66 (2) 165–190

A Contribution to the Phylogeny of the Ciidae and its Relationships with Other Cucujoid and Tenebrionoid Beetles (Coleoptera: Cucujiformia)

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Received 12.vi.2008, accepted 10.x.2008.
Published online at www.arthropod-systematics.de on 05.xii.2008.

> Abstract

In order to study phylogenetic relationships in Ciidae, test its monophyly, and resolve its position within Cucujiformia, we sampled 20 species from 12 genera of Ciidae (*Sphindocis* not included), 27 species from 20 other families of Cucujoidea and Tenebrionoidea, 2 species from the cleroid family Trogossitidae (all Cucujiformia), and a bostrichid (Bostrichiformia). We analysed 18S, COI, and – for Ciidae – COII sequences according to maximum parsimony (fixed alignment with equal and differentiated weighting, and partial direct optimization), maximum likelihood, and Bayesian methodology, all applied to three different subsets of the taxon sample. Saturation curves indicate that 18S, COI, and COII are informative at the systematic levels in question. We demonstrate that the extent to which a particular subgroup is sampled can strongly influence the phylogenetic results, even in distant corners of the tree. Ciidae is obtained as monophyletic. We find non-monophyly for the speciose genus *Cis*, and for *Sulcacis*. Different analyses suggest either *Ropalodontus*, or *Sulcacis fronticornis* + *Xylographus* + *Octotemnus*, or *Sulcacis fronticornis* + *Xylographus* alone as the sister group of the remaining Ciidae. Apart from a clade *Sulcacis fronticornis* + *Xylographus* the results for inter-generic relationships in Ciidae vary strongly with the analytical methods and taxon sampling. Different analyses place Ciidae as sister to Nitidulidae or far basally and isolated in the cucujoid-tenebrionoid assemblage. Regarding the phylogeny of the cucujoid-tenebrionoid assemblage, resolution is mostly conflicting. Yet, monophyly is supported for Sphindidae, Cryptophagidae, Tenebrionidae, Coccinellidae + Endomychidae, and Tetratomidae + Anthicidae + Monotomidae. Altogether, families from Cucujoidea and Tenebrionoidea are fairly mixed up in our trees, and the cleroid Trogossitidae falls within the cucujoid-tenebrionoid assemblage.

> Key words

Cucujiformia, Tenebrionoidea, Cucujoidea, Cleroidea, Ciidae, Cisidae, phylogeny, molecular data.

1. Introduction

1.1. Ciidae

Ciidae (minute tree-fungus beetles) is one of the moderately sized families in the Coleoptera-Cucujiformia and has a worldwide distribution. These beetles (Fig. 1) have a ± cylindrical body 0.5–7 mm long, and most of the ca. 640 known species (Abdullah 1973 and scattered species descriptions thereafter; C. Lopes-Andrade pers. comm.) are mycophagous, living in the basidiocarps of tree fungus (e.g. Orledge & Reynolds 2005). Among the 42 genera distinguished within Ciidae, *Cis* with its ca. 350 species is by far the largest.

Ciidae comprises two subfamilies (Lawrence 1974a,b; Thayer & Lawrence 2002): the species-rich Ciinae, and the Sphindociinae with the single species *Sphindocis denticollis* from Northern California. According to Lawrence (1974b, 1991: 502), their close

relationship may be supported by the presence of a small but distinctive (putative) lacinia in the larval mouthparts (if this a character reversal); however, a similar mouthpart lobe is rare but not unique in Cucujiformia (Anthribidae, LAWRENCE 1991: fig. 34.847) and also occurs, at least, in the Bostrichoidea (LAWRENCE 1991). *Sphindocis* and Ciinae also share a midventral setose, glandular patch (fovea) on the 3rd (= 1st visible) abdominal ventrite of the adult male (Fig. 2), but a fovea with identical location occurs in a number of unrelated cucujiform beetles from Bruchidae, Anthribidae, Tenebrionidae, and Erotylidae (FAUSTINI & HALSTEAD 1982; WĘGRZYNOWICZ 2002). Accordingly, the assignment of *Sphindocis* to Ciidae is quite tentative (LAWRENCE 1974b, pers. comm. 2006).

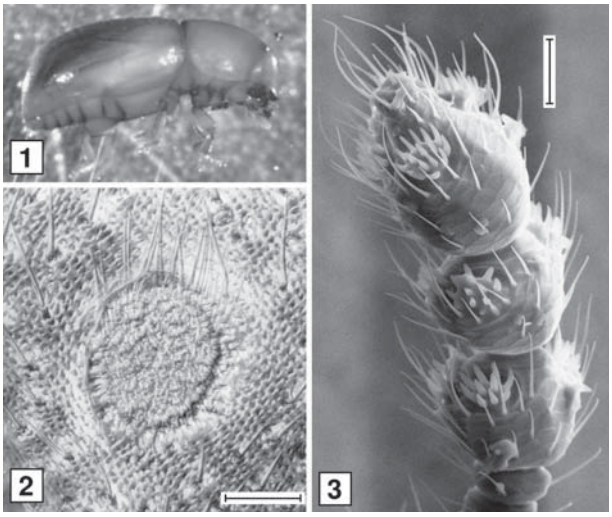
The monophyly of Ciinae was never seriously doubted, although its support is actually quite vague as well. It relies on derived characters that are frequently found in various other cucujiform taxa (see LAWRENCE 1974a,b). An example is the presence of 4 or more compound sensilla on each of the 2 or 3 distal antennomeres (antennal club; Fig. 3); similar structures also occur in, for instance, many Tenebrionidae (MEDVEDEV 1977: antennal tenebrionoid sensoriae) and Bostrichidae (own observations) – while, however, an ultrastructural comparison among these taxa is missing. The internal phylogeny of Ciinae is also highly uncertain. The division into three tribes Ciini, Orophiini, and Xylographellini (the latter erected by KAWANABE & MIYATAKE 1996) is mainly based on surmised apomorphies shared by Xylographellini and Orophiini (strongly projecting forecoxae and strongly spinose foretibiae), or the genera in Xylographellini (distinctive type of antennal club and praementum, and a Y-shaped 9th abdominal segment), while neither Orophiini nor Ciini is characterized by apomorphies. THAYER & LAWRENCE (2002) dismiss any current tribal classification of Ciinae and demand further studies. There have so far been no attempts to classify Ciidae based on cladistic methods, neither using morphology nor molecules.

The phylogenetic relationships of Ciidae to other cucujiform families are also unclarified. Ciidae had long been placed in the Cucujoidea (the "Clavicornia"; e.g. CROWSON 1955). CROWSON (1960) transferred them to the Tenebrionoidea (the "Heteromera") based mainly on characters of the aedeagus and the larval abdomen, and this has been maintained until today (LAWRENCE 1971, 1974a, 1991; LAWRENCE & NEWTON 1995; BEUTEL & LESCHEN 2005). LAWRENCE (1977) tentatively proposed a tenebrionoid subclade including Ciidae, Pterogeniidae, Archeocrypticidae, Tetratomidae, and Mycetophagidae. LAWRENCE & NEWTON (1982), however, set the Tetratomidae in relationship with a grouping Melandryidae + Mordellidae + Rhipiphoridae, while on the other hand they view an assemblage comprising "Pterogeniidae, Archeocrypticidae, and probably Ciidae". Hypotheses on the relationships of Ciidae are generally vaguely formulated and are founded on characters that are highly homoplastic, as evident from their scattered and incongruent occurrence in other cucujiform taxa. Indeed, since the relationships among the family-level lineages of Cucujiformia are altogether very unclear, the affinities of Ciidae might as well lie in any other corner of that taxon. Therefore, the attempt to resolve ciid relationships requires consideration of the entire Cucujiformia.

1.2. Cucujiformia

This most species-rich subgroup of Coleoptera (ca. 207,000; KLAUSNITZER 2005: 489) is classified into the superfamilies Lymexyloidea (ship-timber beetles), Cleroidea (checkered beetles and relatives), Cucujoidea, Tenebrionoidea, Chrysomeloidea (leaf and longhorn beetles), and Curculionoidea (weevils). Its monophyly is clearly supported by the Coleoptera-wide analyses of 18S by VOGLER & CATERINO (2003), VOGLER (2005; 973 coleopteran taxa), and HUNT et al. (2008; 1900 coleopteran taxa, COI and 16S additionally included for part of the taxa). It is also well-supported by morphological apomorphies such as the reduced spiracles of abdominal segment VIII, acone ommatidia with open rhabdomes, reductions in the abdominal segments IX and X, and peculiarities in the metendosternite and aedeagus (e.g. LAWRENCE & NEWTON 1982; LAWRENCE & BRITTON 1991: 644; KLAUSNITZER 2005: 489). Cryptonephridism may furthermore support a close relationship of Cucujiformia to the bostrichiform lineage Bostrichoidea (BEUTEL 1996). This, however, is contradicted by molecular studies (HUNT et al. 2008: Bostrichiformia + Elateriformia + larger part of Staphyliniformia as sister to Cucujiformia). The diversification of Cucujiformia into its family-level subgroups probably occurred during the late Triassic to early Cretaceous (ca. 220–100 MYA; GRIMALDI & ENGEL 2005: 391; HUNT et al. 2008).

Most workers tentatively accept a lineage Cucujoidea + Tenebrionoidea, which includes well over 50,000 species (KLAUSNITZER 2005) in 58 families (according to BEUTEL & LESCHEN 2005, whose classification is followed herein; see also LAWRENCE & NEWTON 1995). Commonly known families are the Coccinellidae (ladybird beetles), Erotylidae (pleasing fungus beetles), Tenebrionidae (darkling beetles), and Meloidae (blister beetles). Other large exemplar groups are the Ciidae, Cucujidae, Endomychidae, Phalacridae, Nitidulidae, Corylophidae, Silvanidae, Cerylonidae, Latridiidae, Mordellidae, Melandryidae, and Oedemeridae, while some families include only one or a few genera. Nonetheless, the monophyly of



Figs. 1–3. Morphology of Ciidae. **1**: A male of *Cis boleti* in lateral view; the specimen is 3.5 mm long. **2**: The glandular fovea on the first visible abdominal ventrite (= coxosternum III) of a male of *Cis nitidus*; scale 40 μm. **3**: Distal antennomeres of *Ropalodontus perforatus*, with compound sensilla; scale 30 μm.

Cucujoidea + Tenebrionoidea as well as Cucujoidea and Tenebrionoidea is hardly supported by conclusive morphological apomorphies. Cleroidea as well as Chrysomeloidea and Curculionoidea might well be nested within the cucujoid-tenebrionoid assemblage (Crowson 1960: 126). The only partly resolved basal cucujiform relationships reported by Hunt et al. (2008: supporting fig. S1) are consistent with these possibilities, and they furthermore even unambiguously suggest that (polyphyletic) Lymexyloidea are closely related to or nested in Tenebrionoidea.

The phylogenetic relationships among the cucujoid and tenebrionoid "families" have remained grossly unclear, as have the monophyly of many of these "families" and the position of a number of individual genera. Recent years have seen numerous systematic rearrangements, such as the inclusion of Alleculidae, Lagriidae, and Nilionidae in Tenebrionidae (see Lawrence & Newton 1995 for references), the inclusion of Languriidae in Erotylidae (Węgrzynowicz 2002; Robertson et al. 2004; Leschen & Buckley 2007), and the erection of separate families for aberrant genera (e.g. Leschen et al. 2005). Altogether, the classification of the entire cucujoid-tenebrionoid(-cleroid) assemblage is still vividly in flux. One reason for this unsatisfactory situation is the high degree of parallel evolution in most morphological characters, which is evident from the vast incongruence of their distribution across taxa (e.g. Ślipiński & Pakaluk 1991). Morphology-based cladistic work is at a very early stage. The most comprehensive approach is that of Leschen et al. (2005), who used a matrix of 99 characters for 37 taxa and focused on the smaller "basal" families of Cucujoidea.

Molecular analyses specifically dedicated to the cucujoid-tenebrionoid(-cleroid) assemblage, or the cucujiforms, are lacking. Robertson et al.'s (2004, 2008) phylogenetic studies of Erotylidae (incl. Languriidae; using 18S and 28S) and of the cerylonid series (Latridiidae, Endomychidae, Coccinellidae, Discolomatidae, Corylophidae, Cerylonidae, Bothrideridae, and Alexiidae), both using 18S and 28S, bear some evidence on interfamilial relationships at the cucujiform level due to rich outgroup sampling also including Ciidae. However, there is no non-cucujiform outgroup taxon included in Robertson et al. (2008). The Coleoptera-wide large scale (1900 taxa) analysis by Hunt et al. (2008) includes most cucujiform families. 18S has therein been used for all included taxa, whereas two other genes (16S, COI) have been sequenced for only some 20% of them (proportionately more in Chrysomelidae). Hunt et al. (2008: Bayesian analysis in supporting fig. S1) find for Cucujiformia a basal polytomy of 5 clades: Sphindidae; Cleroidea (including also Byturidae and Biphyllidae); cerylonid series; Tenebrionoidea (incl. Lymexyloidea); and Chrysomeloidea + Curculionoidea + some cucujoid families; overall, however, basal cucujiform relationships are widely unresolved or represented by weakly supported nodes in that contribution.

1.3. Scope of the study

Our primary objective is (1) to study the internal phylogeny of the Ciidae. For this purpose we have compiled a taxon sample of 20 ciid species. 6 of these belong to the species-rich genus *Cis*, which, however, might be a para- or polyphyletic assemblage lacking the various specialisations that define the other, smaller genera. The other sampled ciids represent the genera *Orthocis*, *Ceracis*, *Octotemnus*, *Ennearthron*, *Neoennearthron*, *Dolichocis*, *Falsocis*, *Ropalodontus*, *Xylographus*, *Sulcacis*, and *Strigocis* (unfortunately, we were not able to obtain sequences from *Sphindocis*). Two further goals of our study are (2) to test the monophyly of Ciidae (or rather Ciinae), and (3) to find indications on which taxa among the Cucujoidea and Tenebrionoidea are their closest relatives. We therefore additionally sequenced 27 species that represent 20 other cucujoid and tenebrionoid families plus 2 species of the cleroid family Trogossitidae. In order to have an unambiguous outgroup for this entire sample we furthermore included a member of Bostrichidae (Bostrichiformia). In this way our taxon sample could also yield some tentative results on family-level relationships in the cucujoid-tenebrionoid assemblage.

We sequenced fragments of the nuclear 18S rDNA (791–837 bp) and the mitochondrial COI (641 bp) for (almost) all taxa. For the ciids and 2 non-ciid out-

group species we additionally included the mitochondrial COII (673–678 bp plus 24–30 bp of the adjacent tRNA-Lys) in order to strengthen resolution within Ciidae. While the CO genes were intended to contribute to the apical parts of our trees, the 18S should yield resolution for the more basal nodes (see VOGLER 2005).

2. Material and methods

2.1. Taxon sampling

The species we studied are listed in Tab. 1, which also includes the classification (according to BEUTEL & LESCHEN 2005), provenience of specimens, and GenBank accession numbers for sequences. The Ciidae are represented by 20 species from a total of 12 genera. All belong to the tribes Ciini and Orophiini of Ciinae, while Ciinae-Xylographellini and Sphindociinae are lacking in the sample. We included 27 further species representing 11 families of Cucujoidea and 9 families of Tenebrionoidea, and 2 species were selected from the cleroid family Trogossitidae. We additionally use *Bostrichus capucinus* (Bostrichiformia: Bostrichidae) as outgroup taxon for the entire cucujoid-tenebrionoid assemblage. This species is phylogenetically clearly (as can be) outside the Cucujiformia, as it lacks the apomorphies supporting this group, and as the Bostrichiformia is a clade distinctly remote from Cucujiformia in the phylogenetic trees in VOGLER (2005) and HUNT et al. (2008). Most of the specimens we sequenced were entirely used up in the extraction procedure; the remaining ones are deposited at the Museum of Zoology Dresden.

The identification of species is based on REITTER (1901), LOHSE (1967), LAWRENCE (1971), and THAYER & LAWRENCE (2002) for Ciidae, and on FREUDE et al. (eds. 1967, eds. 1969), ARNETT et al. (eds. 2002), and DOWNIE & ARNETT (1996) for the remaining beetle families. Further species were identified by specialists of taxa (see Acknowledgements) or regional faunas.

2.2. Extraction of DNA

Total genomic DNA was isolated by an overnight incubation at 55°C in lysis buffer (6% DTAB, 5 M NaCl, 1 M Tris-HCl, 0.5 M EDTA, pH 8.0) including 0.5 mg of proteinase K (Merck), and subsequent purification following the DTAB method (GUSTINCICH et al. 1991). DNA was precipitated from the supernatant with 0.2 volumes of 4 M LiCl and 0.8 volumes of isopropanol, centrifuged, washed, dried, and resuspended in TE buffer.

2.3. PCR and sequencing

Fragments from two genes were amplified for all samples: the (mitochondrial) cytochrome-c-oxidase subunit I, COI (first half), and the (nuclear) small ribosomal subunit, 18S (entire gene, but only the first half was sequenced). In addition, the entire cytochrome-c-oxidase subunit II, COII (including part of the adjacent tRNA-lysine), was amplified for the samples of Ciidae and two outgroup taxa (*Tribolium castaneum*, Tenebrionidae, and *Mallodrya subaenea*, Synchroidae). PCR was performed in a 50 µL volume (50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, and 0.5% Triton X-100, pH 8.5) containing 1 unit of *Taq* DNA polymerase (Bioron), 10 pmol dNTPs (Eppendorf), and 10 pmol of each primer. We used the following primers:

(**1**) 18Sfor [CTCATTAAATCAGTTATGGTTCC] and 18Srev [CACCTCTAACGTCGCAATAC] (after BOPP & CAPESIUS 1996) for the 18S fragment;

(**2**) LCO1490 [GGTCAACAAATCATAAAGATATTGG] and HCO2198 [TAAACTTCAGGGTGACCAAAAAATCA] (FOLMER et al. 1994) for the COI fragment;

(**3**) TL2-J-3037mod.2 [TAATATGGCAGATT(at)(ct)(ag)TG(agct)A(at)TGG] (HUNDSDOERFER et al. 2005) and TK-N-3782 [GAGACCATTACTTGCTTTCAGTCATCT] (SIMON et al. 1994) for the COII gene.

PCR products were sequenced directly with the forward primers on an ABI 3730XL at the DNA Sequencing Facility of the Max Planck Institute of Molecular Cell Biology and Genetics (Dresden, Germany).

2.4. Alignment

Editing of the sequences was performed in BioEdit (HALL 1999) and the "accessory application" ClustalW (THOMPSON et al. 1994) was used for a first alignment. Modifications were undertaken by eye.

No indels were encountered in the COI sequences. In the COII gene *Cis chinensis* and *Sulcacis affinis* contained 6 single base deletions each (alignment positions 367–369 and 373–375 in *C. chinensis* and 379–384 in *S. affinis*), resulting in two missing amino acids. The positioning of the 2×3 gaps each was performed in the most parsimonious way and resulted in autapomorphic deletions only (alignment can be obtained from the authors upon request). The positioning of the gaps in the short fragment of the proximate tRNA-lysine

(3–6, present in all Ciidae when aligned to *Mallodrya subaenea*) was dealt with in the same way.

The 18S sequences contained several hypervariable regions of ambiguous alignment that were excluded from all analyses except for those based on direct optimisation (alignment positions 53–55, 104–139, 175–177, 556–558, 579–580, 594–597, 613–666, 670–677). Alignment lengths, data variability, and nucleotide composition are presented in Tab. 2.

2.5. Phylogenetic analyses

Taxasets. We ran analyses based on three different subsets of our taxon sample (Tab. 3):
(1) Entire sample (E-sample), which includes all taxa we sampled (as in Tab. 1). We used the data from 18S and COI, which are available across (almost) all taxa. Analyses based on this sample are aimed at resolving ciid (non-)monophyly, the placement of Ciidae within the cucujoid-tenebrionoid assemblage, and to some extent the interrelationships among cucujoid-tenebrionoid subgroups.
(2) Reduced sample (R-sample), for which we have excluded most of the Ciidae species (except for *Ennearthron cornutum*, *Octotemnus laevis*, *Falsocis brasiliensis*, and *Cis boleti*) in order to avoid potential bias by over-representation of a single cucujoid-tenebrionoid subgroup. We again used the data from 18S and COI. The analyses are aimed at resolving the position of Ciidae and interrelationships among cucujoid-tenebrionoid subgroups, and at evidencing potential conflicts with analyses under (1).
(3) Ciid sample (C-sample), which includes all sampled Ciidae plus *Scymnus abietis* (Coccinellidae), *Tetratoma fungorum* (Tetratomidae), and *Mallodrya subaenea* (Synchroidae), here acting as outgroup taxa. We used the data from 18S, COI, and COII which are available for (almost) all these taxa. These analyses are aimed at resolving internal relationships in Ciidae.

Analytical procedures. Each of the three taxasets was analysed according to three optimality criteria:
(1) Maximum parsimony (MP) using PAUP* 4.0b10 (Swofford 2002), for which we used the combined data from all included genes. These analyses (command: hs add=cl rearrlimit=10000000 limitperrep=yes rstatus=yes) were first run under equally weighted conditions (ew). In addition, they were run under unequal (differentiated) character weighting (dw), for which average character state change frequencies were converted into weighting factors in the following way (based on the sequence alignment block): factor = 1 / frequency of sites showing the substitution type. The average frequencies of the character state changes A→C, A→G, A→T, C→G, C→T, G→T, as well as C→A, G→A, T→A, G→C, T→C, T→G, were calculated with MEGA (bidirectional, site-by-site nucleotide pair frequencies) and the corresponding weighting factors with Microsoft Excel. The latter were subsequently implemented as usertype step matrices in PAUP. The step matrices were calculated separately for the different gene fragments and, in protein-coding genes, also separately for the 3 codon positions. Invariable positions were excluded.
(2) Bayesian analyses (MB) using MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001), for which we used the combined data from all included genes and additionally analysed the individual genes separately. The run parameter setting commands were the following: mcmcp ngen=10000000 nchains=4 nrun=2 sample=500 temp=0.1 mcmcdiagn=yes Diagnfreq=1000 Swapfreq=1 Nswaps=1 printfreq=500 Savebrlens=yes Startingtree=random. The model of sequence evolution was determined and set separately for the different gene fragments and, in protein-coding genes, also separately for the 3 codon positions. It was established by the Akaike Information Criterion (AIC), as implemented in Modeltest 3.06 (Posada & Crandall 1998). The parameter values were subsequently estimated during tree search (not fixed) in MrBayes. With a burn-in of 500, the first trees before the chains had reached the plateau were excluded from the consensus reconstruction.
(3) Maximum likelihood (ML) using PAUP* 4.0b10, for which we used the combined data from all included genes. The best evolutionary model was again established by the AIC, as implemented in Modeltest 3.06 (Posada & Crandall 1998). These parameters were fixed for the ML calculations.
(4) For the ciid sample we furthermore conducted an analysis following direct optimisation (DO; as implemented in POY 3.0.11) with the commands -buildspersreplicate 50 -replicates 10 and three input files: i) the mitochondrial protein-coding sequence fragments of the COI and COII plus the first 121 unambiguously aligned bp of the 18S (defined as prealigned, the 6 autapomorphic deletions in COII were coded as N's); ii) the remaining (unaligned) part of the 18S including the hypervariable parts; iii) the unaligned 23–29 bp of the tRNA-Lys (sequenced with the COII). Two symmetrical rate matrices were defined with each base change identical, and gap costs set to either 2x or 4x the maximum base change cost. The reason for applying DO was to include evidence from the hypervariable parts in the analysis of relationships among the relatively closely related ciid species.

The phylogenetic analyses thus altogether comprised the 19 reconstructions listed in Tab. 3. Trees were rooted between the sole non-cucujiform beetle (*Bostrichus*) and the members of the cucujoid-tenebrionoid-cleroid assemblage in case of the E- and R-

Tab. 1. Coleoptera species sequenced for this study, with systematic assignment and sequenced genes and their accession numbers (GenBank). * identification by C. Lopes-Andrade (some doubt remaining for *Cis chinensis*); ** *Rhizophagus dispar* or *R. bipustulatus*; $^{+}$ collected in wood imported from Czech Republic (Moravsky Krumlov), provenience thus unclear; $^{++}$ introduced to Brasil from China (C. Lopes-Andrade, pers. comm.). **AF** = abbreviation of family name; **AG** = abbreviation of genus name (only for Ciidae); **SF** = Superfamily: [**B**] = Bostrichoidea; [**M**] = Cleroidea (relatives of Melyridae); [**C**] = Cucujoidea; [**T**] = Tenebrionoidea. **n.a.** = not applicable: either sequencing was not attempted (1), or attempted but not successful (2).

Species	Family(-Tribe)	AF	AG	SF	COI	COII	18S	Provenience
Bostrichus capucinus (L., 1758)	Bostrichidae	BOS		[B]	FM877906	n.a. (1)	FM877860	Germany, Saxonia, Dresden^{+}
Tenebroides corticalis Melsheimer, 1844	Trogossitidae	TRO		[M]	FM877907	n.a. (1)	FM877880	Canada, Ontario, Westport
Thymalus marginicollis Chevrolat, 1842	Trogossitidae	TRO		[M]	FM877908	n.a. (1)	FM877901	Canada, Ontario, Westport
Kateretes rufilabris (Latreille, 1807)	Brachypteridae	BRA		[C]	FM877909	n.a. (1)	FM877887	Germany, Saxonia, Dresden, Keulenberg
Coccinula quatuordecimpustulata (L., 1758)	Coccinellidae	COC		[C]	FM877910	n.a. (1)	FM877889	Germany, Saxonia, Dresden, Keulenberg
Scymnus abietis (Paykull, 1798)	Coccinellidae	COC		[C]	FM877911	n.a. (1)	FM877891	Germany, Saxonia, Dresden, Keulenberg
Atomaria sp. Stephens, 1829	Cryptophagidae	CRY		[C]	FM877912	n.a. (1)	FM877895	Germany, Saxonia, Dresden, Keulenberg
Telmatophilus typhae (Fallén, 1802)	Cryptophagidae	CRY		[C]	FM877913	n.a. (1)	FM877898	Canada, Ontario, Westport
Endomychus biguttatus Say, 1824	Endomychidae	END		[C]	FM877914	n.a. (1)	FM877899	Canada, Ontario, Newborough Lake
Triplax russica (L., 1758)	Erotylidae	ERO		[C]	FM877915	n.a. (1)	FM877878	Germany, Saxonia, Dresden
Cortinicara gibbosa (Herbst, 1793)	Latridiidae	LAT		[C]	FM877916	n.a. (1)	FM877890	Germany, Saxonia, Dresden, Keulenberg
Rhizophagus sp.** Herbst, 1793	Monotomidae	MON		[C]	FM877917	n.a. (1)	FM877900	Germany, Saxonia, Dresden
Omosita discoidea (Fabricius, 1775)	Nitidulidae	NIT		[C]	FM877918	n.a. (1)	FM877886	Germany, Saxonia, Dresden
Pocadius ferrugineus (Fabricius, 1775)	Nitidulidae	NIT		[C]	FM877919	n.a. (1)	FM877884	Germany, Saxonia, Dresden
Olibrus aeneus (Fabricius, 1792)	Phalacridae	PHA		[C]	FM877920	n.a. (1)	FM877888	Germany, Saxonia, Dresden, Keulenberg
Oryzaephilus surinamensis (L., 1758)	Silvanidae	SIL		[C]	FM877921	n.a. (1)	n.a. (2)	Germany, Saxonia, Dresden
Uleiota planata (L., 1761)	Silvanidae	SIL		[C]	FM877922	n.a. (1)	FM877894	Germany, Saxonia, Dresden, Laußnitzer Heide
Aspidiphorus orbiculatus (Gyll., 1808)	Sphindidae	SPH		[C]	FM877923	n.a. (1)	n.a. (2)	Germany, Saxonia, Dresden
Sphindus dubius (Gyll., 1808)	Sphindidae	SPH		[C]	FM877924	n.a. (1)	FM878032 FM877905	Germany, Saxonia, Dresden
Elonus basalis (LeConte, 1855)	Aderidae	ADE		[T]	n.a. (2)	n.a. (1)	FM877896	Canada, Ontario, Westport
Notoxus monocerus (L., 1761)	Anthicidae	ANT		[T]	FM877925	n.a. (1)	FM877892	Germany, Saxonia, Dresden, Keulenberg
Mordella sp. L., 1758	Mordellidae	MOR		[T]	FM877926	n.a. (1)	FM877897	Canada, Ontario, Westport
Mycetophagus sp. Hellwig, 1792	Mycetophagidae	MYC		[T]	FM877927	n.a. (1)	FM877881	Canada, Ontario, Westport

Tab. 1. Continuation.

Species	Family(-Tribe)	AF	AG	SF	COI	COII	18S	Provenience
Diaperis boleti (L., 1758)	Tenebrionidae	TEN		[T]	FM877930	n.a. (1)	FM877883	Germany, Saxonia, Dresden
Eledona agaricola (Herbst, 1783)	Tenebrionidae	TEN		[T]	FM877931	n.a. (1)	FM877882	Germany, Saxonia, Dresden
Tribolium castaneum (Herbst, 1797)	Tenebrionidae	TEN		[T]	FM877932	FM877785	FM877879	Germany, Saxonia, Dresden
Tetratoma fungorum Fabricius, 1790	Tetratomidae	TET		[T]	FM877933	n.a. (1)	FM877885	Germany, Saxonia, Dresden
Ditoma crenata (Fabricius, 1775) (=*Bitoma*)	Zopheridae	ZOP		[T]	FM877934	n.a. (1)	FM877902	Germany, Saxonia, Meißen
Ceracis thoracicornis (Ziegler, 1845)	Ciidae-Ciini	CIS	Ce	[T]	FM877935	FM877787	FM877870	Canada, Ontario, Newborough Lake
Cis boleti (Scopoli, 1763)	Ciidae-Ciini	CIS	Ci	[T]	FM877936	FM877788	FM877866	Germany, Saxonia, Dresden
Cis glabratus Mellié, 1848	Ciidae-Ciini	CIS	Ci	[T]	FM877937	FM877789	FM877868	Germany, Saxonia, Dresden
Cis hispidus (Paykull, 1798)	Ciidae-Ciini	CIS	Ci	[T]	n.a. (2)	FM877790	FM877877	Germany, Saxonia, Dresden
Cis nitidus (Fabricius, 1792)	Ciidae-Ciini	CIS	Ci	[T]	FM877938	FM877791	FM877861	Germany, Saxonia, Dresden
Cis setiger Mellié, 1848	Ciidae-Ciini	CIS	Ci	[T]	FM877939	FM877792	FM877867	France, Grenoble
*Cis chinensis** Lawrence, 1991	Ciidae-Ciini	CIS	Ci	[T]	FM877940	FM877793	FM877874	Brasil, Minas Gerais, Ipatinga^{++}
Dolichocis manitoba Dury, 1919	Ciidae-Ciini	CIS	Do	[T]	FM877941	FM877794	FM877873	Canada, Ontario, Cow Island
Ennearthron cornutum (Gyll., 1827)	Ciidae-Ciini	CIS	En	[T]	FM877942	FM877795	FM877865	Germany, Saxonia, Dresden
*Neoennearthron hisamatsui** Miyatake, 1959	Ciidae-Ciini	CIS	En	[T]	FM877943	n.a. (2)	FM877904	China, Shiwan Dashan, South-Guangxi-Prov.
Falsocis brasiliensis Lopes-Andrade, 2007	Ciidae-Ciini	CIS	Fa	[T]	FM877944	FM877796	FM877875	Brasil, Bahia, Jussari
Orthocis nigrosplendidus (Nobuchi, 1955)	Ciidae-Ciini	CIS	Or	[T]	FM877945	FM877797	FM877871	Japan, Hokkaido, Nopporo Forest Park
Strigocis opacicollis Dury, 1917	Ciidae-Ciini	CIS	St	[T]	FM877946	FM877798	FM877872	Canada, Ontario, Newborough Lake
Sulcacis affinis (Gyll., 1827)	Ciidae-Ciini	CIS	Su	[T]	FM877947	FM877799	FM877864	Germany, Saxonia, Dresden
Sulcacis fronticornis (Panzer, 1809)	Ciidae-Ciini	CIS	Su	[T]	FM877948	n.a. (2)	FM877876	Germany, Saxonia, Dresden
*Xylographus scheerpeltzi** Nobuchi & Wada, 1956	Ciidae-Orophiini	CIS	Xy	[T]	n.a. (2)	n.a. (2)	FM877903	China, Nanning, Guangxi-Province
Octotemnus glabriculus (Gyll., 1827)	Ciidae-Orophiini	CIS	Oc	[T]	FM877949	FM877800	FM877862	Germany, Saxonia, Dresden
Octotemnus laevis Casey, 1898	Ciidae-Orophiini	CIS	Oc	[T]	FM877950	FM877801	FM877869	Canada, Ontario, Cow Island
Ropalodontus harmandi Lesne, 1917	Ciidae-Orophiini	CIS	Ro	[T]	FM877951	n.a. (2)	n.a. (2)	Japan, Hokkaido, Nopporo Forest Park
Ropalodontus perforatus (Gyll., 1813)	Ciidae-Orophiini	CIS	Ro	[T]	FM877952	FM877802	FM877863	Germany, Saxonia, Dresden

samples, and between non-ciids (*Scymnus*, *Mallodrya*, *Tetratoma*) and Ciidae in case of the C-sample. All models of sequence evolution and the respective parameters not reported can be obtained from the authors upon request.

Support values. We calculated bootstrap values for all MP (with PAUP* 4.0b10: nreps=1000) and ML (with GARLI 0.951: bootstrapreps=100 genthreshfortopoterm=5000 {as advised in the manual of the program}; ZWICKL 2006) reconstructions and report the posterior probabilities for the Bayesian reconstructions.

Saturation levels. To assess saturation effects, pairwise comparisons of transitional (s) and transversional (v) changes were plotted against pairwise distances (TN93) in DAMBE version 4.2.13 (XIA & XIE 2001) (Figs. 14–18). Two levels were considered: the family Ciidae (C-sample; on three genes 18S, COI, COII) and the cucujoid-tenebrionoid-cleroid assemblage, including a few Ciidae (R-sample; on two genes 18S, COI).

3. Results and discussion

3.1. Resulting sequences and phylogenetic trees

We were not able to amplify any of the targeted genes in the ciid *Sphindocis denticollis* (preservation probably not adequate for conserving DNA) and the pyrochroid *Pyrochroa coccinea* (freshly killed larva). Even after repeated adaptations and modifications of the PCR program, we also failed to amplify 18S in *Oryzaephilus surinamensis* and *Aspidiphorus orbiculatus*, COI in *Elonus basalis* and *Cis hispidus*, COII in *Neoennearthron hisamatsui* and *Sulcacis fronticornis*, both COII and 18S in *Ropalodontus harmandi*, and both COI and COII in *Xylographus scheerpeltzi*. The sequences that went into our analyses are listed in Tab. 1 by their accession numbers.

Characteristics of the data set, such as length of alignment, variability, and nucleotide composition, are given in Tab. 2.

The resulting phylogenetic trees – at least consensus trees of particular analyses if there were several equally parsimonious trees – are shown in Figs. 4–13 (and more completely in EFigs. E1–E25 of the electronic supplement), including support values (bootstrap or posterior probability) if ≥ 50%. The statistics for the trees resulting from our 19 reconstructions are presented in Tab. 3. The occurrence of selected clades in the trees derived from the various analyses is surveyed in Tab. 4.

3.2. Usefulness of analysed genes

Assessment of information content of data and reliability of trees. Since the phylogenetic relationships among the cucujoid and tenebrionoid families are vastly unclear, and the monophyly of many families is weakly supported, there is hardly any previous evidence upon which we could reflect our phylogenetic results in order, for instance, to search for appropriate analytical procedures. Only to some extent the monophyly of particular families that were represented by more than one taxon in our analyses appears as a useful criterion. Beside Ciidae these are Coccinellidae, Sphindidae, Nitidulidae, Cryptophagidae, Silvanidae, Tenebrionidae, and Trogossitidae (see discussions below). Otherwise we had to rely on statistical examinations of our data: (1) Saturation curves of the genes in the R- and C-samples of our study (Figs. 14–18); (2) occurrence of excessive branch lengths potentially effectuating artefacts like long branch attraction.

18S. VOGLER & CATERINO (2003) and VOGLER (2005) used complete 18S sequences (ca. 1900–2400 bp) for a sample of 795 resp. 973 coleopteran species representing 123 families. They aligned them by ClustalW and then subjected them to parsimony analyses; major presumed monophyla were pre-aligned, before alignment was established for the entire taxon sample. VOGLER (2005) indicates that 18S is useful for the analysis in some subgroups of Coleoptera, but not in others, which only in part depends on the hierarchical level. The gene may be informative at higher and lower levels but not at intermediate ones (we expect this might be due to the composition of 18S of conserved and highly variable portions). One problem with 18S analyses is the strong rate heterogeneity among taxa, which concerns both conserved and hypervariable regions, and causes long branch attraction. Another problem is the great length differences in hypervariable regions. The latter problem can be eliminated by removing the hypervariable parts prior to the analyses, while the former can at most be moderated by this approach. Additional problems are constituted by among-site rate variation and nucleotide compositional biases. In our Bayesian reconstruction based on 18S sequences alone (taxon sample R; see similar situation for sample E in EFig. E7) substantial differences in branch lengths could be observed; however, the level of variation is indicated as adequate for the taxon sample by the linearly ascending shape of the best fit saturation curve (Fig. 17). The latter is also true for the use of 18S sequences within the taxon sample C of Ciidae (Fig. 14).

COI and COII. HOWLAND & HEWITT (1995) have analysed COI for 37 species across the entire Coleoptera, but obtained weak resolution with their neigh-

Tab. 2. Sequence and alignment lengths, data variability, and nucleotide composition of the sequences obtained (calculated with MEGA 3.1), presented for the ciid sample (C-sample without outgroup taxa) and for the cucujoid-tenebrionoid-cleroid sample analysed (R-sample without outgroup taxon *Bostrichus*). For the 18S sequence data, ambiguous alignment positions were excluded. The number behind the alignment lengths, in parentheses, gives the number of positions that include gaps.

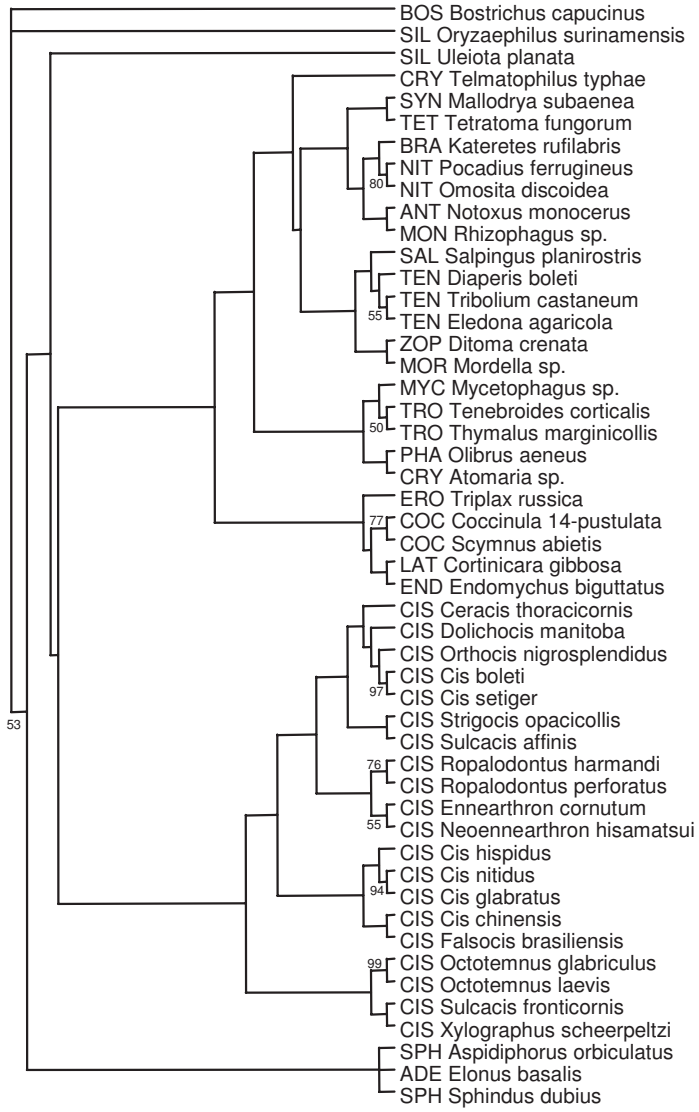
Taxon sample	gene	alignment length	constant	variable	pars.-inf.	T	C	A	G
Ciidae only (C)	18S	740 (2)	706	34	16	24.7	22.3	25.2	27.8
Ciidae only (C)	COI	641 (0)	359	282	235	33.6	19.4	31.8	15.3
Ciidae only (C)	COII	678 (6)	320	358	367	33.1	17.5	38.6	10.8
Ciidae only (C)	tRNA-Lys	27 (3)	21	6	6	25.3	13.7	44.2	16.8
cucujoid/tenebr. assemblage (R)	18S	740 (5)	606	134	55	24.5	22.6	25.2	27.7
cucujoid/tenebr. assemblage (R)	COI	641 (0)	319	322	291	35.7	18.5	29.8	16.0

Tab. 3. Analyses and tree statistics. **CI** = tree consistency index; **RI** = tree retention index; **–lnL** = likelihood score; **Pi** = assumed proportion of invariable sites; **alpha** = shape parameter of gamma distribution; *best length; #value from POY; +sampled for consensus, i.e. after exclusion of burn-in; **E.M.L**. = estimated marginal likelihood. The illustrations are indicated where the trees are shown (**E** = figure of electronic supplement).

Parsimony Analyses	No. trees	Tree length	CI	RI	Illustrations
E-sample-MPew[COI,18S]	24	3723	0.2283	0.3176	– / EFigs. E1, E2
R-sample-MPew[COI,18S]	22	2835	0.2854	0.2694	– / EFigs. E9, E10
C-sample-MPew[COI,COII,18S]	10	2779	0.4109	0.3316	– / EFigs. E16, E17
E-sample-MPdw[COI,18S]	1	6125.66	0.2259	0.3159	Fig. 4 / EFigs. E3, E4
R-sample-MPdw[COI,18S]	1	4557.00	0.2851	0.2708	Fig. 7 / EFigs. E11, E12
C-sample-MPdw[COI,COII,18S]	5	4153.20	0.4169	0.3336	Fig. 10 / EFig. E18
C-sample-DOgap=2x[COI,COII,18S]	1	3001*	14#	58#	Fig. 13 / EFig. E24
C-sample-DOgap=4x[COI,COII,18S]	3	3119*	16#	64#	– / EFig. E25

Maximum Likelihood Analyses	No. trees	–lnL	Pi	alpha	Illustrations
E-sample-ML[COI,18S]	1	15987.12196	0.4765	0.2834	Fig. 5 / EFig. E5
R-sample-ML[COI,18S]	5	12666.69304	0.4466	0.2627	Fig. 8 / EFigs. E13, E14
C-sample-ML[COI,COII,18S]	1	13811.41179	0.552	0.5763	Fig. 11 / EFig. E19

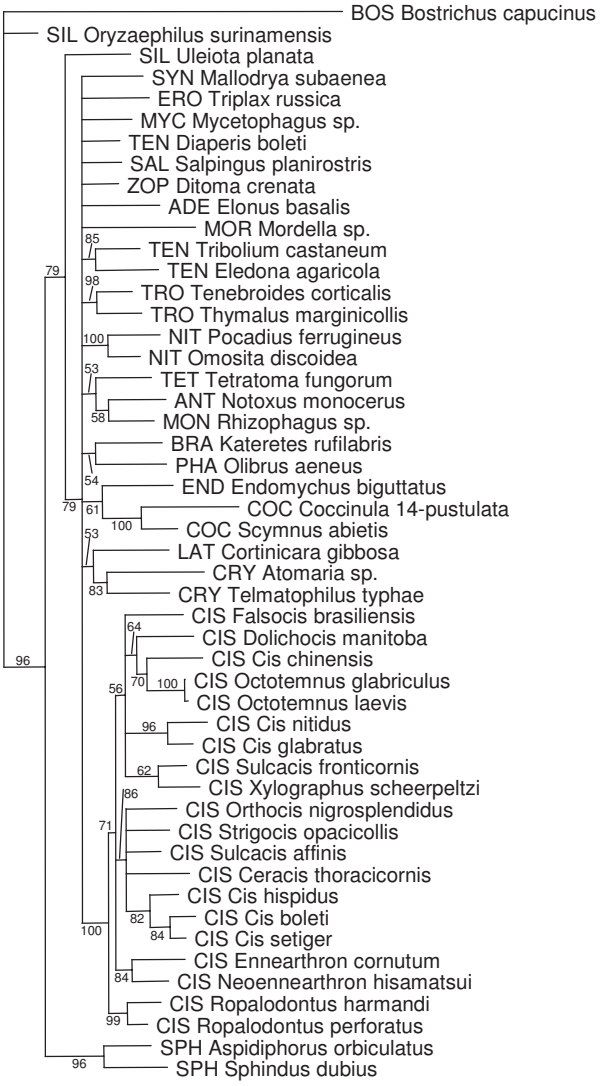
Bayesian Analyses	No. trees+	E.M.L. arithmetic mean	E.M.L. harmonic mean		Illustrations
E-sample-MB[COI,18S]	39002	-15223.39	-15288.56		Fig. 6 / EFig. E6
E-sample-MB[18S]	39002	-2982.16	-3053.53		– / EFig. E7
E-sample-MB[COI]	39002	-12123.52	-12189.15		– / EFig. E8
R-sample-MB[COI,18S]	39002	-11951.95	-12011.85		Fig. 9 / EFig. E15
C-sample-MB[COI,COII,18S]	39002	-12722.99	-12778.89		Fig. 12 / EFig. E23
C-sample-MB[18S]	35002	-1639.88	-1678.99		– / EFig. E20
C-sample-MB[COI]	39002	-5529.36	-5570.83		– / EFig. E21
C-sample-MB[COII]	39002	-5639.61	-5679.00		– / EFig. E22



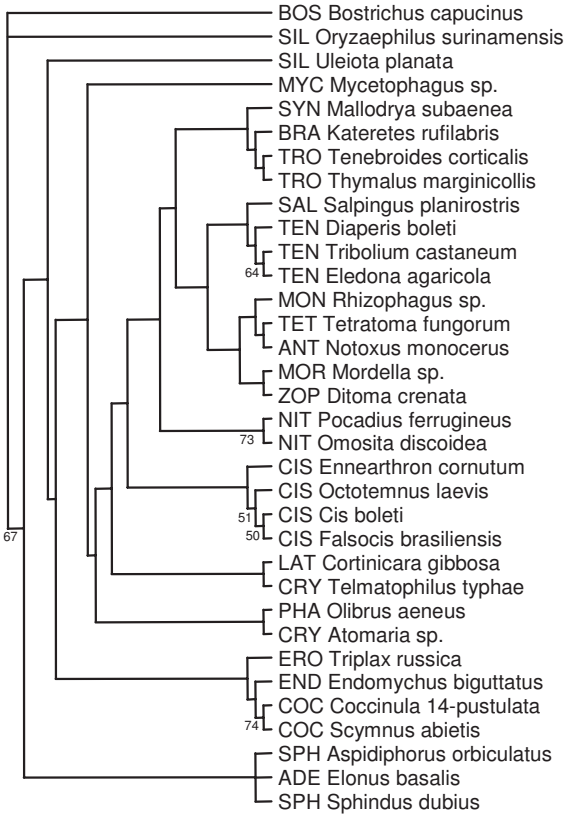
4 E-sample-MPdw[COI,18S](sct)

5 E-sample-ML[COI,18S](1/1)

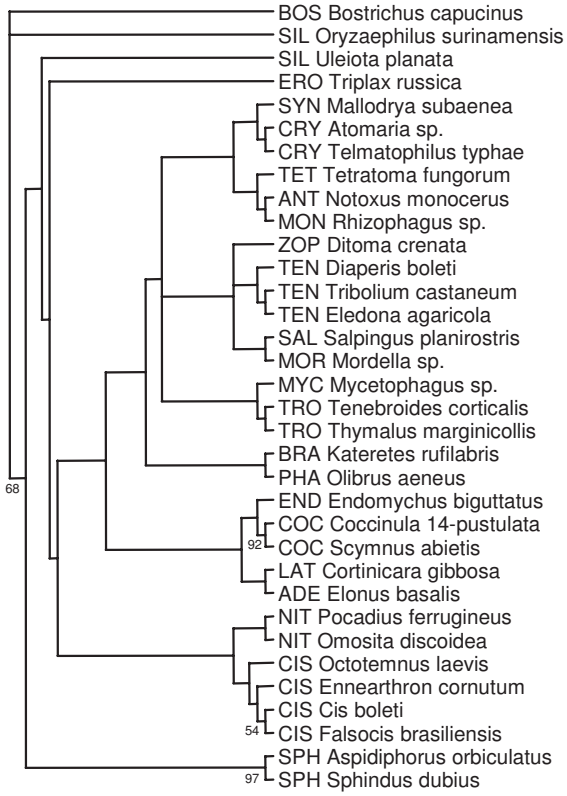
Figs. 4–13. Phylogenetic trees resulting from the various analyses. The trees are designated according to the used taxon sample (E-sample; R-sample; C-sample), the used analytical method (**MPdw** = maximum parsimony based on fixed alignment, under differentiated weighting of characters; **ML** = maximum likelihood; **MB** = MrBayes; **DO** = partial direct optimization, with gap cost 2× that of nucleotide changes), and the included gene fragments (18S, COI, COII, the latter also including part of tRNA-Lys). See chapter 2 for details and Tab. 3 for tree statistics. The last specification, in parentheses, indicates the nature of the tree: (**1/1**) = the single most parsimonious tree is shown; (**sct**) = strict consensus tree; (**50%**) = 50% majority rule tree. Bootstrap values and posterior probabilities of branches are indicated if ≥ 50%. The scale for branch lengths gives a measure for the amount of evolutionary changes (in % of aligned sequences); it is attached to the figures where such measurement is applicable (all but consensus trees and trees derived from DO analyses).



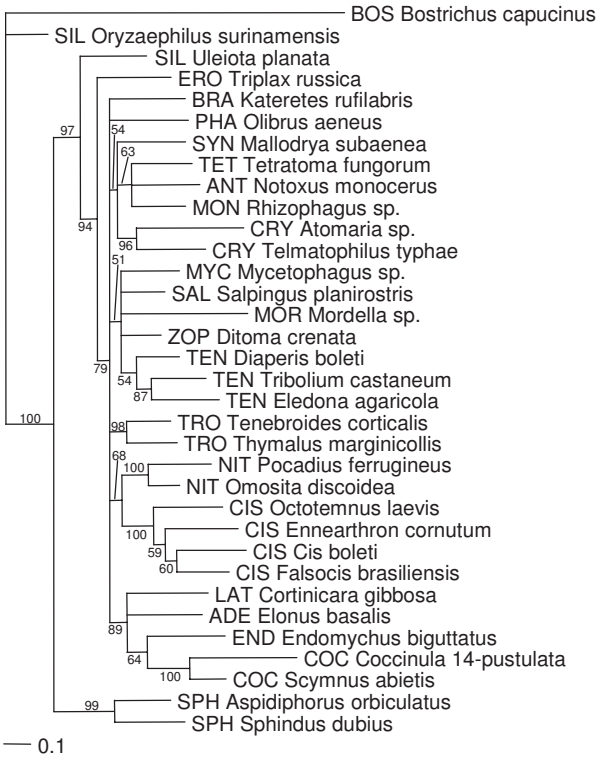
6 E-sample-MB[COI,18S](50%mr)



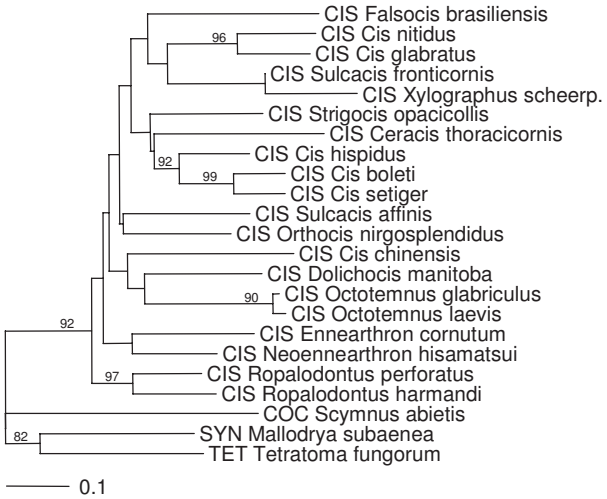
7 R-sample-MPdw[COI,18S](sct)



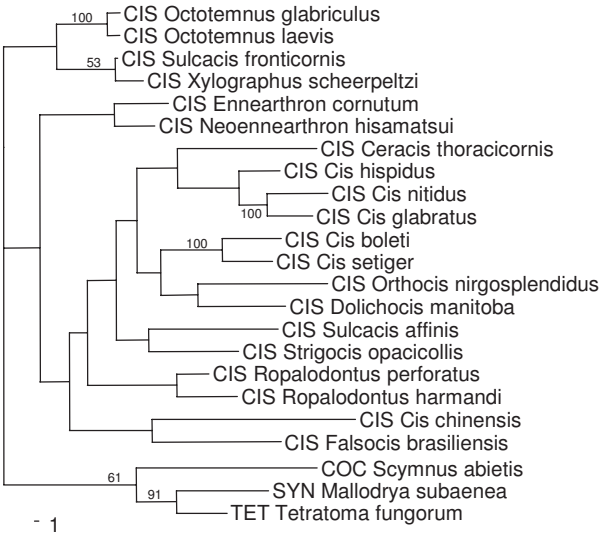
8 R-sample-ML[COI,18S](sct)



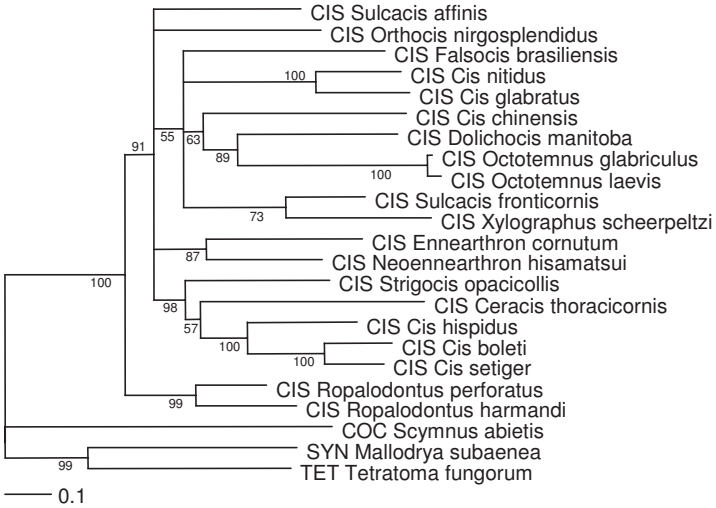
9 R-sample-MB[COI,18S](50%mr)



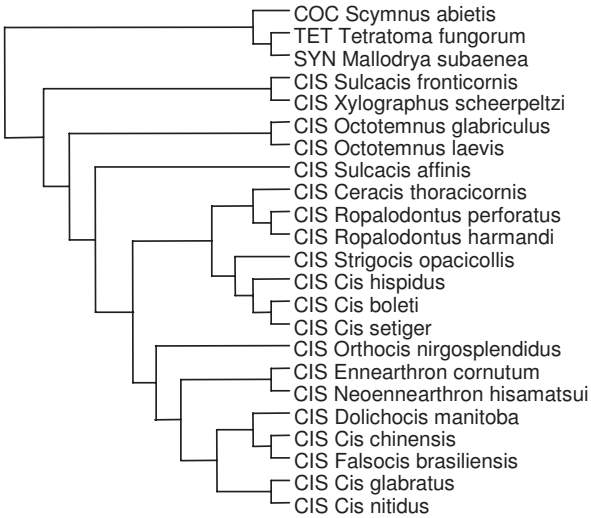
11 C-sample-ML[COI,COII,18S](1/1)



10 C-sample-MPdw[COI,COII,18S](1/1)



12 C-sample-MB[COI,COII,18S](50%mr)



13 C-sample-DOgap2x[COI,COII,18S](sct)

bour-joining analysis. According to these authors and Vogler (2005), COI evolves far too rapidly to provide significant resolution above family-level. We included CO genes hoping they contribute to the apical parts of our trees, and we also applied more sophisticated analytical methods to these sequences. The saturation curve of the COI sequences (taxon sample R, Fig. 18) still indicates sufficient variability without saturation, although the slope of the no. of transitions vs. divergence appears shallower than in the 18S (the slope of the no. of transversions looks similarly steep in the two genes). The curve based on the taxon sample C (Fig. 15) shows that COI is informative and not saturated at the systematic level of Ciidae. The COII curve (only within the Ciidae, taxon sample C; Fig. 16) has not yet reached a plateau either, indicated by still increasing numbers of transitions and transversions with increasing divergence. Based on these statistical results both the COI and COII appear useful for phylogeny reconstruction with regard to the taxon samples for which we used them.

In our analyses the usefulness of the COI sequences is perhaps demonstrated by the separate MB analyses for 18S (EFig. E7) and COI (EFig. E8) data using the entire sample. The clades Nitidulidae, Tenebrionidae, and Coccinellidae + Endomychidae are only retrieved in the COI analysis, while a Cryptophagidae clade is only found in the 18S analysis (Tab. 4) – all with strong support values.

3.3. Usefulness of tree construction methods

MP analyses with equal and unequal (differentiated) weighting. Whether equal or differentiated weighting is used in the analyses (analyses MPew vs. MPdw) has a great effect on the resulting trees. In the analyses for the entire sample (strict consensus; EFig. E2, Fig. 4), monophyletic Trogossitidae are only found in the MPdw analysis. Ciidae are monophyletic in the MPdw analysis but paraphyletic in the MPew analysis (with a clade *Oryzaephilus* + Sphindidae nested in Ciidae). Monophyletic Coccinellidae are obtained by both analyses, but while this clade is in a far basal, isolated position in the MPew analysis, it forms an apical branch associated with its surmised relatives Latridiidae and Endomychidae in the MPdw analysis (i.e., the cerylonid series is retrieved). The clades Tenebrionidae, Nitidulidae, and Nitidulidae + Brachypteridae are detected by both analyses, and monophyletic Cryptophagidae and Silvanidae by none. Considering the reduced sample (EFig. E10, Fig. 7), the problem with mono- vs. polyphyletic Trogossitidae is the same; the taxa sampled from Tenebrionidae and Sphindidae are placed in a basal polytomy in the MPew analysis, while a tenebrionid clade and a sphindid clade (though also including the aderid within a trichotomy) are obtained in the MPdw analysis. A clade comprising Coccinellidae and Endomychidae is only found in the MPdw analysis. In general, while the MPew analysis yields a large basal polytomy, the tree from MPdw is almost completely resolved. Altogether, the MPdw analyses reflect many hypotheses previously derived from morphological data (and other molecular data in case of the cerylonid series), while most of these clades are not obtained in the MPew analysis. This suggests that the MPdw analyses are superior to MPew analyses. A possible explanation is that differentiated weighting as specified in section 2.5. corrects for possible bias through differences in base composition.

MPdw analyses, ML analyses, and MB analyses. The three methods show some differences in the detection of clades that appear reasonable from a morphological point of view. Considering the entire sample (Figs. 4, 5, 6), monophyletic Trogossitidae, Ciidae, Coccinellidae, Nitidulidae, and Sphindidae (though with an unresolved association with the aderid in the MPdw analysis) are detected in all these analyses. The MPdw analysis (strict consensus) additionally finds monophyletic Tenebrionidae, Nitidulidae + Brachypteridae, and Coccinellidae + Latridiidae + Endomychidae (but not Cryptophagidae). Both the ML and MB analyses additionally yield monophyletic Cryptophagidae (but not Tenebrionidae, Nitidulidae + Brachypteridae, and Coccinellidae + Latridiidae + Endomychidae); a Coccinellidae + Endomychidae clade is unambiguous in the MB analysis but additionally includes the aderid in the ML analysis. It may be noted that a monophyletic Silvanidae is not obtained by any of the analyses. There is thus moderate overlap in the detection of such crucial clades. However, results are inconsistent for Tenebrionidae, Cryptophagidae, and Nitidulidae + Brachypteridae, and in the exact composition of the grouping comprising the Coccinellidae, Endomychidae, and Latridiidae (cerylonid series). Based on these results it is difficult to say which of the analytical methods in question here is superior to the others. In general, however, the weak overall resolution in the MB analyses as compared to the ML and MPdw analyses is striking, since as many as 10 million generations were run and the convergence diagnostic (PSRF = potential scale reduction factor, uncorrected) had approached 1, indicating that the runs had converged. On the other hand, the average standard deviation of split frequencies did not always decrease to less than 0.01, suggesting that better resolution could possibly be achieved with even more generations.

DO analysis compared to other analyses for ciid sample. Apart from the analytical difference of partial-

Tab. 4. Occurrence and support of clades observed in our analyses. Only those clades are mentioned that either occur in several of our analyses, or are strongly supported in at least one analysis, or are hypothesised by current classification (mostly taxa included in the same family or tribe). Only the single most parsimonious tree or a consensus tree (strict or majority rule) from all most parsimonious trees is considered for each analysis. Taxon sets: **E** = entire sample; **R** = reduced sample; **C** = ciid sample. Algorithms: **MP** = maximum parsimony based on fixed alignment; **ML** = maximum likelihood; **MB** = MrBayes; **DO** = maximum parsimony based on partial direct optimisation. Weighting: **ew** = equal weighting; **dw** = differentiated weighting; **G=2**, **G=4** = gaps weighted 2× or 4× as compared to maximum cost for nucleotide change. Selected tree or consensus tree: **1/1** = single most parsimonious tree; **sct** = strict consensus (.../24 = out of 24 most parsimonious trees); **50%** = 50% majority rule tree. The results for a clade in the various analyses are either given as a support value (if ≥ 50%; bootstrap for MP and ML analyses; posterior probability for MB analyses), or as "+" (if clade represented in tree but support < 50%), or as "–" (if clade not represented in tree; –* means the taxa of the clade form a paraphyletic assemblage); ** means that in part of the taxa only part of the genes have been sequenced (the respective taxa are given in italics in the first column); 0 means that crucial taxa of the clade are not represented in the taxon set of an analysis, an assessment of the clade not being applicable to an analysis. Taxa outside Ciidae are referred to by their genus names; taxa inside Ciidae by their abbreviated genus names (see Tab. 1) plus species names. "all / ..." and "Ciidae / ..."means all sampled Cucujiformia or Ciidae, respectively, except for those given behind the slash. In column "Fit" it is indicated whether a clade matches with the current classification: if a clade includes all taxa here sampled from a particular genus (concerning Ciidae) or family, the abbreviation for the genus or family name is given (compare Tab. 1; **cers** = cerylonid series).

Analyses																						**Fit**
Taxon set	E	E	E	E	E	E		R	R	R	R		C	C	C	C	C	C	C	C	C	
Algorithm	MP	MP	ML	MB	MB	MB		MP	MP	ML	MB		MP	MP	ML	MB	MB	MB	MB	DO	DO	
Weighting	ew	dw	–	–	–	–		ew	dw	–	–		ew	dw	–	–	–	–	–	G=2	G=4	
Included genes	18S	18S	18S	18S	18S	–		18S	18S	18S	18S		18S	18S	18S	18S	18S	–	–	18S	18S	
	COI	COI	COI	COI	–	COI		COI	COI	COI	COI		COI	COI	COI	COI	–	COI	–	COI	COI	
	–	–	–	–	–	–		–	–	–	–		COII	COII	COII	COII	–	–	COII	COII	COII	
Selected tree or consensus tree	sct/24	sct/3	1/1	50%	50%	50%		sct/22	sct/3	sct/5	50%		sct/10	1/1	1/1	50%	50%	50%	50%	1/1	sct/3	
Figure No. in present paper	–	4	5	6	–	–		–	7	8	9		–	10	11	12	–	–	–	13	–	
Figure No. in electronic supplement	E2	E4	E5	E6	E7	E8		E10	E12	E14	E15		E17	E18	E19	E23	E20	E21	E22	E24	E25	
Clades within Ciidae																						
Ciidae in E-sample	–*	+	+	100**	88**	80**		0	0	0	0		0	0	0	0	0	0	0	0	0	
Ciidae in R-sample (Oc. glabratus + *En. cornutum* + Ci. boleti + Fa. brasiliensis)	0	0	0	0	0	0		+	+	+	100		0	0	0	0	0	0	0	0	0	
Ci. boleti + Ci. setiger	97	97	90	84	85	100		0	0	0	0		100	100	99	100	75	100	100	+	+	
Ci. boleti + Ci. setiger + *Ci. hispidus*	–	–	–	82**	53	0		0	0	0	0		64**	–	92**	100**	71	0	100	+**	+**	
Ci. nitidus + Ci. glabratus	86	94	83	96	98	100		0	0	0	0		96	100	96	100	85	100	100	+	+	
Ci. nitidus + Ci. glabratus + *Ci. hispidus*	–	+**	–	–	–	0		0	0	0	0		–	+**	–	–	–	0	–	–	–	
Ci. chinensis + Fa. brasiliensis	–	+	–	–	–	–		0	0	0	0		–	+	–	–	–	–	–	+	–	
Ci. chinensis + Oc. glabriculus + Oc. laevis	–	–	–*	70	–	85		0	0	0	0		–	–	–*	63/–*	–	83	–	–	–	
Oc. glabriculus + Oc. laevis	99	99	94	100	–	100		0	0	0	0		99	100	90	100	–	100	100	+	+	+ Oc
Ro. perforatus + *Ro. harmandi*	87**	76**	91**	99**	0	100		0	0	0	0		94**	+**	97**	99**	0	100	0	+**	+**	+ Ro
Su. affinis + *Su. fronticornis*	–	–	–	–	–	–		0	0	0	0		–	–	–	–	–	–	0	–	–	+ Su
Su. fronticornis + *Xy. scheerpeltzi*	–	+	+	62**	–	0		0	0	0	0		–	53**	+**	73**	–	0	0	+**	–*	
Su. affinis + St. opacicollis	–	+	–*	–	–	77		0	0	0	0		–	+	–	–	–	77	–	–	–	
Oc. glabriculus + Oc. laevis + *Su. fronticornis* + *Xy. scheerpeltzi*	–	+**	–	–	–	0		0	0	0	0		–	+**	–	–	–	0	0	–*	–	
En. cornutum + Ne. hisamatsui	–	55	62	84	–	99		0	0	0	0		–	+**	+**	87**	–	100	0	+**	–	+
Oc. glabriculus + Oc. laevis + Ro. perforatus + *Ro. harmandi*	–	–	–	–	0	–		0	0	0	0		–	–	–	–	0	–	0	–	–	+

Oc. glabriculus + Oc. laevis + *Xy. scheerpeltzi* + Ro. perforatus + *Ro. harmandi*	–	–	–	–	–	0		0	0	0	0		–	–	–	–	–	0	0	–	–	+
Ciidae / Ro. perforatus, *Ro. harmandi*	–	–	+**	71**	–	74**		0	0	0	0		–	–	+**	91**	–	99**	–	–	–	
Ciidae / *Su. fronticornis*, *Xy. scheerpeltzi*	–	–	–	–	–	–		0	0	0	0		–	–*	–	–	–	–/0	0	+**	–	
Ciidae / *Su. fronticornis*, *Xy. scheerpeltzi*, Oc. glabriculus, Oc. laevis	–	+**	–	–	–	–		0	0	0	0		–	+**	–	–	–	–/0	0	+**	–	
Clades within Cucujoidea/Tenebrionoidea																						
Coccinula + Scymnus	62	77	92	100	92	100		58	74	92	100		0	0	0	0	0	0	0	0	0	+ COC
Coccinula + Scymnus + Endomychus	–*	–*	–*	61	–	100		–	+	+	64		0	0	0	0	0	0	0	0	0	
Coccinula + Scymnus + Endomychus + Cortinicara	–	+	–	–	–	99		–	–	–*	89/–*		0	0	0	0	0	0	0	0	0	+ cers
Sphindus + *Aspidiphorus*	63**	+/–*	94**	96**	0	100		–	+/–*	97**	99**		0	0	0	0	0	0	0	0	0	+ SPH
Sphindus + *Aspidiphorus* + *Elonus*	–	+**	–	–	0	0		–	+**	–	–									0	0	
Pocadius + Omosita	55	80	65	100	–	100		+	73	+	100		0	0	0	0	0	0	0	0	0	+ NIT
Pocadius + Omosita + Kateretes	+	+	–	–	–	–		–	–	–	–		0	0	0	0	0	0	0	0	0	+
Ciidae + Pocadius + Omosita	–	–	–	–	–	–		+	–*	+	68		0	0	0	0	0	0	0	0	0	
Tribolium + Eledona	+	55	+	85	–	58		–	64	+	87		0	0	0	0	0	0	0	0	0	
Tribolium + Eledona + Diaperis	+	+	–	–	–	83		–	+	+	54		0	0	0	0	0	0	0	0	0	+ TEN
Salpingus + Tribolium + Eledona + Diaperis	+	+	–	–	–	–		–	+	–	–		0	0	0	0	0	0	0	0	0	
Telmatophilus + Atomaria	–	–	+	83	87	–		–	–	+	96		0	0	0	0	0	0	0	0	0	+ CRY
Tenebroides + Thymalus	–	50	+	98	–	–*		–	+	+	98		0	0	0	0	0	0	0	0	0	+ TRO
Mallodrya + Tetratoma	–	+	–	–	–	–		–	–	–	–		0	0	0	0	0	0	0	0	0	
Mallodrya + Tetratoma + Telmatophilus + Atomaria	–	–	+	–	–	–		–	–	–*	–*		0	0	0	0	0	0	0	0	0	
Mallodrya + Telmatophilus + Atomaria	–	–	–*	–	–	–		–	–	+	–*		0	0	0	0	0	0	0	0	0	
Rhizophagus + Tetratoma + Notoxus	–	–	–	53	–	–		–	+	+	63		0	0	0	0	0	0	0	0	0	
Rhizophagus + Notoxus	–*	+	–	58	–	–		+	–*	+	–*		0	0	0	0	0	0	0	0	0	
Kateretes + Olibrus	–	–	+	54	–	–		–	–	+	–		0	0	0	0	0	0	0	0	0	
Olibrus + Atomaria	–	+	–	–	–	–		–	+	–	–		0	0	0	0	0	0	0	0	0	
Cortinicara + Endomychus	–	+	–	–	–	99/ –*		–	–	–	–		0	0	0	0	0	0	0	0	0	
Ditoma + Diaperis + Tribolium + Eledona + Mordella	–	–*	–	–	–	–		–	–	–*	–		0	0	0	0	0	0	0	0	0	
Mycetophagus + Tenebroides + Thymalus	–	+	–	–	–	–		–	–	+	–		0	0	0	0	0	0	0	0	0	
Oryzaephilus + Uleiota	–	–	–	–	0	–		–	–	–	–		0	0	0	0	0	0	0	0	0	+ SIL
all / *Oryzaephilus*	–	53**	66**	96**	0	97**		–	67**	68**	100**		0	0	0	0	0	0	0	0	0	
all / *Oryzaephilus*, Sphindus, *Aspidiphorus*	–	–*/**	+**	79**	0	91**		–	–*/**	+**	97**		0	0	0	0	0	0	0	0	0	
all / *Oryzaephilus*, Sphindus, *Aspidiphorus*, Elonus	–	+**	–	–	0	0		–	+**	–	–		0	0	0	0	0	0	0	0	0	
all / *Oryzaephilus*, Sphindus, *Aspidiphorus*, Uleiota	–	–	–	79**	0	56**		–	–	+**	94**		0	0	0	0	0	0	0	0	0	

ly flexible vs. entirely fixed alignment, our DO (direct optimisation) analyses are the only ones in which the hypervariable parts of the 18S were included. Among our two versions of DO, with gap cost 2× or 4× the maximum cost of nucleotide changes (Fig. 13, EFig. E25), the gap=2× analysis yields far better resolution. Most of the few clades found in the gap=4× tree also occur in the gap=2× tree, while the different placements of *Neoennearthron hisamatsui* and *Dolichocis manitoba* are the only exceptions. The better resolution and the finding of a monophylum comprising *Neoennearthron hisamatsui* and *Ennearthron cornutum* (as in the MPdw, ML, and MB analyses, Tab. 4) may let the gap=2× analysis appear more adequate – though these criteria are surely not strong ones. The phylogenetic information added by the hypervariable regions is probably not much – looking at the implied alignments by eye – but is evident at least in one case: Tab. 5 shows a sequence of a few nucleotides that is identical in *Neoennearthron* and *Ennearthron* (GCAA) but different in other Ciidae and outgroup taxa (TTTA, TTAC, TTCG, TCCG, TTAT, TCGT, AATA, TT–T, or –T–T); this probably also yields synapomorphies for a *Neoennearthron* + *Ennearthron* clade. The tree from the DO gap=2× analysis shows all the apical clades (with mostly congeneric taxa) that are common among all or most of the analyses, and DO may thus appear reliable at this level. However, regarding the deeper, mostly intergeneric ciid relationships, there is hardly any evidence allowing scrutinising the rather different results from DO, MPdw, ML, and MB analyses.

Tab. 5. Part of the implied alignment of one hypervariable portion of the 18S rDNA. Nucleotide positions 3–11 of the alignment block are shown as aligned in the analysis with gap cost set at 2x the maximum cost for nucleotide changes (C-sample-DOgap=2×). *Ropalodontus harmandi*: 18S not sequenced.

Position	3------11
CIS_Ennearthron_cornutum	A---GCAA-
CIS_Neoennearthron_hisamatsui	A---GCAA-
CIS_Cis_boleti	A---TTTA-
CIS_Cis_setiger	A---TTTA-
CIS_Cis_hispidus	A---TTTA-
CIS_Cis_glabratus	A---TTTA-
CIS_Cis_nitidus	A---TTTA-
CIS_Sulcacis_fronticornis	AT--TTAC-
CIS_Octotemnus_glabriculus	G---TTCG-
CIS_Ropalodontus_perforatus	A---TCCG-
CIS_Sulcacis_affinis	A---TTTAC
CIS_Octotemnus_laevis	G---TTCG-
CIS_Ceracis_thoracicornis	A-T-TTAT-
CIS_Ropalodontus_harmandi	N---NNNN-
CIS_Orthocis_nirgosplendidus	A---TTTA-
CIS_Strigocis_opacicollis	A--CTCGT-
CIS_Dolichocis_manitoba	A---TTTA-
CIS_Cis_chinensis	A--TAATA-
CIS_Falsocis_brasiliensis	A---TTTAC
CIS_Xylographus_scheerpeltzi	AC--TCGT-
TET_Tetratoma_fungorum	G---TT-T-
COC_Scymnus_abietis	G---TTCG-
SYN_Mallodrya_subaenea	-----T-T-

3.4. The influence of sampling Ciidae

We ran MPew, MPdw, ML, and MB analyses with two datasets that differ only in the inclusion of either all sampled Ciidae (E-sample) or a limited selection of four species from this family (R-sample). One goal of this was to test the influence of extensive sampling of one subgroup. The Ciidae in the R-sample – *Octotemnus laevis*, *Cis boleti*, *Ennearthron cornutum*, and *Falsocis brasiliensis* – represent various major ciid lineages as resulting from the analyses of the E-sample; however, representatives of a putative *Sulcacis fronticornis* + *Xylographus* clade and of *Ropalodontus*, which are obtained sister to the remaining Ciidae in different analyses, are missing in this selection.

First it is evident that depending on their representation in the dataset Ciidae take a different place in the trees. Considering the MP analyses with differentiated weighting (MPdw, Figs. 4, 7), with the E-sample Ciidae form a rather basal clade, while they are much more deeply subordinate in the cucujoid-tenebrionoid assemblage with the R-sample. Considering the ML analyses (Figs. 5, 8), Ciidae form a rather basal clade with both the E- and R-samples; yet, details are different (placement of *Uleiota* and *Triplax*), and only with the R-sample the Ciidae are found associated with Nitidulidae as their sister group. The MB analyses (Figs. 6, 9) show insufficient resolution as to specify the position of the Ciidae, but like in the ML analyses, Ciidae are sister to Nitidulidae only using the R-sample.

Also relationships in other parts of the trees are influenced by the sampling of Ciidae. With the MPdw analyses, the Latridiidae, Tetratomidae, Mycetophagidae, and *Telmatophilus* show very different affinities in the trees derived from the E- and R-samples, and indeed these trees are overall quite different. With the ML analyses, *Uleiota* is obtained as far basal vs. deeply subordinate, the latridiid varies with regard to its affinities to a group comprising Coccinellidae, Endomychidae, and Aderidae, and a tenebrionid clade is only found with the R-sample. Considering these three aspects the tree from the R-sample might appear more reasonable than that from the E-sample. Regarding the MB analyses the trees from the E- and R- samples are more similar to each other, but this is partly due to the poor resolution in these trees. Nonetheless, monophyletic Tenebrionidae and affinities of Latridiidae to Coccinellidae and Endomychidae are, like in the ML analyses, only found with the R-sample.

These differences show that the extent to which some subgroup is sampled can have a great influence on the resulting trees. In this particular case this might be an effect of the absence of the two potential basalmost ciid subgroups in the reduced sample.

3.5. Relationships of the Ciidae

Monophyly of the Ciidae. The Ciidae (or rather Ciinae, as *Sphindocis* has not been sequenced) resulted as monophyletic in nearly all of our reconstructions (Tab. 4; only the analyses based on the entire sample are here considered relevant: EFig. E2, Figs. 4, 5, 6). The bootstrap support values in the MPdw analysis with differentiated weighting and in the ML analysis are < 50%, but in the MB analysis including both 18S and COI there is strong support of 100% posterior probability. From the trees derived from the separate MB analyses of the two genes, it is evident that both support ciid monophyly (posterior probability 88% for 18S and 80% for COI). Only in the MP analysis with equal weighting (MPew) Ciidae appear as paraphyletic due to the inclusion of Sphindidae and the silvanid *Oryzaephilus* (only COI data included for the latter taxon); but there is no meaningful support value for this relationship.

Relationships between Ciidae and other families. In the older literature the affinities proposed for Ciidae vary a lot. After the group had been transferred from Cucujoidea to Tenebrionoidea by Crowson (1960), Lawrence (1977) tentatively indicated an assemblage comprising Ciidae, Pterogeniidae, Archeocrypticidae, Tetratomidae, and Mycetophagidae. For the Tetratomidae, however, Lawrence & Newton (1982) proposed a relationship with a grouping Melandryidae + Mordellidae + Rhipiphoridae, while on the other hand they viewed an assemblage Pterogeniidae + Archeocrypticidae + Ciidae, though with an uncertain inclusion of the latter family. All these hypotheses are founded on morphological apomorphies that show a scattered and incongruent distribution across several cucujiform taxa and are thus strongly homoplastic.

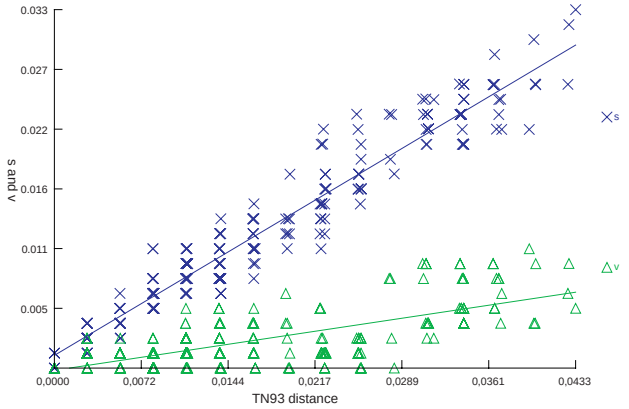
On a molecular basis Robertson et al. (2004, 2008) retrieved Ciidae sister to Tenebrionidae, and these together are related to Zopheridae; no further Tenebrionoidea were included in these papers. Hunt et al. (2008; Pterogeniidae and Archeocrypticidae not included) in their Bayesian analysis (supporting fig. S1 therein) found a weakly supported clade Ciidae + (Anthicidae + Meloidae) as one branch in a large basal polytomy of a tenebrionoid + lymexyloid clade; the tetratomids form two other clades of that polytomy, and the mycetophagids form an additional one. The Maximum Parsimony analysis of these authors (supporting fig. S4 therein) yielded a clade including all Ciidae, some mycetophagids, a lymexyloid, a nitidulid, and the stenotrachelid, which is sister to a clade essentially comprised of the remaining Tenebrionoidea (and Sphindidae).

Our analyses, which also lack Pterogeniidae and Archeocrypticidae, did not provide a clearer picture on this issue, while they offer two essential alternatives. One is that Ciidae forms a rather isolated basal clade in the cucujoid-tenebrionoid assemblage. This results from the MPdw and ML analyses of the entire sample (Figs. 4, 5), where only silvanids and sphindids appear more basal (with differences in the details; the MB analysis lacks resolution, Fig. 6). With the MPew analysis of the entire sample (EFig. E2) the Ciidae are further apical, sister to a clade comprising Brachypteridae, Nitidulidae, Monotomidae, Latridiidae, and Anthicidae. Among the analyses for the reduced sample a similar situation is found in the MPdw analysis (Fig. 7): the ciid sister group comprises most of the aforementioned families plus some additional ones. The second alternative obtained for Ciidae is a sistergroup relationship to Nitidulidae. This is found in the MPew, ML, and MB analyses of the reduced sample (EFig. E10, Figs. 8, 9). However, it should be noted that in none of the trees any sistergroup relationship of Ciidae receives considerable support. It is noteworthy that none of our analyses indicated any relationships between Ciidae and Tetratomidae, or Mycetophagidae, or Tenebrionidae.

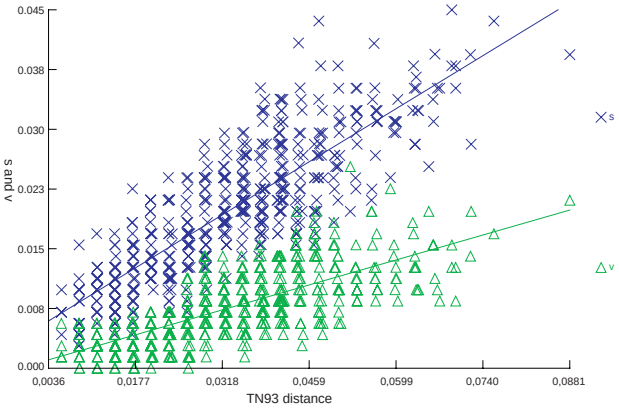
Our results thus leave this issue open, while the indication of ciid-nitidulid relationships might be inspiration for a re-evaluation of morphological characters under this aspect.

Relationships within Ciidae. For this issue the analyses derived from the entire sample (E-sample, including many outgroup taxa to Ciidae but only 18S and COI) and from the ciid sample (C-sample, including few outgroup taxa to Ciidae and the genes 18S, COI, and COII) are relevant.

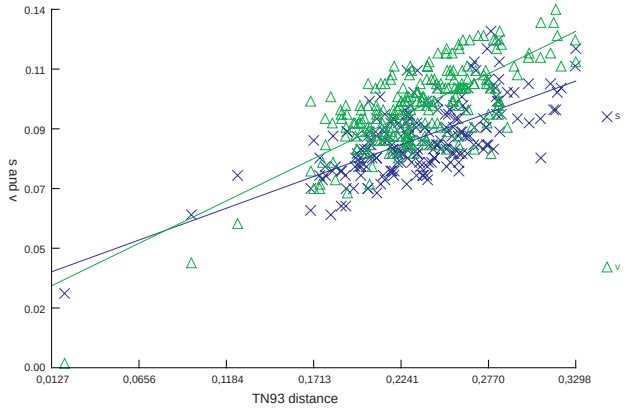
The results from the MP analyses with equal weighting (MPew) are only exceptionally considered here because they show the base of Ciidae as a large polytomy. Analyses based on direct optimisation (DO) were performed only for the C-sample. Considering the other analytical methods (MPdw, ML, and MB), the trees based on the same method but using the E- or C-sample are strikingly different. However, the basalmost split in Ciidae is identical irrespective of whether the E- or the C-sample was used: it is between *Ropalodontus* and the remaining Ciidae in the ML and MB analyses (Figs. 5, 11 and 6, 12), and between a clade *Octotemnus* + *Xylographus* + *Sulcacis fronticornis* and the remaining Ciidae in the MPdw analyses (Figs. 4, 10). This might indicate that different inner-ciid relationships are not a



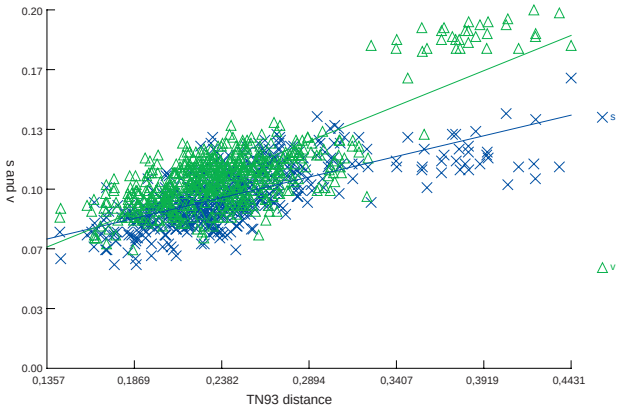
14 18S C-sample



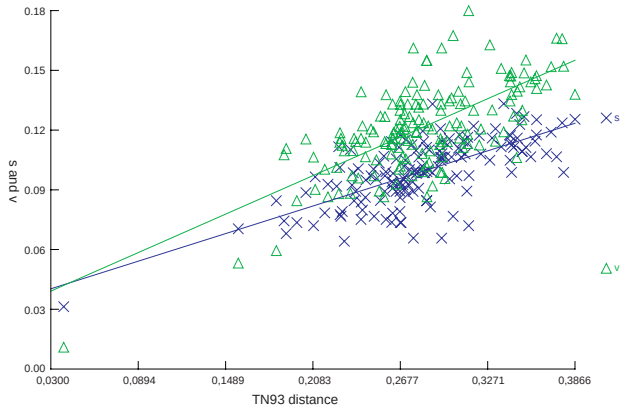
17 18S R-sample



15 COI C-sample



18 COI R-sample



16 COII C-sample

Figs. 14–18. Saturation curves of the sequence data, based on the no. of transitions (= s; curve × in each figure) and transversions (= v; curve Δ in each figure) vs. the ML-corrected distances (TN93: ML-model Tamura-Nei 93). Figs. 14–16 relate to the ciid sample (C-sample); Figs. 17 and 18 relate to the reduced cucujoid-tenebrionoid sample (R-sample). In the calculations for both samples the taxa used as outgroup representatives were included.

result of the grossly different outgroup selection that one could suspect to go along with a different polarisation of characters from the base of Ciidae onward; it rather seems to be an effect of the addition of COII data. The DO analysis with gap=2×, done for the C-sample, shows the *Xylographus* + *Sulcacis fronticornis* clade alone as sister to the remaining Ciidae, but the *Octotemnus* clade follows as the next basal branch (Fig. 13).

More deeply inside the Ciidae, there are few clades that appear consistently in all our trees, each comprising only species of the same genus or morphologically similar genera. The *Octotemnus glabriculus* + *laevis* clade has very high support values when these were measured, and its two species have very short terminal branch lengths. Indeed, also morphologically these two species are very similar to each other, without any differences known including the genitalia (C. Lopes-An-

drade pers. comm. 2007), and they may well represent a single species (Lawrence 1971; Thayer & Lawrence 2002). The values for the *Ropalodontus perforatus* + *harmandi* clade are not that high. Those for the *Neoennearthron* + *Ennearthron* clade are considerably lower (even < 50% in the MPdw and ML analyses of the C-sample, and this clade is even non-monophyletic in the DO analysis with gap=4×, EFig. E25).

Each of the two remaining consistently appearing clades comprises two species of *Cis*: *Cis boleti* + *setiger* and *Cis nitidus* + *glabratus*, both with strong support values throughout. With the exception of *Neoennearthron* + *Ennearthron*, these clades are the few ones that are also detected by the MPew analyses (which otherwise only show a large polytomy of ciid taxa; EFig. E2). The clades *Cis boleti* + *setiger* and *Cis nitidus* + *glabratus* are widely separated in all our trees that provide sufficient inner-ciid resolution, supporting the genus *Cis* to be polyphyletic. This is not unexpected since *Cis* is not clearly supported by apomorphies but rather is a very species-rich assemblage of generalised ciids not showing significant morphological peculiarities. Another species of *Cis*, *Cis hispidus*, variously appears as sister either to the *Cis boleti* + *setiger* clade (ML analysis of C-sample, MB analyses, and DO analyses; Figs. 6, 11, 12, 13, EFig. E25) or the *Cis nitidus* + *glabratus* clade (MPdw analyses; Figs. 4, 10), only the ML analysis of the E-sample shows it remote from all other *Cis* species (Fig. 5: associated with *Dolichocis*); however, only the first-mentioned relationship has high support values. The sixth sampled species of this genus, *Cis chinensis*, is never found associated with other *Cis* species but rather shows affinities to *Falsocis* (MPdw analyses of E- and C-samples, and DO analysis of C-sample with gap=2x, Figs. 4, 10, 13), or *Octotemnus* (MB analysis of E-sample, Fig. 6), or *Octotemnus* + *Dolichocis* (ML analysis of C-sample, Fig. 11).

The two species of *Sulcacis* are always widely separated in our trees, suggesting the polyphyly of this genus. This is not surprising in view of the considerable morphological differences between the two species in our sample, which have been assigned to different subgenera: *S. affinis* to *Sulcacis* s.str., *S. fronticornis* to *Entypocis* (e.g. Lohse 1967). *Sulcacis affinis* usually showed affinities to *Orthocis* (ML analysis of C-sample, Fig. 11), or *Strigocis* (MPdw analyses, Figs. 4, 10), or a larger clade comprising these genera and others (ML and MB analyses of E-sample, Figs. 5, 6), or it appears as a far basal clade following *S. fronticornis* + *Xylographus* and *Octotemnus* (DO analysis with gap=2x, Fig. 13). *Sulcacis fronticornis* was consistently found sister to *Xylographus* (the only exceptions are the MPew and DO gap=4x analyses with their lacking resolution; EFigs. E2, E17, E25). Besides the *Neoennearthron* + *Ennearthron* clade, the *S. fronticornis* + *Xylographus* clade was the only stable one at the suprageneric level, but its support values are low.

The other parts of the ciid trees differ strongly both with the analytical method and the taxon sample used. Hardly any conclusions can thus be drawn on relationships among ciid genera. As mentioned above, at the very base of the Ciidae tree either *Ropalodontus* (ML and MB analyses) or a clade *Octotemnus* + *Xylographus* + *Sulcacis fronticornis* (MPdw analyses), or *Xylographus* + *Sulcacis fronticornis* alone (DO analysis with gap=2x) appears as the sister group to all remaining Ciidae. There is considerable support only for the former relationship, though only from the MB analyses. *Ropalodontus*, *Octotemnus*, and *Xylographus* are the three genera in our sample that have been assigned to the tribe Orophiini. However, in none of our analyses is there a clade formed by these taxa. Furthermore, *Ropalodontus* and *Octotemnus* are usually considered as closely related, supported by the shared possession of a cuticular flap that covers the abdominal fovea from the anterior. But these genera do not appear as sister groups in any of our analyses either. Thus, the Orophiini is suggested to be a polyphyletic assemblage. In trees based on ML and MB analyses *Neoennearthron* + *Ennearthron* appears as another far basal or isolated ciid lineage (Figs. 5, 6), but the relationships in detail vary. It may be noted that *Ropalodontus* and *Neoennearthron* + *Ennearthron* appear as sister groups in a more apical part of the tree in the results from the MPdw analyses using the E-sample (Fig. 4). This might indicate that, using the E-sample, in the MPdw analyses on the one hand and the ML and MB analyses on the other, characters become polarised differently at the base of Ciidae; however, there is no difference regarding the position of the Ciidae in the E-sample based analyses relative to the other groups of the cucujoid-tenebrionoid assemblage that could explain this. Also if re-rooted between *Ropalodontus* and the remaining Ciidae, the trees from the MPdw analyses are very different from those based on ML and MB methods.

3.6. Relationships among cucujoid-tenebrionoid-cleroid subgroups

While the focus of our study was on the relationships of Ciidae, we would also tentatively discuss our results on relationships among subgroups of Cucujoidea and Tenbrionoidea, essentially by scrutinising to what extent specific relationships hypothesised in the previous literature are confirmed or contradicted. Only the trees obtained with our entire and reduced samples (E- and R-samples) are relevant for this issue. The occurrence of many clades in these trees is surveyed in Tab. 4.

Position of Trogossitidae and Cleroidea. The Cleroidea has usually been classified as a separate cucujiform superfamily, with Trogossitidae forming a basal subgroup (Klausnitzer 2005; Lawrence & Newton 1982; Crowson 1964). However, based on larval characters Beutel & Pollock (2000) and Beutel & Ślipiński (2001) suggested Cleroidea to be nested in Cucujoidea. Similarly, Vogler (2005), based on 18S sequences, finds the entire Cleroidea as a subordinate clade of Cucujoidea, sister to the Brachypteridae. In Leschen et al.'s (2005: fig. 11) morphology-based cladistic analysis, however, the Trogossitidae are not unambiguously placed inside the Cucujoidea, though they show a relationship to Boganiidae and Byturidae in some trees. Hunt et al. (2008: supporting figs. S1, S4) obtain a monophyletic Cleroidea (including also Byturidae and Biphyllidae) either as one of five clades of a basal polytomy of Cucujiformia, or as subordinate in Cucujiformia.

In our analyses the two trogossitids, *Thymalus* and *Tenebroides*, form a monophyletic clade in the MB and ML analyses as well as in the MP analyses with differentiated weighting (MPdw; Figs. 4–9), but only in the MB analyses using combined data they receive strong support (98% posterior probability). In the MP analyses with equal weighting (MPew) *Thymalus* and *Tenebroides* are widely separated. All our analyses show the trogossitids deeply nested within the cucujoid-tenebrionoid assemblage, but their relationships vary with the analyses. The sister group of Trogossitidae in the MPdw analyses is Mycetophagidae (E-sample) or Brachypteridae (R-sample), while in the ML and MB analyses the trogossitids are sister to a rather inclusive clade or part of a large polytomy; only the ML analysis for the R-sample again finds Mycetophagidae.

Monophyly of Sphindidae. It has long been disputed whether the genera *Sphindus* and *Aspidiphorus* are closely related and can, together with a few additional genera, be comprised in a single family Sphindidae (Crowson 1955: 101f). McHugh (1993: 86) says there is no reason not to believe that Sphindidae is monophyletic, but it is difficult to find an unambiguous synapomorphy. In their cladistic analyses McHugh (1993) and Chiao & McHugh (2000) obtained *Aspidiphorus* and *Sphindus* as members of a sphindid subgroup that only excludes some basal sphindid genera. In Hunt et al. (2008) *Sphindus* and *Aspidiphorus* are also retrieved as members of a monophyletic Sphindidae, either as one isolated clade of a basal cucujoid polytomy or as deeply subordinate in the tenebrionoid-lymexyloid clade (supporting figs. S1, S4 therein).

We obtain *Aspidiphorus* (only COI data) and *Sphindus* as sister groups in all our ML and MB analyses (Figs. 5, 6, 8, 9), with high support, and can thus confirm the inclusion of at least these genera in the same family. In the MP analyses, under differentiated weighting (dw) (Figs. 4, 7) the sphindids are placed in an unresolved trichotomy with the sampled aderid (only 18S data; note that a relationship aderid + (sphindid + latridiid) is also found in the MB analysis using 18S alone for the E-sample; EFig. E7); under equal weighting (ew) a weakly supported (bootstrap 63%) Sphindidae is recovered using the E-sample (EFig. E2; placed inside Ciidae), but the two sphindids are placed without resolution in a large basal polytomy using the R-sample (EFig. E10).

Monophyly of Cryptophagidae. This family, including the two subfamilies Cryptophaginae and Atomariinae, has usually been considered monophyletic (e.g., Crowson 1955; Leschen 1996). This has been confirmed by the morphological analysis of Leschen et al. (2005), where, as in our study, one representative of each subfamily was included. These authors found considerable statistical support for Cryptophagidae, though not any apomorphies unique to this group. Hunt et al. (2008) also obtained a strongly supported Cryptophagidae (supporting figs. S1, S4; Atomariinae + *Telmatophilus* + *Cryptophagus* in the former).

The sistergroup relationship between Cryptophaginae (represented by *Telmatophilus*) and Atomariinae (*Atomaria*) is also supported by our MB analyses (both the E- and R-samples: 83% resp. 96% posterior probability; Figs. 6, 9). We obtain the same relationship in our ML analyses, but support is < 50% (Figs. 5, 8). On the other hand, the two cryptophagids are widely separated in our MP trees under both modes of weighting (ew and dw; EFigs. E2, E10, Figs. 4, 7). The placement of the two genera in a large polytomy in the Bayesian analysis using COI alone (E-sample; EFig. E8) indicates that data from this gene does not support their relationship.

Monophyly of Silvanidae. The phylogenetic composition and relationships of this family were discussed in Thomas (1984, 2002). The two silvanid genera included in the cladistic analysis of Leschen et al. (2005), *Cryptamorpha* and *Ahasverus*, are assigned to two different subfamilies, Brontinae and Silvaninae, respectively (Thomas 2002), and they form a well-supported clade. Hunt et al. (2008: supporting figs. S1, S4) also obtained a monophyletic clade Silvaninae + Brontinae (but *Oryzaephilus* is not included), either placed in a large polytomy or close to Cerambycidae.

The two genera we have sampled, *Uleiota* (Brontinae) and *Oryzaephilus* (Silvaninae) represent the same two subfamilies, but they were widely separated in all our trees irrespective of the analytical method and ciid sampling (but note that for *Oryzaephilus* only COI data were available). In most of our trees *Oryzaephilus* appears as sister to the entire remainder

of the cucujoid-tenebrionoid assemblage (MPdw, ML, and MB analyses of both the E- and R-samples; Figs. 4–9), and this relationship is strongly supported in some analyses, especially the MB (Tab. 4). The only exceptions are the MPew analyses, where with the E-sample *Oryzaephilus* is placed inside Ciidae (EFig. E2; together with Sphindidae – surely an artefact) and the R-sample shows the base of the cucujoid-tenebrionoid assemblage as a large polytomy (EFig. E10). *Uleiota* also appears as a very basal clade in most of our analyses, following *Oryzaephilus* and Sphindidae as the third-lowest branch (MPdw and MB analyses of E-sample; MPdw, ML, and MB analyses of R-sample; Figs. 4, 6–9). In the ML analysis of the E-sample *Uleiota* is more deeply nested in the tree as sister to Monotomidae (Fig. 5). Altogether the results indicate that Silvanidae is a para- or polyphyletic assemblage of basal cucujiform beetles.

Monophyly of Tenebrionidae, and potentially related families. For this extremely species-rich and habitually highly diverse family monophyly is difficult to demonstrate based on morphology, and there is no comprehensive study yet on the inner-tenebrionid phylogenetic relationships. Following the system of Aalbu et al. (2002) the three genera we have sampled belong to three subfamilies: *Tribolium* to Tenebrioninae, *Eledona* to Bolitophaginae, and *Diaperis* to Diaperinae. Bouchard et al. (2005) include "Bolitophaginae" in Tenebrioninae. Hunt et al. (2008) either find the aforementioned tenebrionid subtaxa inside a strongly supported clade (which, however, does not include some other tenebrionid subgroups, e.g., Pimeliinae; supporting fig. S1), or *Eledona* is far remote from a clade including *Diaperis* and *Tribolium* (supporting fig. S4, with grossly polyphyletic Tenebrionidae).

With all our MPdw, ML, and MB analyses of both the E- and R-samples, and in the MPew analysis of the E-sample, *Tribolium* and *Eledona* appear as sister groups (Figs. 4–9, EFig. E2). *Diaperis* is mostly sister to this clade, but not in the ML and MB analyses of the E-sample. Though support values are in no case significant, this result might be taken as favouring tenebrionid monophyly.

In Hunt et al. (2008: supporting fig. S1) the major tenebrionid clade originates from a large basal polytomy of the Tenebrionoidea + Lymexyloidea clade, the relationships of the Tenebrionidae thus remaining unresolved. In our trees the sampled tenebrionids are found on the same clade with various combinations of other tenebrionoid families. Most frequently Salpingidae is found sister to Tenebrionidae; Mordellidae and Zopheridae are also often part of the same clade, sometimes also Anthicidae and Mycetophagidae, and in one tree (MPdw analysis for R-sample in Fig. 7) a subclade with Monotomidae (Cucujoidea) and Tetratomidae is additionally included. Also for these relationships there are no significant support values.

Monophyly of Nitidulidae, and relationship to Brachypteridae and Phalacridae. Our sample of Nitidulidae, with the genera *Pocadius* and *Omosita*, cannot contribute to the question of nitidulid monophyly, as both belong to the subfamily Nitidulinae (Habeck 2002), for which Leschen (1999) provided a morphology-based cladistic analysis. The two taxa together form a clade in all our analyses of combined data, and this clade is among the most strongly supported ones. The separate analyses of 18S and COI data using MB and the E-sample (EFigs. E7, E8) show that this relationship is highly supported by COI (100% posterior probability) but not at all by 18S.

Nitidulidae and Brachypteridae had long been included in a single family Nitidulidae (Crowson 1955: as subfamilies), and Crowson (1955) furthermore indicates a relationship between these and the Phalacridae (and Smicripidae). A clade including Nitidulidae, Brachypteridae, and Phalacridae (plus some others not sampled in our study) is also obtained by Leschen et al. (2005), but is weakly supported. In Hunt et al. (2008: supporting fig. S1, Brachypteridae not included) the nitidulids form one clade and the Phalacridae belong to another clade of the cucujiform lineage that otherwise also includes Chrysomeloidea and Curculionoidea (this lineage has a large polytomy at its base). In Hunt et al. (2008: supporting fig. S4, Brachypteridae included) the nitidulids are paraphyletic with regard to monotomids and some tenebrionids and widely separated from both Brachypteridae and Phalacridae.

In our study the nitidulid-brachypterid relationship is confirmed in the trees from the MPew and MPdw analyses of the E-sample (Phalacridae far remote; EFig. E2, Fig. 4). On the other hand, a phalacrid-brachypterid relationship is obtained in our ML analyses and the MB analysis using the E-sample (Nitidulidae far remote; Figs. 5, 6, 8). None of the analyses found a clade comprising all three families, and support values are insignificant for all these relationships. Low support is also true for a clade comprising Brachypteridae, Synchroidae, and Trogossitidae (or at least the trogossitid *Thymalus*), as found by the MPew and MPdw analyses of the R-sample (EFig. E10, Fig. 7).

Cerylonid series and Endomychidae + Coccinellidae. Crowson´s (1955, 1960) suggestion that the Cerylonidae, Endomychidae (incl. Merophysiinae), Alexiidae, Corylophidae, Coccinellidae, Discolomatidae, and Latridiidae form a group has become widely accepted. Pal & Lawrence (1986) added the Bothrideridae. The group is considered to be characterised by tarsomeres 4-4-4 or 3-3-3, radial cell of hindwings not closed, some "anal veins" lost, unisetose larval tars-

ungulus, and some other characters. However, there are several cases of members of the cerylonid series lacking some of these apomorphies, and most of the respective apomorphies do also occur in various other subgroups of Cucujiformia (see ŚLIPIŃSKI & PAKALUK 1991; TOMASZEWSKA 2000: 450). The cerylonid series is thus actually, at most, vaguely supported. Within this group a close relationship between Coccinellidae and Endomychidae (and likely Corylophidae) is almost generally assumed (CROWSON 1955, 1960; TOMASZEWSKA 2000), though this is problematic due to the lack of unambiguous synapomorphies and the unclear delimitation of both families concerning some of their "basal" subtaxa (ŚLIPIŃSKI & PAKALUK 1991).

With regard to molecular studies, the cerylonid series is a strongly supported clade in the Bayesian analysis of HUNT et al. (2008: supporting fig. 1), while there is no coccinellid + endomychid clade. The Maximum Parsimony analysis of the same authors (supporting fig. 4 therein) yields both the cerylonid series and (essentially) a coccinellid + endomychid clade. The latter clade also receives some support in the analyses by ROBERTSON et al. (2008).

Concerning the taxa included in our study (Endomychidae, Coccinellidae, Latridiidae), the representatives of Endomychidae and Coccinellidae are almost consistently united: MPdw (with the latridiid inside this clade), ML (with the aderid inside this clade), and MB analyses of E-sample (Figs. 4, 5, 6) as well as MPdw, ML, and MB analyses of R-sample (Figs. 7, 8, 9, all with a monophyletic Coccinellidae + Endomychidae). Though the support values are not high, this may be taken as further confirming a close relationship of the two families. Only the MPew analyses do not yield any coccinellid-endomychid relationship (EFigs. E2, E10). Several analyses furthermore suggest a close relationship of either Latridiidae (*Cortinicara*; MPdw analysis of E-sample; Fig. 4), or Aderidae (*Elonus*; ML analysis of E-sample; Fig. 5), or both (ML and MB analyses of R-sample; Figs. 8, 9) to Coccinellidae and Endomychidae. Yet, all this is weakly supported, and one should note that there is contradictory evidence for a relationship of the tenebrionoid Aderidae to Sphindidae, and of Latridiidae to Cryptophagidae (e.g., MB analysis of E-sample, 53% posterior probability; Fig. 6). The MPdw analyses of the E- and R-samples (Figs. 4, 7) suggest Coccinellidae and Endomychidae, and partly Latridiidae, to form a clade with Erotylidae, which like Aderidae was never considered as pertaining to the cerylonid series.

Anthicidae + Aderidae + Scraptiidae. This grouping was proposed by LAWRENCE & NEWTON (1982). On the other hand, CROWSON (1955: 136) pointed out the distinctive morphological differences between Anthicidae and Aderidae and rather emphasised the cucujoid affinities of Aderidae. This opinion is congruent with our aforementioned results of the aderid in our sample either being associated with Sphindidae or with Coccinellidae + Endomychidae. Our sampled anthicid (*Notoxus*) variously appears in clades together with our monotomid (*Rhizophagus*; MPdw and MB analyses of E-sample; MPew, ML, and MB analyses of R-sample; Figs. 4, 6, 8, 9, EFig. E10), or the tetratomid (*Tetratoma*; MPdw and MB analyses of R-sample; Figs. 7, 9), or the mordellid (*Mordella*; ML analysis of E-sample; Fig. 5). In the MPdw analysis of the R-sample (Fig. 7) Anthicidae, Tetratomidae, and Monotomidae form a monophylum sister to Mordellidae + Zopheridae, and the Anthicidae + Tetratomidae + Monotomidae clade is also found by the ML and MB analyses of the R-sample. This may altogether indicate close affinities between at least the three latter families, but the evidence is altogether weak (no significant support for any of these clades). On the other hand, none of our analyses yielded any evidence for an aderid-anthicid relationship. Such evidence is neither included in HUNT et al. (2008).

Distinction between Cucujoidea, Tenebrionoidea, and other cucujiform "superfamilies". Based on a scarabaeid outgroup taxon and under inclusion of several cucujoid and tenebrionoid families, ROBERTSON et al. (2004; focused on Erotylidae) find their three tenebrionoid taxa (Zopheridae, Ciidae, and Tenebrionidae) to form a clade inside paraphyletic Cucujoidea. The results in ROBERTSON et al. (2008; focused on cerylonid series) are similar (but there is no rooting by an unambiguous non-cucujiform outgroup taxon).

The Bayesian analyses in HUNT et al. (2008: supporting fig. S1) yield a basal polytomy of 5 clades for Cucujiformia: Sphindidae; Cleroidea (including Byturidae and Biphyllidae); cerylonid series; Tenebrionoidea (including Lymexyloidea); and Chrysomeloidea + Curculionoidea + some cucujoid families. In the Maximum Parsimony analyses of these authors (supporting fig. S4) the cerylonid series is sister to the remaining cucujiforms; the next branch comprises the sampled tenebrionoids and lymexyloids plus a deeply subordinate Sphindidae; this is followed by a Cleroidea clade including Byturidae and Biphyllidae; the next branch comprises some cucujoid families (e.g., Nitidulidae, Erotylidae, Phalacridae, Cryptophagidae); the next offshoot is Helotidae + Orsodacnidae; a clade including Chrysomelidae and many Cerambycidae follows; Brachypteridae, Silvanidae, and some Cerambycidae form the next lineage; and most apically there is a sistergroup relationship between the cucujoid Monotomidae and Curculionoidea. Thus, while the Tenebrionoidea (including Lymexyloidea), Cleroidea, Chrysomeloidea, and Curculionoidea ap-

pear essentially as monophyletic, the Cucujoidea are grossly paraphyletic.

In our analyses we also find Cucujoidea to be paraphyletic with regard to Tenebrionoidea (and Cleroidea). Two cucujoid families, i.e., the Silvanidae (or at least *Oryzaephilus*) and Sphindidae, almost consistently form the basalmost clades of the entire cucujoid-tenebrionoid(-cleroid) assemblage. The tenebrionoid Ciidae tends to be the next clade, while in other trees Ciidae groups with the cucujoid Nitidulidae. In the more apical parts of the trees there is a frequent, but variable, grouping of some tenebrionoid families such as Tenebrionidae, Salpingidae, Zopheridae, Mordellidae, Anthicidae, and Tetratomidae, but the cucujoid Monotomidae usually also groups with (some of) these (compare ROBERTSON et al. 2008: fig. 2), and indeed a clade Tetratomidae + Anthicidae + Monotomidae is frequently found by the analyses. There are also larger clades comprising mainly cucujoid families, though with highly variable configurations, and tenebrionoid families may also be nested in these; a good example is the association of Aderidae with either Coccinellidae + Endomychidae, or Sphindidae. Altogether cucujoid and tenebrionoid families appear quite mixed up in all our trees. Thus according to our phylogenetic results there is no positive signal supporting a classification into Cucujoidea and Tenebrionoidea. As mentioned above, the Cleroidea (herein represented by Trogossitidae) also do not appear as phylogenetically distinct. However, it must be kept in mind that our phylogenetic results vary strongly with the analytical methods and ciid sampling, and hardly any clades above family level receive significant support values.

4. Conclusions

In our study we had included a taxon sample of Ciidae representing 20 species and 12 genera as well as 27 species from 20 other families of Cucujoidea and Tenebrionoidea, and 2 species from the cleroid family Trogossitidae; a bostrichid was used as the outgroup. We analysed sequences from 18S, COI, and – for Ciidae – COII using a variety of analytical methods: maximum parsimony based on fixed alignment (with equal or differentiated weighting) or on partial direct optimisation, maximum likelihood, and Bayesian analyses. These methods were applied to three different subsets of the taxon sample (E-sample, R-sample, C-sample). The objectives were to resolve inner-ciid relationships, test the monophyly of Ciidae, and find their closest relatives among the taxa in the cucujoid-tenebrionoid assemblage. The dataset was also expected to yield some evidence on the phylogeny of the cucujoid-tenebrionoid assemblage as a whole.

All sampled ciids belong to the Ciinae, since we did not succeed in sequencing the single species from Sphindociinae, *Sphindocis denticollis*. With this restriction, we clearly obtain Ciidae as a monophyletic taxon. This result is important since the morphological support for this group is weak. Inside this family we find non-monophyly for the huge genus *Cis*, and for *Sulcacis*. Different analyses suggest either *Ropalodontus*, or *Sulcacis fronticornis* + *Xylographus* + *Octotemnus*, or *Sulcacis fronticornis* + *Xylographus* alone as the sister group of the remaining Ciinae. Future studies will have to show whether Ciidae is also monophyletic including *Sphindocis*, and whether this taxon is basal within the family. Apart from the consistently supported clade *Sulcacis fronticornis* + *Xylographus* the results for the relationships among ciid genera vary a lot with the analytical methods and outgroup sampling; no conclusions could be drawn for that systematic level. It has remained unclear which other families from the cucujoid-tenebrionoid assemblage are closest to Ciidae. While some analyses suggest Nitidulidae to be in that position, others favour a far basal position of Ciidae, their sister group being a clade comprising many families. There is no relationship evident between Ciidae and Tetratomidae or Mycetophagidae.

The ambiguous resolution on this latter question is to be seen in the context of the generally highly conflicting resolution on the relationships among cucujoid-tenebrionoid taxa in our analyses, which allows only a few tentative proposals. In the frame of our limited taxon sample monophyly is indicated for Sphindidae and to a lesser extent for Cryptophagidae and Tenebrionidae. We also obtain monophyletic Nitidulidae and Coccinellidae, for which, however, we had sampled only taxa that are quite closely related. In contrast, Silvanidae are not supported as a monophyletic group. On the supra-familial level, a close relationship of Coccinellidae and Endomychidae is supported, and Latridiidae and Aderidae may fall in the same group. A frequently found clade is Tetratomidae + Anthicidae + Monotomidae. The support for clades Latridiidae + Cryptophagidae, Aderidae + Sphindidae, Nitidulidae + Brachypteridae, Brachypteridae + Phalacridae, and Tenebrionidae + Salpingidae is highly ambiguous. Some previously suggested groupings, such as a clade Aderidae + Anthicidae, are not found in any of our trees. Overall, families from Cucujoidea and Tenebrionoidea are fairly mixed up in our trees, which might indicate that this classification is artificial. Our study also supports previous results that the Cleroidea (or at least Trogossitidae) are placed inside the cucujoid-tenebrionoid assemblage.

Our study also shows some interesting methodological aspects. Our saturation curves indicate that 18S,

COI, and COII should all be informative both at the cucujoid-tenebrionoid and the ciid systematic levels, and for some aspects the contribution of CO sequences to tree reconstruction is specifically demonstrated by single-gene analyses. The comparison between analyses based on the E- and R-samples demonstrates that the extent to which a certain subgroup is sampled can have a great influence on the resulting trees; this even concerns parts of the tree that are distant from that particular subgroup.

Analysing multiple genes for a much denser sample of Cucujoidea, Tenebrionoidea, and Cleroidea would surely be of great interest considering the great diversification with regard to life history evident across these groups, for which evolutionary scenarios would be desirable. In addition, the origins of the megadiverse phytophagous clades Curculionoidea and Chrysomeloidea might well lie within this assemblage (as indicated by the analyses of HUNT et al. 2008) and should become an integral part of related studies, which then could lead to a comprehensive view of cucujiform phylogeny and ecological diversification. The most essential point for an improved sampling at that systematic level will be the inclusion of the many small families of Cucujoidea that appear "basal" due to morphological plesiomorphies (see LESCHEN et al. 2005).

5. Supplementary material

Phylogenetic trees from various analyses are associated with the online version of this contribution and freely available at www.arthropod-systematics.de.

6. Acknowledgements

We thank Anke Müller (Museum of Zoology Dresden) for effective help in the molecular lab. We greatly appreciate the help of the following colleagues, who provided specimens for our work: Cristiano Lopes-Andrade (Universidade Federal de Viçosa, Brasil; all Ciidae from Brasil, including their identification), Olaf Jäger (Museum of Zoology Dresden; *Oryzaephilus*), Christian Schmidt (Museum of Zoology Dresden; several species), Tomoyuki Tsuru (Hokkaido University, Sapporo, Japan; *Orthocis nigrosplendidus*, *Ropalodontus harmandi*), and Hans-Peter Reike (associate of Museum of Zoology Dresden; *Cis setiger* and determination of the latridiid). For their hospitability, which enabled us to collect important specimens for this study, we thank Jarmila & Stewart Peck (Ontario, Canada). This is a publication from the "Young Entomologists Group" of the Museum of Zoology Dresden, and we greatly appreciate the financial support of this project by the German Society of General and Applied Entomology (DGaaE) and the Systematics Association/Linnean Society (Systematics Research Fund, about 1200 € for lab costs). Furthermore we also thank Claudia Ketterer and the other organizers of the "Freiwilliges Ökologisches Jahr" in Dresden for their efforts, which enabled two of us (G. Buder, C. Grossmann) to work at the Museum of Zoology Dresden for a whole year.

7. References

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