the late instars and if they pupated within a year of their transfer to the laboratory. As lycids generally have small populations and larvae live cryptically, they are usually collected in small numbers, often in early instars. Therefore, data on their taxonomy and biology have accumulated very slowly (Bocak & Matsuda, 2003). The possibility of extracting DNA from small pieces of tissue enables the identification of lower instars, which were not associated with adults by breeding due to the above described difficulties. Another area calling for DNA-based identification is that of lineages with female neoteny. We know several lycid taxa only in males. Although recently they have been collected in high numbers, larviform females, which do not pupate, remain unassociated with males (Bocak et al., 2008). Among neotenics, both sexes are known only for two species of Duliticola Mjöberg, 1925 (Mjöberg, 1925; Wong, 1996), and in both cases they were identified by locally based entomologists in Borneo after several years of research. Although developmental heterochrony has been studied intensively in many groups, beetles (including Lycidae) have received much less attention (Cicero, 1988; Miller, 1991; Bocak et al., 2008), mainly because of their poorly known biology and the limited knowledge available on larval stages.

The usefulness of molecular markers for species identification and delineation has been advocated by many authors (e.g. Hebert et al., 2003a, b; Proudlove & Wood, 2003; Tautz et al., 2003; Monaghan et al., 2005; Ahrens et al., 2007). The feasibility of DNA-based identification of unknown taxa depends on the availability of sequences in public databases and/or the availability of identified specimens for comparison. Although more sequences have become publicly accessible, taxonomic and geographical coverage varies and the available data are inadequate for some poorly studied groups. Therefore, the chance of identification of an unknown larva with DNA sequences to species level is low and probably will remain low for many groups in the near future. Here, we test the relative usefulness of three mitochondrial markers for the identification of lycid larvae: the large ribosomal unit (rrnL), cytochrome oxydase subunit I (cox1) and NADH dehydrogenase subunit 5 with adjacent tRNAs (nad5). These are widely used in phylogenetic studies and are accessible in public databases. The published sequences representing major lycid lineages (Bocak et al., 2008) form a test database to which larval samples are matched. Unfortunately, no data are available for the 'barcoding' cox1 fragment for Lycidae (Hebert et al., 2003a, b), and we could not test the performance of this fragment under the low-density sampling conditions.

Our intention is to compare the ability of these markers to support monophyly of genera and tribes when the previously published dataset representing major lycid lineages is combined with newly sequenced samples of lycid larvae (Bocak et al., 2008). We chose bootstrap values as an indicator of the robustness of the clades (Moritz & Cicero, 2004). Some of the lycid larvae were identified using morphology (Bocak & Matsuda, 2003) prior to phylogenetic analyses, and such identifications were tested by molecular data. Several larvae belong to lineages with unknown immature stages and their identifications were based solely on phylogenetic analyses. Therefore, additional goals of this study are identification and description of larvae of these lineages, and discussion of the morphological disparity within them.

Material and methods

Larval specimens, DNA extraction, polymerase chain reaction amplification and DNA sequencing

Altogether, 15 larvae of Lycidae and two larvae of Lampyridae were sequenced. These were collected in central Honshu, Japan (11 samples) and in the Indonesian islands of Sumatra, Kalimantan, Java and Sulawesi (five samples; Table 1). The specimens were preserved in 96% alcohol in the field and kept at −20°C until isolation. Total DNA was extracted from the thorax following Vogler et al. (1993), and the rest of specimen was vouchered for morphological study. All voucher specimens are deposited in the collection of the senior author if not stated otherwise.

One rRNA coding (rrnL) and two protein coding (cox1 and nad5) genes were amplified from the mitochondrial genome. All genes were sequenced in both directions in overlapping fragments with primers reported by Bocak et al. (2008). The amplification was carried out using 1 U Taq polymerase (Platinum Taq DNA Polymerase, Invitrogen or BioTaq DNA Polymerase, Bioline), 2 mM $MgCl_2$, 50 mM each dNTP, 0.2 mM each primer and 0.03 mg of template in 50-mL reaction volume. The polymerase chain reactions (PCRs) were performed under the following conditions: initial denaturation for 2 min at 94°C; 40 cycles of 94°C for 1 min, 45°C for 1 min, 72°C for 1–2 min; and a final extension of 10 min at 72°C. The PCR product was purified using the GeneClean III kit (BIO101Systems QBIOgene) and cycle sequenced with the BigDye Terminator v1.1 Cycle Sequencing Kit.

Dataset for comparison

We used the previously published phylogeny of Lycidae (Bocak et al., 2008), which was based on six DNA fragments and a set of cox1 and nad5 sequences of four genera from Sulawesi (rrnL fragment unavailable). Geographical origins of larval samples are given in Table 1. Three fragments, rrnL, cox1 and nad5, were chosen for identification of larval samples. We omitted slowly evolving 18S and 28S rDNA and one protein-coding fragment (cob). All tribes and subtribes of Asian Lycidae were represented in the sequence library by either a few genera or a few species from a single genus. Considering the diversity of Lycidae in the region, the database against which the unknown taxa were assessed represents only a tiny fraction of the diversity of Lycidae. Many lycid genera are yet to be sequenced, and large genera with hundreds of species such as Plateros Bourgeois, 1879

Table 1. List of taxa, geographical origin, designation of samples and GenBank accession numbers of larva specimens used in this study. The accession numbers for the dataset of identified Lycidae were reported by Bocak et al. (2008).

Sample label	Identification	Geographical origin	Voucher UPOLþ	GeneBank accession numbers		
				16S	COI	ND5
A	Platycis sp.	Japan, Shiga Pref., Mikunidake	ZL2008	EF143218	EF143233	EF143247
B	Lyponia sp.	A Japan, Nagano Pref., Mt.Aboyama	ZL2014	FJ390408	FJ390410	FJ390412
C	Lyponia sp. A	Japan, Nagano Pref., Mt.Aboyama	ZL2016	EF143225	EF143240	EF143253
D	Macrolycus sp. A	Japan, Ishikawa Pref., Shiramine, Mt.Hakosan	ZL2005	EF143217	EF143232	EF143246
E	Macrolycus sp. B	Japan, Ishikawa Pref., Shiramine, Mt.Hakosan	ZL2017	EF143226	n.a.	EF143254
F	Cautires sp.	Indonesia, Sumatra, Gn.Talamau, Simpangempat	ZL2009	EF143219	EF143234	EF143248
G	Metriorrhynchini gen.sp.	Japan, Nara Pref., Shakagateyama Asahi Riv.vall.	ZL2015	EF143224	EF143239	n.a.
H	Leptotrichalus sp.	Indonesia, Java, Trawas, Gn.Penanggungan	ZL2002	EF143215	EF143230	EF143244
I	Sulabanus sp.	Indonesia, Sulawesi, Malino, Gn.Lompobatang	ZL2010	EF143220	EF143235	EF143249
J	Platerodrilus sp.	Indonesia, Kalimantan, Muara Teweh	000589	EF143214	EF143229	EF143243
K	Plateros sp. A	Japan, Osaka Pref., Iwawakiyama, Amami	ZL2006	FJ390407	FJ390409	n.a.
L	Plateros sp. A	Japan, Osaka Pref., Iwawakiyama, Amami	ZL2012	EF143222	EF143237	EF143251
M	Plateros sp. B	Japan, Shiga Pref., Mikunidake	ZL2018	EF143227	EF143241	EF143255
N	Dictyopterini gen.sp.	Japan, Osaka Pref., Iwawakiyama, Kagata	ZL2013	EF143223	EF143238	EF143252
O	Macrolibnetis depressus	Malaysia, Cameron Highlands	000515	n.a.	FJ390411	FJ390413
	Lampyridae gen.sp.	Japan, Shiga Pref., Mikunidake	ZL2011	EF143221	EF143236	EF143250
	Lampyridae gen.sp.	Indonesia, Sulawesi, Wasuponda	ZL2019	EF143228	EF143242	EF143256

(over 600 species) or Cautires Waterhouse, 1879 (300 species) were represented by few species.

Alignment and phylogenetic analyses

Sequences were edited using the SEQUENCHER ver. 4.5 software package (Gene Codes Corporation) and aligned using CLUSTALX ver. 1.81 (Thompson et al., 1997) under default settings. The aligned sequences were corrected manually for minor adjustments. The sequences from larval samples were combined with the dataset for comparison. The phylogenetic analyses were performed under parsimony criteria using TNT ver. 1.1 (Goloboff et al., 2003). The new technology search algorithm was applied and the shortest tree was found 25 times. The characters were given equal weights. Ochotyra sp. (Rhagophthalmidae) was designated as outgroup when trees were rooted. The strict consensus and majority-rule trees were used for evaluation of the relationships of samples representing larvae, and bootstrap analyses were used to estimate the robustness of the lineages. We performed 1000 pseudoreplicates, with the search stopped when the shortest tree was found three times. All analyses were conducted on partial matrices of rrnL, cox1 and nad5 mtDNA, on all possible combinations of two fragments, and on the full dataset (Table 2). Altogether, 18 monophyletic groups were defined based on morphology (Bocak & Bocakova, 2008) and the previous analysis of the phylogeny of Lycidae (Bocak et al., 2008), which supported their monophyly. The robustness of these predefined clades was then evaluated with bootstrap proportions returned by analyses of the partial datasets described above (Table 3).

Abbreviations. BL, length of body; PL, length of pronotum; PW, width of pronotum; T1–3, thoracic segments; A1–A9, abdominal segments.

Table 2. Results of individual analyses.

Fragments	Number of					Tree scores			Number of ingroup nodes		
	taxa	characters	constant characters	informative characters	trees	length	CI	RI	in the strict consensus tree/maximum	in the majority consensus tree/maximum	with bootstrap support over 50%
rrnl	94	519	188	288	5	3320	0.215	0.521	82/86	86/86	46
cox1	104	731	261	433	3	7639	0.127	0.379	71/96	96/96	35
nad5	103	1246	175	971	1	15 443	0.166	0.421	95/95	95/95	67
rrnL, cox1	105	1250	449	721	5	11 195	0.151	0.418	93/97	96/97	53
rrnL, nad5	105	1765	363	1259	6	18 978	0.173	0.439	84/97	95/97	69
cox1, nad5	105	1977	436	1404	7	23 401	0.151	0.401	82/97	96/97	68
rrnL, cox1, nad5	105	2496	624	1692	4	26 904	0.158	0.415	84/97	91/97	71

Table 3. Bootstrap support of selected clades in individual analyses and the grouping of larval samples with these clades. (p) designates support values for Metriorrhynchini (17 taxa) when sample G was found outside the clade (see Results for details). Designation of samples is given in Table 1.

Genes	rrnL		cox1		nad5		rrnL cox1		rrnL nad5		cox1 nad5		rrnL cox1 nad5	
Lineages	%	Larvae	%	Larvae	%	Larvae	%	Larvae	%	Larvae	%	Larvae	%	Larvae
Dictyopterinae	71	N	–		79	N	93	N	–	–	–	–	–	–
Dictyopterini	76	N	–		79	N	93	N	91	N	56	N	69	N
Platerodrilini	–		–		87	JP	70	JP	86	JP	94	JP	95	JP
Macrolibnetis	n.a.		100	P	100	P	100	P	100	P	100	P	100	P
Platerodrilus	–		–		100	J	–		58	J	67	J	71	J
Metriorrhynchini	69	FGH	–		93	FHI	80	FGHI	99(p)	FHI	99(p)	FHI	76(p)	FHI
Leptotrichalus	100	H	100	H	100	H	100	H	100	H	100	H	100	H
Cautires/Xylob.	65	FG	–		78	F	72	FG	–		–		–	
Metriorrhynchini	69	FGH	–		93	FHI	80	FGHI	99(p)	FHI	99(p)	FHI	76(p)	FHI
Erotini	51	A	–		53	A	–		88	A	51	A	85	A
Platycis	53	A	–		98	A	–		99	A	96	A	97	A
Lyponia	92	BC	–		100	BC	96	BC	100	BC	100	BC	100	BC
Macrolycus	87	DE	55	D	98	DE	99	DE	100	DE	100	DE	100	DE
Platerodini	96	JKL	–		96	KL	78	JKL	–		72	JKL	95	JKL
Lycinip Calopt.	–	n.a.	–	n.a.	–	n.a.	–	n.a.	–	n.a.	–	n.a.	–	n.a.
Calochromini	74	n.a.	51	n.a.	98	n.a.	87	n.a.	98	n.a.	99	n.a.	99	n.a.
Dihammatini	62	n.a.	–	n.a.	86	n.a.	–	n.a.	98	n.a.	64	n.a.	90	n.a.
Ateliini	–	n.a.	–	n.a.	96	n.a.	–	n.a.	93	n.a.	96	n.a.	95	n.a.
No. clades > 50%	12/16		4/17		15/18		11/18		12/18		14/18		14/18	
Average support	56.0		18.0		75.7		53.8		61.7		66.3		70.7	
Identified larvae	11/14		3/12		13/14		14/15		11/15		14/15		14/15	
Lycidae-support	76		70		76		97		97		94		99	

Results

Molecular data

The aligned rrnL, cox1 and nad5 sequences for all taxa formed an alignment of 2496 homologous positions, of which 1692 were parsimony-informative. The fragments were variable in length and number of parsimony-informative characters. We found 288 informative characters in the rrnL alignment, 433 in cox1, and 971 in nad5 (Table 2).

Phylogenetic analyses

The partial analyses of individual genes returned one to seven most parsimonious trees (Table 2), but bootstrap proportions were generally low (Fig. 1; Table 3). The bootstrap values were the lowest in the partial analysis of cox1, and only four of the evaluated clades had a bootstrap proportion over 50% (Table 3). Higher bootstrap values and more clades with support over 50% were returned by the partial analysis of rrnL (12 of 16 evaluated clades). The analysis of nad5 recovered 15 of 18 evaluated clades. Combinations of two fragments in partial analyses returned more robust topologies in most cases (Tables 2 and 3). All taxa were combined in two-fragment datasets, including those for which only one fragment was available. As a consequence, these sets included various proportions of missing data. These affect the bootstrap analyses (Heath et al., 2008), and therefore the absolute proportions of individual clades cannot be compared. The complete dataset returned 14 of 18 evaluated clades and showed high average bootstrap support for most evaluated clades (Table 3). The combined dataset provided lower support for Metriorrhynchini, as sequences of rrnL were not available for several species of this lineage.

All 15 samples of lycid larvae can be identified to various levels on the basis of the parsimony analyses. We sequenced both sexes of the same species in two cases and we associated the female neotenic larva and adult male of respective species (Platerodrilus sp. from Kalimantan, sample J and Macrolibnetis depressus from Peninsular Malaysia, sample O, Figs 2–4). The sample of Platerodrilus sp. was grouped with adult male unambiguously by high similarity of all three fragments (uncorrected infraspecific pairwise distances: rrnL 0.00%; cox1 two variable bases of 731, 0.27%; nad5 0/1160 bp, 0.00%). Macrolibnetis depressus showed higher diversity of mitochondrial haplotypes (rrnL data not available; cox1 8/731 bp, 1.09%; nad5 30/1136 bp, 2.64%). Given that Macrolibnetis is a monotypic genus and that much higher uncorrected pairwise distances were found among species of Platerodrilus spp. (rrnL 8.64–20.04%; cox1 19.01–23.94; nad5 19.25–27.12%), we provisionally identify the larval and adult samples as conspecific.

Further larval samples were identified with variable support from topologies inferred from parsimony and bootstrap

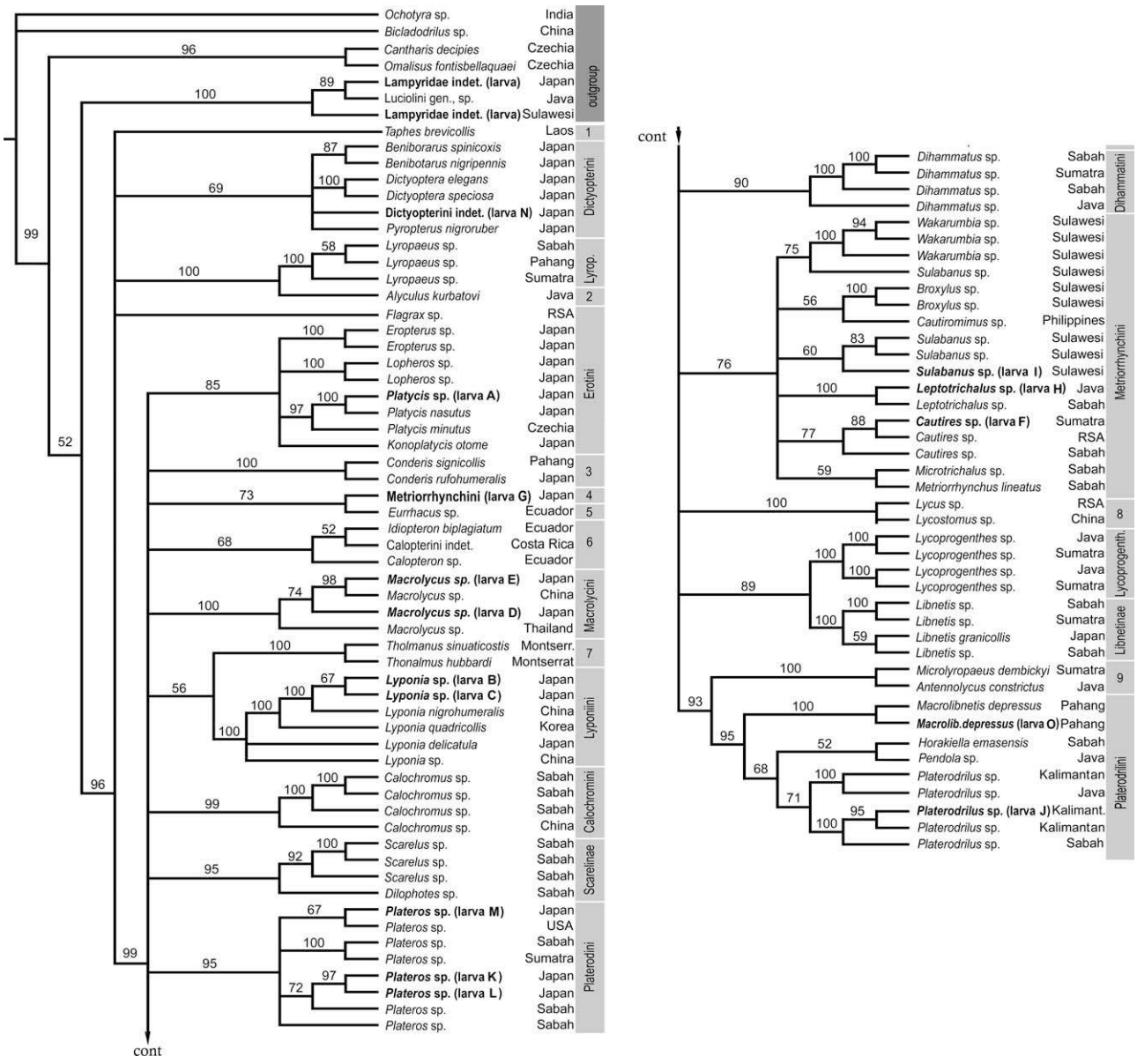
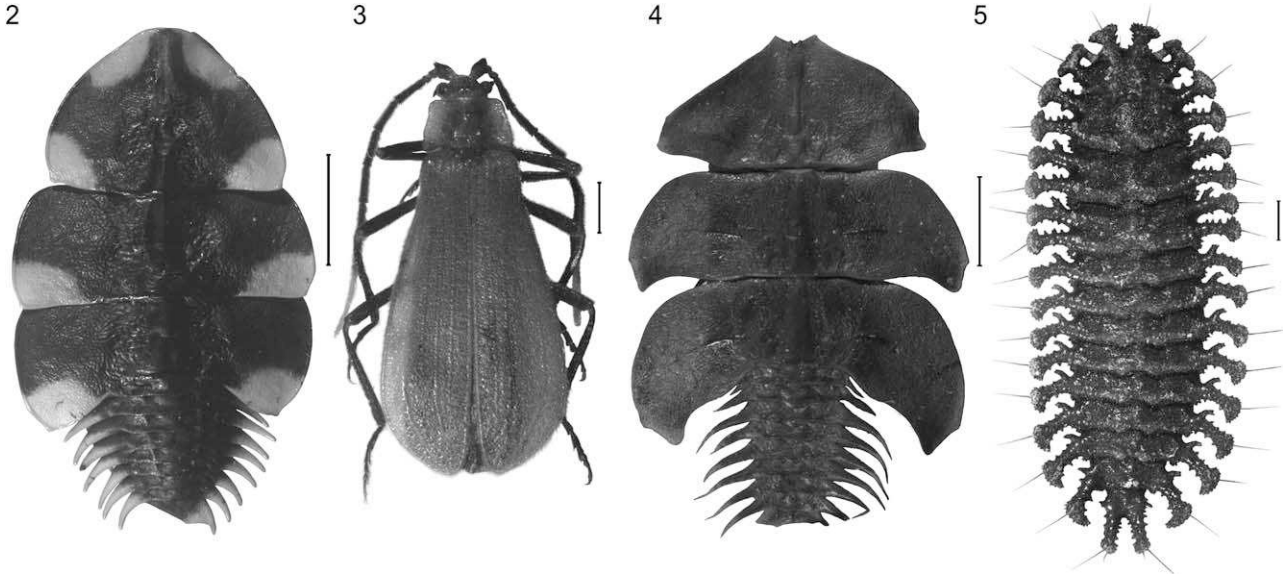


Fig. 1. Majority tree returned by bootstrap analysis of the combined dataset of rrnL, cox1 and nad5 genes and 107 taxa. Tribes: 1, Taphini; 2, Alyculini; 3, Conderini; 4, Metriorrhynchini (part); 5, Eurrhacini; 6, Calopterini; 7, Thonalmini; 8, Lycini; 9, Antennolycini.

analyses (Fig. 1). Only two samples of larvae, Macrolycus (samples D, E) and Leptotrichalus (sample H), were embedded by all analyses in the respective clades with a bootstrap proportion of 100% in all cases (Leptotrichalus) or with a bootstrap proportion of 55–100% (Macrolycus; Table 3). High support was also obtained for the membership of samples B and C in the clade of Lyponia (six of seven analyses, bootstrap proportions 92–100%). By contrast, three identified species and one larva (sample I) of Sulabanus never formed a clade. The larval sample grouped with at least some Sulabanus species in most analyses and never with other genera of the Metriorrhynchini. All lineages of Metriorrhynchini from Sulawesi were present in the library, and therefore we consider sample I as identified to the genus Sulabanus. Plateros, Platerodrilus and Platycis were returned as monophyletic clades by six-gene analyses of Lycidae (Bocak et al., 2008), and when some partial analyses reported here did not support the monophyly of these genera, the larval samples grouped with some species belonging to the respective genus and did not form a clade with any other taxon (Table 3).

Other samples can be identified only to the tribal level. The larva of a dictyopterine (sample N) was placed consistently as a member of the Dictyopterini clade, with support of 56–93% (Dictyopterini returned by all analyses except cox1, Table 3). Although two sympatrically occurring genera were present in the analyses, the larval sample did not form a clade with



Figs 2–5. General appearance. 2, Macrolibnetis depressus Pic, female (sample O); 3, ditto, male; 4, Platerodrilus sp. (conspecific with sample J); 5, Metriorrhynchini indet. from Madagascar. Scales: 5 mm (Figs 2, 4), 1 mm (Figs 3, 5).

either of them. Sample G of metriorrhynchine larva may be a species of either Xylobanus or Cautires, as suggested by analyses of rrnL and rrnL/cox1 datasets and the distribution of Metriorrhynchini in Japan, but is not supported by the dataset combining all fragments (Fig. 1). Xylobanus and Cautires are the only genera of Metriorrhynchini in the region, where sample G was collected, but no sequence of Xylobanus was given in the library set.

Support often decreased at deeper taxonomic levels (Table 3). The Dictyopterinae (Taphes þ Dictyopterini) were returned only twice. The Erotini were found by five of seven analyses, but the support was often very low. The erotine genus Platycis, represented by P. minutus, P. nasutus, and one larval sample (sample A), was also returned by five analyses, but with much higher bootstrap proportions, and the Platycis larva formed a clade with Platycis nasutus in all analyses with 100% bootstrap support. Although no larvae were available for Lycini þ Calopterini, Calochromini, Dihammatini and Ateliini (clades well supported by morphology and previous analyses, Bocak et al., 2008), we evaluated support for these clades from various partial datasets. Lycini þ Calopterini were never recovered as a clade, despite their close relationship and highly similar larvae (Bocak & Matsuda, 2003), and, similarly, Dihammatini and Ateliini were not supported by some analyses (Table 3).

Discussion

The library dataset from identified adults of Lycidae is extensive in comparison with data available for many beetle families. Ninety-two taxa in the library dataset represent over 2% of species described in Lycidae. However, the dataset includes only 41 genera of about 160, and the species often originate from zoogeographical regions different from those where larvae were collected. These conditions differ from situations in which numerous larvae and adults are collected simultaneously and DNA data are used in order to confirm or refute the conspecifity of adult and larval samples (e.g. Miller et al., 2005). Our principal aim was to identify larvae to the genus or tribe level using distant relatives for which data are currently available. We suggest that this is the more typical situation, given the current state of knowledge of beetle immature stages and the extent of DNA libraries.

We show here that molecular markers provide a powerful tool for the identification of immature stages, but that the task of identifying unknown samples is prone to failure when only distantly related taxa are represented in the databases. We found that single short fragments cannot reliably identify many samples, or produce only an ambiguous indication of relationships (Table 3). Therefore, combined analyses of several DNA fragments should be employed to increase the reliability of identification, especially when closely related species and/or genera are unrepresented in the library. Multiple fragments can also lower the chance of false identification, owing to the amplification of nuclear mitochondrial pseudogenes (Song et al., 2008). We observed apparent differences in the performance of individual markers. The widely used cox1 mtDNA had the poorest performance in our dataset (Table 3). We sequenced here a part of cox1 different from that used in DNA barcode projects (Hebert et al., 2003a, b), but we assume a similar performance across the whole of cox1. Roe & Sperling (2007) studied patterns of nucleotide divergence within cox1–cox2 and did not identify any optimally informative part of these genes. Similar findings were reported for a fragment used for the identification of chironomid midges (Ekrem et al., 2007).

Two cases in our data provided an opportunity to associate adult males and larvae of neotenic females. Even short and highly variable gene fragments can identify reliably members of a population represented by several specimens, as the intraspecific variation is regularly much lower than the interspecific (see Platerodrilus sp. in the Results section or Monaghan et al., 2005; Vogler & Monaghan, 2006; Ahrens et al., 2007). Such data can solve the long-standing problem of unassociated females and males of neotenic taxa in Lycidae, such as Lyropaeus, Scarelus, Platerodrilus and Macrolibnetis (Bocak et al., 2008). As both species were represented in our analyses by only a pair of specimens we cannot discuss the limits of the intraspecific variability, and the identification is based on the shared haplotype in the case of Platerodrilus sp. and on the high similarity of haplotypes of the monotypic genus Macrolibnetis. Extensive sequencing is needed to study the delimitation of species in lineages where females are larviform, and strong genetic structure can be expected in populations with extremely low dispersal ability (Bocak et al., 2008).

The barcoding procedures were originally proposed to cope with decline in the number of taxonomists, the nonexistence of identification keys, and the poor taxonomic framework for many important groups of animals (Hebert et al., 2003a, b). These claims were challenged, with many authors pointing out that DNA taxonomy cannot replace the traditional morphological approach (e.g. Lipscomb et al., 2003; Will et al., 2005; Wheeler, 2008). The above demonstrated ambiguity in identifications calls for building extensive DNA libraries that include both slowly and rapidly evolving DNA fragments. The effectiveness of identification depends also on the extent of the libraries for comparison. Therefore, a proportional sample of whole beetle diversity (i.e., all major lineages) from all zoogeographical regions is essential for building sequence libraries. These databases could be universally used in taxonomy for the identification of unknown samples. Using molecular techniques, it is possible to build combined morphological and molecular datasets and make available new information on morphological evolution and natural history that may be of interest for evolutionary studies. Single fragments cannot provide robust results unless the diversity of a lineage is densely sampled and species are represented by data from distant populations. Such a goal is unattainable for the extremely diverse tropical faunas in the near future.

Taxonomy

Subfamily Dictyopterinae

Tribe Dictyopterini.

Dictyopterini gen. sp. (Figs 7–9)

Material examined. One specimen, JAPAN: Osaka Pref., Iwawakiyama, Kagata, 10–23 Apr 2003 (L. Bocak) (ZL2013); 1 specimen, ditto, Amami, 10–16 Nov 2002, 500 m (L. Bocak).

Diagnosis. The larva is characterized by a simple pygidium and the absence of all processes. The functional metathoracic spiracles and the long urogomphi are known in related Lycoprogenthes.

Description. Early instar larva. Body slightly flattened, widest in basal part of abdomen, sclerites light brown, moderately sclerotized, membranes restricted to intersegmental regions (Fig. 7). Head transverse, lateral part of epicranium membranous. Eyes absent. Mandibles slender, long, slightly curved. Mala long, slender. Maxillary palpi slender, as long as palpifer. Apical palpomere very slender, parallel-sided, 1.5 longer than preceding. Labial palpi minute, slender (Fig. 9). Tergites formed by one sclerite, with longitudinal keel at midline (Fig. 7). Prothoracic tergite longest, without any process, roughly punctured. Tergites T2 and T3 transverse. Prosternum small, subtriangular, precoxale T1 free, triangular. Sterna T2 and T3 small, transverse. Spiracular plate T2 located ventrolaterally. Abdominal tergites transverse, with straight frontal and posterior margins, without processes. Segment A9 small, slender, without urogomphi (Fig. 8).

Measurements. BL 3.45 mm, PL 0.63 mm, PW 0.87 mm.

Remark. The larva was consistently found as a member of Dictyopterini, but we are not able to identify it further than to the tribe level. Only two dictyopterine genera, Dictyoptera and Benibotarus, are common in central Honshu (Nakane, 1969), where we collected the analysed specimen, and each was represented by two species in the library dataset. Nevertheless, there is no indication of which genus this larva belongs to. The morphology is similar to that of larva of D. aurora from Europe (see diagnosis below); therefore, we base the association with Dictyopterini on both morphological similarity and molecular data.

Functional metathoracic spiracles are present only in a few lycid genera. In addition to Lycoprogenthes (Dictyopterinae: Lycoprogenthini) and the related Lyropaeinae (Platerodrilus, Duliticola) they are known also in Lyponiini, which are classified in Lycinae (Bocak & Bocakova, 2008). Dictyopterini, which are closely related to Lycoprogenthes, have functional spiracles only in the mesothorax. Although larval metathoracic spiracles are not known in other beetle families, they define no monophyletic lineage in Lycidae and may have evolved several times.

Dictyoptera aurora (Herbst, 1784)

Material examined. Two specimens, SCOTLAND: Aviemore, Inverness-shire, G. C. G. (G. C. Champion), B. M. 1964–540 (deposited in the Natural History Museum, London).

Diagnosis. The larva of D. aurora is similar to the unidentified dictyopterine larva from Honshu, but differs in the smooth surface of all tergites, very fine longitudinal midline, and wide tergite A9.

Measurements. BL 15.1 mm, PL 1.33 mm, PW 2.58 mm.

Remark. There are available two larvae of D. aurora, which were found in the collection of the Natural History Museum in London. They are similar to the larva that was identified as a member of Dictyopterini using molecular markers. Given that only two species of Dictyopterini occur in Great Britain and that the larva of Pyropterus nigroruber De Geer, 1774 is known (Bocak & Matsuda, 2003), the identification of the larva as D. aurora is reliable.

Although Pyropterus and Dictyoptera are closely related, their larvae are substantially different. The tergites are continuous in Dictyoptera, and only a shiny midline lies in the place of the division of tergites in two sclerites in Pyropterus. Continuous tergites resemble those of Lycoprogenthes (cited as Pseudosynchonnus by Bocak & Matsuda, 2003). These two genera differ in the presence or absence of urogomphi. The observed high morphological disparity agrees with the presumed basal position of Dictyopterinae in the lycid phylogeny and with the ancient origin of these lineages (Bocak et al., 2008).

Subfamily Lyropaeinae

Tribe Platerodrilini.

Platerodrilus sp. (Figs 4, 6)

Material examined. Eight female larvae, one male adult. INDONESIA: Kalimantan, Muara Teweh (000588, 589).

Diagnosis. The larvae share all unique characters of Platerodrilus and Duliticola as described by Bocak & Matsuda (2003). The sequenced species has characteristic narrow transverse ridges in the thoracic tergites (Fig. 4).

Description. Mature female larva. Body very flat, light brown coloured. Frontal margin of T1 with four small tubercles, lateral margins emarginate, those of T2 and T3 projected, similarly emarginate at apex as T1. T2 and T3 with narrow, transverse shining ridges beside midline. Lateral processes of A1–A8 very long, slender; A9 wide, transverse (Fig. 4). Male larva. Unknown. Pupa. Neotenic females do not pupate and remain larviform after the last ecdysis (Wong, 1996).

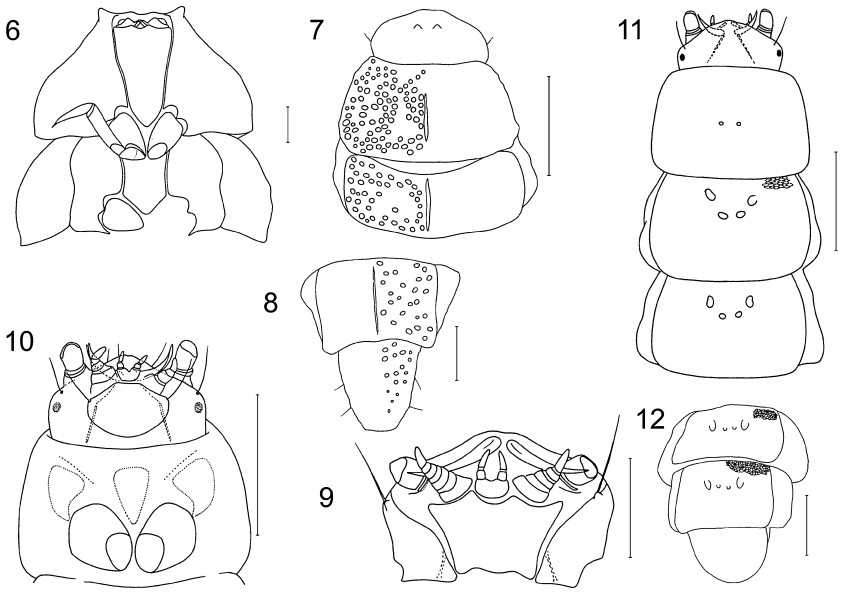
Measurements. BL 26.7 mm, PL 6.8 mm, PW 14.9 mm.

Remark. The study of neoteny in Lycidae is complicated by the fact that we know most described species only in the male semaphoront, and, although females are represented in collections, we are not able to associate them with conspecific males. Furthermore, only large-bodied female larvae are known, and the male larva has not yet been described (Bocak & Bocakova, 2008). The accumulation of DNA sequences is necessary for building a stable classification of the lineage to enable identification of all semaphoronts and set a basis for evolutionary studies.

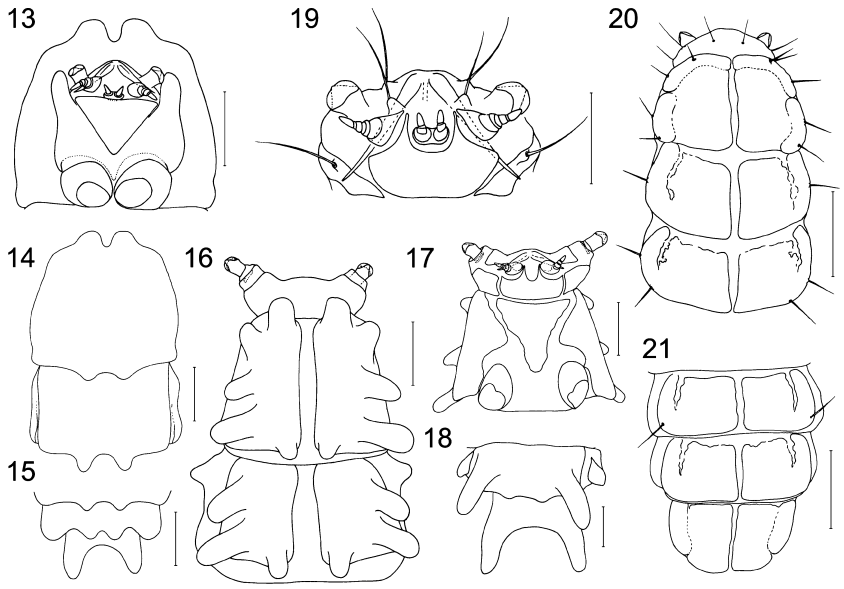
Numerous species of Platerodrilus occur in Southeast Asia (Wong, 1998) and they can be recognized by differences in the shape of thoracic and abdominal tergites, the presence of tubercles in the surface of thoracic tergites, and colouration. The sequenced species does not belong to any species that has been described based on female larva in Lycidae (Wong, 1998). Further species were described as males in the family Drilidae by Maurice Pic (Wittmer, 1944) without reference to females. The classification is chaotic and needs thorough revision.

Macrolibnetis depressus Pic, 1938. (Figs 2, 3)

Material examined. One female larva. MALAYSIA: Cameron Highlands, Tanah Rata env. (000515); one male adult, same locality data (000L21).



Figs 6–12. 6, Platerodrilus sp. (sample J), pro- and mesothorax, ventral view; 7–9 Dictyopterini gen., sp. (sample N); 7, head, pro- and mesothorax, dorsal view; 8, terminal abdominal segments, dorsal view; 9, head, ventral view; 10–12 Platycis sp. (sample A); 10, head and prothorax, ventral view; 11, head and thorax, dorsal; 12, terminal abdominal segments, dorsal view. Scales: 0.5 mm.



Figs 13–21. 13–15 Cautires sp. (sample F); 13, head and prothorax, ventral view; 14, pro- and mesothorax, dorsal view; 15, terminal abdominal segments, dorsal view. 16–18 Leptotrichalus sp. (sample H); 16, head, pro-and mesothorax, dorsal view; 17, head, prothorax, dorsal view; 16, head and prothorax, ventral view; 18, terminal abdominal segments, dorsal view. 19–21 Sulabanus sp. (sample I); 19, head, ventral view; 20, head and prothorax, ventral view; 21, terminal abdominal segments, dorsal view. Scales: 0.5 mm.

Diagnosis. Female larvae of M. depressus resemble the larvae of Platerodrilus and Duliticola in diagnostic characters given by Bocak & Matsuda (2003). The female larva is easily recognizable by the body shape and colouration (Fig. 2).

Description. Female mature larva. Body flat, dark brown to black coloured, yellow at anterior and posterolateral margins of segment T1, posterolateral margins of T2 and T3 and lateral processes of all abdominal segments (Fig. 2). Dorsal sclerites without ridges or prominent shining tubercles, roughly structured along midline (Fig. 2). Male larva. Unknown. Pupa. Absence of pupal stage is supposed (Wong, 1996).

Measurements. BL 22.1 mm, PL 7.3 mm, PW 10.8 mm.

Remark. The molecular markers enabled the association of the female larva (Fig. 2) with Macrolibnetis depressus, which was described based solely on the male semaphoront (Fig. 3). Previously, the larva was classified in Platerodrilus (Wong, 1998), despite the different shape of the body (Figs 2, 4). The differences in external morphology of the female larvae of Platerodrilus and Macrolibnetis support their distant position inferred from molecular markers (Bocak et al., 2008).

Subfamily Lycinae

Tribe Metriorrhynchini

Leptotrichalus sp. (Figs 16–18)

Material examined. One specimen, INDONESIA: E Java, E slope Gn. Penanggungan, 6–9 May 2001, 1000 m (ZL2002).

Diagnosis. The larva of Leptotrichalus has the reduced mala, which enables its classification to Metriorrhynchini (Bocak & Matsuda, 2003), and it differs from the related genera in the presence of numerous fixed processes in the thoracic and abdominal tergites (Figs 16–18).

Description. Mature larva. Body moderately flat, widest in basal part of abdomen, sclerites small, connected by extensive membranes, dark brown to black. Head transverse, with produced frontolateral part forming antennal tubercles (Fig. 17). Lateral part of epicranium membranous. Eyes small. Mandibles slender, long, slightly curved. Mala vestigial, small membranous tubercle with apical seta present at base of palpifer. Maxillary palpi slender, slightly longer than palpifer. Labial palpi minute. Tergites T1–T3 divided into two small tergites (Fig 16), tergites A1–A9 undivided. Prothoracic tergites largest, each with four processes. Tergites T2 and T3 with three processes. Prosternum large, subtriangular (Fig. 17). Sterna T2 and T3 small, weakly sclerotized. Spiracular plate T2 located on ventral side of body, small, simple, with functional spiracles. Abdominal tergites A1–A8 transverse, with posterolateral fixed processes. Upper pleurites with spiracles at posterodorsal margin and similar process behind pleurite as tergites (Fig. 18). Segment A9 with stout, fixed urogomphi (Fig. 18).

Measurements. BL 9.3 mm, PL 1.4 mm, PW 1.8 mm.

Remark. The larva was mentioned as unidentified Metriorrhynchini by Bocak & Matsuda (2003). Only molecular markers enabled the classification of the larva to Leptotrichalus. It belongs to Trichalina, a group of metriorrhynchines with shortened elytral primary costa 1. It is a very unusual larva, which at present cannot be compared with any closely related lineage.

Sulabanus sp. (Figs 19–21)

Material examined. INDONESIA: S Sulawesi, Malino, Gn. Lompobatang, 1800 m, July 2001, 199.53.31E, 5.17.50S (ZL2010).

Diagnosis. The thoracic tergites of Sulabanus are divided longitudinally in two sclerites as in most metriorrhynchine genera, and the vestigial mala enables classification in Metriorrhynchini. Unlike the case for other known genera, the sclerites of Sulabanus are simple and no processes are present at margins or attached to membranes. The pronotum of Sulabanus is characteristic in a depression along frontal and lateral margins (Fig. 20).

Description. Larva, instar 2 or 3. Body widest in basal part of abdomen, sclerites light brown, very lightly sclerotized in depression along margins. Membranes extensive, yellowish white. Lateral part of epicranium membranous. Eyes small. Mandibles slender, long, slightly curved. Mala vestigial, detached from palpifer, lightly sclerotized, triangular, with long seta at apex (Fig. 19). Maxillary palpi slender, labial palpi minute. Tergites T1–T3 and A1–A9 divided into two small tergites (Figs 20, 21). Prothoracic tergites large, without processes. Prosternum pale, indistinct, ventral part of body membranous. Abdominal tergites A1–A8 transverse, with similar lightly sclerotized depression along lateral margins (Fig. 21). Segment A9 rounded at apex, without urogomphi.

Measurements. BL 6.2 mm, PL 0.59 mm, PW 0.87 mm.

Remark. Only one specimen of a lycid larva was collected in the Gunung Lompobatang area in 2001. Several subfamilies of Lycidae occur in the region, and no larvae were previously described from Sulawesi. The identification is based solely on the sequenced DNA fragments. As Metriorrhynchini are the most common lycid group in Sulawesi, we compared the unknown larva with Wakarumbia Bocak, 1999, Broxylus Waterhouse, 1879, Cautiromimus Kleine, 1926, Metriorrhynchus Gemminger & Harold, 1869, and Sulabanus Dvorak & Bocak, 2007. The larva was consistently a member of the metriorrhynchine clade and grouped in the trees inferred from individual DNA fragments and their combinations with some species of Sulabanus represented in the dataset.

The morphology of the mala, which is typical for Metriorrhynchini, supports the identification based on DNA markers. However, the general appearance of the larva is unlike that of any known metriorrhynchine larva (Bocak & Matsuda, 2003), and the sclerotized processes, which were earlier considered as typical for Metriorrhynchini (Bocak & Matsuda, 2003), are absent in Sulabanus. Metriorrhynchini have very variable morphology of tergites (Bocak & Matsuda, 2003), but we have not found any indication that these morphological differences indicate deeper relationships. Movable processes are present in some Xylobanus, Cautires, Metanoeus and Metriorrhynchus, and fixed processes in some Cautires and Porrostoma (Bocak & Matsuda, 2003). A peculiar metriorrhynchine larva with fixed processes was collected by M. Ivie in Madagascar (Fig. 5). We found that the related genera Porrostoma, Metriorrhynchus and Sulabanus differ substantially in larval stages. The differences in external larval morphology may be a result of adaptation to a whole spectrum of conditions from the very humid environment in the mountains of Sulawesi to the semiarid conditions in Australia.

Cautires sp. (Figs 13–15)

Material examined. Cautires sp., INDONESIA: Sumatra, Gn. Talamau, Simpangempat (ZL2009).

Remark. The larva resembles the previously described larvae of C. pulcher Kleine, 1926 and C. asper Kleine, 1928 (Bocak & Matsuda, 2003). These species share the undivided tergites (Figs 14–15) and differ in general appearance from C. yuasai Nakane, 1969. As few larvae are known and the monophyly of Cautires has not been tested by morphology of adults, we cannot say if these differences indicate relationships.

Metriorrhinchini gen. sp.

Material examined. Metriorrhynchini gen. sp. JAPAN: Nara Pref., Shakagateyama, Asahi Riv. (L. Bocak) (ZL2015).

Remark. We were not able to identify this specimen to genus level, and it may belong either to Cautires or to Xylobanus, the only metriorrhynchine genera that occur in the region and formed a clade in the previous analysis (Bocak, 2002). The larva resembles those of Cautires yuasai as described by Bocak & Matsuda (2003).

Tribe Erotini.

Platycis sp. (Figs 10–12)

Material examined. JAPAN: Shiga Pref., Mikunidake (L. Bocak) (ZL2008).

Remark. A larva of Platycis sculptilis (Say) from the U.S.A. was described by McCabe & Johnson (1979). The morphology of the specimen is similar to that of P. sculptilis.

Tribe Lyponiini.

Lyponia sp.

Material examined. Lyponia sp., two specimens, JAPAN: Nagano Pref., Mt. Aboyama (L. Bocak) (ZL2014, 2016).

Tribe Macrolycini.

Macrolycus spp.

Material examined. Macrolycus sp. A, one specimen, JAPAN: Ishikawa Pref., Shiramine, Mt. Hakosan (L. Bocak) (ZL2005). Macrolycus sp. B, one specimen, JAPAN: Ishikawa Pref., Shiramine, Mt. Hakosan (L. Bocak) (ZL2017).

Tribe Platerodini

Plateros spp.

Material examined. Plateros sp. A, two specimens, JAPAN: Osaka Pref., Iwawakiyama, Amami (L. Bocak) (ZL2006, ZL2012). Plateros sp. B, JAPAN: Shiga Pref., Mikunidake (L. Bocak) (ZL2018).

Remark. All here identified Lyponia (one species), Macrolycus (two species) and Plateros (two species) belong to genera with known larvae (Hayashi, 1954; Hayashi & Takenaka, 1960; Bocak & Matsuda, 2003). The morphology of studied specimens is similar to those of previously described species. Their generic identification is unambiguous, but although some Japanese species were present in the dataset for comparison, we are unable to identify any of them to the species level.

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Materials and Methods
Figs. S1 to S8
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A Comprehensive Phylogeny of Beetles Reveals the Evolutionary Origins of a Superradiation

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Beetles represent almost one-fourth of all described species, and knowledge about their relationships and evolution adds to our understanding of biodiversity. We performed a comprehensive phylogenetic analysis of Coleoptera inferred from three genes and nearly 1900 species, representing more than 80% of the world's recognized beetle families. We defined basal relationships in the Polyphaga supergroup, which contains over 300,000 species, and established five families as the earliest branching lineages. By dating the phylogeny, we found that the success of beetles is explained neither by exceptional net diversification rates nor by a predominant role of herbivory and the Cretaceous rise of angiosperms. Instead, the pre-Cretaceous origin of more than 100 present-day lineages suggests that beetle species richness is due to high survival of lineages and sustained diversification in a variety of niches.

The extraordinary diversity of beetles has long fascinated evolutionary biologists (*1*). The strongly sclerotized front wings defining the order Coleoptera (the beetles), which provide protection while retaining the ability of powered flight with the membranous hindwings, may be an evolutionary novelty that promoted extensive diversification (*2*). Beetles appeared around 285 million years ago (Ma) (*2*, *3*), followed by radiations of wood-boring (suborder Archostemata), predacious (Adephaga), and fungivorous (Polyphaga) lineages (*4*) present in the fossil record from the middle Triassic on (*2*, *3*). Their species richness is associated with extreme morphological, ecological, and behavioral diversity (*4*), and diversification of the most species-rich extant lineages may have been driven by co-radiations with angiosperms (*5*) and/or mammals (*6*) and/or geological and climatic change (*7*) occurring since the Cretaceous (145 to 65 Ma).

Studies of phylogenetic relationships within the Coleoptera resulted in a preliminary consensus on the classification, defining 4 suborders, 17 superfamilies, and 168 families (*8*–*10*). However, formal phylogenetic analyses of morphological characters (*11*, *12*) and more recently molecular data (*5*, *13*, *14*) have been limited to subgroups at the family or superfamily level. Because of the sheer size of the group and the complexity of morphological character systems, these analyses have not been applied to the entire order.

We compiled a three gene data matrix providing a complete taxonomic representation for all suborders, series and superfamilies; >80% of recognized families; and >60% of subfamilies (*9*, *10*), which together contain >95% of described beetle species. Sequences for the small subunit ribosomal RNA (18 *S* rRNA) were obtained for 1880 species from de novo sequencing and existing databases. Mitochondrial 16S rRNA (*rrnL*) and cytochrome oxidase subunit I (*cox1*) sequences were added for nearly half of these taxa (table S1) to create a data matrix of rapid, medium, and slowly evolving sequences. Phylogenetic analysis of the combined matrix was performed with a fragment-extension procedure for global sequence alignment followed by tree searches with fast parsimony algorithms (*15*). We tested for long-branch attraction, i.e., the spurious pairing of rapidly evolving lineages, by removing taxa terminal to long branches and assessing trees with a retention index (RI) measure of fit to the traditional classification (table S2) (*15*). The resulting parsimony tree largely agrees with the existing classification at the family and superfamily levels [on average, 95.7% of terminals assigned to a family were recovered as monophyla (table S2)], although our taxon sampling was not comprehensive in some families. Model-based Bayesian methods were applied to a 340-taxon representative subset at the subfamily level.

The trees (Figs. 1 and 2) were rooted with the neuropterid orders, the presumed sister to the Coleoptera (*16*), and recovered the major subdivisions of Adephaga [37,000 known species; posterior probability (*pp*) = 1.0] and Polyphaga (>300,000 species; *pp* = 1.0) as sisters to the Myxophaga (94 species) plus Archostemata (40 species) (*8*). The Adephaga was divided into two clades containing an aquatic (Hydradephaga; diving beetles and whirligig beetles; *pp* = 0.90) and a terrestrial (Geadephaga; ground beetles and tiger beetles; *pp* = 1.0) lineage, supporting a single terrestrial-to-aquatic transition in this suborder (*13*).

In the strongly supported suborder Polyphaga, five families occupied the basal nodes (Figs. 1 and 2) (*pp* = 1.0). These families include the Decliniidae; the Scirtidae, with aquatic larvae; the Derodontidae, an ecologically diverse family from global temperate zones; and the Eucinetidae and the Clambidae. These ancestral five families were previously considered basal Elateriformia (superfamily Scirtoidea), except for Derodontidae,

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which has been associated with Bostrichiformia (*9*, *10*). All five families exhibit archaic morphological features shared only with Archostemata and Adephaga (*8*, *17*). Their basal position was stable (always *pp* = 1.0) (table S3) when trees were rooted with the neuropterid orders or only with Myxophaga or Adephaga as outgroups.

All superfamilies of Polyphaga were previously grouped into five series (*4*, *9*), of which only the Scarabaeiformia (*pp* = 1.0) and the Cucujiformia (*pp* = 1.0) were strongly supported as monophyletic in this study. Staphyliniformia comprised a paraphyletic basal grade, and both Bostrichiformia and Elateriformia were polyphyletic. Relationships among the five series were poorly supported or unresolved in the consensus tree (fig. S1). Nosodendridae, usually included in Bostrichiformia near Derodontidae (*4*, *9*) but recently associated with Scirtoidea on the basis of thoracic characters (*18*), grouped instead with the nonscirtoid Elateriformia, albeit with low support (fig. S1) (*pp* = 0.59).

Within Elateriformia, the superfamilies Buprestoidea (jewel beetles; *pp* = 1.0), Dascilloidea (*pp* = 1.0), and Elateroidea (click beetles and allies; *pp* = 0.72) were supported. Our data showed that Byrrhoidea, sensu Lawrence and Newton (*9*), is paraphyletic, supporting the division of this clade (*8*) into Byrrhoidea (Byrrhidae, moss beetles; *pp* = 1.0) and Dryopoidea (riffle beetles and water pennies). The Cantharoidea (soldier beetles, fireflies, etc.) fell inside the Elateroidea, and our tree supported that bioluminescence arose repeatedly in beetles, in agreement with structural differences in luciferases (*19*). Scarabaeiformia (chafers, stag beetles, and dung beetles; *pp* = 1.0) is thought to be related to the Staphyliniformia (*4*, *14*, *20*). In our trees, it was part of an unresolved paraphyletic Staphyliniformia including the superfamilies Histeroidea (clown beetles; *pp* = 1.0); Hydrophiloidea (*pp* = 1.0), a clade of both Leiodidae and Agyrtidae (*pp* = 1.0); the Staphylinidae (rove beetles including Silphidae and carrion beetles; *pp* = 0.86); and the Hydraenidae as sister (*pp* = 0.74) to the Ptiliidae (featherwing beetles).

The hyperdiverse Cucujiformia, representing more than half of all beetles and 90 families, was strongly supported as monophyletic (Figs. 1 and 2; *pp* = 1.0). Among the seven established superfamilies, the Lymexyloidea (ship-timber beetles) was found near the base of the Tenebrionoidea (30 families; *pp* = 0.76). The Cleroidea (checkered beetles and allies) was monophyletic (*pp* = 0.70) only when including the Biphyllidae plus Byturidae (*pp* = 1.0). The latter two were formerly classified as Cucujoidea, but their association with Cleroidea is supported by genitalic characters (*11*). The Cucujoidea, comprising 34 families, was polyphyletic, but the Cerylonid series (Figs. 1 and 2 and fig. S3) (*pp* = 1.0) consisting of eight families (*21*) was monophyletic. Apart from the Sphindidae (*pp* = 1.0), the remaining cucujoid families formed a monophyletic clade (*pp* = 0.72) together with the species-rich Curculionoidea (weevils and bark beetles; *pp* = 0.73) and Chrysomeloidea (leaf beetles and longhorns).

Once the relationships among coleopteran families and superfamilies were established, we investigated the origins of beetle diversity. Diversification may be driven by feeding strategy, and we tested the hypothesis that feeding on plants (herbivory), and specifically flowering plants (angiosperms), explains the diversity of beetles (*5*). Predominantly herbivorous clades tend to contain

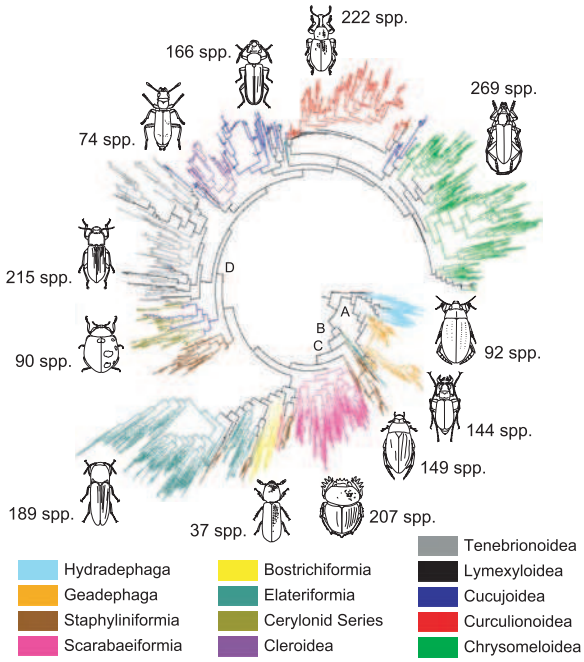

Fig. 1. One of 27 most parsimonious trees obtained from the aligned 1880-taxon matrix. The number of representatives from each major lineage analyzed (in colors) is given. Major clades are denoted by letters: A, Adephaga; B, Polyphaga; C, Polyphaga minus the ancestral five families; and D, Cucujiformia. For full details of the tree, see fig. S4.

Table 1. Comparisons of species richness between clades feeding on living plants and their sister clades with alternative feeding strategies. Restricting the comparisons to those feeding on angiosperms removes contrast 4 and adds two contrasts of angiosperm- versus gymnosperm-feeding lineages within Curculionoidea and two within Chrysomeloidea [table S4; see also (*5*)]. Plant-feeding clades include taxa feeding mainly on rotting vegetation in contrast 7 or in recently dead wood in contrast 8, but probably >70% of species in both clades are herbivorous. Excluding the last two contrasts increases the probability under a Wilcoxon test to $P = 0.28$.

	Plant-feeding	Diet	No. of species	Non–plant-feeding	Diet	No. of species
1	Byturidae	Fruits, flowers	16	Biphyllidae	Fungivorous	195
2	Languriinae	Stem borers	800	Xenoscelinae	Fungivorous, decaying vegetation	100
3	Chrysomeloidea	Herbivorous xylophagous	53,442	Nitidulidae plus Erotylid plus Cucujid series	Mostly fungivorous	7743
4	Curculionoidea	Herbivorous xylophagous	59,340	Brontinae plus Silvaninae plus Priasilphinae	Fungivorous	480
5	Epilachninae	Herbivorous	1051	Coccidulinae plus Chilocorinae plus Scymninae	Predacious	3900
6	Dascillinae	Roots	80	Rhipiceridae	Ectoparasitic on cicadas	57
7	Melolonthinae plus Orphninae plus Rutelinae plus Dynastinae	Herbivorous (and saprophagous)	16,329	Cetoniinae	Saprophagous (detritus)	4121
8	Buprestidae	Xylophagous, herbivorous, roots, leaf miners	14,000	Dryopoidea	Saprophagous, algivorous	3242

more species than nonherbivorous sister clades, but this difference was not significant [Table 1; one-tailed Wilcoxon test on contrasts in log (no. of species), $P = 0.13$] even when we distinguished between angiosperm and gymnosperm feeders ($P = 0.06$) (table S4). Similarly, of 21 significant shifts in diversification rate inferred with a robust equal rates null model (*22*, *23*), only two characterize transitions between angiosperm and gymno-

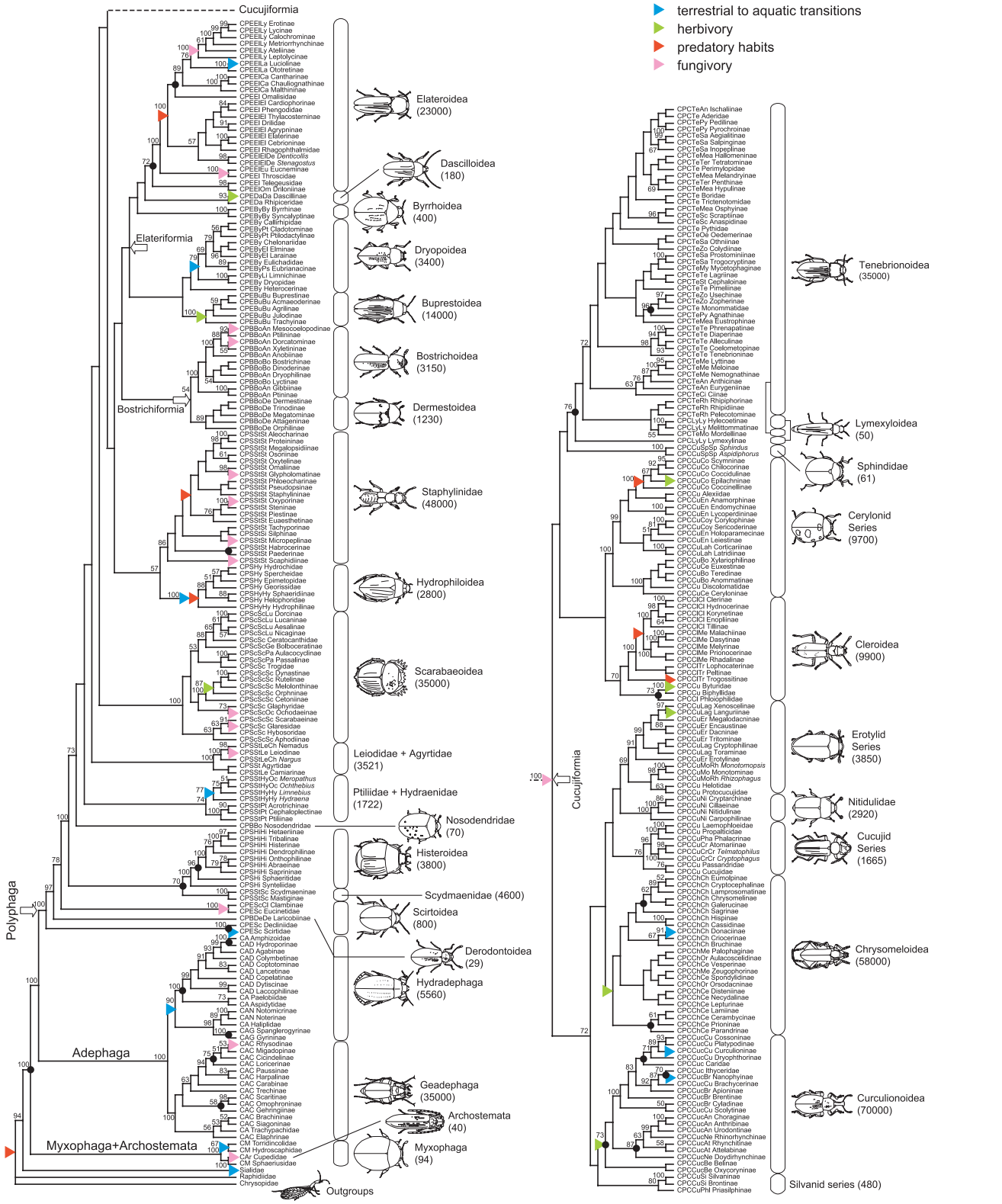

Fig. 2. The phylogeny of Coleoptera at the subfamily level. The tree was selected from the 340-taxon Bayesian analysis based on maximum congruence with the majority-rule consensus (fig. S1). Posterior probability clade support values indicated at nodes >0.5. Approximate known species numbers in terminal taxa are given in parentheses. Black circles mark significant shifts in diversification rate of sister clades (table S5). Colored triangles mark character transitions in lifestyles inferred by parsimony optimization (see figs. S2 and S3 for details).

sperm feeders, whereas the remainder showed no association with transitions to feeding on angiosperms or seed plants (table S5). A significant increase in diversification rate was inferred near the base of the Polyphaga whether herbivorous taxa were included or excluded from the analyses (table S5). Herbivory has played a role in the diversification of some beetle lineages, but the trait per se does not explain why beetles are so diverse.

Fast diversification rates also do not explain beetle diversity. Dating the tree with fossil calibration and penalized likelihood rate-smoothing (Fig. 3 and table S6) (*15*), we estimated net diversification rates across terminal taxa of 0.048 to 0.068 Myear^{-1} (table S7), slightly lower than comparable measures for the angiosperms (0.077 Myear^{-1}) (*24*). However, more than 100 modern beetle lineages were present at the first appearance of crown-group angiosperms dated to <140 Ma on the basis of pollen records (*25*), and less than one-third of extant beetle species are associated with angiosperms (table S8 and fig. S3). Therefore, the extreme diversity of beetles reflects the Jurassic origin of numerous modern lineages, high lineage survival, and the diversification into a wide range of niches, including the utilization of all parts of plants. These switches into new niches occur repeatedly as, for example, the multiple shifts from terrestrial to aquatic habits in the evolutionary history of beetles, which occurred at least 10 times (Fig. 2 and fig. S2).

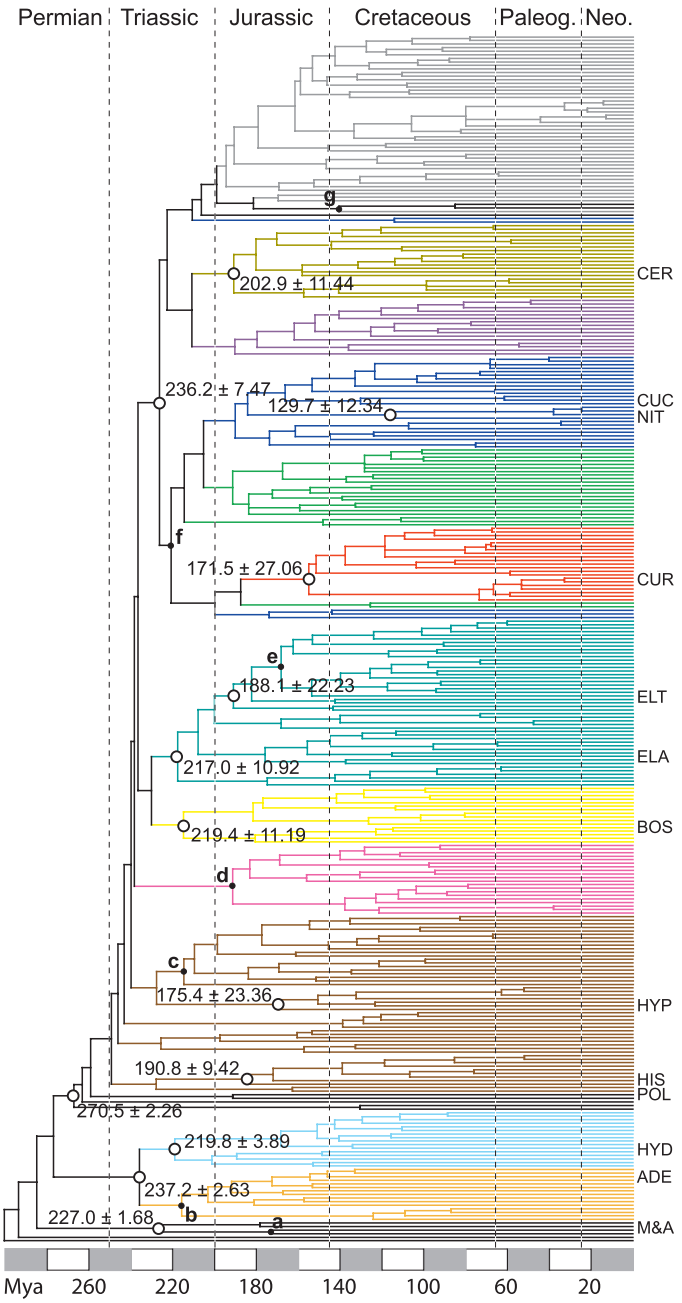

Fig. 3. A dated 340-taxon "all-compatible" consensus tree of Coleoptera from Bayesian analysis was dated with penalized likelihood placing the origin of Coleoptera at 285 Ma (*15*). Estimated number of lineages present at 200 Ma, 36; at 140 Ma, 145; and at 65 Ma, 301 (see also table S7). Colors correspond to the same groups as in Fig. 1. Numbers refer to average ages and 95% confidence intervals (*15*) of selected clades (open circles): CER, Cerylonid series; CUC, Cucujiformia; NIT, Nitidulidae; CUR, Curculionoidea; ELT, Elateroidea; ELA, Elateriformia; BOS, Bostrichiformia; HYP, Hydrophiloidea; HIS, Histeroidea; POL, Polyphaga; HYD, Hydradephaga; ADE, Adephaga; and M&A, Myxophaga and Archostemata. Seven fossil calibration points (table S6) were used to cross-validate rate-smoothing parameters (optimal value = 100) (*15*): point a, Cupedidae; b, *Sogdodromeus* (Geadephaga); c, Staphylinidae; d, *Holcorobeus* (Scarabaeoidea); e, *Elaterophanes* (Elateridae); f, *Cerambycomima* (Chrysomeloidea); and g, *Praemordella* (Mordellidae).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/318/5858/1913/DC1
Materials and Methods
Figs. S1 to S5
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Alignment files S1 and S2

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