Zoological Journal of the Linnean Society, 2019, **187**, 1259–1277. With 6 figures.

One less mystery in Coleoptera systematics: the position of Cydistinae (Elateriformia *incertae sedis*) resolved by multigene phylogenetic analysis

ROBIN KUNDRATA[1,*], STEPHAN M. BLANK[2], ALEXANDER S. PROSVIROV[3], ELISKA SORMOVA[1], MATTHEW L. GIMMEL[4], DOMINIK VONDRÁČEK[5,6] and KATJA KRAMP[2]

[1]*Department of Zoology, Faculty of Science, Palacky University, 17. listopadu 50, 771 46, Olomouc, Czech Republic*
[2]*Senckenberg Deutsches Entomologisches Institut, Eberswalder Strasse 90, 15374 Müncheberg, Germany*
[3]*Department of Entomology, Faculty of Biology, Moscow State University, Leninskie gory 1/12, 119234, Moscow, Russia*
[4]*Santa Barbara Museum of Natural History, 2559 Puesta del Sol Rd, Santa Barbara, CA 93105-2998, USA*
[5]*Department of Entomology, National Museum, Cirkusová 1740, 193 00, Praha 9 – Horní Počernice, Czech Republic*
[6]*Department of Zoology, Faculty of Science, Charles University in Prague, Viničná 7, 128 43, Praha 2, Czech Republic*

Received 27 March 2019; revised 9 July 2019; accepted for publication 20 August 2019

Cydistinae are a rare monogeneric beetle lineage from Asia with a convoluted history of classification, historically placed in various groups within the series Elateriformia. However, their position has never been rigorously tested. To resolve this long-standing puzzle, we are the first to present sequences of two nuclear and two mitochondrial markers for four species of Cydistinae to determine their phylogenetic position. We included these sequences in two rounds of analyses: one including a broad Elateriformia dataset to test placement at the superfamily/family level, and a second, including a richer, targeted sampling of presumed close relatives. Our results strongly support Cydistinae as sister to Phengodidae in a clade with Rhagophthalmidae. Based on our molecular phylogenetic results and examination of morphological characters, we hereby transfer the formerly unplaced Cydistinae into Phengodidae and provide diagnoses for the newly circumscribed Phengodidae, Cydistinae and *Cydistus*. Since both Phengodidae and Rhagophthalmidae have bioluminescent larvae and strongly neotenic females, similar features can be hypothesized for Cydistinae. Additionally, *Cydistus minor* is transferred to the new genus ***Microcydistus***.

ADDITIONAL KEYWORDS: bioluminescence – classification – Elateroidea – morphology – neoteny – Phengodidae – Rhagophthalmidae – systematics.

INTRODUCTION

Coleoptera (beetles) is the most diverse group of insects on Earth. About 400 000 species have been described and many others still remain to be discovered (Ślipiński *et al.*, 2011). Therefore, it is not surprising that beetle classification has changed dramatically since the era of early systematists (see e.g. Lawrence *et al.*, 1995; Lawrence, 2016a). Both morphology-based studies (Crowson, 1955; Lawrence & Newton, 1982, 1995; Lawrence *et al.*, 2011) and those using molecular data (e.g. Hunt *et al.*, 2007; Bocak *et al.*, 2014; McKenna *et al.*, 2015; Zhang *et al.*, 2018) made tremendous

*Corresponding author. E-mail: robin.kundrata@upol.cz.
[Version of record, published online 19 October 2019; http://zoobank.org/urn:lsid:zoobank.org:pub: A49A0216-FB08-46B0-AEA8-A151FF681326]

progress on the phylogeny and classification of this insect order. However, some branches in the beetle tree of life remain unresolved.

Within the series Elateriformia, the classification has always been complicated by extremely diverse morphologies of included groups, particularly of soft-bodied lineages (e.g. Crowson, 1955; Lawrence, 1988; Beutel & Leschen, 2005). This beetle series or infraorder consists of Buprestoidea (jewel beetles; about 15 000 species), Dascilloidea (about 150 species), Rhinorhipoidea (one species), Byrrhoidea (pill beetles, riffle beetles, etc.; about 4000 species) and Elateroidea (e.g. soft-bodied soldier-beetles, fireflies and net-winged beetles, as well as fully sclerotized click-beetles and a few other families; about 25 000 species) (Ślipiński *et al.*, 2011). Despite recent progress in Elateriformia classification and phylogeny (Bocakova *et al.*, 2007; Timmermans & Vogler, 2012; Kundrata *et al.*, 2014, 2017; Bocak *et al.*, 2016; Kusy *et al.*, 2018), relationships among the deep splits have not yet been fully resolved. Although the family limits remain roughly stable, a few enigmatic lineages are classified as Elateriformia *incertae sedis* or placed only tentatively within the morphologically most similar groups (Lawrence & Newton, 1995; Lawrence *et al.*, 2010). These *incertae sedis* lineages have mostly been well examined morphologically, but due to a number of homoplastic characters in the group, their position in Elateriformia cannot be clearly established based on morphology alone (Lawrence *et al.*, 2011). In cases when morphology reaches its limits in reliable systematic placement of certain taxa, molecular data provides a powerful independent source of evidence for testing phylogenetic relationships (e.g. Resch *et al.*, 2014; Derocles *et al.*, 2016).

Lawrence & Newton (1995) classified the monotypic Rhinorhipidae and Podabrocephalidae in Elateriformia *incertae sedis*. Later, Lawrence (2010) included Rhinorhipidae in Elateroidea, and Kusy *et al.* (2018) erected for it a new superfamily Rhinorhipoidea based on the results of a molecular phylogeny. In the *Handbook of zoology*, Lawrence *et al.* (2010) treated Ototretinae, Pterotinae, *Harmatelia* Walker, 1858, *Stenocladius* Fairmaire, 1878, Podabrocephalidae, *Neocrowsonia* Kistner & Abdel-Galil, 1986 and Cydistinae as Elateriformia *incertae sedis*. The first four taxa are currently classified in the elateroid family Lampyridae (e.g. Sagegami-Oba *et al.*, 2007; Martin *et al.*, 2017) and Podabrocephalidae have been synonymized with the byrrhoid Ptilodactylidae (Lawrence *et al.*, 2016a; Kundrata *et al.*, 2019). The monotypic termitophilous *Neocrowsonia* was originally classified with Throscidae and, although this placement is questionable, it is most probably a modified member of one of the clicking elateroid families (Lawrence & Newton, 1995; Lawrence *et al.*, 2010). Hence, the only remaining taxon with unresolved phylogenetic placement at the superfamily level is the monogeneric Cydistinae.

Cydistus Bourgeois, 1908 includes seven described species distributed in Asia Minor, the Levant and Iran (Wittmer, 1979). It is sparsely represented in collections, and all available specimens are adult males. They are small to medium-sized, with a weakly sclerotized body, bipectinate antennae and distinctly shortened elytra exposing several abdominal segments. Adult females, immature stages and natural history of *Cydistus* are unknown. This genus has a convoluted history of classification and, due to its peculiar morphology, it was placed in various groups within Elateriformia. Bourgeois (1885) compared this genus with the New World *Phengodes* Illiger, 1807 and suggested its position near Drilidae. Subsequent authors (e.g. Olivier, 1910; Wittmer, 1944) followed the placement of *Cydistus* in Drilidae, which was at that time a kind of 'trash can' for various unrelated soft-bodied lineages (Crowson, 1972; Kundrata & Bocak, 2011). Drilids have recently been incorporated into Elateridae as Agrypninae: Drilini and their definition has been considerably restricted, such that they include taxa morphologically clearly different from *Cydistus* (Kundrata & Bocak, 2011, 2017). Crowson (1955) hypothesized *Cydistus* might be an intermediate form between Karumiidae and Phengodidae, both at that time regarded as families in the soft-bodied Cantharoidea. Karumiidae were later moved to Dascilloidea based mainly on the morphology of the corpotentorium, their wing venation, serrate tibial spurs and the bilobate median lobe (Crowson, 1971), and they are currently classified as a subfamily of Dascillidae (e.g. Lawrence, 1988, 2016b; Lawrence & Newton, 1995; Kundrata *et al.*, 2017). Paulus (1972) revised Karumiidae, including *Cydistus*, for which he erected a separate subfamily Cydistinae. However, Crowson (1972) transferred *Cydistus*, together with genera usually classified with the Asian family Rhagophthalmidae, into Phengodidae *s.l.*, which was followed by several other authors (Wittmer, 1979; Lawrence & Newton, 1995; Bocak, 2007). Currently, Rhagophthalmidae and the exclusively New World Phengodidae are treated as closely related families (Kawashima *et al.*, 2010; Kundrata *et al.*, 2014). Both families are characterized by soft-bodied males with large eyes, usually bipectinate antennae comprising 12 antennomeres, leathery, usually shortened and narrowed elytra, larviform females and larvae that possess bioluminescent organs and feed on millipedes. Currently, Cydistinae are treated as Elateriformia *incertae sedis* (Lawrence *et al.*, 1999, 2010; Lawrence, 2016a).

Most elateriform lineages were included in the largest molecular phylogeny of the series to date (Kundrata

et al., 2017). However, no representative of Cydistinae had been sequenced so far. Here, we generated four widely used nuclear and mitochondrial markers for four Cydistinae species from Israel and Iran. The main aims of our study were to (1) test the phylogenetic position of Cydistinae within Elateriformia, (2) examine the phylogenetic structure of the group, (3) assess its morphological diversity and distribution and (4) modify the classification of the group based on the combination of molecular phylogeny and detailed investigation of its morphology.

MATERIAL AND METHODS

Taxon sampling and laboratory methods

We sequenced four *Cydistus* specimens representing the following species: *C.* cf. *chindaaricus* Bolívar y Pieltain, 1913, *C. minor* Bolívar y Pieltain, 1913, *C.* cf. *persicus* Bolívar y Pieltain, 1913 (all from Iran) and *C. reitteri* Bourgeois, 1908 (type species, from Israel; Supporting Information, Table S1). Additionally, we sequenced three species of Phengodidae: *Cenophengus debilis* LeConte, 1881, *Distremocephalus opaculus* (Horn, 1895) and *Zarhipis integripennis* (LeConte, 1874) (all from USA: California; Supporting Information, Table S1). Voucher specimens are stored in pure ethanol and are deposited in the collection of the Department of Zoology, Palacky University in Olomouc, Czech Republic. Whole genomic DNA was extracted either using the Genomic DNA Mini Kit (Tissue) (Geneaid Biotech Ltd, Taiwan) according to the manufacturer protocol but with incubation with GT buffer prolonged to 3 h, incubation with GBT buffer prolonged to 1 h and the elution performed with 40 µL of Elution buffer each, or the E.Z.N.A Tissue DNA Kit (Omega Bio-tek Inc. Norcross, USA) following standard protocol but with the overnight incubation and elution performed twice with 100 µL Elution buffer each. Amplifications were performed either using Qiagen Multiplex PCR Plus Master Kit (Qiagen, Hilden, Germany) or PPP Master Mix (Top-Bio, Czech Republic) according to the manufacturer protocols. The PCR amplification conditions and list of primers used are given in Supporting Information, Table S2. Two nuclear markers, 18S rRNA (~1850 bp) and 28S rRNA (~660 bp), and two fragments of the mitochondrial genome, *rrnL* (~520 bp) and *COI* mtDNA (~723 bp), were sequenced for the phylogenetic analysis. These markers have been widely used in Coleoptera and Elateriformia phylogenenetics (e.g. Bocakova *et al.*, 2007; Hunt *et al.*, 2007; Bocak *et al.*, 2014, 2016; Kundrata *et al.*, 2014, 2017; Kusy *et al.*, 2018) and, therefore, enable us to test the position of Cydistinae using as many representatives of Karumiinae, Phengodidae and Rhagophthalmidae from previous studies as possible. The PCR products were purified either using the ethanol precipitation method or Exonuclease I and FastAP Thermosensitive Alkaline Phosphatase (Life Technologies, Darmstadt, Germany), and subsequently sequenced with the ABI3730XL sequencer using Big Dye v.3.1 Terminator Kit (Thermo Fisher Scientific, Darmstadt, Germany) by Macrogen Europe, Netherlands. GenBank accession numbers of newly generated sequences are given in Supporting Information, Table S1.

Dataset assembly and phylogenetic analyses

In our study, we analysed two different datasets including Cydistinae. First, we investigated the position of this group within Elateriformia using the Cydistinae plus the 251-taxon dataset from the most comprehensive phylogenetic study of Elateriformia to date by Kundrata *et al.* (2017) (henceforth the 'Elateriformia dataset'). It contained scirtiform (outgroup) and elateriform terminals with all four markers present except for a few taxonomically important lineages for which some fragments were missing (Supporting Information, Table S1). Because Cydistinae were recovered as part of the robustly supported clade formed by Phengodidae and Rhagophthalmidae, we created a second dataset to examine the interrelationships within the Phengodidae + Cydistinae + Rhagophthalmidae clade (henceforth the 'Phengodidae dataset'). As outgroups, we used ten representatives of Elateridae, which were found to be closely related to Phengodidae and Rhagophthalmidae (e.g. Bocakova *et al.*, 2007; Amaral *et al.*, 2014; Bocak *et al.*, 2014; Kundrata *et al.*, 2014; McKenna *et al.*, 2015; Zhang *et al.*, 2018). We did not include more outgroups, e.g. from Lampyridae, in order to avoid complexity of alignment of the length-variable rRNA sequences (see: Kundrata *et al.*, 2014). As ingroups, we used all publicly available data for Phengodidae and Rhagophthalmidae (32 terminals each with at least two markers present), three newly sequenced phengodid genera, including the systematically important genus *Cenophengus* LeConte, 1881 (see: Zaragoza-Caballero & Zurita-García, 2015), and four species of Cydistinae (Supporting Information, Table S1).

Sequences were edited using GENEIOUS 7.1.7 (www.geneious.com; Kearse *et al.*, 2012) and aligned using MAFFT 7.157 at default parameters (Katoh & Standley, 2013). Alignments of the length-invariable protein-coding *COI* sequences were checked by amino acid translation. To evaluate the occurrence of substitution saturation in our data, we performed Xia's nucleotide substitution saturation test (Xia *et al.*, 2003) implemented in DAMBE 5.6.14 (Xia & Lemey, 2009) for each non-coding gene and each position of the protein-coding *COI* mtDNA. We estimated the

empirical proportion of invariant sites from the data and used 10 000 replicates on the fully resolved sites. Basic sequence statistics were calculated using MEGA 6.06 (Tamura *et al.*, 2013). The best-fit partitioning schemes and partition-specific substitution models were tested using a greedy algorithm in PartitionFinder 1.1.1 (Lanfear *et al.*, 2012) under the corrected Akaike information criterion (AICc).

Maximum likelihood (ML) analyses were conducted using RAxML 8.2.9 (Stamatakis, 2006) via the CIPRES web server (www.phylo.org; Miller *et al.*, 2010). We applied the GTR+I+G model and the partitioning by genes and codons as defined by PartitionFinder. Branch supports were calculated using the Rapid Bootstrap algorithm (Stamatakis *et al.*, 2008) with 1000 bootstrap replicates. Bayesian inference (BI) was performed using MrBayes 3.2.6 (Huelsenbeck & Ronquist, 2001) on the CIPRES portal (Miller *et al.*, 2010), with the nucleotide substitution model GTR+I+G for most partitions (SYM+I+G for 28S rRNA in the Elateriformia dataset) and the partitioning by genes and codon positions as identified in PartitionFinder. Four chains were run for 40 million generations using the Markov chain Monte Carlo method. Stationary phase and convergence were checked using TRACER 1.7.1 (Rambaut *et al.*, 2018) and the first 20% of generations were discarded as burn-in. The 50% majority-rule consensus tree was constructed to determine the posterior probabilities (PP) from the remaining trees. For the Phengodidae dataset we also performed a maximum parsimony (MP) analysis. This was conducted in TNT 1.1 (Goloboff *et al.*, 2008) using Sectorial Search, Ratchet, Drift and Tree fusing methods under New Technology Search, with 'init. level' = 100 (14 initial addseqs), 'find min. length' = 25 and gaps treated as a fifth character state. Clade support was assessed by standard bootstrap with a traditional search strategy using 1000 pseudoreplicates. The final trees were edited in FigTree 1.3.1 (Rambaut, 2010).

Morphology

For a detailed morphological examination of Cydistinae and their putative relatives (see habitus images of selected genera in Fig. 1), we used specimens from the following collections: Naturhistorisches Museum, Basel, Switzerland (NHMB); The Natural History Museum, London, UK (BMNH); Muséum national d'Histoire naturelle, Paris, France (MNHN); collection of Robin Kundrata, Olomouc, Czech Republic; collection of Alexander S. Prosvirov, Moscow, Russia; collection of Rudolf Schuh, Wiener Neustadt, Austria; Hungarian Natural History Museum, Budapest, Hungary (HNHM); Naturalis Biodiversity Center, Leiden, the Netherlands (RMNH); Santa Barbara Museum of Natural History, Santa Barbara, California, USA (SBMNH); Naturhistorisches Museum, Vienna, Austria (MHMW); National Museum, Prague, Czech Republic (NMPC); and Senckenberg Deutsches Entomologisches Institut, Müncheberg, Germany (SDEI) (see Supporting Information, Table S3 for a list of examined Cydistinae). The original descriptions and detailed photographs of type specimens deposited in the Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN) were also examined. Additional information on the morphological characters of the examined groups were taken from recent literature (Costa & Zaragoza-Caballero, 2010; Lawrence *et al.*, 2010; Zaragoza-Caballero & Pérez Hernández, 2014; Lawrence, 2016b). Classification of Phengodidae follows Zaragoza-Caballero & Zurita-García (2015).

Body length was measured from frontal region of head to apex of abdomen (although the abdomen is highly flexible and capable of elongation/contraction), body width at the widest part of the body, pronotal length at midline and pronotal width at the widest part. Genitalia were dissected after a short treatment in 10% hot KOH. For the purpose of light microscopic image capture, complete specimens and dissected parts preserved in ethanol were rehydrated and mounted in K-Y Lubricating Jelly Sterile (Reckitt Benckiser Healthcare, UK), which prevents drift during image capture. Hind wings were temporarily embedded in glycerine and expanded between a microscopic slide and a cover slide. Images were taken with a Leica DFC450 camera attached either to a Leica M205 C stereo microscope (Leica Microsystems, Switzerland) (most images) or to a BX51 microscope (Olympus, Japan) applying phase-contrast illumination (Fig. 5L), applying the software Leica Application Suite (LAS v.4.9.0). Stacked photographs were combined with the software CombineZP (Hadley, 2010).

For subsequent investigation with the scanning electron microscope, the specimens were rinsed in deionized water, gradually dehydrated in ethanol and, finally, in 100% acetone, and critical point dried with liquid CO_2 using a Polaron CPD7501 critical point dryer (Quorum Technologies, UK). The specimens were mounted with polycarbonate tabs ('Leit-Tabs', Plano, Germany) or glued with water-based Berlese Mixture (Waldeck, Germany) on plain stubs and sputtered with Au/Pd using a Polaron SC7640 sputter coater. Images were taken with a JSM-6060LV scanning electron microscope (Jeol, Japan), usually at 6.0 kV acceleration voltage.

RESULTS

Alignment parameters

The alignment of the Elateriformia dataset includes 4681 homologous positions (2244, 1119, 595 and 723

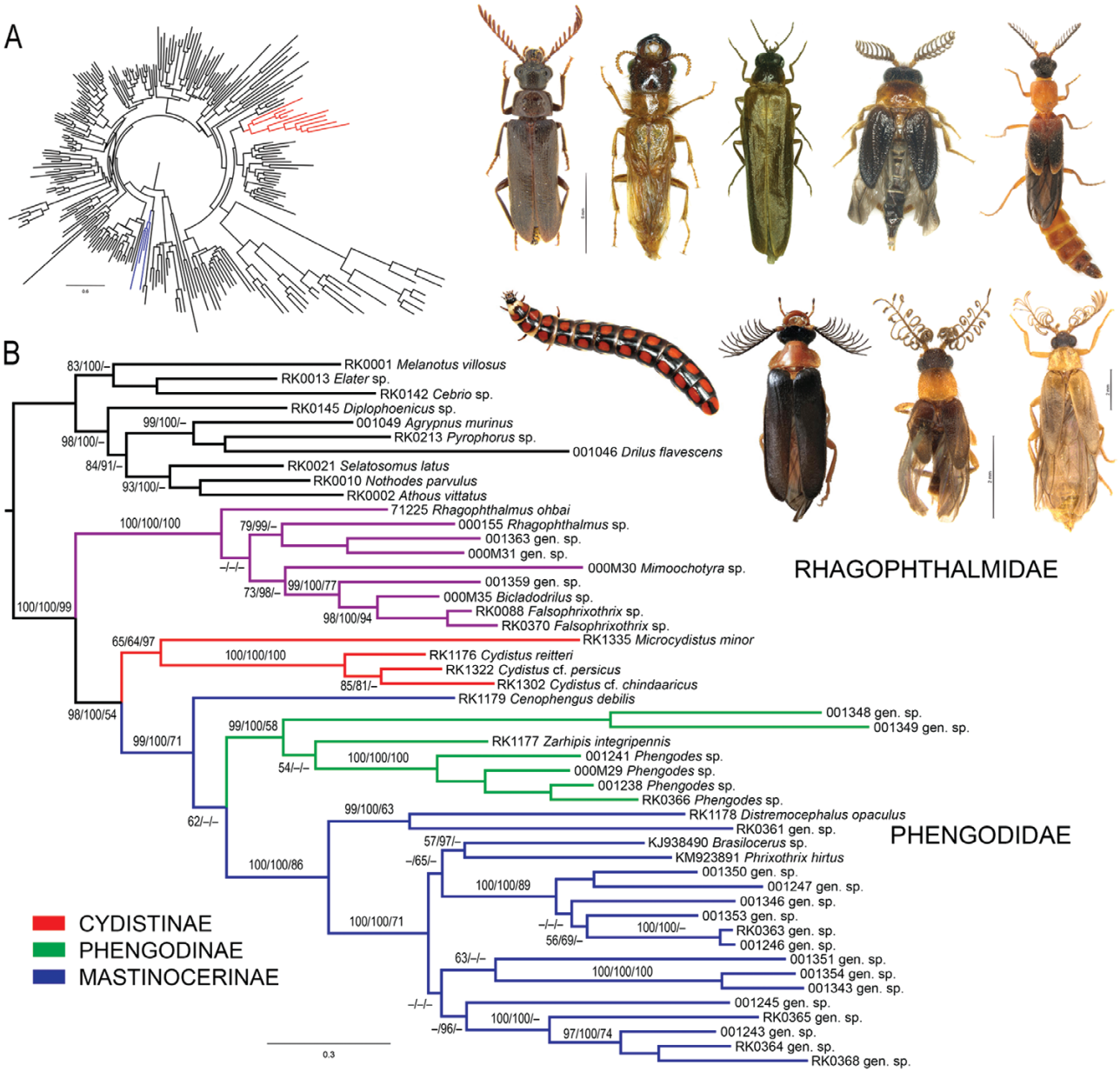

Figure 1. Phylogenetic hypothesis for Cydistinae. A, phylogenetic positions of Dascillidae including Karumiinae (blue) and Phengodidae + Cydistinae (red) within Elateriformia based on the ML analysis performed on the 255-taxon dataset aligned by MAFFT. The full-resolution tree with taxon names and statistical support for branches is given in Supporting Information (Fig. S1). B, phylogenetic hypothesis of Phengodidae + Rhagophthalmidae based on the ML analysis performed on the 49-taxa dataset aligned by MAFFT, with Elateridae as an outgroup. Numbers associated with the branches indicate ML bootstrap values, Bayesian posterior probabilities and MP bootstrap values, respectively. Only values above 50% are shown. Habitus images represent select investigated taxa (top row, left to right): *Genecerus* cf. *cervinus*, *Karumia* sp. (both Dascillidae: Karumiinae), *Rhagophthalmus* sp., *Falsophrixothrix* sp. (both Rhagophthalmidae), *Cydistus reitteri* (Phengodidae: Cydistinae; credit: Amir Weinstein, Israel); (lower row, left to right) *Phengodes* sp. (credit: Patrick Coin, USA), *Zarhipis integripennis* (credit: Lucie Gimmel, USA; both Phengodidae: Phengodinae), *Stenophrixothrix* sp., *Howdenia fischeri* (both Phengodidae: Mastinocerinae). All images are males except *Phengodes*; not to scale.

positions for 18S, 28S, *rrnL* and *COI*, respectively), and contains 2102 conserved, 2393 variable and 1897 parsimony informative characters. The alignment of the Phengodidae dataset includes 3868 homologous positions (1905, 689, 545 and 729 positions for 18S, 28S, *rrnL* and *COI*, respectively), and contains 2738 conserved, 1067 variable and 856 parsimony informative characters. Only little substitution saturation is detected except for the highly improbable asymmetrical topology for the 3rd codon positions of the *COI* fragment (Supporting Information, Table S4).

Phylogenetic analyses

Both maximum likelihood and Bayesian analyses of the Elateriformia dataset, comprising 255 taxa, recovers Cydistinae as sister to Phengodidae (98% BS, 100% PP) in a clade with Rhagophthalmidae. This clade obtains the maximal statistical support and is nested within the strongly supported, more advanced 'higher elateroids' *sensu* Kundrata *et al.* (2014), far from Dascillidae: Karumiinae (Fig. 1; Supporting Information, Fig. S1). The maximum likelihood, Bayesian and maximum parsimony analyses of the Phengodidae dataset, including 49 taxa, yield similar tree topologies. In order to summarize results and congruence among analyses, we map support values on the phylogenetic tree inferred from the ML analysis (Fig. 1). Rhagophthalmidae are recovered sister to a robustly supported clade formed by Cydistinae + Phengodidae. Cydistinae are always monophyletic, although usually without strong statistical support. In all analyses, *Cydistus minor* is sister to the remainder of Cydistinae and *Cydistus reitteri* is sister to the remaining species. Within Phengodidae, *Cenophengus* is found either sister to all remaining Phengodidae (ML and MP analyses) or sister to the remaining Mastinocerinae (BI analysis). Therefore, Mastinocerinae are only monophyletic in the BI analysis (87% PP). Both Phengodinae and Mastinocerinae, excluding *Cenophengus*, are monophyletic with strong support (weaker in MP analysis) in all analyses, although interrelationships in these subfamilies varied depending on the analytical approach.

Systematics

In the light of the results obtained from the molecular phylogeny (Fig. 1; Supporting Information, Fig. S1) and detailed morphological investigation (see below), we propose to place Cydistinae as a subfamily in Phengodidae. Additionally, we describe the new genus, *Microcydistus*, to accommodate *Cydistus minor*, which is genetically and morphologically distant from the remaining species of *Cydistus* (Figs 1–6). Therefore, the definitions of Phengodidae, Cydistinae and *Cydistus* need to be modified accordingly. In order to avoid excessively verbose and lengthy morphological descriptions repeating characters typical for their respective higher taxonomic ranks, we here provide concise diagnostic (re)descriptions for the newly erected and redefined taxa using primarily major diagnostic characters.

Phengodidae LeConte, 1861

(Fig. 1)

Type genus: Phengodes Illiger, 1807.

Diagnostic redescription: Adult male with body elongate, soft, flattened, smooth, in some New World taxa with light-emitting organs on head, prothorax or abdomen; head usually subquadrate; antennal insertions usually widely separated (rarely approximate); antennal tubercles absent; antennae usually with 12 antennomeres (rarely ten or 11); antennomeres II and III minute, short, almost subequal in length, antennomere III wider than long, antennomeres IV to penultimate one bipectinate, with more or less long rami arising subbasally; eyes lateral; mouthparts prognathous; mandibles falcate, usually distinctly elongate (reduced in *Microcydistus*); galea well-developed; pronotal disc usually more or less flattened, without distinctly prominent posterior angles; prosternum usually short, transverse; scutellar shield flattened, usually apically truncate; elytra shortened to various extent, exposing several to all tergites, somewhat leathery, often narrowed towards apex; hind wing not folded under elytron, radial cell elongate, wedge cell absent; tibial spurs usually absent; tarsi 5-5-5; abdomen with eight free ventrites connected by extensive membranes; aedeagus trilobate (median lobe divided into dorsal and ventral lobes in Cydistinae); phallobase usually small (larger in *Cydistus*); endophallus (flagellum) usually conspicuous, not encapsulated inside median lobe and freely extensible (not visible in Cydistinae). Adult female and larva (known only for Phengodinae and Mastinocerinae) elongate, orthosomatic, larviform, more or less flattened, bioluminescent, with prognathous head; antennae with three antennomeres; single lateral stemma; falcate and internally grooved mandibles; and short legs with a single claw per leg.

Composition: Phengodinae (four genera, 61 species), Mastinocerinae (26 genera, 212 species, Cydistinae (two genera, seven species). For the revised and updated supraspecific classification of Phengodidae

see Table 1.

Distribution: Neotropical and Nearctic regions (Phengodinae, Mastinocerinae), Palaearctic region (Cydistinae).

CYDISTINAE PAULUS, 1972

(FIGS 1–6)

Type genus: Cydistus Bourgeois, 1885.

Diagnostic redescription: Adult male with body about 3.5–10.0 mm long, about 4.5–6.0 times as long as wide, without luminous organs, coloration usually yellowish to light brown, head dark brown, elytra often bicoloured; head prognathous, subquadrate; frontoclypeal region declined anteriorly or projected at apex; eyes strongly protuberant, entire; antennal insertions moderately widely separated; antenna with 12 antennomeres; antennomeres II and III short, antennomeres IV–XI elongate, bipectinate, with rami slender or stout, slightly flattened to subcircular in cross-section, terminal antennomere longer than wide, suboval, flattened, apically narrowly rounded or slightly emarginate; labrum free or fused; mandible unidentate, elongate, sickle-shaped or distinctly reduced; maxilla with both galea and lacinia densely setose; maxillary palp with four articles, as long as or longer than head, palpomere I short, strongly transverse, palpomeres II and III elongate, terminal palpomere elongate-securiform, apically obliquely cut; labial palp with one or three articles; gula longer than wide, gular sutures relatively narrowly separated; prothorax 1.0–1.2 times as long as wide; pronotal disc narrower than elytra at humeri, anterior margin almost straight to widely rounded, sides subparallel or widely rounded, posterior margin slightly emarginate; prosternum transverse, in front of coxae usually longer than diameter of procoxal cavity, up to about 2.5 times as wide as long, anterior portion produced to form chin piece; prosternal process short, flat, broadly rounded or truncate; protrochantins exposed, plate-like, elongate; procoxal cavities transverse, contiguous, widely open; scutellar shield flat, posteriorly truncate to widely emarginate; elytra shortened, not covering whole abdomen, together 1.3–1.7 times as long as wide, about 1.6–2.0 times as long as pronotum, with apices independently rounded; mesoventrite short, widely V-shaped, anteriorly more or less deeply emarginate, medially longitudinally depressed, posteriorly with short and widely rounded mesoventral process; mesocoxae conical, projecting; mesocoxal cavitites contiguous, closed by mesanepisternum and mesepimeron; metaventrite large, with short discrimen; metacoxae narrowly separated, obliquely transverse, extending laterally to meet elytra; metacoxal plates weakly developed; hind wing not folded under elytra, about 2.1–2.5 times as long as wide, apical field short, with three elongate sclerotizations, radial cell closed, about 2.3–4.5 times as long as wide, r3 short, AP_{3+4} simple or distinctly forked, legs moderately long; tibia elongate, without tibial spurs, with crown of thickened setae apically; tarsomeres without ventral lobes but with several apical spines; tergites IX and X connected by membrane; sternite IX elongate; aedeagus trilobate, symmetrical; phallobase longer than wide to transverse; parameres robust, elongate; median lobe divided into dorsal and ventral lobes, ventral lobe slightly shorter to distinctly reduced. Adult female and immature stages unknown.

Comparison: Cydistinae differ from the remaining Phengodidae (i.e. Phengodinae and Mastinocerinae) in having the prosternum extending well in front of procoxae (Fig. 3C; other phengodids usually have a short, transverse prosternum, often reduced to a slender transverse strip of cuticle, although this is slightly longer in *Cenophengus*), and a median lobe divided into dorsal and ventral lobes (Figs 4E–G, I, J, 6F–L; this character has never been reported for any other phengodid).

Composition: Two genera: *Cydistus* (six species) and *Microcydistus* (monotypic).

Distribution: Asia Minor, the Levant, Iraq, Iran.

CYDISTUS BOURGEOIS, 1885

(FIGS 2–4)

Type species: Cydistus reitteri Bourgeois, 1885; by monotypy.

Diagnostic redescription: Adult male with body about 5.5–10.0 mm long; frontoclypeal region declined anteriorly; antennomeres IV–XI with rami slender, more than six times as long as wide, more than twice as long as their respective stems; labrum free, strongly transverse, fully sclerotized or partly membranous; mandible elongate, narrow, sickle-shaped, slightly widened basally, moderately curved mesally, acute apically; labial palp with three articles, palpomere I strongly transverse, palpomere II about as long as wide or slightly longer than wide, terminal palpomere elongate, fusiform; prothorax 1.0–1.2 times as long as wide; pronotal disc of variable shape; elytra shortened to various extent, together 1.4–1.7 times as long as

Figure 2. Habitus images of Cydistinae adult males. A, *Cydistus reitteri*; dorsal view. B–D, *Cydistus* cf. *persicus*; dorsal, ventral and lateral view, respectively. E, *Cydistus* cf. *chindaaricus*; dorsal view. F–H, *Microcydistus minor*; dorsal, ventral and lateral view, respectively. Scale bars: A–E, 2.0 mm; F–H, 1.0 mm.

wide, about 1.6–2.0 times as long as pronotum, more or less dehiscent; hind wing about 2.2–2.5 times as long as wide, radial cell about 4.5 times as long as wide, AP_{3+4} distinctly forked; sternite IX 1.75–2.20 times as long as wide, apically rounded or truncate; aedeagus with phallobase longer than wide; median lobe divided

Figure 3. Body parts of *Cydistus* cf. *persicus*, adult male. A, forebody; dorsal view. B, C, head and prothorax; lateral and ventral view, respectively. D–F, mouthparts; dorso-frontal, fronto-ventral and frontal view, respectively. G, antenna; lateral view. H, detail of antenna; ventral view. I, detail of scutellar shield; dorsal view. J, hind leg, detail of apex of tibia and tarsus; lateral view. K, meso- and metathorax; ventral view. L, hind-wing venation. Scale bars: A, K, 1.0 mm; B, C, F, 0.5 mm; E, G, I, J, 0.2 mm; D, H, L, 0.1 mm.

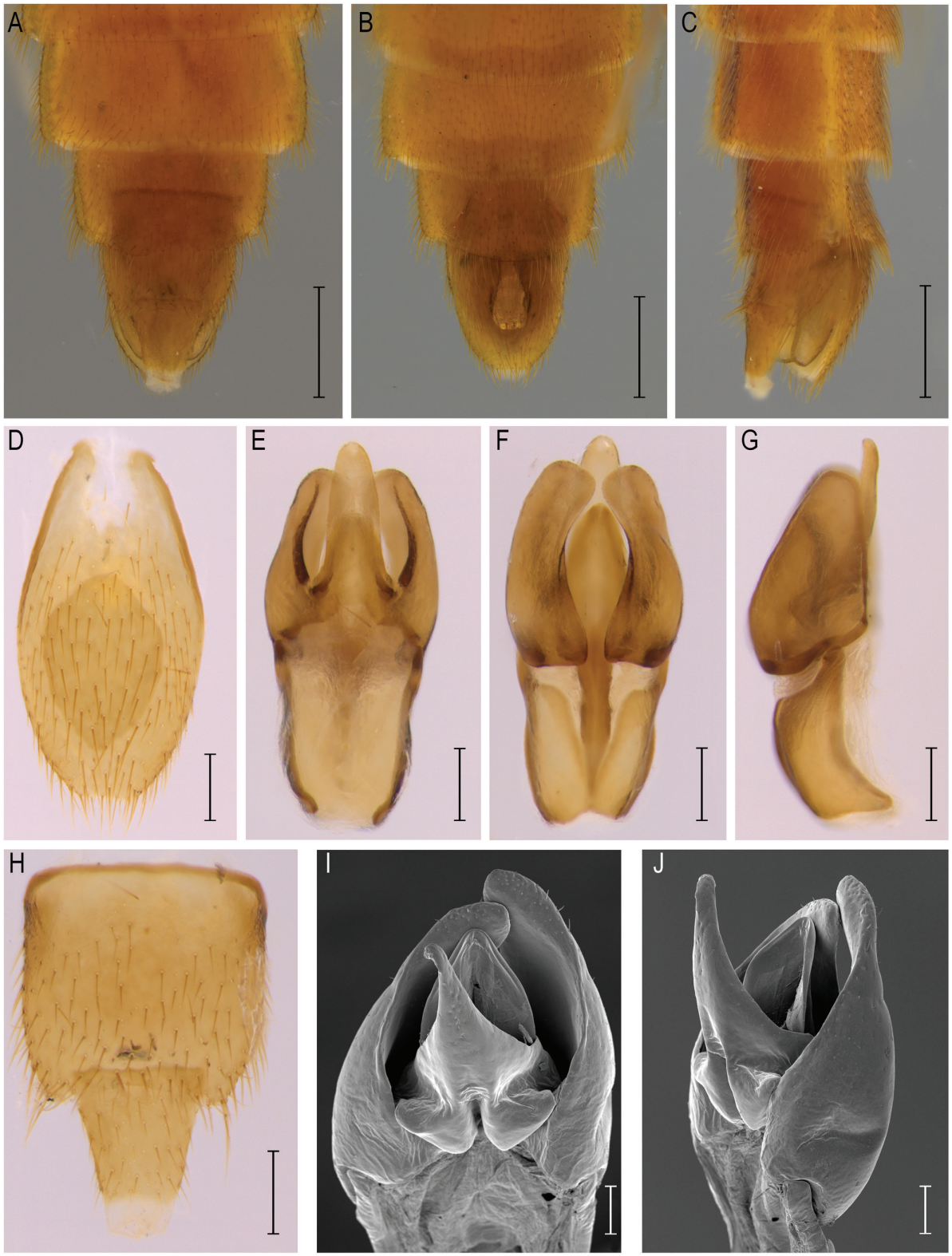

Figure 4. Distal abdominal segments and aedeagus of *Cydistus* cf. *persicus*, adult male. A–C, apical abdominal segments; dorsal, ventral and lateral view, respectively. D, sternite IX; ventral view. E–G, aedeagus; dorsal, ventral, lateral view, respectively. H, tergites IX and X; dorsal view. I, J, details of aedeagus; fronto-ventral, ventro-lateral view, respectively. Scale bars: A–C, 0.5 mm; D–H, 0.2 mm; I, J, 0.1 mm.

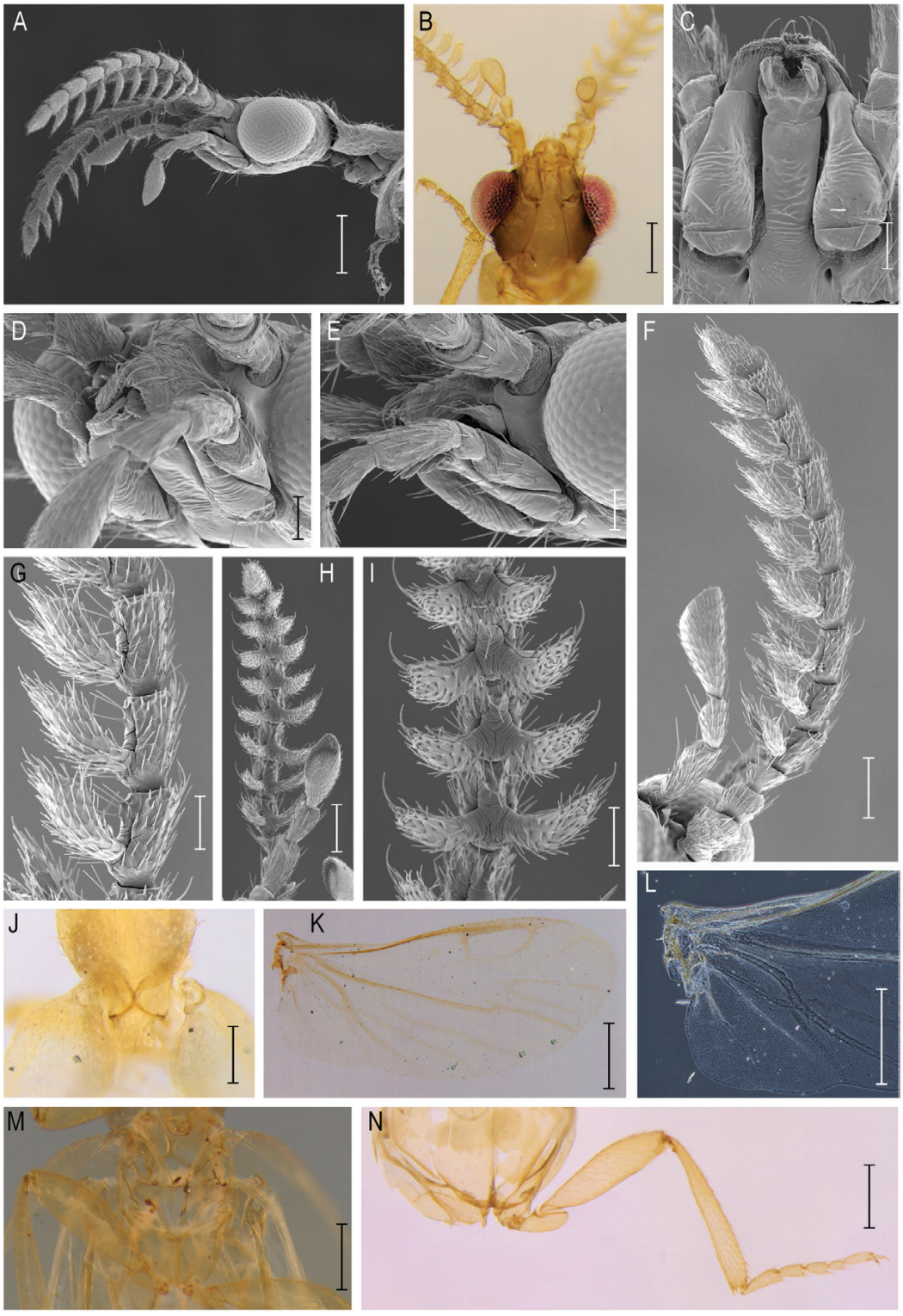

Figure 5. Body parts of *Microcydistus minor*, adult male. A, B, head with mouthparts; lateral and ventral view, respectively. C, mouthparts; ventral view. D, E, detail of head with mouthparts; fronto-lateral and lateral view, respectively. F, H, antenna; lateral and ventral view, respectively. G, I, detail of antenna; lateral and ventral view, respectively. J, detail of scutellar shield; dorsal view. K, hind-wing venation. L, detail of base of hind-wing venation. M, detail of mesothorax, ventral view. N, hind leg; lateral view. Scale bars: K, 0.5 mm; A, B, J, M, N, 0.2 mm; F, H, 0.1 mm; C–E, G, I, 0.05 mm.

Table 1. List of genera and subgenera classified in Phengodidae, with numbers of described species [modified from Zaragoza-Caballero and Pérez-Hernández (2014)]

Phengodidae LeConte, 1861	
Cydistinae Paulus, 1972	
Cydistus Bourgeois, 1885	6 spp.
Microcydistus Kundrata, Blank & Prosvirov	1 sp.
Mastinocerinae LeConte, 1881	
Akamboja Roza, Quintino, Mermudes & Silveira, 2017	5 spp.
Brasilocerus Wittmer, 1963	9 spp.
Cenophengus LeConte, 1881	23 spp.
Cephalophrixothrix Wittmer, 1976	3 spp.
Decamastinocerus Wittmer, 1988	2 spp.
Distremocephalus Wittmer, 1976	11 spp.
Eurymastinocerus Wittmer, 1976	8 spp.
Euryognathus Wittmer, 1976	2 spp.
Euryopa Gorham, 1881	8 spp.
Howdenia Wittmer, 1976	10 spp.
Mastinocerus Solier, 1849	
subgenus *Mastinocerus* Solier, 1849	18 spp.
subgenus *Paramastinocerus* Wittmer, 1976	8 spp.
Mastinomorphus Wittmer, 1976	15 spp.
Mastinowittmerus Zaragoza-Caballero, 1984	2 spp.
Neophengus Wittmer, 1976	3 spp.
Nephromma Wittmer, 1976	2 spp.
Oxymastinocerus Wittmer, 1963	9 spp.
Paraphrixothrix Zaragoza-Caballero, 2010	1 sp.
Paraptorthodius Schaeffer, 1904	3 spp.
Phrixothrix Olivier, 1909	17 spp.
Pseudomastinocerus Wittmer, 1963	9 spp.
Ptorthodiellus Wittmer, 1976	2 spp.
Ptorthodius Gorham, 1881	3 spp.
Spangleriella Wittmer, 1988	1 sp.
Steneuryopa Wittmer, 1986	1 sp.
Stenophrixothrix Wittmer, 1963	19 spp.
Taximastinocerus Wittmer, 1963	18 spp.
Phengodinae LeConte, 1861	
Microphengodes Wittmer, 1976	2 spp.
Phengodes Illiger, 1807	
subgenus *Phengodella* Wittmer, 1975	20 spp.
subgenus *Phengodes* Illiger, 1807	10 spp.
Pseudophengodes Pic, 1930	26 spp.
Zarhipis LeConte, 1881	3 spp.

into distinct dorsal and ventral lobes.

Composition: Six described and several undescribed species. Described species: *Cydistus chindaaricus* Bolívar y Pieltain, 1913, *C. escalerai* Bolívar y Pieltain, 1913, *C. nigripennis* Wittmer, 1979, *C. persicus* Bolívar y Pieltain, 1913, *C. reitteri* Bourgeois, 1885, *C. zurcheri* Bourgeois, 1908 (= *C. reitteri* Reitter, 1908).

Distribution: Southern and eastern Turkey, Jordan, Israel, Iraq, Iran. Its occurrence in Syria is highly likely, but has not been confirmed.

MICROCYDISTUS KUNDRATA *ET AL.*, GEN. NOV.

(FIGS 2, 5, 6)

URN:LSID:ZOOBANK.ORG:ACT:8C286DEB-E556-448F-A2C8-F7FBBB9896D8

Type species: Cydistus minor Bolívar y Pieltain, 1913; here designated.

Diagnostic description: Adult male with body about 3.5–4.8 mm long, about 4.5–5.0 times as long as wide; coloration yellowish to light brown, head darker, elytral apices pale yellow, several apical abdominal segments dark brown; frontoclypeal region projecting at apex; antennomeres IV to XI with rami stout, less than three times as long as wide, only slightly longer than their respective stems; terminal antennomere suboval, apically narrowly rounded; labrum fused; mandible considerably reduced, barely visible, short, subtriangular, apically sharp; labial palp with one article; prothorax 1.2 times as long as wide; pronotal disc widest anteriorly, gradually slightly narrowed posteriorly, anterior margin widely rounded, anterior angles subrectangular, posterior angles inconspicuous, blunt; scutellar shield posteriorly emarginate; elytra shortened, exposing at least half of abdomen, together about 1.3 times as long as wide, about 1.6 times as long as pronotum, slightly dehiscent; hind wing about 2.1 times as long as wide, radial cell about 2.3 times as long as wide, AP_{3+4} simple, not forked; sternite IX about 2.3 times as long as wide, apically narrowed, subacute; aedeagus with phallobase reduced, transverse, widely U-shaped; parameres robust, elongate, median lobe divided into dorsal and ventral lobes, ventral lobe distinctly reduced, transverse, T-shaped.

Comparison: This genus differs from *Cydistus* in having a smaller body, frontoclypeal region produced anteriorly (short and wide in *Cydistus*; Figs 3B, D, F, 5A, D, E), antennal rami relatively shorter and stouter (Figs 3A, B, G, H, 5A, E–I), labrum fused (free in *Cydistus*; Figs 3D, F, 5D, E), labial palp with one article (with three articles in *Cydistus*; Figs 3C, E, 5B, C), reduced hind-wing venation with a shorter radial cell (Figs 3L, 5K), AP_{3+4} simple, not forked (formed by

Figure 6. Distal abdominal segments and aedeagus of *Microcydistus minor*, adult male. A–C, apical abdominal segments; ventral, dorsal, lateral view, respectively. D, sternite IX; ventral view. E, tergites IX and X; dorsal view. F–H, aedeagus; ventral, dorsal, lateral view, respectively. I–L, details of aedeagus; ventral, posteroventral, posterodorsal, lateral view, respectively. Scale bars: A–E, 0.2 mm; F–H, 0.1 mm; I–L, 0.05 mm.

two branches in *Cydistus*; Figs 3L, 5K, L), abdominal sternite IX apically narrowed (rounded or truncate in *Cydistus*; Figs 4D, 6D), phallobase small, transverse (robust, longer than wide in *Cydistus*) and a fairly reduced ventral lobe on the median lobe (ventral lobe usually shorter than dorsal lobe but always distinct in *Cydistus*; Figs 4E–G, I, J, 6F–L).

Etymology: The generic name *Microcydistus* refers to the small size of this genus (from Greek μικρός, small) and its relationship to *Cydistus*. Gender: masculine.

Composition: Monotypic, with only ***Microcydistus minor* (Bolívar y Pieltain, 1913), comb. nov.** included. lsid: urn:lsid:zoobank.org:act:8C286DEB-E556-448F-A2C8-F7FBBB9896D8

Distribution: Iran.

DISCUSSION

Phylogenetic position of Cydistinae in Elateriformia

The attempts to investigate relationships in Elateriformia based on morphological characters have always been hampered by the high number of homoplastic characters in this group (Lawrence, 1988; Beutel, 1995; Lawrence *et al.*, 2011). The soft-bodied lineages formerly included in the now-dissolved superfamily Cantharoidea share similar morphological features, such as a soft body, variously reduced mouthparts, elytra and hind wings, freely movable abdominal segments and larviform females, but these characters have evolved independently in distantly related groups (Kundrata & Bocak, 2011; Bocak *et al.*, 2018). Cydistinae present a mosaic of characters found in various other soft-bodied lineages with modified morphology, so it is not surprising that their position in Elateriformia has always been controversial. They were at various times classified in Drilidae *s.l.*, Rhagophthalmidae, Phengodidae and Karumiinae (e.g. Lawrence & Newton, 1995; Lawrence *et al.*, 2010).

Until the current study, the placement of this group remained problematic based on the results of examination of morphology alone (Lawrence *et al.*, 1999, 2010; Lawrence, 2016a). Here, our molecular phylogenetic analyses recovered Cydistinae deeply nested in Elateroidea, in a robustly supported clade with Phengodidae and Rhagophthalmidae (Fig. 1; Supporting Information, Fig. S1). Therefore, the morphological characters earlier reported as supporting the placement of *Cydistus* in Dascillidae, such as the forked vein AP_{3+4} in the hind wing and the median lobe divided into dorsal and ventral lobes, are apparently homoplastic. Lawrence (2016b) already questioned the placement of *Cydistus* in Dascillidae, because it lacks the dascillid-like broad tentorium and modified tibial spurs, and has an elongate radial cell similar to various lineages of Elateroidea rather than Dascilloidea. After the assignment of Rhinorhipidae (Kusy *et al.*, 2018), Podabrocephalidae (Kundrata *et al.*, 2019) and now Cydistinae to their proper taxonomic ranks based on strong molecular phylogenetic evidence, no further suprageneric taxa remain as Elateriformia *incertae sedis*.

Phylogeny and morphology of the Rhagophthalmidae + Cydistinae + Phengodidae clade

The close relationships of Phengodidae and Rhagophthalmidae were proposed based on the similar morphology of their larvae and females (Crowson, 1972; Lawrence & Newton, 1995; Costa & Zaragoza-Caballero, 2010), and this was later supported by molecular studies using a variety of sampling, types of datasets and analytical approaches (e.g. Bocakova *et al.*, 2007; Sagegami-Oba *et al.*, 2007; Bocak *et al.*, 2014; Kundrata *et al.*, 2014; McKenna *et al.*, 2015; Amaral *et al.*, 2016; Zhang *et al.*, 2018). The exact position of the Phengodidae + Rhagophthalmidae clade within Elateroidea remains unclear. It is usually found either in a clade with Lampyridae (McKenna *et al.*, 2015; Timmermans *et al.*, 2015) or near Elateridae and Omalisidae (Bocak *et al.*, 2014; Kundrata *et al.*, 2014; Zhang *et al.*, 2018; this study).

Rhagophthalmidae were sometimes treated as a subfamily of Phengodidae, with *Cydistus* classified there, presumably because both Rhagophthalmidae and Cydistinae are from Asia, whereas Phengodidae, as originally defined, are restricted to the New World (e.g. Lawrence & Newton, 1995; Bocak, 2007). However, results of our molecular phylogeny strongly suggest a sister-group relationship between Cydistinae and Phengodidae, and this is also supported by the morphology of their antennae. Antennomeres II and III are both short and simple, i.e. never with rami, in Phengodidae and Cydistinae, whereas Rhagophthalmidae have antennomere III longer than antennomere II, and in cases where the antennae are serrate or pectinate, antennomere III is not simple, i.e. serration or rami begin on antennomere III. Other characters supporting the placement of Cydistinae closer to Phengodidae than to Rhagophthalmidae are variable, but can be used as additional support. Phengodidae, including Cydistinae, have usually long maxillary palpomeres (short in Rhagophthalmidae), the pronotum is usually about as long as wide or longer (distinctly transverse in Rhagophthalmidae, only

rarely transverse in Phengodidae), the scutellar shield is relatively wide, U-shaped, posteriorly emarginate (often narrowed posteriorly in Rhagophthalmidae) and the tibial spurs are usually absent (present in Rhagophthalmidae) (Costa & Zaragoza-Caballero, 2010; Kawashima *et al.*, 2010; pers. observ.). Cydistinae differ from Phengodidae in several morphological characters, such as a longer prosternum and an aedeagus with a bilobate median lobe and without apparent flagellum. Other characters previously used for the separation of Cydistinae from Phengodidae are the presence of the forked vein AP_{3+4} and long, well-separated gular sutures (Lawrence *et al.*, 2010). However, the vein AP_{3+4} formed by two branches is present only in *Cydistus*, but not in *Microcydistus* (Figs 3L, 5K, L), and an apparently forked vein AP_{3+4} can be found in at least some *Cenophengus* species, not including the type species *C. debilis* (Zaragoza-Caballero & Pérez Hernández, 2014; pers. observ.). Similarly, the gular sutures are highly variable in Phengodidae, ranging from complete to incomplete, and well separated to nearly contiguous (Costa & Zaragoza-Caballero, 2010; pers. observ.). Therefore, we consider the above-mentioned characters not sufficient for erecting a new family in Elateroidea and, instead, we subsume Cydistinae within Phengodidae.

It is common in lineages affected by neoteny that males greatly outnumber females in collections or that females are not known at all (e.g. Bocak *et al.*, 2016; Kundrata & Bocak, 2017). The latter is the case in Cydistinae, for which we examined >170 male specimens from major European collections (Supporting Information, Table S3), but their immature stages and females remain unknown. However, all known larvae and neotenic larva-like females of both Rhagophthalmidae and Phengodidae are bioluminescent, live in leaf litter and soil, and feed on millipedes (e.g. Costa *et al.*, 1999; Kawashima *et al.*, 2010), so we can hypothesize a similar biology and ecology for Cydistinae. Bioluminescence was also reported for males in some genera of Phengodidae and Rhagophthalmidae, but Cydistinae males do not bear any light-emitting organs. Discovery of bioluminescent larvae or females of Cydistinae would offer additional strong evidence of their phylogenetic position within the Rhagophthalmidae + Phengodidae clade.

Phylogeny and suprageneric classification of Phengodidae

Until recently, Phengodidae were divided into three subfamilies: Phengodinae, Mastinocerinae and Penicillophorinae (Paulus, 1974; Wittmer, 1976; O'Keefe, 2002; Costa & Zaragoza-Caballero, 2010). Zaragoza-Caballero & Pérez Hernández (2014) published the most recent systematic overview of a family with such limits. The first morphology-based phylogenetic hypothesis for the family was introduced by Zaragoza-Caballero & Zurita-García (2015). They removed Penicillophorini from Phengodidae and placed them into Telegeusidae, which in turn have been recently treated as a subfamily of Omethidae (Kundrata *et al.*, 2014; Bocak *et al.*, 2016). Further, they revealed the reciprocal paraphyly of both Phengodinae and Mastinocerinae, and they identified *Cenophengus* (Mastinocerinae) as sister to the remaining phengodids.

Our molecular phylogeny recovered a monophyletic Phengodidae including Cydistinae. The deepest split in the family conforms to biogeographic lines, with the Cydistinae exclusively Old World and Phengodinae + Mastinocerinae exclusively New World. Phengodinae were shown to be monophyletic in all analyses, but in line with the results by Zaragoza-Caballero & Zurita-García (2015), two of our three analyses placed *Cenophengus* outside of the bulk of Mastinocerinae as sister to Phengodinae + Mastinocerinae (Fig. 1). It is beyond the scope of this study to discuss the phylogenetic relationships within Phengodinae + Mastinocerinae. Unfortunately, most phengodid sequences in GenBank are not identified to genus level, because they were used for higher phylogeny of Elateroidea (Kundrata *et al.*, 2014). Furthermore, the genus *Cenophengus* itself is a complex assemblage of 23 species, even showing great variability in hind-wing venation (Zaragoza-Caballero & Pérez Hernández, 2014; Zaragoza-Caballero & Zurita-García, 2015); our analysis included only the type species, *C. debilis*. However, the placement of *Cenophengus* bridging a grade between Cydistinae and 'higher' Phengodidae is morphologically coherent. For example, adult male *Cenophengus* possess a larger prosternal plate than other Phengodidae, but not as large as that of Cydistinae, and at least one species has vein AP_{3+4} apparently forked as in *Cydistus* (see fig. 21 in Zaragoza-Caballero & Pérez Hernández, 2014).

The placement of the various species of *Cenophengus* and paraphyly of Mastinocerinae as it relates to *Cenophengus* is the chief outstanding issue in the higher classification of Phengodidae, and must be deferred to a future molecular phylogenetic study with a more comprehensive sampling of genera and multiple species of *Cenophengus*. Because of the great morphological plasticity resulting in a high level of homoplasy of characters within soft-bodied elateroids including Phengodidae (e.g. Crowson, 1972; Lawrence, 1988; Bocakova *et al.*, 2007; Kundrata & Bocak, 2011; Bocak *et al.*, 2016, 2018), this molecular phylogenetic investigation will be crucial in placing the higher classification of Phengodidae on firm footing.

Systematics and diversity of Cydistinae

Both molecular phylogeny and examination of diagnostic characters of Cydistinae revealed that *Cydistus minor* is genetically and morphologically divergent from other *Cydistus* species, and we have erected the new genus *Microcydistus* to accommodate this species. Although the statistical support for Cydistinae is low, no alternative placement of *Cydistus* and *Microcydistus* was proposed based on the molecular phylogeny. Therefore, we retain the position of both genera in the newly circumscribed Cydistinae without establishing any suprageneric rank for the new genus. Furthermore, *Microcydistus* has a minute body, and its several genus-specific diagnostic characters, such as the reduced mouthparts (e.g. labrum, mandibles and labial palpi) and hind-wing venation, are probably connected with body miniaturization. A similar tendency of males towards miniaturization and simplification or reduction of particular morphological structures was reported for various other soft-bodied neotenic lineages within Elateroidea (e.g. Bocak *et al.*, 2016; Kundrata & Bocak, 2017).

Our study revealed that both genetic and morphological diversity within Cydistinae is much higher than expected. In addition, this study at least partially uncovered the relationships among *Cydistus*, with two species from Iran sister to *Cydistus reitteri* from Israel. Additional species, especially from Turkey, should be included in a future molecular phylogeny to cover the entire distributional range of the genus. Further, an alpha-taxonomic revision of *Cydistus* should be carried out, including the descriptions of several new species identified by the first author in the examined collections. Another goal for future students of this group is to discover the immature stages and adult females in order to understand their biology and ecology, which would help increase our knowledge of the natural history and taxonomy of this group.

ACKNOWLEDGEMENTS

We thank I. Zürcher-Pfander (NHMB), J. Hájek (NMPC), M. Geiser (BMNH), A. Mantilleri, A. Taghavian (MNHN), R. Schuh (Austria), M. Jäch, H. Schillhammer, I. Plonski (NHMW), O. Merkl, T. Németh (HNHM) and H. Huijbregts (RMNH) for the loan of material used in this study, L. Friedman (The Steinhardt Museum of Natural History (SMNH), Tel Aviv University, Tel Aviv, Israel) for providing us with the DNA-grade specimen of *Cydistus reitteri*, M. París (MNCN) for the photographs of type material of Cydistinae deposited in MNCN, A. Weinstein (SMNH) for the photograph of *C. reitteri* in Figure 1, L. Gimmel (USA) for the photograph of *Zarhipis integripennis*, P. Coin (USA) for the photograph of the female *Phengodes* in Figure 1 and A. S. Roza (Brazil) for help with identification of *Stenophrixothrix* sp. This study was supported in part by the DAAD (German Academic Exchange Service) scholarship nr. 57378441 (RK), the IGA project of the UP Olomouc, Czech Republic (IGA_PrF_2019_024; RK, ES), the Moscow State University research project No. AAA A-A16-116021660095-7 (AP), the Schlinger Foundation (MLG), the Ministry of Culture of the Czech Republic (DKRVO 2019-2023/5.I.a, National Museum, 00023272; DV), and Charles University in Prague (SVV 260 434/2019; DV). We have no conflict of interest to declare.

REFERENCES

Amaral DT, **Arnoldi FGC**, **Rosa SP**, **Viviani VR. 2014.** Molecular phylogeny of Neotropical bioluminescent beetles (Coleoptera: Elateroidea) in southern and central Brazil. *Luminescence* **29:** 412–422.

Amaral DT, **Mitani Y**, **Ohmiya Y**, **Viviani VR. 2016.** Organization and comparative analysis of the mitochondrial genomes of bioluminescent Elateroidea (Coleoptera: Polyphaga). *Gene* **586:** 254–262.

Beutel RG. 1995. Phylogenetic analysis of Elateriformia (Coleoptera: Polyphaga) based on larval characters. *Journal of Zoological Systematics and Evolutionary Research* **33:** 145–171.

Beutel RG, **Leschen RAB. 2005.** Elateriformia Crowson, 1960. Introduction, phylogeny (Table 1). In: Beutel RG, Leschen RAB, eds. *Handbook of zoology, Arthropoda: Insecta; Coleoptera, beetles, Vol. 1: morphology and systematics (Archostemata, Adephaga, Myxophaga, Polyphaga partim)*. Berlin: Walter de Gruyter, 427–429.

Bocak L. 2007. Phengodidae. In: Löbl I, Smetana A, eds. *Catalogue of Palaearctic Coleoptera, Vol. 4: Elateroidea – Derodontoidea – Bostrichoidea – Lymexyloidea – Cleroidea – Cucujoidea*. Stenstrup: Apollo Books, 224–225.

Bocak L, **Barton C**, **Crampton-Platt A**, **Chesters D**, **Ahrens D**, **Vogler AP. 2014.** Building the Coleoptera tree-of-life for >8000 species: composition of public DNA data and fit with Linnaean classification. *Systematic Entomology* **39:** 97–110.

Bocak L, **Kundrata R**, **Andújar Fernández C**, **Vogler AP. 2016.** The discovery of Iberobaeniidae (Coleoptera: Elateroidea), a new family of beetles from Spain, with immatures detected by environmental DNA sequencing. *Proceedings of the Royal Society B: Biological Sciences* **283:** 20152350.

Bocak L, **Motyka M**, **Bocek M**, **Bocakova M. 2018.** Incomplete sclerotization and phylogeny: The phylogenetic classification of *Plastocerus* (Coleoptera: Elateroidea). *PLoS ONE* **13:** e0194026.

Bocakova M, **Bocak L**, **Hunt T**, **Teraväinen M**, **Vogler AP. 2007.** Molecular phylogenetics of Elateriformia (Coleoptera): evolution of bioluminescence and neoteny. *Cladistics* **23:** 477–496.

Bourgeois J. 1885. Description d'un genre nouveau et d'une espèce nouvelle de Malacodermes de la faune paléarctique. *Annales de la Société Entomologique de France* **5:** 272–274.

Costa C, Vanin SA, Casari SA, Viviani VR. 1999. Larvae of Neotropical Coleoptera. XXVII. *Phrixothrix hirtus*: immatures, neotenic female, adult male and bionomic data (Phengodinae, Phengodidae, Coleoptera). *Iheringia, Série Zoologia* **86**: 9–28.

Costa C, **Zaragoza-Caballero S. 2010.** Phengodidae LeConte, 1861. In: Leschen RAB, Beutel RG, Lawrence JF, eds. *Handbook of zoology, Arthropoda: Insecta; Coleoptera, beetles, Vol. 2: morphology and systematics (Elateroidea, Bostrichiformia, Cucujiformia partim*). Berlin: Walter de Gruyter, 126–135.

Crowson R. 1955. The natural classification of the families of Coleoptera. London: Nathaniel Lloyd & Co.

Crowson R. 1971. Observations on the superfamily Dascilloidea (Coleoptera: Polyphaga), with the inclusion of Karumiidae and Rhipiceridae. *Zoological Journal of the Linnean Society* **50:** 11–19.

Crowson R. 1972. A review of the classification of Cantharoidea (Coleoptera), with the definition of two new families: Cneoglossidae and Omethidae. *Revista de la Universidad de Madrid* **21:** 35–71.

Derocles SAP, **Plantegenest M**, **Rasplus JY**, **Marie A**, **Evans DM**, **Lunt DH**, **Le Ralec A. 2016.** Are generalist Aphidiinae (Hym. Braconidae) mostly cryptic species complexes? *Systematic Entomology* **41:** 379–391.

Goloboff PA, **Farris JS**, **Nixon KC. 2008.** TNT, a free program for phylogenetic analysis. *Cladistics* **24:** 774–786.

Hadley A. 2010. Combine ZP, Image Stacking Software. Available at: https://www.computerbild.de/download/CombineZP-5695354.html. (accessed 4 September 2014).

Huelsenbeck JP, **Ronquist F. 2001.** MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17:** 754–755.

Hunt T, **Bergsten J**, **Levkanicova Z**, **Papadopoulou A**, **John OS**, **Wild R**, **Hammond PM**, **Ahrens D**, **Balke M**, **Caterino MS**, **Gómez-Zurita J**, **Ribera I**, **Barraclough TG**, **Bocakova M**, **Bocak L**, **Vogler AP. 2007.** A comprehensive phylogeny of beetles reveals the evolutionary origins of a superradiation. *Science (New York, N.Y.)* **318:** 1913–1916.

Katoh K, **Standley DM. 2013.** MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30:** 772–780.

Kawashima I, **Lawrence JF**, **Branham MA. 2010.** Rhagophthalmidae Olivier, 1907. In: Leschen RAB, Beutel RG, Lawrence JF, eds. *Handbook of zoology, Arthropoda: Insecta; Coleoptera, beetles, Vol. 2: morphology and systematics (Elateroidea, Bostrichiformia, Cucujiformia partim*). Berlin: Walter de Gruyter, 135–140.

Kearse M, **Moir R**, **Wilson A**, **Stones-Havas S**, **Cheung M**, **Sturrock S**, **Buxton S**, **Cooper A**, **Markowitz S**, **Duran C**, **Thierer T**, **Ashton B**, **Meintjes P**, **Drummond A. 2012.** Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28:** 1647–1649.

Kundrata R, **Bocak L. 2011.** The phylogeny and limits of Elateridae (Insecta, Coleoptera): is there a common tendency of click beetles to soft-bodiedness and neoteny? *Zoologica Scripta* **40:** 364–378.

Kundrata R, **Bocak L. 2017.** Taxonomic review of Drilini (Elateridae: Agrypninae) in Cameroon reveals high morphological diversity, including the discovery of five new genera. *Insect Systematics and Evolution* **48:** 441–492.

Kundrata R, **Bocakova M**, **Bocak L. 2014.** The comprehensive phylogeny of the superfamily Elateroidea (Coleoptera: Elateriformia). *Molecular Phylogenetics and Evolution* **76:** 162–171.

Kundrata R, **Jäch MA**, **Bocak L. 2017.** Molecular phylogeny of the Byrrhoidea-Buprestoidea complex (Coleoptera, Elateriformia). *Zoologica Scripta* **46:** 150–164.

Kundrata R, **Ivie MA**, **Bocak L. 2019.** *Podabrocephalus* Pic is the morphologically modified lineage of Ptilodactylinae (Coleoptera: Elateriformia: Ptilodactylidae). *Insect Systematics and Evolution* **50**: 147–161.

Kusy D, **Motyka M**, **Andujar C**, **Bocek M**, **Masek M**, **Sklenarova K**, **Kokas F**, **Bocakova M**, **Vogler AP**, **Bocak L. 2018.** Genome sequencing of *Rhinorhipus* Lawrence exposes an early branch of the Coleoptera. *Frontiers in Zoology* **15:** 21.

Lanfear R, **Calcott B**, **Ho SY**, **Guindon S. 2012.** Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* **29:** 1695–1701.

Lawrence JF. 1988. Rhinorhipidae, a new beetle family from Australia, with commention the phylogeny of the Elateriformia. *Invertebrate Taxonomy* **2:** 1–53.

Lawrence JF. 2010. Rhinorhipidae Lawrence, 1988. In: Leschen RAB, Beutel RG, Lawrence JF, eds. *Handbook of zoology, Arthropoda: Insecta; Coleoptera, beetles, Vol. 2: morphology and systematics (Elateroidea, Bostrichiformia, Cucujiformia partim)*. Berlin: Walter de Gruyter, 38–42.

Lawrence JF. 2016a. Classification (families & subfamilies). In: Beutel RG, Leschen RAB, eds. *Handbook of zoology, Arthropoda: Insecta; Coleoptera, beetles, Vol. 1: morphology and systematics (Archostemata, Adephaga, Myxophaga, Polyphaga partim), 2nd edn*. Berlin: Walter de Gruyter, 13–22.

Lawrence JF. 2016b. Dascillidae Guérin-Méneville, 1843. In: Beutel RG, Leschen RAB, eds. *Handbook of zoology, Arthropoda: Insecta; Coleoptera, beetles, Vol. 1: morphology and systematics (Archostemata, Adephaga, Myxophaga, Polyphaga partim), 2nd edn*. Berlin: Walter de Gruyter, 531–537.

Lawrence JF, **Newton AF. 1982.** Evolution and classification of beetles. *Annual Reviews of Ecology and Systematics* **13:** 261–290.

Lawrence JF, **Newton AF. 1995.** Families and subfamilies of Coleoptera (with selected genera, notes, references and data on family-group names). In: Pakaluk J, Ślipiński SA, eds. *Biology, phylogeny, and classification of Coleoptera. Papers celebrating the 80th birthday of Roy A. Crowson, Vol. 1–2*. Warsaw: Muzeum i Instytut Zoologii PAN, 779–1083.

Lawrence JF, **Ślipiński SA**, **Pakaluk J. 1995.** From Latreille to Crowson: a history of the higher-level classification of beetles. In: Pakaluk J, Ślipiński SA, eds. *Biology, phylogeny, and classification of Coleoptera. Papers celebrating the 80th birthday of Roy A. Crowson, Vol. 1–2*. Warsaw: Muzeum i Instytut Zoologii PAN, 87–154.

Lawrence JF, **Hastings AM**, **Dallwitz MJ**, **Paine TA**, **Zurcher EJ. 1999.** *Beetles of the world: a key and information system for families and subfamilies. CD-ROM, version 1.0 for MS-Windows.* Melbourne: CSIRO Publishing.

Lawrence JF, **Kawashima I**, **Branham MA. 2010.** Elateriformia *Incertae sedis*. In: Leschen RAB, Beutel RG, Lawrence JF, eds. *Handbook of zoology, Arthropoda: Insecta; Coleoptera, beetles, Vol. 2: morphology and systematics (Elateroidea, Bostrichiformia, Cucujiformia partim)*. Berlin: Walter de Gruyter, 162–177.

Lawrence JF, **Ślipiński A**, **Seago AE**, **Thayer MK**, **Newton AF**, **Marvaldi AE. 2011.** Phylogeny of the Coleoptera based on morphological characters of adults and larvae. *Annales Zoologici* **61:** 1–217.

Martin GJ, **Branham MA**, **Whiting MF**, **Bybee SM. 2017.** Total evidence phylogeny and the evolution of adult bioluminescence in fireflies (Coleoptera: Lampyridae). *Molecular Phylogenetics and Evolution* **107:** 564–575.

McKenna DD, **Wild AL**, **Kanda K**, **Bellamy CL**, **Beutel RG**, **Caterino MS**, **Farnum CW**, **Hawks DC**, **Ivie MA**, **Jameson ML**, **Leschen RAB**, **Marvaldi AE**, **McHugh JV**, **Newton AF**, **Robertson JA**, **Thayer MK**, **Whiting MF**, **Lawrence JF**, **Ślipiński A**, **Maddison DR**, **Farrell BD. 2015.** The beetle tree of life reveals that Coleoptera survived end-Permian mass extinction to diversify during the Cretaceous terrestrial revolution. *Systematic Entomology* **40:** 835–880.

Miller MA, **Pfeiffer W**, **Schwartz T. 2010.** Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing Environments Workshop (GCE)*, 14 Nov. 2010, New Orleans, Louisiana, 1–8.

O'Keefe ST. 2002. Phengodidae LeConte 1861. In: Arnett RH, Thomas MC, Skelley PE, Frank JH, eds. *American beetles, Vol. 2. Polyphaga: Scarabaeoidea through Curculionoidea*. Boca Raton: CRC Press LLC, 181–186.

Olivier E. 1910. Rhagophthalmidae, Drilidae. In: Schenkling S, ed. *Coleopterorum catalogus, Pars 10*. Berlin: W. Junk, 1–10.

Paulus HF. 1972. Die systematische und phylogenetische Stellung der Karumiidae, mit einer Beschreibung von *Escalerina serraticornis* n. sp. aus S-Persien. *Senckenbergiana Biologica* **53:** 37–54.

Paulus HF. 1974. *Penicillophorus ctenotarsus* n. gen. et n. sp. aus Kolumbien, mit einer Beschreibung einer neuen Tribus Penicillophorini der Phengodidae (Col., Polyphaga, Cantharoidea). *Zeitschrift der Arbeitsgemeinschaft Österreichischer Entomologen* **25:** 69–80.

Rambaut A. 2010. *FigTree v.1.3.1*. Edinburgh: Institute of Evolutionary Biology, University of Edinburgh. Available at: http://tree.bio.ed.ac.uk/software/figtree/ (accessed 4 September 2014).

Rambaut A, **Drummond AJ**, **Xie D**, **Baele G**, **Suchard MA. 2018.** Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* **67:** 901–904.

Resch MC, **Shrubovych J**, **Bartel D**, **Szucsich NU**, **Timelthaler G**, **Bu Y**, **Walzl M**, **Pass G. 2014.** Where taxonomy based on subtle morphological differences is perfectly mirrored by huge genetic distances: DNA barcoding in Protura (Hexapoda). *PLoS ONE* **9:** e90653.

Sagegami-Oba R, **Takahashi N**, **Oba Y. 2007.** The evolutionary process of bioluminescence and aposematism in cantharoid beetles (Coleoptera: Elateroidea) inferred by the analysis of 18S ribosomal DNA. *Gene* **400:** 104–113.

Ślipiński SA, **Leschen RAB**, **Lawrence JF. 2011.** Order Coleoptera Linnaeus, 1758. In: Zhang ZQ, ed. *Animal biodiversity: an outline of higher-level classification and survey of taxonomic richness. Zootaxa* **3148:** 203–208.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22:** 2688–2690.

Stamatakis A, **Hoover P**, **Rougemont J. 2008.** A rapid bootstrap algorithm for the RAxML Web servers. *Systematic Biology* **57:** 758–771.

Tamura K, **Stecher G**, **Peterson D**, **Filipski A**, **Kumar S. 2013.** MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30:** 2725–2729.

Timmermans MJ, **Vogler AP. 2012.** Phylogenetically informative rearrangements in mitochondrial genomes of Coleoptera, and monophyly of aquatic elateriform beetles (Dryopoidea). *Molecular Phylogenetics and Evolution* **63:** 299–304.

Timmermans MJ, **Barton C**, **Haran J**, **Ahrens D**, **Culverwell CL**, **Ollikainen A**, **Dodsworth S**, **Foster PG**, **Bocak L**, **Vogler AP. 2015.** Family-level sampling of mitochondrial genomes in Coleoptera: compositional heterogeneity and phylogenetics. *Genome Biology and Evolution* **8:** 161–175.

Wittmer W. 1944. Catalogue des Drilidae E. Oliv. (Coleoptera - Malacodermata). *Revista de la Sociedad Entomológica Argentina* **12:** 203–221.

Wittmer W. 1976. Arbeiten zu einer Revision der Familie Phengodidae (Coleoptera). *Entomologische Arbeiten aus dem Museum G. Frey Tutzing bei München* **27:** 415–524.

Wittmer W. 1979. 64. Beitrag zur Kenntnis der palaearktischen Cantharidae Phengodidae und Malachiidae (Col.). *Entomologica Basiliensia* **4:** 327–346.

Xia X, **Lemey P. 2009.** Assessing substitution saturation with DAMBE. In: Lemey P, Salemi M, Vandamme AM, eds. *The phylogenetic handbook: a practical approach to DNA and protein phylogeny, 2nd edn*. Cambridge: Cambridge University Press, 615–630.

Xia X, **Xie Z**, **Salemi M**, **Chen L**, **Wang Y. 2003.** An index of substitution saturation and its application. *Molecular Phylogenetics and Evolution* **26:** 1–7.

Zaragoza-Caballero S, **Pérez Hernández CX. 2014.** *Sinopsis de la familia Phengodidae (Coleoptera): trenecitos, bigotudos, glow-worms, railroad-worms o besouros trem de ferro*. México, D.F.: Instituto de Biología, Universidad Nacional Autónoma de México.

Zaragoza-Caballero S, **Zurita-García ML. 2015.** A preliminary study on the phylogeny of the family Phengodidae (Insecta: Coleoptera). *Zootaxa* **3947:** 527–542.

Zhang SQ, **Che LH**, **Li Y**, **Dan Liang**, **Pang H**, **Ślipiński A**, **Zhang P. 2018.** Evolutionary history of Coleoptera revealed by extensive sampling of genes and species. *Nature Communications* **9:** 205.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Figure S1. Phylogenetic hypothesis for Elateriformia based on the ML analysis performed on the 255-taxa dataset aligned by MAFFT. Numbers associated with the branches indicate ML bootstrap values and Bayesian posterior probabilities, respectively. Only values above 50% are shown.
Table S1. List of sequences used in the analysis of the 'Phengodidae' dataset, with GenBank accession numbers and voucher numbers.
Table S2. Primers used for PCR amplification of the studied gene fragments.
Table S3. List of Cydistinae material examined in this study.
Table S4. Results of Xia's nucleotide substitution saturation test in DAMBE.