

The phylogeny and limits of Elateridae (Insecta, Coleoptera): is there a common tendency of click beetles to soft-bodiedness and neoteny?

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Phylogenetic relationships in Elateroidea were investigated using partial 18S and 28S rDNA and *rrnl* and *cox1* mtDNA sequences with special interest in the phylogeny of Elateridae and the position of soft-bodied lineages Drilidae and Omalisidae that had been classified as families in the cantharoid lineage of Elateroidea until recently. Females in these groups are neotenic and almost completely larviform (Drilidae) or brachypterous (Omalisidae). The newly sequenced individuals of Elateridae, Drilidae, Omalisidae and Eucnemidae were merged with previously published datasets and analysed matrices include either 155 taxa with the complete representation of fragments or 210 taxa when some fragments were missing. The main feature of inferred phylogenetic trees was the monophyly of Phengodidae + Rhagophthalmidae + Omalisidae + Elateridae + Drilidae with Omalisidae regularly occupying a basal node in the group; Drilidae were embedded as a terminal lineage in the elaterid subfamily Agrypninae and soft-bodied Cebriioninae were a part of Elaterinae. The soft-bodied males and incompletely metamorphosed females originated at least three times within the wider Elateridae clade. Their atypical morphology has been considered as a result of long evolutionary history and they were given an inappropriately high rank in the previous classifications. The frequent origins of these modifications seem to be connected with modifications of the hormonal regulation of the metamorphosis. The superficial similarity with other soft-bodied lineages, such as Cantharidae, Lycidae, Lampyridae, Phengodidae and Rhagophthalmidae is supposed to be a result of homoplasious modifications of the ancestral elateroid morphology due to the incomplete metamorphosis. The results of phylogenetic analyses are translated in the formal taxonomic classification. Most Drilidae are placed in Elateridae as a tribe Drilini in Agrypninae, whilst *Pseudeuonoma* and *Euanoma* are transferred from Drilidae to Omalisidae. The subfamily Cebriioninae is placed in Elaterinae as tribes Cebriionini and Aplastini. Oxynopterini, Pityobiini and Semiotini are lowered from the subfamily rank to tribes and classified in Denticollinae.

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Introduction

Elateroidea represent a very diverse lineage with a long evolutionary history (Whalley 1985; Hunt *et al.* 2007), but relationships between 17 recognized families remain contentious due to presence of the morphologically distant forms, well-sclerotized true elateroids and soft bodied and often neotenic cantharoids, which are supposed to be a result of the heterochronic modifications (Lawrence 1988; Bocakova *et al.* 2007; Bocak *et al.* 2008). The modifications

of morphology have presented systematic biologists with a number of special challenges and the morphology-based classifications lack consensus (Crowson 1955, 1960, 1972, 1973; Lawrence 1988; Beutel 1995; Muona 1995; Branham & Wenzel 2003; Beutel *et al.* 2007).

The soft-bodied beetles were for a long-time classified Malacodermata, but the relationships between cantharoid and elateroid families were recognized as the beginning of the phylogenetic approach to beetle classification (for a

review Lawrence *et al.* 1995b) and Cantharoidea were classified in Elateriformia as a superfamily in a sister position to Elateroidea (Crowson 1955, 1960, 1972). Later, both morphologically disparate lineages were placed in Elateroidea (Lawrence 1988; Lawrence & Newton 1995). Despite the general acceptance of the wider definition of Elateroidea, most recent studies have addressed separately either the ‘elateroid’ or ‘cantharoid’ lineage (Calder *et al.* 1993; Muona 1995; Branham & Wenzel 2003; Lawrence *et al.* 2007; Stanger-Hall *et al.* 2007) or soft-bodied cantharoid families were represented by a single lineage in the analyses of morphological datasets (Lawrence 1988; Lawrence *et al.* 1995a). Only Beutel (1995) analysed the larval morphology of both lineages. Bocakova *et al.* (2007) and Sagegami-Oba *et al.* (2007b) proposed phylogenetic hypotheses on the evolution of Elateroidea based on molecular datasets, but also their sampling was biased to the soft-bodied lineages. Both the authors demonstrated monophyly of widely defined Elateroidea and Omalidae and Drilidae were recovered as close relatives of Elateridae, but they were represented by a single species each and Elateridae were represented only by a few taxa. These studies showed that the soft-bodied forms originated several times in unrelated lineages. Female neoteny, as a pronounced form of the same modification of ontogeny, also evolved multiple times even within a single family (Bocak *et al.* 2008). Herein, having a newly produced dataset, we are focussing on a number of soft-bodied lineages in the vicinity of Elateridae, whose males are soft-bodied and females are brachypterous (Elateridae: Cebrioninae, Omalidae) or larviform (Drilidae).

Click beetles with some 10 000 described species are the largest family of Elateroidea. Their morphology-based classification remains uncertain and relies mainly on older studies (e.g. Hyslop 1917; Stibick 1979) or partial analyses focusing on position and phylogeny of a single lineage (Calder *et al.* 1993; Muona 1995; Lawrence *et al.* 2007; Vahtera *et al.* 2009). Conflicting opinions were published on the relationships of Throscidae, Eucnemidae and Elateridae, the position of Lissominae or Thylacosterninae, and definitions of the subfamilies of Elateridae (for a review Costa *et al.* 2010). Soft-bodied Cebrioninae were classified either as a family or click beetle subfamily and numerous elaterid lineages were given subfamily status despite their unclear relationships. Sagegami-Oba *et al.* (2007a) used partial 28S rDNA sequences to investigate molecular phylogeny of Elateridae and identified four major clades representing (i) Elaterinae, (ii) Agrypninae, (iii) Denticollinae and (iv) Negastrinae and Cardiophorinae.

Drilidae and Omalidae have similarly contentious position and limits. Despite low numbers of species, only eight

species of Omalidae and about 120 species of Drilidae, the limits of both families remain uncertain and neither of these families has been defined by a clear synapomorphy (Lawrence 1988; Bocak 2007; Kundrata & Bocak 2007; Bocak & Brlik 2008; Bocak *et al.* 2010). Traditionally, numerous genera were classified in Drilidae (Wittmer 1944) until Crowson (1972) redefined the family. Unfortunately, Crowson studied in detail only a fraction of the 35 genera listed by Wittmer and therefore many genera remained in an uncertain position. Several genera were transferred to Rhagophthalmidae or Lampyridae, *Drilonius* to Omethidae and *Platerodrillus* to Lycidae (Crowson 1972). Further, *Pseudeuanoma* was transferred from Omalidae to Drilidae. Lawrence & Newton (1995) listed only genera *Drilus*, *Selasia* and *Pseudeuanoma* in Drilidae, but *Malacogaster*, closely related to *Drilus*, must be classified here (Kundrata & Bocak 2007). Bocak & Brlik (2008) revised Omalidae.

Elateroid females often retain apparent larval traits when sexually mature and these heterochronic modifications deserve attention when morphology is analysed (Lawrence 1988; Bocakova *et al.* 2007; Bocak *et al.* 2008). There is a wide variability in the completeness of the metamorphosis. The modifications range from the females with vestigial wings and almost adult-like thorax (Omalidae, Lampyridae part, Elateridae: Cebrioninae, part), through wingless females (Lampyridae part), larviform females with only mouthparts and the head adult-like (Drilidae) to the completely larviform females (Lycidae part). Despite such variability of the neotenic modifications in females, the soft-bodied appearance of all males was perceived as a synapomorphy of Cantharoidea (Crowson 1972) and the characters potentially affected by neoteny were widely employed for definitions of the higher taxa. The hypothesis on frequent shifts to the neotenic development pathway opens a possibility that the morphological disparity supporting the concepts of the families like Drilidae and Omalidae or the elateroid subfamily Cebrioninae is not a result of an independent evolutionary history, but a consequence of the modified endocrine system. Postembryonic development is regulated through the actions of ecdysone and the juvenile hormone and the modifications of the hormonal system easily lead to morphologically distant forms (Erezylmaz *et al.* 2006; Konopova & Jindra 2007, 2009). Therefore, we suggest that multiple neotenic shifts may result in homoplasious modifications of the morphology in unrelated lineages and that relationships of the affected lineages are difficult to interpret using morphology-based analyses.

Considering the number of unscorable characters due to the absence of metamorphosed females, unknown larvae and uncertain homology assessments we prefer to analyse

molecular data as the only source of information. We intend to test the hypothesis that (i) the soft-bodied lineages classified in Drilidae are a part of the click beetles lineage; (ii) Omalidae are closely related to Elateridae and consequently, (iii) the modification of ontogeny is a common phenomenon in click beetles and closest relatives. With a large dataset proportionally representing most elateroid families, we expect from this phylogenetic investigation the reassessment of the phylogeny of Elateroidea and Elateridae and better understanding of the evolution of neoteny. The proposed classification is likely to be controversial because some lineages, such as Omalidae, Drilidae and Phengodidae superficially resemble fireflies or net-winged beetles, but they are now classified as closely related to click beetles exclusively on the basis of molecular data. We argue that the previous classifications were based on highly homoplasious characters influenced by the neotenic reprogramming.

Materials and methods

Sampling and dataset assembling

To investigate the alternative hypotheses on the position of elateroid soft-bodied families, we produced a new extensive sampling of click beetles and Drilidae and we explored previously produced Elateroidea datasets. Altogether 11 elateroid and 15 outgroup families (Scirtoidea, Dascilloidea, Byrrhoidea and Buprestoidea) were included in the analyses (Tables 1 and S1). The substantial part of the previously published sequences was produced by the A. P. Vogler's group (Ignacio Ribera, unpublished data deposited in Genbank; Caterino *et al.* 2002; Bocakova *et al.* 2007; Hunt *et al.* 2007; Bocak *et al.* 2008). All these taxa were included in the analyses and they are listed in the Table S1 (Supporting information). The sequences of 18S rDNA published by Sagegami-Oba *et al.* (2007b) do not have any other marker and the partial sequences of 18S rDNA published by Stanger-Hall *et al.* (2007) are short (800 bp) and therefore were omitted. We produced a new set of 18S rDNA, 28S rDNA, *rrnL* and *cox1* mtDNA sequences for Elateroidea (Table 1). Drilidae were represented by *Selasia* (two species), *Drilus* (six species) and *Pseudeuanoia* (two species); Omalidae by *Omalisus fontisbellaquaei* and *O. sanguinipennis*.

Two datasets were assembled from the available data. The complete dataset included all taxa with full-length 18S rDNA and at least two additional markers (210 taxa) and the pruned dataset included only taxa with all four genes available but omalids and drilids were kept in the set to investigate their position even when some fragment was missing (155 taxa). Although a total evidence approach may be preferred, it was shown that high level of ambiguity in data might lead to incorrect phylogeny esti-

mation (Lemmon *et al.* 2009). Both datasets were analysed in full length and with 18S and 28S length variable regions excluded as their alignment is often ambiguous.

DNA extraction, PCR amplification and sequencing

All specimens were preserved in 96% alcohol in the field and kept in -20°C until isolation of DNA. Total DNA was extracted from thoracic muscles and legs using the phenol-chloroform protocol (Vogler *et al.* 1993). The 18S rDNA (1 900 bp) was amplified as four overlapping fragments. The other markers were amplified in a single fragment: a portion of 28S rDNA (420–810 bp), *rrnL* mtDNA (640 bp) and *cox1* mtDNA (810 bp). The fragments were amplified using 0.5–0.6 U Taq polymerase (BioTaq DNA Polymerase, Bionline), 1 mM MgCl_2 , 50 μM each dNTP, 0.2 μM each primer and typically 0.03 μg of the template in 50 μL reaction volume. Cycle conditions were generally 2 min at 94°C ; 30–60 s at 94°C , 30–60 s at $45\text{--}52^{\circ}\text{C}$ (depending on the melting temperatures of primer pair used), 1–2 min at 72°C (repeated for 30–40 cycles) and 10 min at 72°C . The primers are listed in the Table S2. The amplification products were purified using a Gene CleanIII kit (Bio 101 Systems/Qbiogene, Inc., Solon, OH, USA). The ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit was used for cycle sequencing. Fragments were sequenced using an ABI 3130 genetic analyser.

Sequence handling and alignment

Sequences were edited using the SEQUENCHER 4.7 software package (Gene Codes Corp., Ann Arbor, MI, USA). As insertion-deletion differences were common in all ribosomal genes, two approaches were applied to the alignment of ribosomal genes. The *cox1* mtDNA sequences were aligned under default settings and attached to the datasets. All four markers were combined for the final analyses.

The rDNA sequences were aligned by CLUSTALX version 1.8 (Thompson *et al.* 1997) under a range of five settings (opening: extension gap penalty 5:1, 10:6.66, 15:6.66, 20:6.66 and 25:6.66). The variable regions were ambiguously aligned under all settings and subsequent parsimony analyses showed inconsistent phylogenetic signal across individual alignments. Therefore, we did not explore the alignment parameters further, used only alignment under the default setting 15:6.66, and excluded length variable regions in most analyses of Clustal alignments. We created four datasets aligned by CLUSTALX: (i) the full number of 210 taxa and complete sequences including ambiguously aligned indels, (ii) the full number of 210 taxa and without ambiguously aligned indels of 18S and 28S rDNA, (iii) the dataset without taxa with missing fragments (155 taxa) and including ambiguously aligned loop regions and (iv) the same set of 155 taxa without ambiguously aligned loops.

Table 1 The list of the sequenced samples. The Genbank voucher numbers have format “UPOL” and the six digit designation listed below. Previously reported sequences used in the analyses are given in the Supplementary Table S1.

Subfamily tribe	Genus/species	Geographic origin	Markers				Specimen voucher
			18S	28S	<i>rnl</i>	<i>cox1</i>	
Elateridae							
Agrypninae							
Agrypnini	<i>Agrypnus</i> sp.	Japan	HQ333757	HQ333852	HQ333676	HQ333941	RK0004
Agrypnini	<i>Agrypnus</i> sp.	Indonesia	HQ333783	HQ333878	HQ333697	HQ333965	RK0030
Agrypnini	<i>Agrypnus</i> sp.	Japan	HQ333810	HQ333905	HQ333719	HQ333991	RK0057
Agrypnini	<i>Agrypnus</i> sp.	Indonesia	HQ333820	HQ333915	HQ333727	HQ334000	RK0067
Agrypnini	<i>Adelocera</i> sp.	Japan	HQ333772	HQ333867	HQ333689	HQ333955	RK0019
Agrypnini	<i>Adelocera</i> sp.	Japan	HQ333778	HQ333873	HQ333694	HQ333961	RK0025
Agrypnini	<i>Adelocera</i> sp.	Indonesia	HQ333794	HQ333889	N	HQ333976	RK0041
Agrypnini	<i>Adelocera</i> sp.	Indonesia	HQ333806	HQ333901	HQ333715	HQ333987	RK0053
Agrypnini	<i>Adelocera</i> sp.	Indonesia	HQ333815	HQ333910	N	N	RK0062
Agrypnini	<i>Adelocera</i> sp.	Japan	HQ333817	HQ333912	HQ333724	HQ333997	RK0064
Agrypnini	<i>Adelocera</i> sp.	Malaysia	HQ333818	HQ333913	HQ333725	HQ333998	RK0065
Agrypnini	<i>Lacon</i> sp.	Indonesia	HQ333789	HQ333884	N	HQ333971	RK0036
Monocrepidiiini	<i>Conoderus</i> sp.	Panama	HQ333746	HQ333841	HQ333665	HQ333931	001416
Monocrepidiiini	indet.	Panama	HQ333747	HQ333842	HQ333666	HQ333932	001417
Monocrepidiiini	<i>Drasterius bimaculatus</i>	Slovakia	HQ333793	HQ333888	HQ333704	HQ333975	RK0040
Monocrepidiiini	<i>Drasterius</i> sp.	Morocco	HQ333816	HQ333911	HQ333723	HQ333996	RK0063
Monocrepidiiini	indet.	Malaysia	HQ333798	HQ333893	HQ333708	HQ333980	RK0045
Platycrepidiiini	<i>Platycrepidius</i> sp.	Panama	HQ333748	HQ333843	HQ333667	HQ333933	001418
Pyrophorini	<i>Pyrophorus</i> sp.	Panama	HQ333751	HQ333846	HQ333670	HQ333936	001421
Pyrophorini	<i>Pyrophorus</i> sp.	Panama	HQ333753	HQ333848	HQ333672	N	001423
Hemirhipini	<i>Chalcolepidius</i> sp.	Panama	HQ333752	HQ333847	HQ333671	HQ333937	001422
Hemirhipini	<i>Cryptalaus</i> sp.	Japan	HQ333768	HQ333863	HQ333685	HQ333951	RK0015
Hemirhipini	<i>Cryptalaus</i> sp.	Indonesia	HQ333781	HQ333876	N	N	RK0028
Hemirhipini	<i>Cryptalaus</i> sp.	Malaysia	HQ333834	HQ333926	HQ333740	HQ334014	RK0082
Drilini	<i>Selasia</i> sp.	South Africa	HQ333824	HQ333919	HQ333731	HQ334004	RK0071
Drilini	<i>Selasia</i> sp.	South Africa	HQ333825	HQ333920	HQ333732	HQ334005	RK0072
Drilini	<i>Drilus</i> sp.	Greece	HQ333826	HQ333921	HQ333733	HQ334006	RK0073
Drilini	<i>Drilus concolor</i>	Hungary	HQ333827	N	HQ333734	HQ334007	RK0074
Drilini	<i>Drilus</i> sp.	Turkey	N	N	HQ333739	HQ334013	RK0081
Drilini	<i>Drilus</i> sp.	Spain	HQ333836	HQ333927	HQ333742	HQ334015	RK0084
Drilini	<i>Drilus</i> sp.	Spain	HQ333837	HQ333928	HQ333743	HQ334016	RK0085
Cardiophorinae							
	<i>Cardiophorus erichsoni</i>	Slovakia	HQ333790	HQ333885	HQ333701	HQ333972	RK0037
	<i>Dicronychus rubripes</i>	Slovakia	HQ333764	HQ333859	N	HQ333947	RK0011
	<i>Dicronychus cinereus</i>	Czech Republic	HQ333776	HQ333871	N	HQ333959	RK0023
	indet.	Indonesia	HQ333784	HQ333879	N	HQ333966	RK0031
	indet.	Indonesia	HQ333788	HQ333883	N	HQ333970	RK0035
	indet.	Morocco	HQ333823	HQ333918	HQ333730	HQ334003	RK0070
Denticollinae							
Denticollini	<i>Athous vittatus</i>	Czech Republic	HQ333755	HQ333850	HQ333674	HQ333939	RK0002
Denticollini	<i>Denticollis</i> sp.	Japan	HQ333759	HQ333854	N	HQ333943	RK0006
Denticollini	<i>Nothodes parvulus</i>	Slovakia	HQ333763	HQ333858	HQ333681	N	RK0010
Denticollini	<i>Limonium quercus</i>	Czech Republic	HQ333775	HQ333870	HQ333692	HQ333958	RK0022
Denticollini	<i>Cidnopus pilosus</i>	Slovakia	HQ333792	HQ333887	HQ333703	HQ333974	RK0039
Ctenicerini	<i>Anostirus purpureus</i>	Slovakia	HQ333761	HQ333856	HQ333679	HQ333945	RK0008
Ctenicerini	<i>Neopristilophus serrifer</i>	Japan	HQ333765	HQ333860	HQ333682	HQ333948	RK0012
Ctenicerini	<i>Selatosomus latus</i>	Czech Republic	HQ333774	HQ333869	HQ333691	HQ333957	RK0021
Ctenicerini	indet.	Japan	HQ333787	HQ333882	HQ333700	HQ333969	RK0034
Dimini	<i>Platiana</i> sp.	Indonesia	HQ333782	HQ333877	HQ333696	HQ333964	RK0029
Hypnoidini	<i>Hypolithus</i> sp.	Japan	HQ333795	HQ333890	HQ333705	HQ333977	RK0042
Semiotini	<i>Semiotus</i> sp.	Chile	HQ333799	HQ333894	HQ333709	HQ333981	RK0046
Oxynopterini	<i>Oxynopterus</i> sp.	Philippines	HQ333800	HQ333895	HQ333710	HQ333982	RK0047
Elaterinae							

Table 1 Continued.

Subfamily tribe	Genus/species	Geographic origin	Markers				Specimen voucher
			18S	28S	<i>mtl</i>	<i>cox1</i>	
Aplastini	<i>Octinodes</i> sp.	Panama	HQ333749	HQ333844	HQ333668	HQ333934	001419
Elaterini	<i>Elater</i> sp.	Japan	HQ333766	HQ333861	HQ333683	HQ333949	RK0013
Elaterini	<i>Tomicephalus</i> sp.	Panama	HQ333750	HQ333845	HQ333669	HQ333935	001420
Elaterini	<i>Mulsanteus</i> sp.	Panama	HQ333744	HQ333839	N	HQ333929	001414
Elaterini	indet.	Indonesia	HQ333773	HQ333868	HQ333690	HQ333956	RK0020
Elaterini	indet.	Indonesia	HQ333819	HQ333914	HQ333726	HQ333999	RK0066
Elaterini	<i>Ludioschema</i> sp.	Japan	HQ333777	HQ333872	HQ333693	HQ333960	RK0024
Elaterini	<i>Ludioschema</i> sp.	Indonesia	HQ333808	HQ333903	HQ333717	HQ333989	RK0055
Dicrepidini	<i>Anoplischius</i> sp.	Panama	HQ333745	HQ333840	HQ333664	HQ333930	001415
Ampedini	<i>Ampedus</i> sp.	Japan	HQ333758	HQ333853	HQ333677	HQ333942	RK0005
Ampedini	<i>Amp. sanguinolentus</i>	Slovakia	HQ333760	HQ333855	HQ333678	HQ333944	RK0007
Ampedini	<i>Ampedus rufipennis</i>	Slovakia	HQ333762	HQ333857	HQ333680	HQ333946	RK0009
Ampedini	<i>Ampedus</i> sp.	Japan	HQ333771	HQ333866	HQ333688	HQ333954	RK0018
Ampedini	<i>Ampedus sinuatus</i>	Slovakia	HQ333791	HQ333886	HQ333702	HQ333973	RK0038
Ampedini	<i>Ampedus sinuatus</i>	Czech Republic	HQ333822	HQ333917	HQ333729	HQ334002	RK0069
Agriotini	<i>Agriotes acuminatus</i>	Czech Republic	HQ333756	HQ333851	HQ333675	HQ333940	RK0003
Agriotini	<i>Agriotes ustulatus</i>	Czech Republic	HQ333786	HQ333881	HQ333699	HQ333968	RK0033
Agriotini	<i>Agriotes obscurus</i>	Czech Republic	HQ333805	HQ333900	N	N	RK0052
Megapenthini	indet.	Japan	HQ333767	HQ333862	HQ333684	HQ333950	RK0014
Adrastini	indet.	Japan	HQ333769	HQ333864	HQ333686	HQ333952	RK0016
Adrastini	indet.	Japan	HQ333770	HQ333865	HQ333687	HQ333953	RK0017
Adrastini	indet.	Japan	HQ333779	HQ333874	HQ333695	HQ333962	RK0026
Adrastini	indet.	Japan	HQ333780	HQ333875	N	HQ333963	RK0027
Adrastini	indet.	Malaysia	HQ333801	HQ333896	HQ333711	HQ333983	RK0048
Adrastini	indet.	Malaysia	HQ333812	HQ333907	HQ333720	HQ333993	RK0059
Melanotini	<i>Melanotus villosus</i>	Czech Republic	HQ333754	HQ333849	HQ333673	HQ333938	RK0001
Melanotini	<i>Priopus ornatus</i>	Laos	HQ333785	HQ333880	HQ333698	HQ333967	RK0032
Melanotini	<i>Priopus humeralis</i>	Indonesia	HQ333821	HQ333916	HQ333728	HQ334001	RK0068
Physorhinini	<i>Anchastus</i> sp.	Indonesia	HQ333804	HQ333899	HQ333714	HQ333986	RK0051
Physorhinini	<i>Anchastus</i> sp.	Indonesia	HQ333809	HQ333904	HQ333718	HQ333990	RK0056
Physorhinini	<i>Anchastus</i> sp.	Indonesia	HQ333813	HQ333908	HQ333721	HQ333994	RK0060
Physorhinini	<i>Anchastus</i> sp.	Indonesia	HQ333814	HQ333909	HQ333722	HQ333995	RK0061
Lissominae	<i>Drapetes</i> sp.	Czech Republic	HQ333828	HQ333922	HQ333735	HQ334008	RK0075
Negastrinae	<i>Zorochros</i> sp.	Malaysia	HQ333796	HQ333891	HQ333706	HQ333978	RK0043
	<i>Zorochros</i> sp.	Malaysia	HQ333797	HQ333892	HQ333707	HQ333979	RK0044
	<i>Quasimus</i> sp.	Malaysia	HQ333802	HQ333897	HQ333712	HQ333984	RK0049
	<i>Quasimus</i> sp.	Malaysia	HQ333803	HQ333898	HQ333713	HQ333985	RK0050
	indet.	Malaysia	HQ333811	HQ333906	N	HQ333992	RK0058
Omalisidae	<i>Omalisus sanguinipennis</i>	Croatia	HQ333835	N	HQ333741	N	RK0083
	<i>Pseudeuonoma</i> sp.	Greece	HQ333832	N	HQ333738	HQ334011	RK0079
	<i>Pseudeuonoma</i> sp.	Turkey	HQ333833	N	N	HQ334012	RK0080
Eucnemidae	<i>Anischia</i> sp.	New Caledonia	HQ333838	N	EU128149*	HQ334017	RK0086
Anischiinae	indet.	Indonesia	HQ333807	HQ333902	HQ333716	HQ333988	RK0054
	indet.	Indonesia	HQ333829	HQ333923	HQ333736	HQ334009	RK0076
	indet.	Indonesia	HQ333830	HQ333924	HQ333737	HQ334010	RK0077
	indet.	Indonesia	HQ333831	HQ333925	N	N	RK0078

*From GenBank (Vahtera et al. 2009).

Further, the datasets were aligned by BlastAlign using default parameters. The BlastAlign was used to align nucleic rDNA sequences with large indels that are difficult

to align globally (Belshaw & Katzourakis 2005). jMODELTEST v. 0.1.1 was used for model selection (Posada 2008).

Phylogenetic analyses

All alignments were subsequently analysed using parsimony, maximum likelihood and Bayesian methods. The phylogenetic analyses of all datasets described above were carried out under the parsimony criterion (MP) using TNT 1.0 (Goloboff *et al.* 2003). Equal weights were given to all positions and gaps were treated as a fifth character. New technology search was applied to find the shortest tree 10 times. The trees were subsequently rooted by *Declinia versicolor* or *Cyphon hilaris*, which are hypothesized as members of basal Polyphaga (Hunt *et al.* 2007). We used the bootstrap values to measure branch support. For bootstrap analysis 500 pseudoreplicates were generated with the shortest tree found three times in each search. Each marker, genomic rDNA and mtDNA markers were analysed separately using the same settings of the parsimony analysis as described above to investigate the potential conflict in the signal.

The datasets of 155 and 210 taxa produced by CLUSTALX without ambiguously aligned loop regions and by BlastAlign were analysed using MRBAYES v. 3.1.2 (Huelsenbeck & Ronquist 2001), where tree topology and evolutionary model parameters were permuted using a Markov chain Monte Carlo method (MCMC). The MCMC was set for independent variability of parameters in individual coding and non-coding genes under the GTR + I + G model (partitions: 18S, 28S, *rnl*, *cox1* – first, second, and third positions, model parameters partitioned by genes and regions). Four chains ran simultaneously for 10⁷ generations, with trees being sampled each 1000 generations, all partitions unlinked. The first 3000 trees were discarded as burn-in and we used the resulting 14 000 trees to estimate posterior probabilities and to obtain a 50% majority-rule consensus tree using PAUP* 4.03b10.

In addition, the same datasets were analysed under maximum likelihood criterion using RAXML v. 7.2.3 (Stamatakis 2006; Stamatakis *et al.* 2008) with the GTR-CAT algorithm and with each fragment and each codon position of the protein coding *cox1* mtDNA partitioned during analysis as in the Bayesian analysis above.

Preliminary analyses indicated a placement of drilids in Elateridae: Agrypninae and *Cebrio* in Elateridae: Elaterinae. This prompted likelihood based statistical testing of competing topological hypotheses using constrained searches performed on a pruned dataset aligned by BlastAlign. We performed four ML searches to obtain the best tree under (i) the constrained Elateridae in the narrow sense without drilids and *Cebrio*, (ii) Elateridae without drilids but with *Cebrio* in a sister position to them, (iii) the constrained clades of all soft- and hard bodied families and (iv) the unconstrained analysis. The constrained topologies were then assessed by a comparison of likelihoods,

with statistical significance determined by an individual site bootstrapping procedure implemented in ConSel (Shimodaira & Hasegawa 2001). The best topology obtained for each hypothesis was input into RAXML to calculate the likelihoods at each individual site. The resulting per site likelihoods were then input into ConSel, where multiscale bootstrapping was performed.

Results

The alignment of the dataset of 210 taxa and the four genes produced by CLUSTALX contained 4363 homologous positions. The ambiguously aligned positions of 18S and 28S rDNA were removed after eye inspection to avoid their impact on the topology and only 3803 and 3743 positions remained for analysis in the 155 and 210 taxa datasets respectively (Table S3). As an alternative we used BlastAlign, which keeps only the more conserved regions in the resulting alignment. The resulting matrix contained 4787 homologous positions. The maximum uncorrected pairwise distances between ingroup taxa varied between 12.7% for 18S rDNA and 36.3% for *cox1* mtDNA in the CLUSTALX alignment.

All analyses recovered Elateroidea as a monophylum (except *Telegeusis* placed in outgroup in some analyses) and most families were monophyletic (Figs 1 and 2). The Elateroidea obtained low-bootstrap support values (BS) and posterior probabilities (PP) in topologies produced from all datasets (Figs 1 and 2). However, the monophyly of the clade was not contradicted because alternative groupings did not receive significant support.

The morphologically delimited families were regularly recovered by the analyses of most datasets and under various optimality criteria. Telegeusidae and Omethidae were inferred as a sister group of remaining Elateroidea. Throscidae were placed in a monophylum or paraphylum with Eucnemidae (Figs 1 and 2, Table 2). The families Lycidae, Cantharidae and Lampyridae formed either a monophylum (six analyses) or a paraphylum (five analyses, Table 2). The Phengodidae and Rhagophthalmidae were always found as a monophylum but their position was unstable and they were found as a sister group of the widely defined Elateridae clade or in relationships to the Lycidae + Lampyridae + Cantharidae group. There was no support for the close relationships of the Throscidae, Eucnemidae and Elateridae (Figs 1 and 2).

The clade of Omalidae + drilids + Elateridae was found in most topologies (Table 2) but without sufficient support (Fig. 2). Drilid genera *Drilus* and *Selasia* were inferred within the elaterid subfamily Agrypninae (PP 92%, BS 71%) and *Pseudeuanoma* formed a clade with *Omalisus* (PP 94%, BS 48%, Table 2). *Cebrio* was found as one of the Elaterinae lineages (PP 100%, BS 88%), but

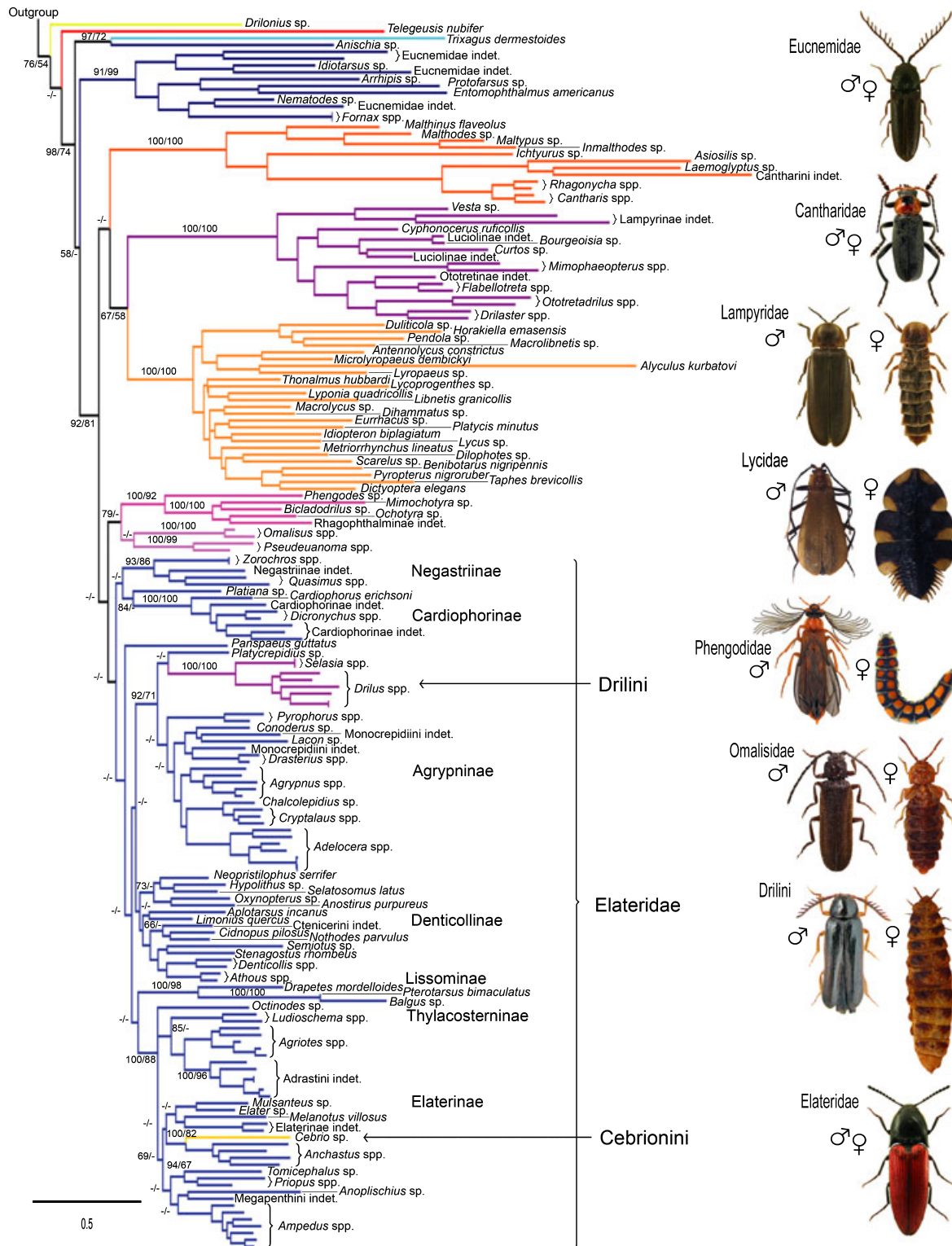


Fig. 1 Phylogenetic hypothesis for Elateroidea based on an analysis of all taxa (210 taxa, 18S and 28S rDNA, *cox1*, and *rrn1* mtDNA) with length variable fragment aligned by BlastAlign and the alignment analysed by RAXML. Numbers at the branches are bayesian, and likelihood frequencies (selected branches only).

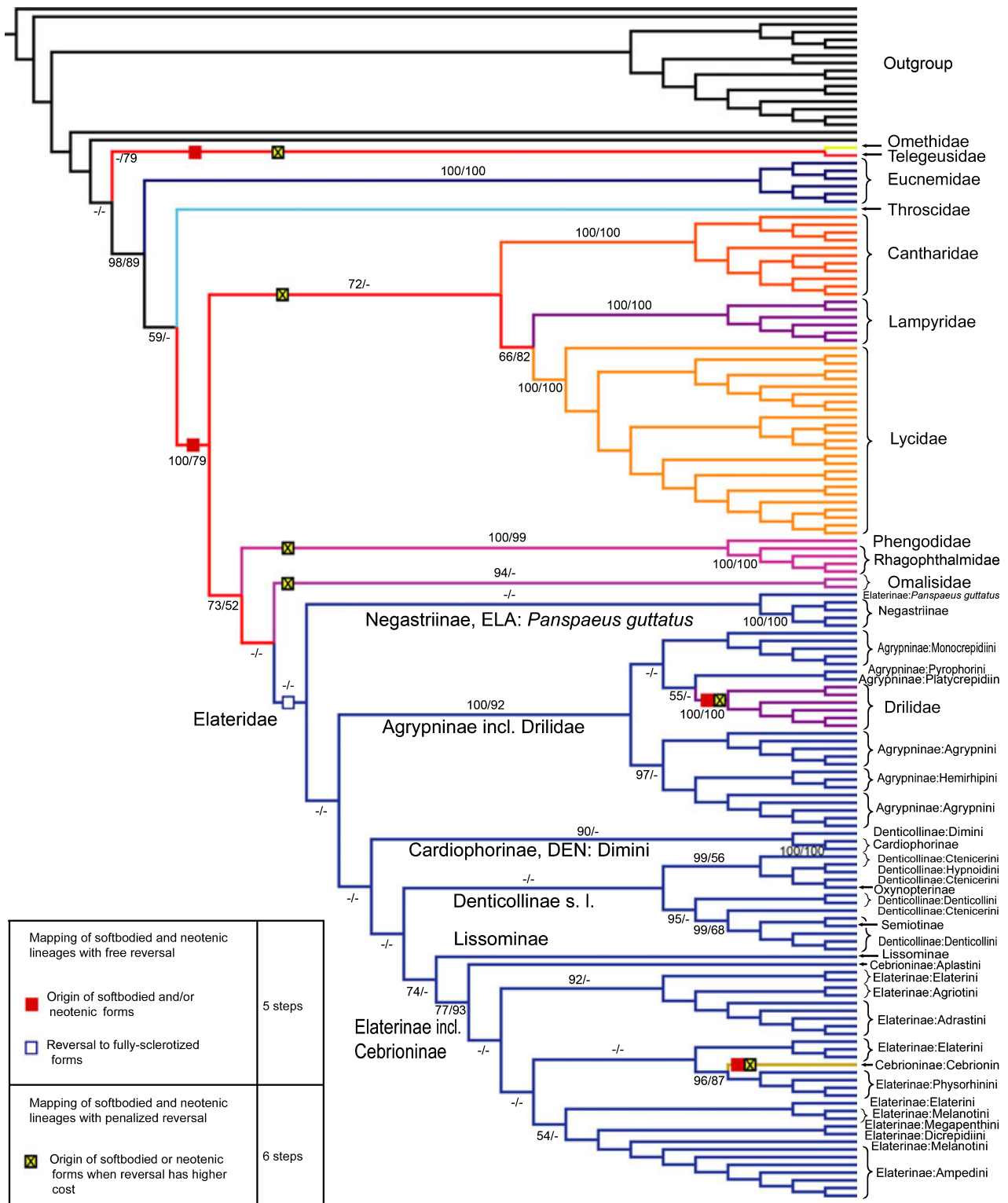


Fig. 2 Phylogenetic hypothesis for Elateroidea based on an analysis of all taxa represented by four fragments (155 taxa, 18S and 28S rDNA, *cox1*, and *rnr1* mtDNA) with length variable fragments aligned by BlastAlign and the alignment analysed by RAxML. Numbers at the branches are bayesian, and likelihood frequencies (selected branches only).

Table 2. The frequency of selected groups of taxa under different alignments and datasets

Alignments method/dataset	Telegeusidae + Omethidae (basal position)	Throscidae + Eucnemidae	Cantharidae + Lampyridae + Lycidae	Phen + Rhago + Elater (including Omalisidae & Drilidae)	Omalisidae + Elateridae (including Drilidae)	<i>Omalisus</i> + <i>Pseudeuana</i>	Drilidae within Elateridae	Drilidae + Agrypninae
Clustal/210 taxa								
Parsimony (A)	M	M	-	P	M	-	M	M
Parsimony (B)	M	M	P	P	M	-	M	M
Bayesian analysis (b)	M	M	P	P	P	M	M	M
Likelihood analysis (b)	M	P	-	P	P	M	M*	M
Clustal/155 taxa								
Parsimony (A)	M**	M	P	M	P	-	M	M
Parsimony (B)	M	-	P	P	M	M	M	M
Bayesian analysis (B)	M	M	M	P	M	M	M	M
Likelihood analysis (b)	P	P	M	M	M	M	M	M
BlastAlign/210 taxa								
Parsimony	M**	-	M	P	P	M	M	P
Bayesian analysis	M	P	P	P	M*	M	M*	M
Likelihood analysis	M	M	P	M	M	M	M	M
BlastAlign/155 taxa								
Parsimony	M	-	M	M	M	M	M	M
Bayesian analysis	P	P	M	M	M	M	M	M
Likelihood analysis	M	P	M	M	M	M	M	M

M, selected taxa form a monophylum; P, paraphylum.

*Drilidae or Omalisidae formed a clade only with a part of Elateridae.

**The clade Telegeusidae + Omethidae present but not in the position of the sister group of remaining Elateroidea.

Dataset aligned by Clustal were analysed either in the full length (A) or ambiguously aligned length variable regions were omitted (B).

regularly in a distant position from *Octinodes*, which was placed as one of the basal lineages of Elaterinae (Fig. 1).

The monophyly of Elateridae s.l. (i.e. including Drilidae) was supported in analyses of the datasets aligned by BlastAlign, but received low-posterior probabilities and bootstrap values in all analyses due to the unstable position of a few taxa (Table 2). Although the monophyly of widely defined Elateridae was regularly recovered, the topology of basal elaterid lineages was variable and all obtained only low support (Figs 1 and 2). We did not find any support for the independent positions of subfamilies Semiotinae and Oxynopterinae. The genera of these families were regularly recovered within Denticollinae. The close relationships of Thylacosterninae and Lissominae were supported by all analyses, where both taxa were included (PP 100%, BS 98%) and they were found regularly in a close position to Elaterinae (Fig. 1).

The partitioned analyses of all four genes were conducted to investigate the potential incongruence of the signal from individual fragments. Although most topologies were poorly resolved, all suggested similar position of Drilidae in Agrypninae and Omalisidae in close relationships to Elateridae. Similarly, the position of *Cebrio* in Elaterinae obtained support from all partial datasets (Table S4). The positions of all neotenic lineages, *Drilus*, *Selasia*, *Pseudeuana* and *Cebrio* inferred from combined

datasets of all nuclear and mitochondrial genes were mostly in agreement with the result of total evidence analyses (Figs 1 and 2, Table S4).

Likelihoods for the best unconstrained topology and the best topology under each constraint were compared by multiscale bootstrapping, with approximately unbiased (AU) *P*-values given for each topology. AU *P*-values for individual analyses were 99.8 (unconstrained topology), 0.003 (drilides and *Cebrio* excluded from Elateridae), 0.001 (drilides excluded from Elateridae and *Cebrio* in the sister position with Elateridae s.str.), and 0.00008 (soft- and hard bodied families in separate clades). The values <0.05 indicate the significant rejection of the hypothesis.

Discussion

The ribosomal DNA has been widely used for the investigation of beetle phylogeny including Elateroidea (Caterino *et al.* 2002; Bocakova *et al.* 2007; Hunt *et al.* 2007; Stanger-Hall *et al.* 2007; Bocak *et al.* 2008) and we consider these genes as appropriate for the study of family level relationships, especially when hypervariable loop regions are excluded from analyses (Sagegami-Oba *et al.* 2007b). These genes were used also in previous studies on deeper phylogeny of beetles and proved their usefulness (e.g. Hunt *et al.* 2007). Nevertheless, the inferred relationships among major elateroid lineages obtained low bootstrap

and posterior probabilities value. On the other hand, all partial analyses, different alignments and methods of tree building suggest similar topologies and did not produce an alternative with similar support. Although the present dataset is much larger than previously analysed data (Bocakova *et al.* 2007; Sagegami-Oba *et al.* 2007a, b; etc.) further data are needed to obtain a more robust hypothesis.

Monophyly of Elateroidea and family level relationships

The comprehensive dataset confirms wide definition of Elateroidea (Lawrence 1988) as an assemblage containing both well-sclerotized and soft-bodied lineages and confirms multiple origins of the neotenic forms in Elateroidea (Bocakova *et al.* 2007; Sagegami-Oba *et al.* 2007b). The distinction between the elateroid and cantharoid lineages of Elateroidea had been artificial and based on traits resulting from homoplasious modification of the ancestral elateroid forms (Fig. 1). We hypothesize the parallel evolution of some structures, e.g. the loss of clicking mechanism, short, transverse prosternum or elytral costae with a strengthening function. The monophyly of Cantharoidea as redefined by Crowson (1972) was rejected also by previous studies and discussed in detail by Bocakova *et al.* (2007). The monophyly of most large families of Elateroidea is well-supported and this finding is well in agreement with previous results based on the molecular data (Bocakova *et al.* 2007; Sagegami-Oba *et al.* 2007b). Our dataset with more extensive sampling provides the stronger signal for some relationships. Omethidae and Telegeusidae are consistently a basal lineage and sister group of the remaining Elateroidea (Figs 1 and 2, Table 2). Throscidae and Eucnemidae form either a monophylum or are placed at the base of Elateroidea, but we did not obtain any support for close relationships among Eucnemidae, Throscidae and Elateridae despite the presence of similar clicking mechanism in these families (Figs 1 and 2, Table 2; Bocakova *et al.* 2007), but for different opinion see Lawrence (1988), Calder *et al.* (1993), Lawrence *et al.* (1995a), Muona (1995), and Vahtera *et al.* (2009).

The species-rich, soft-bodied families Cantharidae, Lampyridae and Lycidae form a clade or a paraphylum in a similar position within the tree (Figs 1 and 2, Table 2). As no previous scholar analysing morphology of these groups suggested the relationships of Cantharidae and Lampyridae, Stanger-Hall *et al.* (2007) proposed that higher substitution rate caused the attraction of long branches and rejected their relationships as an artefact of the analysis. Here, having much larger dataset, we found repeatedly support for their relationships (Figs 1 and 2).

Phengodidae and Rhagophthalmidae are closely related and in most analyses of the dataset with complete representation of fragments form a clade with Elateridae,

Omalisidae and Drilidae. Lampyridae and Rhagophthalmidae were lumped together by Suzuki (1997) on the basis of the *rrnl* sequence, but the multiple marker dataset refutes this hypothesis (Figs 1 and 2). Omalisidae are consistently a sister group of widely defined Elateridae (Fig. 2) or part of Elateridae in some analyses. The position of *Pseudeuonoma* previously classified in Drilidae or Omalisidae has been another long-standing uncertainty in the classification of Elateroidea. The analyses consistently prefer the position of *Pseudeuonoma* in Omalisidae (Fig. 1, Tables 2 and S4), where the genus was classified until Crowson (1972) transferred it to Drilidae. Its position is well-supported by much longer prosternum than in *Drilus* and *Selasia* (Kundrata & Bocak 2007). In contrast to morphological similarity, Drilidae, represented by *Drilus* and *Selasia*, are always found as an internal lineage within Elateridae and in close relationships with Agrypninae (Figs 1 and 2, Tables 2 and S4). Another genus classified at present in the redefined Drilidae is *Malacogaster*, which is undoubtedly closely related to *Drilus*. *Paradrilus* remains in Drilidae, but its position should be investigated if the DNA becomes available as this genus differs in several morphological characters from other drilides (Kundrata & Bocak 2007).

The only available morphological analysis testing the monophyly of selected cantharoid families was published by Branham & Wenzel (2003) and summarized by Lawrence *et al.* (2010). These authors proposed some unexpected groupings, such as polyphyly of Lampyridae, paraphyly of Cantharidae and close relationships of Telegeusidae, Omethidae and Cantharidae. These conclusions are in conflict with the results of all analyses of molecular data (Bocakova *et al.* 2007; Sagegami-Oba *et al.* 2007b; Stanger-Hall *et al.* 2007; and this study). The analysis of Branham & Wenzel (2003) relied heavily on the morphology of antennae. As pheromone communication plays a significant role in Lampyridae (Branham & Wenzel 2003) and very probably also in the related families, the structure of antennae is under strong selection and may evolve very quickly. Therefore, the morphology-based conclusions on the evolution of bioluminescence (Branham & Wenzel 2003) are in a need of deep revision.

A possible affiliation between Drilidae and Elateridae has been proposed only recently (Bocakova *et al.* 2007) and there is no apparent signal from morphology to support it. Although we did not observe long branches in Elateridae and Drilidae or Omalisidae grouped together (Fig. 1), we took the lack of support from morphology as a potential indication of the long branches attraction (Bergsten 2005) or a random artefact of the analyses. The results, however, show the weak but consistent support from various datasets (Tables 2 and S4) and we never

found a grouping of omalidid or drilid taxa with Lampyridae, Cantharidae, Phengodidae, Telegeusidae or Lycidae, which were long considered as their closest relatives in previous classifications (e.g. Crowson 1972).

Phylogeny of Elateridae

The basal phylogeny of Elateridae remains contentious as various alignment methods and optimality criteria yielded different topologies. Nevertheless, some relationships were repeatedly found. We primarily focused on the position of the soft-bodied, neotenic drilides, which had been until recently considered unrelated to Elateridae. Here, we found that Agrypninae regularly contain Drilidae as a terminal branch and we suggest that the morphological differences are a consequence of heterochronic modifications in the drilid lineage. Their position is also supported by the partial analyses and considering the same position of drilides in analyses based on mitochondrial and nuclear markers we consider their position as strongly supported (Figs 1 and 2, Tables 2 and S4). The position of Drilidae within widely defined Elateridae was inferred also by Bocakova *et al.* (2007), Sagegami-Oba *et al.* (2007b) and Hunt *et al.* (2007), but in all cases with poorly sampled Elateridae and other lineages within Elateroidea and therefore, no conclusions have been made by these authors. Branham & Wenzel (2003) considered drilides as a basal lineage of the cantharoid clade, but no Elateridae were included in their study and their analysis could not solve the position of drilides.

Another lineage with some females partly metamorphosed and the morphologically atypical males are cebrioinines. Their morphological distinctiveness led students of Elateroidea to give them either the family or subfamily rank or to consider them as the most primitive Elateridae (Calder *et al.* 1993; Costa *et al.* 2010; etc.). The analyses showed that they are part of Elateridae as was found already by Bocakova *et al.* (2007) and Hunt *et al.* (2007) and with extensive sampling, we inferred their close relationships with Elaterinae (Figs 1 and 2, Table S4). Two cebrioinine lineages were present in our dataset – *Octinodes* (Aplastini) and *Cebrio* (Cebrioinini), both placed in Cebrioininae in the most recent classification (Costa *et al.* 2010). No analysis supported their close relationships and we found only *Octinodes* as a sister group or one of basal lineages of Elaterinae (Figs 1 and 2). *Cebrio* formed the clade with Physorhinini or some other genera (Figs 1 and 2, Table S4), but always in a distant position to *Octinodes*. The relationships of Aplastini and with some reservations also of *Cebrio* with Elaterinae were suggested already by Stibick (1979). He based his conclusion on several larval and adult characters and considered Aplastinae as a link between Elateridae and Cebrioinidae. Therefore, not only

molecular analyses but also morphological data support their position.

Denticollinae, a very species-rich lineage of click beetles, was a consistently recovered branch and it also included Oxynopterinae and Semiotinae, which were previously considered as independent lineages (Costa *et al.* 2010). Both results presented here and those of Sagegami-Oba *et al.* (2007a) reject their independent position and these smaller lineages are regularly in the terminal position in Denticollinae (Figs 1 and 2). Another widely recognized lineage, Pityobiinae, was found within Denticollinae by Sagegami-Oba *et al.* (2007a).

Negastriinae and Cardiophorinae were inferred from all analyses, but their position was unstable (Figs 1 and 2) and they formed a monophylum as proposed by Sagegami-Oba *et al.* (2007a) only in some analyses (Fig. 1). *Panspaeus guttatus*, which was classified in Elateridae *incertae sedis* (Cate 2007) was found as a sister group of Negastriinae (Fig. 2) or as one of basal elaterid lineages (Fig. 1). Dimini classified by Costa *et al.* (2010) in Denticollinae were found as a sister group of Cardiophorinae. More comprehensive sampling will be necessary for more robust results in these cases.

Thylacosterninae and Lissominae were previously classified in Eucnemidae or Throscidae (Crowson 1955; Calder *et al.* 1993) and only recently transferred to Elateridae by Lawrence *et al.* (2007) on the basis of morphology and *cox1* mtDNA sequences. Unfortunately, only three species of Elateridae were included in their analyses and therefore Lissominae and Thylacosterninae were inferred as basal lineages of Elateridae. These two lineages formed a clade in the present analyses and were found in a sister-group position with Elaterinae (Figs 1 and 2).

Evolution of neoteny

The results strongly support multiple origins of the soft-bodiedness and neotenic development in Elateroidea as has already been suggested by Bocakova *et al.* (2007). Under parsimony reconstruction an independent origin of the feebly sclerotized body and incompletely metamorphosed females is inferred in (i) Omethidae + Telegeusidae, (ii) Cantharidae + Lampyridae + Lycidae, (iii) drilides and (iv) Elateridae: Cebrioininae (Fig. 2). Nevertheless, conclusions about the origin of these modifications need caution. The most parsimonious explanation suggests four origins and one reversal to the fully metamorphosed forms or alternatively six origins are needed under the no-reversal scenario (Fig. 2). The principal question is, if the origin and reversal are equally probable and hence parsimony mapping provides adequate evolutionary scenario. If reversals are weighted higher by a factor of two than the forward ontogenetic shifts leading to neotenic features

then both explanations are equally parsimonious; any greater imbalance of weights favours multiple origins over reversals. The neotenic modifications result from the modification of the ontogenetic programme and e.g. females of *Omalisus* remind in some aspects, e.g. short appendages, less diversified meso- and metathorax and larviform abdomen, the phenotypes produced by the manipulations with hormonal regulation of the metamorphosis (Konopova & Jindra 2007). Whiting *et al.* (2003) discussed a similar problem of character reconstruction to assess the origin of winglessness in Phasmatodea. Although they advocated the reversal to winged forms, the criticism pointed to the unknown probabilities of the origins and reversals (Trueman *et al.* 2004). Any dysfunction of the hormonal system produces incompletely metamorphosed forms, but the reversal needs to re-establish the endocrine system to the previous form. Although less parsimonious under equal probability scenario, the multiple shift to incomplete metamorphosis remains as a plausible explanation.

Similar uncertainty remains, when the origin of the clicking mechanism is considered. Vahtera *et al.* (2009) refused the possibility of its multiple origin due to its complex structure. Comparing the hypotheses of multiple loss and origins, we face the same problem of potentially unequal but unknown probabilities as in the case of soft-bodiedness and neoteny.

For the first time, the more extensive sampling produced a phylogeny of the Omalisidae + Elateridae + Drilidae clade and we found the multiple origin of the variable degree of incompleteness of metamorphosis in the widely defined Elateridae similar to the results found in Lycidae (Bocak *et al.* 2008) (Figs 1 and 2). The elaterid clade contains three groups with incompletely metamorphosed females: Omalisidae, *Drilus* + *Selasia* and Elateridae: Cebriioninae. Males in these lineages substantially differ in the degree of the heterochronic modifications but all are soft-bodied and winged (Bocakova *et al.* 2007). Their degree of dissimilarity with sister lineages is correlated with the degree of modifications of females when presence of the higher number of larval traits in females usually means less sclerotized integument in males. We argue that the morphological disparity of these soft-bodied lineages is a result of the shift to modified ontogeny pathway, and therefore cannot be used to support their high rank in the classification (Tables 2 and S5). Their close relationships with Elateridae were also supported by some morphological characters, e.g. the shape of male genitalia or pronotum (Kundrata & Bocak 2007).

Evolution of bioluminescence

Bocakova *et al.* (2007) and Sagegami-Oba *et al.* (2007b) showed that luminescence evolved multiple times in

Elateroidea and our results strongly support this hypothesis. Vahtera *et al.* (2009) argued that the predisposition for luminescence had developed in the ancestral, clicking forms, but ancestral to non-elateroid luminescent beetles are only Throscidae and Eucnemidae, which are not luminescent (Figs 1 and 2). The authors also relate the origin of bioluminescence to the loss of the clicking mechanism, but two luminescent lineages of Elateridae, i.e. Thylacosterninae: *Balgus* and Agrypninae: Pyrophorini have kept the click mechanism. Therefore, the hypothesis on the causal relationships between the loss of clicking mechanism and bioluminescence does not get any support from the phylogeny. Their hypothesis of the secondary transfer of luminous organs to other body parts is also in conflict with our results. Most luminescent beetles belong to Lampyridae, which are distantly related with Elateridae and never have light organs in thoracic segments. Phengodidae + Rhagophthalmidae are preferred as a sister group of Omalisidae + Elateridae (including Drilidae) and have light organs in numerous body segments. Therefore, we infer that luminescence was independently obtained in some Thylacosterninae and Agrypninae and the position of their luminescent organs in pronotum cannot be considered as an ancestral state under present phylogenetic hypotheses.

Taxonomy

Omalisidae. The inferred position of Omalisidae justifies their family rank as a convenient expression of their morphological and biological distinctiveness. Although once found as an internal clade in Elateridae, the result was based on the dataset, which contained two species with highly incomplete fragments from both *Omalisus* and *Pseudeuanoma*. The incompleteness of sequences is a probable cause for anomalous position of Omalisidae in this analysis. The traditional relationships between Omalisidae and Lycidae are refuted (Kleine 1933; Crowson 1955, 1972). *Pseudeuanoma* is transferred to Omalisidae and the original concept of Omalisidae is restored (Kleine 1933; Crowson 1955) and Crowson's subsequent delineation of Omalisidae refuted (Crowson 1972). The closely related *Euanoma* was not available for the DNA analysis, but based on the similar morphology (Kundrata & Bocak 2007) the genus is also transferred to Omalisidae. The revised definition of Omalisidae means that the family contains five genera: *Omalisus*, *Phaeopterus*, *Thilmanus*, *Euanoma* and *Pseudeuanoma*. The genus and species level classification was revised by Bocak & Brlik (2008) and Kundrata & Bocak (2007).

Elateridae. The subfamily level classification of Elateridae has been very unstable and 17 subfamilies were recog-

nized by Costa *et al.* (2010) and further more than 50 subfamily level names were proposed for various lineages (Table S5). Already Stibick (1979) tried to propose a conservative classification with a limited number of subfamilies, but the tendency of high rank assigned to each lineage prevailed in the most recent studies. The results published by Sagegami-Oba *et al.* (2007a) and the present analyses enable us to propose a classification reflecting the relationships and does not give a high rank to each lineage with modified morphology, but nested within lineages of the same rank. We analysed the relationships of 10 of 17 subfamilies recognized by Costa *et al.* (2010) and our results agree in most aspects with classification used by Johnson (2002) in the review of the North American fauna of Elateridae. Although seven subfamilies were not available, they represent in total about 150 species, i.e. 1.5% of the species level diversity of Elateridae and their position will have to be investigated later. Sagegami-Oba *et al.* (2007a) studied the representatives of seven subfamilies; one of these was unavailable to us. Unfortunately, these two datasets could not be combined as Sagegami-Oba *et al.* (2007a) used only one, non-overlapping fragment of 28S rDNA. On the other hand, the independent sources of the phylogenetic signal and independent analyses provide more robust support for repeatedly found relationships among elaterid lineages. Considering available information on the molecular phylogeny of Elateridae and high similarity of these results with the morphology-based approach published by Johnson (2002), we propose the following modifications of the elaterid classification published by Costa *et al.* (2010).

Agrypninae. The analyses by Bocakova *et al.* (2007), Sagegami-Oba *et al.* (2007b) and this study suggests wider definition of Elateridae. The substantial part of Drilidae was nested within Agrypninae in the present analyses (Figs 1 and 2, Tables 2 and S4) and we propose to classify these taxa as a tribe Drilini with the following genera: *Drilus*, *Selasia*, *Malacogaster* and *Paradrilus*.

Elaterinae. All analyses support an internal position of Cebriioninae within Elaterinae. Cebriionines represent a lineage with neotenic females and considerably modified morphology of males and due to morphological disparity they were considered as an independent family (Crowson 1972) or later as a subfamily of Elateridae (Lawrence 1988; Costa *et al.* 2010). Already Stibick (1979) pointed to some morphological similarities between Elaterinae and Cebriioninae and he erected a subfamily Aplastinae for *Aplastus*, *Octinodes* and related genera. Our analyses do not show close relationships between Aplastini and Cebriionini, but both are consistently found as a part of the Elaterinae

clade (Figs 1 and 2, Table S4). Therefore, these lineages are proposed as tribes within Elaterinae.

Denticollinae. Oxynopterinae and Semiotinae, two lineages recognized as subfamilies by Costa *et al.* (2010), were found consistently as internal branches within Denticollinae (Figs 1 and 2). Oxynopterinae were represented in both datasets and they were never found outside Denticollinae, similarly with Semiotinae in our analyses [they were unavailable for Sagegami-Oba *et al.* (2007a)]. These results are far from surprising and close relationships of these lineages with Denticollinae were proposed e.g. by Johnson (2002). The relationships between Semiotinae and Oxynopterinae were suggested by Stibick (1979). Pityobiini were inferred as an internal branch of Denticollinae by Sagegami-Oba *et al.* (2007a). Although their analyses were based on a single fragment and therefore are in need of further investigation, the agreement with the classification proposed by Johnson (2002) indicates the highly probable position of Pityobiinae in Denticollinae. Based on these results, Denticollinae are widely defined here and we propose to classify these three lineages as tribes Oxynopterini, Semiotini and Pityobiini in Denticollinae. The summary of the proposed subfamily and tribe level classification of Elateridae is given in the Supplements (Table S5).

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References

- Belshaw, R. & Katzourakis, A. (2005). BlastAlign: a program that uses blast to align problematic nucleotide sequences. *Bioinformatics*, *21*, 122–123.
- Bergsten, J. (2005). A review of long-branch attraction. *Cladistics*, *21*, 163–193.
- Beutel, R.G. (1995). Phylogenetic analysis of Elateriformia (Coleoptera: Polyphaga) based on larval characters. *Journal of Zoological Systematics and Evolutionary Research*, *33*, 145–171.

- Beutel, R.G., Bocak, L. & Bocakova, M. (2007). Are Polyphaga (Coleoptera) really a basal neopteran lineage – a reply to Kazantsev. *Acta Zoologica*, 88, 153–158.
- Bocak, L. (2007). Drilidae, Omalidae. In I. Löbl & A. Smetana (Eds) *Catalogue of Palaearctic Coleoptera, Vol. 4. Elateroidea – Derodontoidea – Bostrichoidea – Lymexyloidea – Cleroidea – Cucujoidea* (pp. 209–211, 224–225). Stenstrup: Apollo Books.
- Bocak, L. & Brlik, M. (2008). Revision of the family Omalidae (Coleoptera, Elateroidea). *Insect Systematics and Evolution*, 46, 189–212.
- Bocak, L., Bocakova, M., Hunt, T. & Vogler, A.P. (2008). Multiple ancient origins of neoteny in Lycidae (Coleoptera): consequences for ecology and macroevolution. *Proceedings of the Royal Society B*, 275, 2015–2023.
- Bocak, L., Branham, M.A. & Kundrata, R. (2010). Family Drilidae Blanchard, 1845. In J. F. Lawrence & R. A. B. Leschen (Eds) *Handbook of Zoology, Volume IV* (ed. by N. P. Kristensen & R. G. Beutel). Part 38, Volume 2: Coleoptera, Polyphaga, part (pp. 104–110). New York, Berlin: Walter de Gruyter.
- Bocakova, M., Bocak, L., Hunt, T. & Vogler, A.P. (2007). Molecular phylogenetics of Elateriformia (Coleoptera): evolution of bioluminescence and neoteny. *Cladistics*, 23, 477–496.
- Branham, M.A. & Wenzel, J.W. (2003). The evolution of photic behavior and the evolution of sexual communication in fireflies (Coleoptera: Lampyridae). *Cladistics*, 19, 1–22.
- Calder, A.A., Lawrence, J.F. & Trueman, J.W.H. (1993). *Austrelater*, gen. nov. (Coleoptera: Elateridae), with a description of the larva and comments on elaterid relationships. *Invertebrate Taxonomy*, 7, 1349–1394.
- Cate, P.G. (2007). Family Elateridae. In I. Löbl & A. Smetana (Eds) *Catalogue of Palaearctic Coleoptera, Vol. 4. Elateroidea – Derodontoidea – Bostrichoidea – Lymexyloidea – Cleroidea – Cucujoidea* (pp. 89–209). Stenstrup: Apollo Books.
- Caterino, M.S., Shull, V.L., Hammond, P.M. & Vogler, A.P. (2002). Basal relationships of Coleoptera inferred from 18S rDNA sequences. *Zoologica Scripta*, 31, 41–49.
- Costa, C., Lawrence, J.F. & Rosa, S.P. (2010). Elateridae Leach, 1815. In J. F. Lawrence & R. A. B. Leschen (Eds) *Handbook of Zoology, Volume IV* (ed. by N. P. Kristensen & R. G. Beutel). Part 38, Volume 2: Coleoptera, Polyphaga, part (pp. 75–103). New York, Berlin: Walter de Gruyter.
- Crowson, R.A. (1955). *The Natural Classification of the Families of Coleoptera*. London: Nathaniel Lloyd & Co., Ltd., pp. 187.
- Crowson, R.A. (1960). The Phylogeny of Coleoptera. *Annual Review of Entomology*, 5, 111–134.
- Crowson, R.A. (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.
- Crowson, R.A. (1973). On a new superfamily Artematopoidea of polyphagan beetles, with the definition of two new fossil genera from the Baltic amber. *Journal of Natural History*, 7, 225–238.
- Erezyilmaz, D.F., Riddiford, L.M. & Truman, J.W. (2006). The pupal specifier broad directs progressive morphogenesis in a direct-developing insect. *Proceedings of the National Academy of Sciences of the USA*, 103, 6925–6930.
- Goloboff, P., Farris, S. & Nixon, K. (2003). TNT (tree analysis using new technology). *Cladistics*, 20, 84.
- Huelsenbeck, J.P. & Ronquist, F. (2001). MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17, 754–755.
- Hunt, T., Bergsten, J., Levkanicova, Z., Papadopoulou, A., St-John, O., Wild, R., Hammond, P.M., Ahrens, D., Balke, M., Caterino, M.S., Gómez-Zurita, J., Ribera, I., Barraclough, T.G., Bocakova, M., Bocak, L. & Vogler, A.P. (2007). A comprehensive phylogeny of beetles reveals the evolutionary origins of a superradiation. *Science*, 318, 1913–1916.
- Hyslop, J.A. (1917). The phylogeny of the Elateridae based on larval characters. *Annals of the Entomological Society of America*, 10, 241–263.
- Johnson, P.J. (2002). Elateridae Leach 1815. In R. H. Arnett Jr, M. C. Thomas, P. E. Skelley & J. H. Frank (Eds) *American Beetles, Vol. 2* (pp. 160–173). Boca Raton, FL: CRC.
- Kleine, R. (1933). Pars 128: Lycidae. In S. Schenckling (Ed.) *Coleopterorum Catalogus auspiciis et auxilio* (pp. 145). Berlin: W. Junk.
- Konopova, B. & Jindra, M. (2007). Juvenile hormone resistance gene Methoprene-tolerant controls entry into metamorphosis in the beetle *Tribolium castaneum*. *Proceedings of the National Academy of Sciences of the USA*, 104, 10488–10493.
- Konopova, B. & Jindra, M. (2009). Broad-complex acts downstream of Met in juvenile hormone signaling to coordinate primitive Holometabolism metamorphosis. *Development*, 135, 559–568.
- Kundrata, R. & Bocak, L. (2007). A revision of *Euanoma* and *Pseudeuanoma* (Coleoptera: Drilidae). *Annales Zoologici*, 57, 427–441.
- Lawrence, J.F. (1988). Rhinorhipidae, a new beetle family from Australia, with comments on the phylogeny of the Elateriformia. *Invertebrate Taxonomy*, 2, 1–53.
- Lawrence, J.F. & Newton, A.F., Jr (1995). Families and subfamilies of Coleoptera (with selected genera, notes, references and data on family-group names). In J. Pakaluk & S. A. Ślipiński (Eds) *Biology, Phylogeny, and Classification of Coleoptera: Papers Celebrating the 80th Birthday of Roy A. Crowson* (pp. 779–1006). Warszawa, Poland: Muzeum i Instytut Zoologii PAN.
- Lawrence, J.F., Nikitsky, N.B. & Kirejtshuk, A.G. (1995a). Phylogenetic position of Decliniidae (Coleoptera: Scirtoidea) and comments on the classification of Elateriformia (sensu lato). In J. Pakaluk & S. A. Ślipiński (Eds) *Biology, Phylogeny and Classification of Coleoptera: Papers Celebrating the 80th Birthday of Roy A. Crowson* (pp. 375–410). Warszawa, Poland: Muzeum i Instytut Zoologii PAN.
- Lawrence, J.F., Ślipiński, S.A. & Pakaluk, J. (1995b). From Latreille to Crowson: a history of the higher-level classification of beetles. In J. Pakaluk & S. A. Ślipiński (Eds) *Biology, Phylogeny and Classification of Coleoptera: Papers Celebrating the 80th Birthday of Roy A. Crowson* (pp. 87–155). Warszawa, Poland: Muzeum i Instytut Zoologii PAN.
- Lawrence, J.F., Muona, J., Teräväinen, M., Ståhls, G. & Vahtera, V. (2007). *Anischia*, *Perothops* and the phylogeny of Elateroidea (Coleoptera: Elateriformia). *Insect Systematics and Evolution*, 38, 205–239.
- Lawrence, J.F., Bocak, L., Bocakova, M., Beutel, R.G. & Muona, J. (2010). Elateroidea. In J. F. Lawrence & R. A. B. Leschen

- (Eds) *Handbook of Zoology, Volume IV* (ed. by N. P. Kristensen & R. G. Beutel). Part 38, Volume 2: Coleoptera, Polyphaga, part (pp. 35–37). New York, Berlin: Walter de Gruyter.
- Lemmon, A.R., Brown, J.M., Stanger-Hall, K. & Lemmon, E.M. (2009). The effect of ambiguous data on phylogenetic estimates obtained by maximum likelihood and Bayesian inference. *Systematic Biology*, 58, 130–145.
- Muona, J. (1995). The phylogeny of Elateroidea (Coleoptera), or which tree is the best today? *Cladistics*, 11, 317–341.
- Posada, D. (2008). jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution*, 25, 1253–1256.
- Sagegami-Oba, R., Oba, Y. & Ôhira, H. (2007a). Phylogenetic relationships of click beetles (Coleoptera: Elateridae) inferred from 28S ribosomal DNA: Insights into the evolution of bioluminescence in Elateridae. *Molecular Phylogenetics and Evolution*, 42, 410–421.
- Sagegami-Oba, R., Takahashi, N. & Oba, Y. (2007b). The evolutionary process of bioluminescence and aposematism in cantharoid beetles (Coleoptera: Elateroidea) inferred by the analysis of 18S ribosomal DNA. *Gene*, 400, 104–113.
- Shimodaira, H. & Hasegawa, M. (2001). ONSEL: for assessing the confidence of phylogenetic tree selection. *Bioinformatics*, 17, 1246–1247.
- 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*, 75, 758–771.
- Stanger-Hall, K.F., Lloyd, J.E. & Hillis, D.M. (2007). Phylogeny of North American fireflies (Coleoptera: Lampyridae): Implications for the evolution of light signals. *Molecular Phylogenetics and Evolution*, 45, 33–49.
- Stibick, J.N.L. (1979). Classification of the Elateridae (Coleoptera): relationships and classification of the subfamilies and tribes. *Pacific Insects*, 20, 145–186.
- Suzuki, H. (1997). Molecular phylogenetic studies of Japanese fireflies and their mating systems (Coleoptera: Cantharoidea). *Tokyo Metropolitan University Bulletin of Natural History*, 3, 1–53.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, 25, 4876–4882.
- Trueman, J.W.H., Pfeil, B.E., Kelchner, S.A. & Yeates, D.K. (2004). Did stick insects really regain their wings? *Systematic Entomology*, 29, 138–139.
- Vahtera, V., Muona, J. & Lawrence, J.F. (2009). Phylogeny of the Thylacosterninae (Coleoptera, Elateridae). *Cladistics*, 25, 147–160.
- Vogler, A.P., De Salle, R., Assmann, T., Knisley, C.B. & Schultz, T.D. (1993). Molecular population genetics of the endangered tiger beetle, *Cicindela dorsalis* (Coleoptera: Cicindelidae). *Annals of the Entomological Society of America*, 86, 142–152.
- Whalley, P.E.S. (1985). The systematic and palaeogeography of the Lower Jurassic insects from Dorset, England. *Bulletin of the British Museum of Natural History (Geology)*, 39, 107–189.
- Whiting, M.F., Bradler, S. & Maxwell, T. (2003). Loss and recovery of wings in stick insects. *Nature*, 421, 264–267.
- Wittmer, W. (1944). Catalogue des Drilidae E. Oliv. (Coleoptera – Malacodermata). *Revista de la Sociedad Entomológica Argentina*, 12, 203–221.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. The list of examined specimens.

Table S2. Primers used for PCR amplifications.

Table S3. Numbers of taxa and characters in the analysed datasets.

Table S4. Results of the parsimony analysis of the partial analyses of the dataset of 155 taxa aligned by ClustalX under default settings and with variable regions omitted.

Table S5. An overview of the classification of Elateridae.

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Supplementary Information

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Table S5. An overview of the classification of Elateridae

Supplementary Table 1: List of GenBank sequences

Superfamily	Family	Subfamily	Genus/Species	Geographic Origin	18S rDNA	28S rDNA	rrnl mtDNA	cox1 mtDNA	Source
Outgroup									
Scirtoidea									
	Decliniidae		<i>Declinia versicolor</i>	Japan	AY745556	DQ198696	-	-	Bocakova <i>et al.</i> 2007
	Eucinetidae		<i>Eucinetus</i> sp.	USA	AF427609	DQ198697	-	DQ198541	Bocakova <i>et al.</i> 2007
	Scirtidae		<i>Cyphon hilaris</i>	United Kingdom	AF201419	DQ198698	DQ198620	DQ198542	Bocakova <i>et al.</i> 2007
	Scirtidae		<i>Scirtes hemisphericus</i>	United Kingdom	AF451937	DQ198699	-	-	Bocakova <i>et al.</i> 2007
Dascilloidea									
	Dascillidae		<i>Dascillus cervinus</i>	United Kingdom	AY745558	DQ198700	DQ198621	DQ198543	Bocakova <i>et al.</i> 2007
Buprestoidea									
	Buprestidae	Agrilinae	<i>Agrilus sinuatus</i>	United Kingdom	AF451934	-	-	-	Bocakova <i>et al.</i> 2007
	Buprestidae	Agrilinae	<i>Agrilus cuprescens</i>	Czech Republic	-	DQ198701	DQ198622	DQ198544	Bocakova <i>et al.</i> 2007
	Buprestidae	Buprestinae	<i>Anthaxia hungarica</i>	France	DQ100484	DQ198702	DQ198623	DQ198545	Bocakova <i>et al.</i> 2007
	Buprestidae	Julodinae	indet.	South Africa	AF451935	DQ198703	DQ198624	DQ198546	Bocakova <i>et al.</i> 2007
	Buprestidae	Agrilinae	<i>Trachys minutus</i>	Russia	AF451936	DQ198704	-	DQ198547	Bocakova <i>et al.</i> 2007
Byrrhoidea									
	Byrrhidae	Byrrhinae	<i>Byrrhus pilula</i>	United Kingdom	AF427604	DQ198705	DQ198625	DQ198548	Bocakova <i>et al.</i> 2007
	Byrrhidae	Syncalyptinae	<i>Chaetophora spinosa</i>	United Kingdom	AF451929	DQ198706	-	-	Bocakova <i>et al.</i> 2007
	Byrrhidae	Syncalyptinae	<i>Curimopsis setigera</i>	United Kingdom	AF451930	DQ198707	-	-	Bocakova <i>et al.</i> 2007
	Dryopidae		<i>Pomatinus substriatus</i>	United Kingdom	AF451924	DQ198708	DQ198626	DQ198549	Bocakova <i>et al.</i> 2007
	Elmidae	Elminae	<i>Elmis maugetti</i>	Slovakia	AF451916	DQ198709	-	-	Bocakova <i>et al.</i> 2007
	Elmidae	Elminae	<i>Homalosolus hospitalis</i>	Malaysia	AF451921	DQ198710	-	-	Bocakova <i>et al.</i> 2007
	Elmidae	Elminae	<i>Limnius volckmari</i>	Spain	AF451914	DQ198712	DQ198627	DQ198550	Bocakova <i>et al.</i> 2007
	Elmidae	Elminae	<i>Macron quadritubercu.</i>	Hungary	AF451920	DQ198713	-	-	Bocakova <i>et al.</i> 2007
	Elmidae	Elminae	<i>Oulimnius rivularis</i>	Portugal	AF451913	DQ198714	DQ198628	DQ198551	Bocakova <i>et al.</i> 2007
	Elmidae	Larinae	<i>Potamodytes</i> sp.	South Africa	AF451912	DQ198715	DQ198629	DQ198552	Bocakova <i>et al.</i> 2007
	Elmidae	Elminae	<i>Stenelmis canaliculata</i>	France	AF451919	DQ198716	-	-	Bocakova <i>et al.</i> 2007
	Heteroceridae		<i>Augyles maritimus</i>	Morocco	AF451927	DQ198717	-	-	Bocakova <i>et al.</i> 2007

Heteroceridae		<i>Heterocerus</i> sp.	Germany	AF451928	-	-	-	Bocakova <i>et al.</i> 2007
Heteroceridae		<i>Heterocerus</i> sp.	Slovakia	-	DQ198718	DQ198630	DQ198553	Bocakova <i>et al.</i> 2007
Limnichidae		<i>Limnichus pygmaeus</i>	United Kingdom	AF451923	DQ198719	DQ198631	DQ198554	Bocakova <i>et al.</i> 2007
Psephenidae	Eubrianacinae	<i>Eubrianax edwardsi</i>	USA	AF451933	DQ198720	-	-	Bocakova <i>et al.</i> 2007
Psephenidae	Eubrianacinae	<i>Eubrianax</i> sp.	Indonesia	DQ100485	DQ198721	DQ198632	DQ198555	Bocakova <i>et al.</i> 2007
Ptilodactylidae	Cladotominae	<i>Paralichas pectinatus</i>	Japan	DQ100486	DQ198722	DQ198633	DQ198556	Bocakova <i>et al.</i> 2007
Ptilodactylidae	Ptilodact.	<i>Ptilodactyla serricornis</i>	Japan	AF451932	DQ198723	DQ198634	DQ198557	Bocakova <i>et al.</i> 2007
Chelonariidae		<i>Chelonarium</i> sp.	Indonesia	DQ100488	DQ198724	DQ198635	DQ198558	Bocakova <i>et al.</i> 2007
Eulichadidae		<i>Eulichas</i> sp.	Malaysia	DQ100489	DQ198725	DQ198636	DQ198559	Bocakova <i>et al.</i> 2007
Callirhipidae		indet.	Malaysia	DQ100490	DQ198726	DQ198637	DQ198560	Bocakova <i>et al.</i> 2007

Ingroup

Elateroidea

Drilidae		<i>Drilus flavescens</i>	Malta	DQ100501	DQ198748	DQ198657	DQ198579	Bocakova <i>et al.</i> 2007
Omalisidae		<i>Omalisus fontisbellaquei</i>	Czech Republic	AF451948	DQ198749	DQ198658	DQ198580	Bocakova <i>et al.</i> 2007
Omethidae	Driloniinae	<i>Drilonius</i> sp.	Indonesia	DQ100502	DQ198750	DQ198659	DQ198581	Bocakova <i>et al.</i> 2007
Telegeusidae		<i>Telegeusis nubifer</i>	USA	DQ100503	DQ198751	DQ198660	DQ198582	Bocakova <i>et al.</i> 2007
Throscidae		<i>Trixagus dermestoides</i>	United Kingdom	AF451950	DQ198747	DQ198656	DQ198578	Bocakova <i>et al.</i> 2007
Eucnemidae	Melasinae	<i>Entomophth. americanus</i>	Bolivia	DQ100491	DQ198727	DQ198638	-	Bocakova <i>et al.</i> 2007
Eucnemidae	Macraulacinae	<i>Fornax</i> sp.	Bolivia	-	DQ198728	DQ198639	DQ198561	Bocakova <i>et al.</i> 2007
Eucnemidae	Macraulacinae	<i>Fornax</i> sp.	Bolivia	DQ100492	DQ198729	DQ198640	DQ198562	Bocakova <i>et al.</i> 2007
Eucnemidae	Eucneminae	<i>Idiotarsus</i> sp.	Bolivia	DQ100493	DQ198730	DQ198641	DQ198563	Bocakova <i>et al.</i> 2007
Eucnemidae	Macraulacinae	<i>Nematodes</i> sp.	Bolivia	DQ100495	DQ198731	DQ198642	DQ198564	Bocakova <i>et al.</i> 2007
Eucnemidae	Melasinae	<i>Protofarsus</i> sp.	Bolivia	DQ100496	DQ198732	-	DQ198565	Bocakova <i>et al.</i> 2007
Eucnemidae	Melasinae	<i>Arrhipis</i> sp.	Bolivia	-	DQ198745	DQ198654	DQ198577	Bocakova <i>et al.</i> 2007
Phengodidae	Phengodinae	<i>Phengodes</i> sp.	USA	DQ100504	DQ198752	DQ198661	DQ198583	Bocakova <i>et al.</i> 2007
Rhagophthalm.		<i>Mimochotyra</i> sp.	Malaysia	DQ100505	DQ198753	DQ198662	DQ198584	Bocakova <i>et al.</i> 2007
Rhagophthalm.		indet.	Indonesia	DQ100506	DQ198754	DQ198663	DQ198585	Bocakova <i>et al.</i> 2007
Rhagophthalm.		<i>Bicladodrilus</i> sp.	China	DQ100507	DQ198755	DQ198664	DQ198586	Bocakova <i>et al.</i> 2007
Rhagophthalm.		<i>Ochotyra</i> sp.	India	DQ100508	DQ198756	DQ198665	DQ198587	Bocakova <i>et al.</i> 2007

Lampyridae	Lampyrinae	indet.	Indonesia	DQ100509	DQ198759	DQ198667	DQ198590	Bocakova <i>et al.</i> 2007
Lampyridae	Lampyrinae	indet.	Indonesia	DQ100510	-	DQ198668	DQ198591	Bocakova <i>et al.</i> 2007
Lampyridae	Lampyrinae	<i>Vesta</i> sp.	Indonesia	DQ100511	DQ198760	DQ198669	DQ198592	Bocakova <i>et al.</i> 2007
Lampyridae	Cyphonocer.	<i>Cyphon. ruficollis</i>	Japan	DQ100512	-	DQ198670	DQ198593	Bocakova <i>et al.</i> 2007
Lampyridae	Luciolinae	<i>Curtos</i> sp.	Indonesia	DQ100513	DQ198761	DQ198671	DQ198594	Bocakova <i>et al.</i> 2007
Lampyridae	Luciolinae	indet.	Indonesia	DQ100514	DQ198762	DQ198672	DQ198595	Bocakova <i>et al.</i> 2007
Lampyridae	Luciolinae	<i>Bourgeoisisa</i> sp.	Indonesia	DQ100515	-	DQ198673	DQ198596	Bocakova <i>et al.</i> 2007
Lampyridae	Luciolinae	indet.	Malaysia	DQ100516	-	DQ198674	DQ198597	Bocakova <i>et al.</i> 2007
Lampyridae	Ototretinae	<i>Drilaster</i> sp.	Indonesia	DQ100517	-	DQ198675	DQ198598	Bocakova <i>et al.</i> 2007
Lampyridae	Ototretinae	<i>Mimophaeopterus</i> sp.	Indonesia	DQ100518	-	DQ198676	DQ198599	Bocakova <i>et al.</i> 2007
Lampyridae	Ototretinae	<i>Flabell. obscuriceps</i>	Indonesia	DQ100519	-	DQ198677	DQ198600	Bocakova <i>et al.</i> 2007
Lampyridae	Ototretinae	<i>Flabellotreta</i> sp.	Indonesia	DQ100520	DQ198763	DQ198678	DQ198601	Bocakova <i>et al.</i> 2007
Lampyridae	Ototretinae	<i>Mimophaeopterus</i> sp.	Indonesia	DQ100521	DQ198764	DQ198679	DQ198602	Bocakova <i>et al.</i> 2007
Lampyridae	Ototretinae	<i>Drilaster borneensis</i>	Indonesia	DQ100522	-	DQ198680	DQ198603	Bocakova <i>et al.</i> 2007
Lampyridae	Ototretinae	indet.	Malaysia	DQ100523	-	DQ198681	DQ198604	Bocakova <i>et al.</i> 2007
Lampyridae	Ototretadrilinae	<i>Ototretadrilus</i> sp.	India	DQ100524	DQ198765	DQ198682	DQ198605	Bocakova <i>et al.</i> 2007
Lampyridae	Ototretadrilinae	<i>Ototratadrilus</i> sp.	India	DQ100525	DQ198766	DQ198683	DQ198606	Bocakova <i>et al.</i> 2007
Cantharidae	Cantharinae	<i>Cantharis rufa</i>	United Kingdom	-	DQ198767	DQ198684	DQ198607	Bocakova <i>et al.</i> 2007
Cantharidae	Cantharinae	<i>Cantharis decipiens</i>	Czech Republic	DQ100526	DQ198768	DQ198685	DQ198608	Bocakova <i>et al.</i> 2007
Cantharidae	Cantharinae	<i>Rhagonycha nigriceps</i>	France	DQ100527	DQ198769	DQ198686	DQ198609	Bocakova <i>et al.</i> 2007
Cantharidae	Cantharinae	<i>Rhagonycha lignosa</i>	United Kingdom	AF451939	DQ198770	DQ198687	DQ198610	Bocakova <i>et al.</i> 2007
Cantharidae	Silinae	<i>Laemoglyptus</i> sp.	Indonesia	DQ100528	DQ198771	DQ198688	DQ198611	Bocakova <i>et al.</i> 2007
Cantharidae	Cantharinae	Cantharini indet.	Indonesia	DQ100529	DQ198772	DQ198689	DQ198612	Bocakova <i>et al.</i> 2007
Cantharidae	Silinae	<i>Asiosilis</i> sp.	Indonesia	DQ100530	DQ198773	DQ198690	DQ198613	Bocakova <i>et al.</i> 2007
Cantharidae	Chauliognathinae	<i>Ichtyurus</i> sp.	Indonesia	DQ100531	DQ198774	DQ198691	DQ198614	Bocakova <i>et al.</i> 2007
Cantharidae	Malthininae	<i>Malthinus flaveolus</i>	United Kingdom	AF451938	DQ198775	DQ198692	DQ198615	Bocakova <i>et al.</i> 2007
Cantharidae	Malthininae	<i>Malthodes</i> sp.	France	DQ100532	DQ198776	DQ198693	DQ198616	Bocakova <i>et al.</i> 2007
Cantharidae	Malthininae	<i>Maltypus</i> sp.	Indonesia	DQ100533	DQ198777	DQ198694	DQ198617	Bocakova <i>et al.</i> 2007
Cantharidae	Malthininae	<i>Inmalthodes</i> sp.	Indonesia	DQ100534	DQ198778	DQ198695	DQ198618	Bocakova <i>et al.</i> 2007
Elateridae	Thylacosterninae	<i>Balgus</i> sp.	Bolivia	DQ100500	DQ198746	DQ198655	-	Bocakova <i>et al.</i> 2007
Elateridae	Elaterinae	<i>Agriotes lineatus</i>	United Kingdom	-	DQ198733	DQ198643	DQ198566	Bocakova <i>et al.</i> 2007
Elateridae	Elaterinae	<i>Agriotes obscurus</i>	United Kingdom	-	DQ198734	DQ198644	-	Bocakova <i>et al.</i> 2007

Elateridae	Agrypninae	<i>Agrypnus murinus</i>	United Kingdom	AF451943	-	-	-	Bocakova et al. 2007
Elateridae	Agrypninae	<i>Agrypnus murinus</i>	Slovakia	-	DQ198735	DQ198645	DQ198567	Bocakova et al. 2007
Elateridae	Elaterinae	<i>Ampedus balteatus</i>	United Kingdom	AF427605	DQ198736	DQ198646	DQ198568	Bocakova et al. 2007
Elateridae	Denticollinae	<i>Aplotarsus incanus</i>	United Kingdom	-	DQ198737	DQ198647	DQ198569	Bocakova et al. 2007
Elateridae	Denticollinae	<i>Ath. haemorrhoidalis</i>	United Kingdom	AF451944	DQ198738	DQ198648	DQ198570	Bocakova et al. 2007
Elateridae	Cardiophorinae	indet.	Namibia	AF451942	DQ198739	DQ198649	DQ198571	Bocakova et al. 2007
Elateridae	Cebrioninae	<i>Cebrio</i> sp.	Spain	DQ100497	DQ198740	DQ198650	DQ198572	Bocakova et al. 2007
Elateridae	Denticollinae	<i>Denticollis linearis</i>	Czech Republic	DQ100498	DQ198741	DQ198651	DQ198573	Bocakova et al. 2007
Elateridae	incertae sedis	<i>Panspaeus guttatus</i>	United Kingdom	DQ100499	DQ198742	DQ198652	DQ198574	Bocakova et al. 2007
Elateridae	Thylacostern.	<i>Pterotars. bimaculatus</i>	Bolivia	-	DQ198743	-	DQ198575	Bocakova et al. 2007
Elateridae	Denticollinae	<i>Stenagostus rhombeus</i>	United Kingdom	AF451945	DQ198744	DQ198653	DQ198576	Bocakova et al. 2007
Lycidae	Libnetinae	<i>Libnetis granicollis</i>	Japan	DQ181107	DQ181181	DQ181033	DQ181255	Bocak et al. 2008
Lycidae	Dictyopterinae	<i>Lycoprogenthes</i> sp.	Indonesia	DQ181070	DQ181144	DQ180996	DQ181218	Bocak et al. 2008
Lycidae	Dictyopter.	<i>Taphes brevicollis</i>	Laos	DQ181098	DQ181172	DQ181024	DQ181246	Bocak et al. 2008
Lycidae	Dictyopter.	<i>Dictyoptera elegans</i>	Japan	DQ181073	DQ181147	DQ180999	DQ181221	Bocak et al. 2008
Lycidae	Dictyopter.	<i>Benib. nigripennis</i>	Japan	DQ181075	DQ181149	DQ181001	DQ181223	Bocak et al. 2008
Lycidae	Dictyopter.	<i>Pyropt. nigroruber</i>	Japan	DQ181077	DQ181151	DQ181003	DQ181225	Bocak et al. 2008
Lycidae	Lyropaeinae	<i>Alyculus kurbatovi</i>	Indonesia	DQ181072	DQ181146	DQ180998	DQ181220	Bocak et al. 2008
Lycidae	Lyropaeinae	<i>Lyropaeus</i> sp.	Malaysia	DQ181042	DQ181116	DQ180968	DQ181190	Bocak et al. 2008
Lycidae	Lyropaeinae	<i>Antenn. constrictus</i>	Malaysia	DQ181051	DQ181125	DQ180977	DQ181199	Bocak et al. 2008
Lycidae	Lyropaeinae	<i>Microlyr. dembickyi</i>	Indonesia	DQ181071	DQ181145	DQ180997	DQ181219	Bocak et al. 2008
Lycidae	Lyropaeinae	<i>Platerodrilus</i> sp.	Malaysia	DQ181037	DQ181111	DQ180963	DQ181185	Bocak et al. 2008
Lycidae	Lyropaeinae	<i>Macrolibnetis</i> sp.	Malaysia	DQ181050	DQ181124	DQ180976	DQ181198	Bocak et al. 2008
Lycidae	Lyropaeinae	<i>Pendola</i> sp.	Indonesia	DQ181058	DQ181132	DQ180984	DQ181206	Bocak et al. 2008
Lycidae	Lyropaeinae	<i>Horakiella emasensis</i>	Malaysia	DQ181110	DQ181184	DQ181036	DQ181258	Bocak et al. 2008
Lycidae	Ateliinae	<i>Dilophotes</i> sp.	Malaysia	DQ181066	DQ181140	DQ180992	DQ181214	Bocak et al. 2008
Lycidae	Ateliinae	<i>Scarelus</i> sp.	Malaysia	DQ181085	DQ181159	DQ181011	DQ181233	Bocak et al. 2008
Lycidae	Lycinae	<i>Dihammatus</i> sp.	Malaysia	DQ181043	DQ181117	DQ180969	DQ181191	Bocak et al. 2008
Lycidae	Lycinae	<i>Eurrhacus</i> sp.	Ecuador	DQ181056	DQ181130	DQ180982	DQ181204	Bocak et al. 2008
Lycidae	Lycinae	<i>Macrolycus</i> sp.	China	DQ181049	DQ181123	DQ180975	DQ181197	Bocak et al. 2008
Lycidae	Lycinae	<i>Thonalmus hubbardi</i>	Montserrat	DQ181094	DQ181168	DQ181020	DQ181242	Bocak et al. 2008
Lycidae	Lycinae	<i>Lyponia quadricollis</i>	Korea	DQ181101	DQ181175	DQ181027	DQ181249	Bocak et al. 2008

Lycidae	Lycinae	<i>Lycus</i> sp.	South Africa	DQ181039	DQ181113	DQ180965	DQ181187	Bocak <i>et al.</i> 2008
Lycidae	Lycinae	<i>Idiopt. biplagiatum</i>	Ecuador	DQ181057	DQ181131	DQ180983	DQ181205	Bocak <i>et al.</i> 2008
Lycidae	Lycinae	<i>Metriorrh. lineatus</i>	Malaysia	DQ181040	DQ181114	DQ180966	DQ181188	Bocak <i>et al.</i> 2008
Lycidae	Lycinae	<i>Platycis minutus</i>	Czech Republic	DQ181069	DQ181143	DQ180995	DQ181217	Bocak <i>et al.</i> 2008

Some 18S rDNA sequences were originally published by Caterino *et al.* (2002) or submitted to Genbank by I. Ribera (BMNH London). The authors were cited by Bocakova *et al.* (2007).

Supplementary Table 2. Primers used for PCR amplifications

Fragment	Code	-mer	Sequence (5' >> 3')
18S rRNA	5'	24	GACAACCTGGTTGATCCTGCCAGT
	b5.0	19	TAACCGCAACAACCTTTAAT
	ai	22	CCTGAGAAACGGCTACCACATC
	b2.5	20	TCTTTGGCAAATGCTTTCGC
	a1.0	20	GGTGAAATTCTTGGACCGTC
	bi	20	GAGTCTCGTTCGTTATCGGA
	a2.0	19	ATGGTTGCAAAGCTGAAAC
	3'I	24	CACCTACGGAAACCTTGTTACGAC
28S rRNA	ff	20	TTACACACTCCTTAGCGGAT
	dd	19	GGGACCCGTCTTGAAACAC
16S rDNA	16a	20	CGCCTGTTTAACAAAAACAT
	16b	22	CCGGTCTGAACTCAGATCATGT
	ND1A	27	GGTCCCTTACGAATTTGAATATATCCT
	ND1-2	24	ATCAAAAAGGAGCTCGATTAGTTTC
<i>cox1</i> mtDNA	Jerry	23	CAACATTTATTTTGATTTTTTGG
	Pat	25	TCCATTGCACTAATCTGCCATATTA

Supplementary Table S3. Numbers of taxa and characters in the analyzed datasets

Alignment method size of dataset	Number of characters	Constant characters	Variable uninformative	Parsimony informative
Clustal				
210 taxa dataset				
full length	4363	1865	289	2209
variable out	3743	1837	289	1617
155 taxa dataset				
full length	4363	2250	398	1715
variable out	3803	2102	273	1428
BlastAlign				
210 taxa dataset	4787	2757	520	1510
155 taxa dataset	4787	2927	486	1374

Supplementary Table S4. Results of the parsimony analysis of the partial analyses of the dataset of 155 taxa aligned by ClustalX under default settings and with variable regions omitted. The positions were inferred from the majority consensus trees (M - taxa form a monophylum, P - paraphylum).

	Omalisidae+ Elateridae+ Drilidae	<i>Omalisus</i> + <i>Pseudeuanoma</i>	Drilidae+ Elateridae	Drilidae+ Agrypninae	<i>Cebrio</i> in Elaterinae	<i>Cebrio</i> + Physorhinini
18S	P*	-	M**	M	M	P
28S	P	n. a.	-	-	-	-
16S	M	M	M	M	M	P#
coi	P***	-	M	P****	P##	-
nucl. rDNA	P*	-	P	P	M	P
mtDNA	M	M	M	M	P###	M

* Phengodidae+Rhagophthalmidae found as a part of the clade Omalisidae + Elateridae + Drilidae

** Drilidae formed a clade with a part of Elateridae

*** Several unrelated taxa found within the clade

**** Drilidae formed a clade with Agrypninae and other lineage of Elateridae

Cebrio formed a clade with a part of Physorhinini

Cebrio in the clade with a part of Elaterinae and some other subfamilies of Elateridae

Cebrio in the clade with a part of Elaterinae

n.a. The sequence of 28S rDNA was unavailable for *Pseudeuanoma*

Supplementary Table S5. An overview of the classification of Elateridae (changes in the status refer to Costa *et al.* 2010)

Agrypninae Candèze, 1857 (~120 genera, 2500 species; cosmopolitan)

- =Adelocerinae Du Buysson, 1893
 - =Alauinae Laurent, 1973
 - =Cavicoxidae Arnett, 1962
 - =Conoderinae Fleutiaux, 1919
 - =Hemirhipinae Candèze, 1857
 - =Octocryptinae Candèze, 1892
 - =Pachyderinae Fleutiaux, 1919
 - =Pangaurinae Gistel, 1856
 - =Phyllophoridae Hope, 1842
 - =Pyrophorinae Candèze, 1863
 - =Tetralobinae Laporte, 1840
 - =Drilidae Blanchard, 1845
 - Agrypnini Candèze, 1857
 - Anaissini Golbach, 1984
 - Cleidecostini Johnson, 2002 (=Heligmini Costa, 1975)
 - Hemirhipini Candèze, 1857
 - Monocrepidiini Candèze, 1859 (=Conoderini Fleutiaux, 1919; Oophorini Gistel, 1856)
 - Platycrepidiini Costa & Casari-Chen, 1993
 - Pseudomelanactini Arnett, 1967
 - Pyrophorini Candèze, 1863
 - Tetralobini Laporte, 1840
- Drilini Blanchard, 1845 - new status**

Campyloxeninae Costa, 1975 (1, 1; Neotropical region)

Cardiophorinae Candèze, 1859 (~35, 800; cosmopolitan)

- =Esthesopinae Fleutiaux, 1919

Denticollinae Stein & Weise, 1877 (~170, 1700; cosmopolitan)

- =Athoinae Candèze, 1859
- =Campsosterninae Fleutiaux, 1927
- =Campylidae Gistel, 1856
- =Corymbitinae Candèze, 1863
- =Crepidomeninae Candèze, 1863
- =Ctenicerinae Fleutiaux, 1936
- =Dendrometrinae Gistel, 1856
- =Diminae Candèze, 1863
- =Hemicrepidinae Champion, 1894

- =Hypnoidinae Schwarz, 1906
- =Hypolithinae Fleutiaux, 1928
- =Lepturoidinae Schwarz, 1906
- =Melanactinae Candèze, 1857
- =Morostominae Dolin, 2000
- =Oxynopterinae Candèze, 1857
- =Pityobiinae Hyslop, 1917
- =Pleonominae Semenov & Pjatakova, 1936
- =Prosternidae Gistel, 1856
- =Rostricephalinae Fleutiaux, 1947
- =Semiotinae Jacobson, 1913
- =Senodoniinae Schenkling, 1927
 - Ctenicerini Fleutiaux, 1936 (=Prosternini Gistel, 1856)
 - Crepidomenini Candèze, 1863
 - Denticollini Stein & Weise, 1877 (=Athoini Candèze, 1859; Dendrometrini Gistel, 1856)
 - Dimini Candèze, 1863
 - Hypnoidini Schwarz, 1906
- Oxynopterini Candèze, 1857 - new status**
- Pityobiini Hyslop, 1917 - new status**
- Pleonomini Semenov & Pjatakova, 1936
- Semiotini Jacobson, 1913 - new status**
- Senodoniini Schenkling, 1927

Elaterinae Leach, 1815 (~200, 3500; cosmopolitan)

- =Adrastinae Candèze, 1863
- =Agriotinae Champion, 1894
- =Ampedidae Gistel, 1856
- =Amphilabridae Gistel, 1856
- =Aplastinae Stibick, 1979
- =Athoomorphinae Laurent, 1966
- =Cardiorhininae Candèze, 1891
- =Cebriognathinae Paulus, 1981
- =Cebriionidae Latreille, 1802
- =Cratonychidae Gistel, 1956
- =Dicrepidinae Candèze, 1859
- =Melanotinae Candèze, 1859
- =Plastoceridae LeConte, 1861 (not Crowson, 1972)
- =Physorhininae Candèze, 1859
- =Steatoderidae Gistel, 1856
- =Synaptidae Gistel, 1856

- Adrastini Candèze, 1863
- Agriotini Champion, 1894
- Ampedini Gistel, 1856
- Dicrepidini Candèze, 1859
- Elaterini Leach, 1815
- Megapenthini Gurjeva, 1973
- Melanotini Candèze, 1859
- Odontonychini Girard, 1972
- Physorhinini Candèze, 1859
- Pomachiliini Candèze, 1859
- Cebriionini Latreille, 1802 - new status**
- Aplastini Stibick, 1979 - transf. from Cebriioninae**

Eudicronychinae Girard, 1971 (3, 29; Afrotropical region)

- =Dicronychidae Schwarz, 1897

Hemiopinae Fleutiaux, 1941 (4, 30; Oriental, Palaearctic and Afrotropical regions)

Lissominae Laporte, 1835 (~10, 150; cosmopolitan)

- =Oestodinae Hyslop, 1917
- =Protelateridae Schwarz, 1902
 - Lissomini Laporte, 1835
 - Oestodini Hyslop, 1917
 - Protelaterini Schwarz, 1902

Morostominae Dolin, 2000 (2, 15; Afrotropical region)

Negastriinae Nakane & Kishii, 1956 (~29, 570; cosmopolitan)

Physodactylinae Lacordaire, 1857 (~8, 40; Neotropical, Oriental and Afrotropical regions)

- =Toxognathinae Fleutiaux, 1941

Subprotelaterinae Fleutiaux, 1920 (1, 4; Palaearctic region - Japan and New Caledonia)

Thylacosterninae Fleutiaux, 1920 (~5, 45; Australian, Oriental, Afrotropical, and Neotropical regions)

- =Balginae Fleutiaux, 1926
- =Soleniscinae Lameere, 1900