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Towards a phylogeny of the Tenebrionoidea (Coleoptera)

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ABSTRACT

The phylogenetic relationships of the beetle superfamily Tenebrionoidea are investigated using the most comprehensive genetic data set compiled to date. With ~34,000 described species in approximately 1250 genera and 28 families, Tenebrionoidea represent one of the most diverse and species-rich superfamilies of beetles. The interfamilial relationships of the Tenebrionoidea are poorly known; previous morphological and molecular phylogenies recovered few well-supported and often conflicting relationships between families. Here we present a molecular phylogeny of Tenebrionoidea based on genes commonly used to resolve family and superfamily-level phylogenies of beetles (18S, 28S, 16S, 12S, tRNA Val and COI). The alignment spanned over 6.5 KB of DNA sequence and over 300 tenebrionoid genera from 24 of the 28 families were sampled. Maximum Likelihood and Bayesian analysis could not resolve deeper level divergences within the superfamily and very few relationships between families were supported. Increasing gene coverage in the alignment by removing taxa with missing data did not improve clade support but when rogue taxa were removed increased resolution was recovered. Investigation of signal strength suggested conflicting phylogenetic signal was present in the standard genes used for beetle phylogenetics, even when rogue taxa were removed. Our study of Tenebrionoidea highlights that even with relatively comprehensive taxon sampling within a lineage, this standard set of genes is unable to resolve relationships within this superfamily.

1. Introduction

The Tenebrionoidea is one of the largest superfamilies in the animal kingdom with approximately 34,000 described species and 28 currently recognized families (see Lawrence and Newton, 1995, Lawrence et al., 2010; Slipinski et al., 2011). In the tenebrionoid lineage almost two-thirds of the richness belongs to the single family Tenebrionidae (20,000 spp.), with five other species-rich families Anthicidae (3000 spp.), Meloidae (3000 spp.), Mordellidae (1500 spp.), Zopheridae (1700 spp.) and Aderidae (1000 spp.) accounting for another 38% of the diversity.

Morphologically the superfamily is very diverse but are characterized by having heteromerous tarsi with 5-5-4 tarsomeres in both sexes, occasionally reduced to 4-4-4, 3-3-3 or 3-4-4 in males, and never having a 5-5-5 tarsal formula. Generalizing about tenebrionoid biology is also difficult given the wide range of feeding strategies. Members of many families are fungus feeders but feeding on wood or decaying plant material is also common. Many oedemerids and anthicids are known to feed on pollen, while some mycterids feed within palms and grasses. Very few tenebrionoids are predators or feed on living plant tissue. Larvae of ripiphorids are ectoparasites of wood-boring beetles, aculeate Hymenoptera, and cockroaches. The superfamily is conspicuously diverse in arid environments.

Despite having few features common in all families, Lawrence and Newton (1995) considered the superfamily to be well-defined and monophyletic. Recent phylogenetic analyses of morphological characters of larvae supported the monophyly (Beutel and Friedrich, 2005) yet Schunger et al. (2003) pointed to the absence of autapomorphies inferred from a comprehensive cladistic analysis. A comprehensive morphological phylogeny of the Coleoptera based on both larval and adult characters also did not recover a monophyletic Tenebrionoidea (Lawrence et al., 2011). Instead a clade containing the cerylonid series (Cucujoidea) and Rentoniinae (Trogossitidae) were nested within a clade containing four tenebrionoid lineages. No molecular phylogeny has yet to focus solely

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on a molecular relationships of this superfamily. When tenebrionoids were included in the molecular phylogeny of beetles, these datasets either rejected the monophyly of the Tenebrionoidea, as Lymexyloidea nested within Tenebrionoidea (Hunt et al., 2007), or found Tenebrionoidea to be sister to Lymexyloidea (Bocak et al., 2014).

The relationships between families within the Tenebrionoidea have received little attention. The studies of Crowson (1966), Beutel and Friedrich (2005), Hunt et al. (2007), Lawrence et al. (2011) and Bocak et al. (2014) are the most comprehensive to date, although most had a wider focus on beetle phylogeny. Table 1 lists lineages recovered by various studies and highlights that few common relationships are recovered between studies. Additionally, classification of families into natural groupings remains problematic with various families frequently recovered as poly- or paraphyletic.

To gain insight into the relationships of tenebrionoid taxa we analyzed a robust multi-gene dataset. As the issue of monophyly of the Tenebrionoidea with respect to the Lymexyloidea has yet to be conclusively resolved, we first analyzed a broader dataset that included over 102 outgroup species representing 32 families (mostly cleroid and cucujoid families). Once the relationships between the Tenebrionoidea and Lymexyloidea were established, the distantly related out-group taxa were removed and the 330 ingroup species intensively analyzed. The influence of rogue taxa (*sensu* Wilkinson, 1996) and of missing loci on nodal support and topological stability was assessed by removing offending species. As our molecular datasets used standard loci which have dominated previous higher level phylogenetic studies of the beetle super-families, this study thus allowed us to examine the effect of greatly expanded taxon sampling. This is in contrast to the numerous studies which have expanded the number and/or type of molecular loci for smaller exemplar datasets (e.g. Wild and Maddison, 2008; Song et al., 2010; Timmermans et al., 2010).

2. Materials and methods

2.1. Taxon sampling

In total, 326 species representing 302 tenebrionoid genera were collected from Asia, Australia and Europe and preserved in 96% undenatured ethanol. Taxon sampling included species from 24 of 28 tenebrionoid families and 48 of 71 subfamilies (only 17 families contain subfamilial classifications). A list of taxa, localities, code names, voucher location and GenBank accession numbers is provided in Table S1.

2.2. Taxon Table, DNA amplification and sequencing

DNA was extracted from the head and thorax and/or metathoracic muscles of specimens using a QIAGEN DNeasy tissue kit as per standard protocols. The mitochondrial genes, *16S* rDNA, *12S* rDNA and cytochrome *c* oxidase subunit I (*COI*), and the nuclear gene, *28S* (LSU) rDNA and 18S (SSU) rDNA were amplified using the primers and protocols of Levkanicova (2009) and Gunter et al. (2013). All sequences are available from GenBank (see Supplementary Table 1).

2.3. Phylogenetics

The primary aim of this paper was to examine the relationships within the superfamily Tenebrionoidea. However to test these, the relationship between Tenebrionoidea and Lymexyloidea must first be resolved. Previous molecular phylogenies have suggested that the Lymexyloidea were nested within the Tenebrionoidea with the Cleroidea and a part of Cucujoidea also forming close relationships (Hunt et al., 2007; Timmermans et al., 2010) or Lymexyloidea were sister to Tenebrionoidea (Bocak et al., 2014). To test the phylogenetic position of the Lymexyloidea, a large data set comprising over 100 outgroup taxa primarily representing cleroid and cucujoid lineages was examined

Sequences of each of the 4 gene fragments were aligned separately using default parameters of MUSCLE in Geneious (v5.6; Drummond et al., 2012). Each alignment was edited by eye before concatenation; final dataset size was ~6600 nt. The full dataset consisted of 428 taxa with sequence data for at least 1000 bp of data and comprised 353 *16S* sequences (of which 241 are complete to the adjacent gene *12S*), 371 *18S* sequences, 348 *28S* sequences and 363 *COI* sequences. Total gene coverage in the alignment was approximately 73%.

The program PartitionFinder (Lanfear et al., 2012) was used to determine the best partitioning strategy and nucleotide substitution models for the analysis. The optimal partitioning scheme for both the 428 and 330 taxon datasets divided the data into seven partitions, one for each rRNA gene (*tRNA Val* combined with *12S*), plus separate codon positions for *COI*.

As outlined above two datasets were prepared, a 428 taxa set which included 102 outgroup species and a 330 taxon set which was restricted to members of the Tenebrionoidea and Lymexyloidea. Given the size of these data sets, only Maximum-Likelihood analyses were performed on the preliminary analyses. Maximum Likelihood (ML) analyses were performed using the RaxML black-box cluster on the CIPRES portal (Miller et al., 2010, 2013). The following strategies were also investigated in an attempt to improve bootstrap support (i) removal of all taxa not represented by COI, 18S, 28S and 16s (ii) removal of transitional taxa identified by RogueNaRok (Aberer et al., 2011, 2013). Of the above strategies similar results were recovered with the removal of rogue taxa recovering the most support. Bayesian analysis was only preformed on this data set and consisted of 50 million generations with a random starting tree, and two simultaneous runs with four Markov chains sampled every 1000 generations were conducted with unlinked partitions. Stationarity in MCM chains was determined in Tracer (Rambaut and Drummond, 2007), and burn-in

Table 1
Relationships recovered in past phylogenies.

	Lineages	Characters
Crowson (1966)	Anthicidae + Aderidae + Meloidae; Pythidae + Pyrochroidae + Mycteridae + Boridae + Salpingidae; Synchroidae + Zopheridae + Tenebrionidae; Melandryidae + Mordellidae + Ripiphoridae + Scraptiidae	Adult morphology
Beutel and Friedrich (2005)	Ripiphoridae + Meloidae; Trictenotomidae + Pythidae; Anthicidae + Euglenidae (Aderidae) + Scraptiidae; Mycteridae + Boridae (+Prostomidae in 1/2 analyses)	Larval morphology
Hunt et al. (2007)	Mordellidae + Ripiphoridae + Lymexyloidea; Ciidae + Anthicidae + Meloidae	Molecular (supported only)
Lawrence et al. (2011)	Zopheridae + Trictenotomidae; Promecheilidae (Perimylopidae) + Prostominiinae (Salpingidae) + Inopeplinae (Salpingidae)	Adult and larval morphology
Bocak et al. (2014)	Lymexyloidea + Tenebrionoidea; Ciidae + Tenebrionidae; Ripiphoridae (Meloidae + Mordellidae); Trictenotomidae + Boridae + Salpingidae	Molecular

was set appropriately. A majority-rule consensus tree was obtained from the two combined runs to establish the posterior probabilities of clades.

Nucleotide substitution saturation was tested for each gene as well as by codon for COI, as implemented in DAMBE (Xia and Xie, 2001). Transitions and transversions were plotted against pair-wise genetic distance for visualization of the level of nucleotide substitution saturation.

To visualize the signal content in the dataset neighbournet networks were generated in Splitstree (Huson, 1998; Huson and Bryant, 2006). Neighbournet networks were generated for each gene, with 18s and 16s divided into two fragments to minimize affect of sequence length and overlap on the networks. To visualize the signal of the concatenated gene the original dataset and a modified one that only contained overlapping regions (~3500 bp, $n = 120$) were examined.

3. Results

3.1. DNA data and alignments

Lengths of the amplified fragments varied from 536 to 1264 bp for *COI*, 391–1739 bp for *16S* (sometimes amplified in combination with *tRNA* and *12S*), 747–1979 bp for *18S* and 466–714 bp for *28S*. The total aligned length of the concatenated dataset was 6986 bp. Base frequencies were almost equal in the nuclear genes *18S* (A = 24.9, C = 23.3, G = 27.3, T = 24.5) and *28S* (A = 26.5, C = 22.3, G = 29.5, T = 21.7), whereas the mitochondrial genes *16S* (A = 44.6, C = 13.6, G = 6.2, T = 35.6), tRNA Val (A = 43.1, C = 12.1, G = 6.2, T = 38.6), *12S* (A = 40.0, C = 15.8, G = 8.9, T = 35.3) and *COI* (A = 30.3, C = 17.5, G = 14.8, T = 37.3) showed a higher A–T bias. Substitution saturation was observed in most genes (Iss. significantly higher than Iss.c) (See Supplementary Table 2).

Maximum likelihood analysis of the partitioned 428 taxon dataset recovered the Tenebrionoidea and Lymexyloidea as a monophyletic lineage (BS 53). The Lymexyloidea ($n = 4$, BS = 23) formed a sister relationship to the Tenebrionoidea ($n = 226$, BS = 7) however bootstrap support was limited. Within the Tenebrionoidea almost no bootstrap support was recovered for any of the backbone within ML analysis (See Supplementary Fig. 1). This was somewhat surprising as the ingroup taxa were better sampled than all outgroup families, yet all nodes within the outgroup clades showed significantly higher bootstrap support than the in group clades. Only the families Scraptiidae ($n = 6$, BS = 21), Oedemeridae ($n = 8$, BS = 96), Meloidae ($n = 8$, BS = 100), Mycetophagidae ($n = 4$, BS = 32), Mordellidae ($n = 13$, BS = 100) and Ciidae ($n = 7$, BS = 81) were recovered as monophyletic lineages. Most Salpingidae ($n = 8$, BS = 60) formed a clade to the exclusion of Inopeplinae while the majority of Aderidae ($n = 8$, BS = 98) formed a clade to the exclusion of an unidentified aderid "ZL0168". Similarly almost all darkling beetles (Tenebrionidae) formed an unsupported clade (BS = 4) which was rendered paraphyletic to the inclusion of *Neotrichus serraticollis* (Zopheridae) and *Neosteriopalus niponicus* (Anthicidae). Only two tenebrionid taxa were excluded from the main Tenebrionidae clade. *Tanylypa* (Tenebrionidae: Zolodininae) formed a weakly supported relationship with *Docalis* (Zopheridae) (BS = 12) near the base of a clade containing the majority of zopherids in the analysis and *Nilio* sp. (Tenebrionidae: Nilioninae) which clustered with *Cephaloon pallens* (Stenotrachelidae) and Boridae ($n = 2$) in a poorly supported clade (BS = 1). The families Zopheridae, Melandryidae and Tetratomidae were recovered as polyphyletic lineages scattered throughout the tree whereas Rhipiphoridae, Anthicidae and Pyrochroidae were also paraphyletic lineages but near to one another in the phylogeny.

By removing more distant out-groups, we expected to reduce noise within the alignment, however, due to the limited support for a sister relationship between lymexyloids and tenebrionoids the analysis of the 330-taxon set was kept unrooted. Bootstrap support was almost identical between the 428 and 330-taxon sets with minimal backbone support and no additional well-supported lineages were recovered (See Supplementary Fig. 2). Within the ingroup, the topologies were similar in regards to which clades were supported vs. para- and polyphyletic lineages but the positions of clades across the unsupported backbone varied significantly. For example the "sister" lineage to the Tenebrionoidae was Ciidae + Serropalpini melandryids in 428- taxon set but in 330- taxon a trifurcating clade containing Tenebrionidae, Zopheridae + *Anaplopus* (Pythidae) + Trictenotomidae + Stenotrachelidae and *Pytho* (Pythidae) + *Tetratoma* (Tetratomidae) + the majority of melandryines (Melandryidae) was recovered. However, both of these relationships with the Tenebrionidae had no bootstrap supported (BS = 0). This lack of bootstrap support prompted the investigation into the signal strength in the data set and was examined by removing taxa with missing data and rogue taxa. All strategies were investigated using the Tenebrionoidea + Lymexyloidea only data set (taxa = 330, nt = 6,600) and was modified accordingly.

Some improvement in bootstrap support was recovered by removing taxa with missing data (see Supplementary Fig. 3). However, removal of rogue taxa significantly improved bootstrap values in some areas of the tree including the split between the Lymexyloidea (B = 87) and Tenebrionoidea (BS = 87). Fig. 1 shows the unrooted topology of the maximum likelihood analysis once rogue taxa were removed, see Supplementary Fig. 4 for exact bootstrap results. The Tenebrionidae was recovered as a supported lineage when missing data were removed ($n = 66$, BS = 31) and when rogue taxa were removed ($n = 119$, BS = 72). Within the Tenebrionidae taxa clustered in subfamily groups with Lagriinae, Pimeliinae and Stenochiinae recovered as monophyletic lineages, while Tenebrioninae, Alleculinae and Diaperinae remained paraphyletic.

Bayesian analysis was performed on the Tenebrionoidea + Lymexyloidea minus rogue taxa data set, which showed the most promising results of resolving relationships. The 2 runs of the 50 million generation analysis did not converge until approximately 23 million generations, so the last 25 million were used to build the consensus tree. The topology of the Maximum Likelihood and Bayesian analyses varied with Bayesian analysis recovering fewer well-supported clades (See Supplementary Fig. 5). Bayesian analysis separated the taxa into 3 well supported lineages representing the Lymexyloidea ($n = 3$, PP = 1), Ciidae ($n = 5$, PP = 1) and the rest of the Tenebrionoidea ($n = 322$, PP = 1). This tenebrionoid lineage was largely a comb comprising 22 clades. These clades represented monophyletic families (Oedemeridae, Salpingidae, Aderidae and Pyrochroidae), 8 tenebrionid clades (Tenebrioninae + Alleculinae + Stenochiinae, Lagriinae, 2× Tenebrioninae, Tenebrioninae + Diaperinae, 2× Pimeliinae, Diaperinae), 2 zopherid and melandryid lineages and 4 clades representing multiple families (i) Ripiphoridae + Mordellidae + *Macratria* (Anthicidae), (ii) Anthicidae + Meloidae, (iii) Archeocrypticidae + Mycetophagidae + Ulodidae + Promecheilidae, and (iv) Scraptiidae + Melandryidae. All the above clades were recovered in Maximum Likelihood analyses. The position of *Penthe* (Tetratomidae) and Pythidae could not be resolved.

3.2. Neighbournet networks

Neighbournet networks were produced for each gene fragment (see Supplementary Fig. 6) as well as for the concatenated data sets (Fig. 2a–c). These networks are not phylogenies but instead used to visualize differences in clade support patterns. In general the more tree-like the structure the more signal information the data set contains while net-like structures indicate contradicting evidence. In the networks, splits are visualized using a series of parallel edges

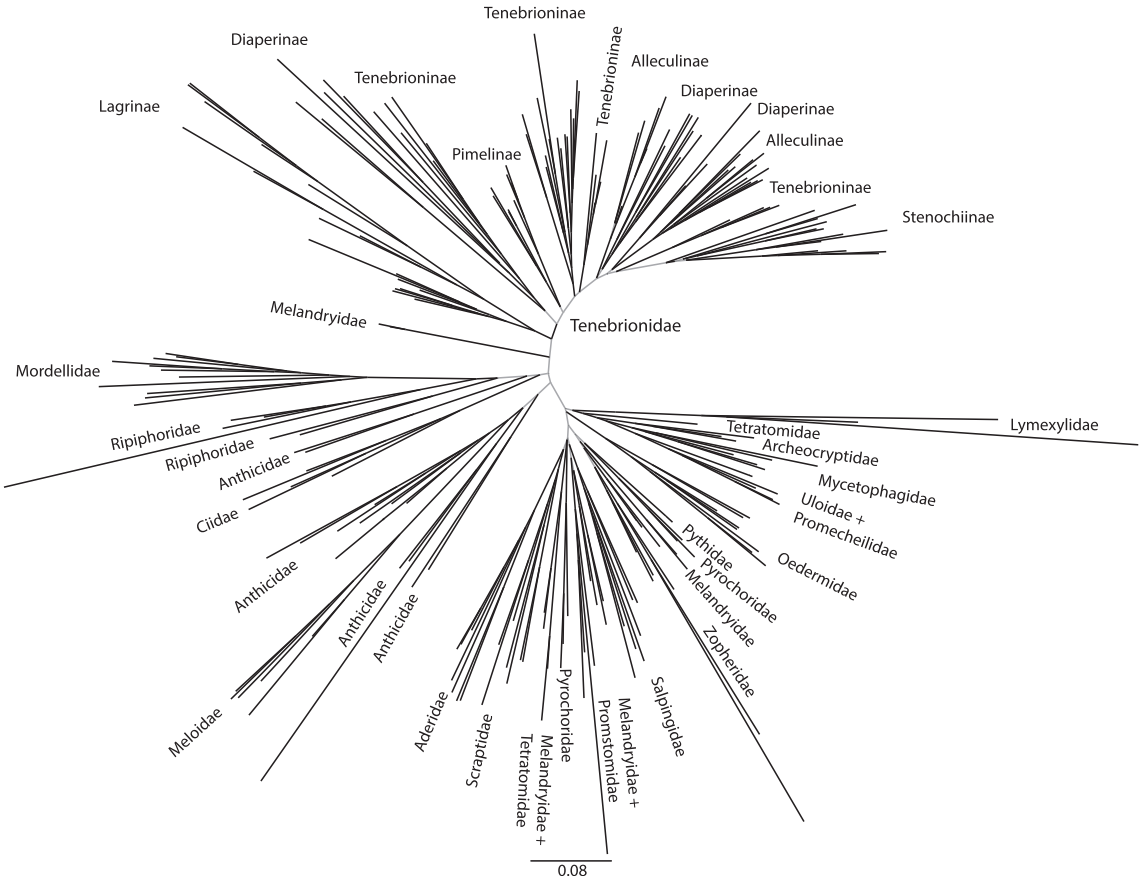

Fig. 1. Unrooted maximum-likelihood phylogeny of the Tenebrionoidea with rogue taxa removed. The phylogenetic tree is based on a partitioned six gene dataset. Nodes that are supported are represented in black while unsupported clades are in gray. Exact bootstrap values are given in Supplementary Fig. 4.

with edge length proportional to the weight of the split (Huson and Bryant, 2006). The neighbournet networks for each gene all contained various degrees of internal net-like structures indicating conflicting signal within the data (See Supplementary Fig. 6). As they are calculated using distance methods the networks are greatly affected by sequence length and overlap so the original alignment was modified to only contain regions that overlapped. Fig. 2a depicts the signal of all overlapping regions of the alignment (n = 172, 3294 nt) compared to the networks for the original alignment (n = 330, 6600 nt) (Fig. 2b) and when rogue taxa were removed (n = 232, 6600 nt) (Fig. 2c). All alignments produced networks that can be generally characterized as having long terminal branches and almost no distinct internal branches. Such patterns indicate the phylogenetic signal is limited and/or contradictory (Wägele and Mayer, 2007).

4. Discussion

We present the largest molecular phylogeny of the Tenebrionoidea to date; examining relationships between 326 species representing 302 genera from 24 families that belong to the superfamily. To date, the only other molecular phylogeny with comprehensive tenebrionoid sampling was that of Hunt et al. (2007) that contained 217 species representing 154 genera from 19 families as part of a phylogeny of the Coleoptera. The phylogeny of Hunt et al. (2007) was predominately based on 18S sequence data, however approximately 25% of the Tenebrionoidea in the data set were also represented by 16s and COI data with only 44 taxa (~20%) with all 3 genes sampled. The phylogeny that contains the full dataset was not bootstrapped instead a smaller representative dataset containing 48 tenebrionoids was bootstrapped. The relationships recovered by Hunt et al. (2007) and in our analyses differ but in general all were poorly supported.

To date, no phylogeny based on either morphological or molecular characters has successful been able to resolve the interfamilial relationships between tenebrionoids. The few clades recovered in the major phylogenies of Crowson (1966), Beutel and Friedrich (2005), Hunt et al. (2007), Lawrence et al. (2011) often provide conflicting relationships. Both the molecular phylogeny of Hunt et al. (2007) and our analyses were unable to recover much backbone support for the superfamily or definitively be able to conclude the basal lineages within the superfamily or its relationship with Lymexyloidea. The inability of these molecular data to resolve the higher-level relationships of the Tenebrionoidea may be due to inappropriate data for resolving the evolutionary history of the group (i.e. insufficient taxon sampling or conflicting signal) or a rapid radiation within the lineage.

Taxon sampling is known to be one of the most important determinants of accurate phylogenetic inference, particularly in species rich lineages (Zwickl and Hillis, 2002; Rosenberg, 2007; Heath et al., 2008). Within this dataset, the taxon sampling is relatively proportional to the richness and diversity of the superfamily. The richest families Tenebrionidae, Anthicidae, Meloidae, Mordellidae, Zopheridae, Oedemeridae and Aderidae account for 63%, 10%, 10%, 5%, 5%, 5%, 3.3% of the diversity respectively and make up 50%, 5.5%, 2.5%, 4%, 5.5%, 2.5% and 2.7% of taxa in the original data set (before rogue taxa were removed). However as this dataset is sampled at a generic level it is also important to consider the diversity of families. There are just over 1250 genera within the

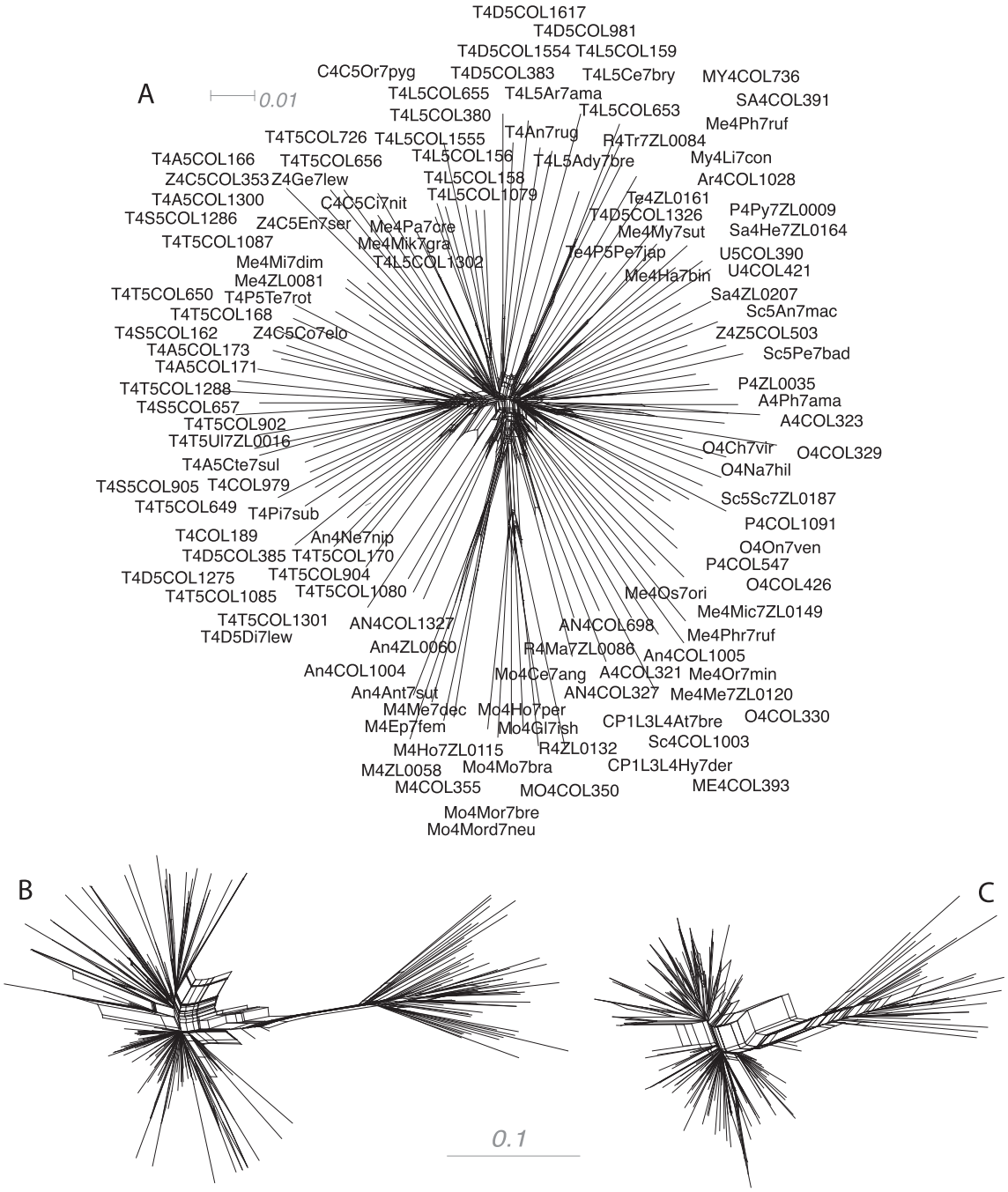

Fig. 2. Neigbour-nets showing conflicting splits when (A) all overlapping regions of the alignment are included (n = 172, 3294 nt); (B) when all tenebrionoid taxa from the original alignment are included (n = 330, 6600 nt); and (C) when rogue taxa are removed (n = 232, 6600 nt).

Tenebrionoidea and our dataset contains approximately 300 genera representing approximately a quarter of described genera. These genera are fairly proportional to the diversity of the lineages with the exception of the Zopheridae, which are underrepresented with only 18 of 190 genera included in this analysis. The high diversity relative to richness within the Zopheridae (1,500 spp. in 190 genera) maybe an artifact of taxonomy and we do not believe the zopherids in our analysis are significantly under sampled as they represent ~5.5% of the data set and 5% of the richness of the Tenebrionoidea. Our dataset also includes 24 of the 28 families within the Tenebrionoidea with only the smallest and least common families absent. Inclusion of members of the families Pterogeniidae, Chalcodryidae, Trachelostenidae and Synchroidae may help with the placement of a few families but it is unlikely that they will provide enough signal to stabilize the backbone as in total they only represent 11 genera. On the basis of relatively comprehensive taxon sampling representing ~25% of the generic diversity of Tenebrionoidea with proportional distribution among families, we believe that taxon sampling should be sufficient to recover relationships within Tenebrionoidea if phylogenetic signal was present. However, future efforts to improve taxon sampling may help resolve the position of the transitional or rogue taxa identified in this dataset.

Ninety-seven rogue taxa were identified from the Tenebrionoidea in our data set. The phylogenetic position of rogue taxa cannot be resolved, assuming varying and often contradictory position in a tree set which substantially reduced the bootstrap or posterior probability of a consensus tree (Aberer et al., 2013). Ambiguous

or insufficient phylogenetic signal is generally the cause of this phenomenon (Sanderson and Shaffer, 2002). The taxa identified by RogueNaRok included many taxa from families well represented in the tree plus a few that were poorly sampled. In total 55 tenebrionids, 9 zopherids, 4 melandryids and ripiphorids, 2 aderids, anthicids, borids, ciids, meloids, mordellids, salpingids and tetratomids and 1 ischaliine, mycterid, mycetophagid, promecheilid, prostomid, pyrochroid, pythid, scraptiid, stenotracheloid and trictenotomid were excluded from the 330-taxon dataset. Of these taxa all Boridae ($n = 2$), Ischalinae ($n = 1$), Stenotrachelidae ($n = 1$) and Trictenotomidae ($n = 1$) species were identified as rogue taxa and therefore these groups were entirely removed from the final analyses. These represent some of the smallest and least diverse families within the superfamily (Boridae: 3 genera, 4 spp.; Ischaliinae: 1 genus, 41 spp.; Stenotracheloidae: 7 genera, 19 spp.; Trictenotomidae: 2 genera, 13 spp. (see Slipinski et al., 2011)), however other species poor families (i.e. Uloididae, Pythidae, Promecheilidae and Prostomidae) remained in the analysis.

Removal of rogue taxa did improve the support values recovered in the analyses, however neighbournet networks generated in Splitstree revealed a large amount of noise was still present in the data once these taxa were removed (see Fig. 2b and c). Large net like structures are observed centrally in the networks and indicate conflict in signal. These net-like structures are also present to various extents in all genes included in the alignment (see Supplementary Fig. 6) and suggest that noise is not an artefact of conflict between one or more genes. The genes included in this analysis are some of the most commonly used to resolve the phylogeny of various beetle families. They were able to recover well-supported relationships of the out-group taxa in the original alignment suggesting that there are no major issues with the alignment. The lack of phylogenetic signal present in this dataset highlights the limitations of sequencing standard sets of mitochondrial and ribosomal genes currently used for beetle phylogenetics. Next gene sequencing promises to deliver a quick and cost-effective way to generate sequence data and it is generally thought that the inclusion of more genetic data will eventually overcome conflicting signal present in a few genes. Whole mitochondrial genomes may also help resolve the relationships between the families, however past phylogenies that have used these standard set of mitochondrial and ribosomal genes compared to whole mitochondrial genomes recover superficially similar topologies (e.g. for Curculionidae compare McKenna et al. (2009) and Haran et al. (2013)). Recovering phylogenetic relationships within beetles using whole mitochondrial genomes have been shown to be greatly affected by base compositional heterogeneity and when the data are biased, standard phylogenetic inference methods consistently perform poorly (Sheffield et al., 2009; Song et al., 2010). Slowly evolving nuclear protein coding genes are less prone to base-composition bias than the mitochondrial markers (Lin and Danforth, 2004) and present fewer alignment issues than ribosomal rDNA (Danforth et al., 2005) have more potential for recovering deep divergences within the Tenebrionoidea. Candidate genes including Arginine Kinase, Alpha spectrin, CAD, Wingless and PepCK have been shown to reconstruct deeper divergences slightly more accurately than shallower divergences at least within a test dataset dominated by carabid beetles (Wild and Maddison, 2008). Sequencing the transcriptomes of Tenebrionoidea may help identify new genes with strong phylogenetic signal.

Despite being unable to recover inter-family relationships within the superfamily, the current standard set of mitochondrial and ribosomal genes proved useful at a lower level. Only 17 of 28 tenebrionoid families are divided into subfamilies, however, our phylogeny includes 48 of the 71 tenebrionoid subfamilies; 27 subfamilies are represented by more than a single genus. Within the 428 and 330 taxon data set the vast majority of nodes within family level clades are well-supported. Posterior probability values in the Bayesian analysis were generally strongly supported (PP > 95). Of the 17 families currently classified using subfamily rank, 12 were represented by at least 2 subfamilies. Taxon sampling is not comprehensive enough to comment on validity of the current classification as many subfamilies are only represented by a single genus. Certainly there are examples where the current classification does not reflect natural groupings (e.g. Salpinginae is rendered paraphyletic by the inclusion of Aegialitinae) or validate rank debates (e.g. Nacerdinae vs. Oedemerinae: Nacerdini with results confirming it is more suited as tribal rank as *Agamosium* (Nacerdinae *sensu* Arnett, 1951) is nested deeply within the Oedemerinae). The strong node support observed at lower levels suggest that these genes will prove useful for refining subfamily and tribal limits and offers a basis on which to choose exemplar taxa for sequencing additional genes to resolve family level relationships as outlined above

4.1. Lymexyloidea?

Our analysis could not definitively confirm the sister relationship between the Lymexyloidea and Tenebrionoidea due to low bootstrap support but was always recovered as a distinct and monophyletic lineage. Regardless our results confirm that of Hunt et al. (2007) that Lymexyloidea are more closely related to Tenebrionoidea than to Cleroidea or Cucujoidea as previously suggested by Crowson (1955, 1960, 1981) and Lawrence and Newton (1982). Our results conflict with the phylogenetic position of Lymexyloidea in Hunt et al. (2007) which recover it as a paraphyletic lineage at base of the Tenebrionoidea, with some taxa forming a weak relationship with Mordellidae in an unsupported clade also containing the Ripiphoridae. Lymexylidae are short-lived as adults and feed on fungus; their larvae are wood-boring and have evolved the ability to cultivate fungus in their tunnels (Wheeler, 1986). While mycophagy is a common lifestyle within the Tenebrionoidea (Lawrence et al., 2010), wood-boring larvae are rare with only mordellids, oedemerids and some tenebrionid and melandryid species feeding on dead wood and in rotten stems of herbaceous plants (Lawrence, 1991). Lymexylids differ from all tenebrionoids in having 5-5-5 tarsi with almost all tenebrionoids have a 5-5-4 formula, although some taxa have reduced numbers of tarsomeres (4-4-4, 3-3-3 or 3-4-4) (Lawrence et al., 2010). Whether the Lymexyloidea deserve separate superfamily status or are a basal family within the Tenebrionoidea remains debatable; however on the basis of current evidence there appears no reason to make any changes to their classification.

4.2. Inter-family relationships

Phylogenetic relationships between families within the Tenebrionoidea remain elusive despite analyzing a fairly comprehensive data set, with only four relationships linking families supported in all our analyses. The plausibility of these relationships are discussed below with confidence values provided from the dataset with rogue taxa removed (see Supplementary Figs. 4 and 5).

4.2.1. Ripiphoridae + Mordellidae

A supported relationship (BS = 52, PP = 0.99) was always recovered between a grade of Ripiphorids and the monophyletic Mordellidae. The relationship between Mordellidae + Ripiphoridae was recognized over 150 years ago when Lacordaire (1859) placed the family Ripiphoridae near Mordellidae. The sister relationship has never been confirmed by molecular analysis and is often debated. On the basis of morphology mordellids are generally considered to be closely related to melandryids (Crowson, 1966; Lawrence and Newton, 1982). Ripiphorids have been linked to meloids on the

basis of larval characters (Beutel and Friedrich, 2005) and mordellids on the basis of adult characters (Falin, 2003). Crowson (1966) also considered Ripiphoridae and Scraptiidae to belong to this lineage while Lawrence and Newton (1982) excluded Scraptiidae from their tentative groupings and placed Melandryidae, Mordellidae and Ripiphoridae together.

4.2.2. Anthicidae (Anthicinae, Lemodinae, Notoxinae and Tomoderinae) + Meloidae

The majority of anthicid subfamilies included in the analysis formed a paraphyletic lineage with a monophyletic Meloidae nested within the clade. Two other anthicid subfamilies were included in the analyses but the position of Eurygeniinae remained unresolved while Macratriinae occasionally formed a poorly supported relationship with the Ripiphoridae + Meloidae. The anthicid subfamilies Copobaeninae and Steropinae were not included in this analysis. The monophyly of the Anthicidae has been debated over the years and subfamilies Lagrioidinae, Afreminae and Ischaliinae previously included are often considered *incertae sedis* within the Tenebrionoidea (Lawrence et al., 2010). To date no phylogeny has examined the relationships of the Anthicidae but on the basis of morphology (particularly of the Steropinae) it is considered to be closely related to the Meloidae (Abdullah, 1965, 1967; Hemp and Dettner, 1997). This hypothesis is strongly supported by Bayesian analysis (PP = 1) and Maximum Likelihood (BS = 81). Lawrence (1977) also considered the Aderidae (Euglenidae) to be part of this lineage, however although aderids were recovered as a monophyletic lineage its phylogenetic position remained unresolved. Hunt et al. (2007) also recovered the relationship between Anthicidae + Meloidae but also placed Ciidae at the base of the clade. The phylogenetic position of Ciidae remained unresolved in our analyses.

4.2.3. Archeocrypticidae + Mycetophagidae + Ulodidae + Promecheilidae (+Tetratomidae)

A clade containing the Archeocrypticidae, Mycetophagidae, Ulodidae and Promecheilidae (Perimylopidae) was recovered in both Bayesian and Maximum Likelihood analyses (BS = 78, PP = 0.5). In Maximum likelihood analysis *Penthe japana* (Tetratomidae) was also recovered at the base of this clade (BS = 61). Morphologically Archeocrypticidae and Mycetophagidae share many features and their similarities have been discussed by Lawrence (1991). These families are thought to belong to the basal tenebrionoid lineages of Crowson (1966), also containing Tetratomidae, Ciidae and Pterogeniidae (Lawrence, 1977; Lawrence and Newton, 1982, 1995). The relationship with a tetratomid was recovered only in Maximum likelihood analysis; however the phylogenetic position of Ciidae remained unresolved in our analyses and no pterogeniids were included. The Promecheilidae have either been recovered as the sister group to Tenebrionidae + Chalcodryidae or nested within a paraphyletic Ulodidae in cladistics analyses of larval and adult characters with differing results recovered when multistate characters were ordered (Lawrence, 1994). Our results support the latter hypothesis with two promecheilids nested between the ulodid genera *Meryx* and *Ulodes* (BS = 90, PP = 1). Although the relationships between Archeocrypticidae + Mycetophagidae and Ulodidae + Promecheilidae have been suggested before, this is the first evidence that these groups are related to each other. Hunt et al. (2007) recovered alternate relationships but did not include Archeocrypticidae or Ulodidae in their analysis. Instead Hunt et al. (2007) recover an unsupported relationship between Mycetophagidae and Salpingidae, while Promecheilidae (Perimylopidae) forms an unsupported clade with Tetratominae and Hallomeninae tetratomids (not Penthinae). On the absence of archeocrypticids and ulodids in the Hunt et al. (2007) data set and on the basis of plausible morphological links, it is likely that the relationships recovered in our analysis reflect more natural groupings than those recovered in Hunt et al. (2007).

4.2.4. Scraptiidae + Eustrophinae (Tetratomidae?) + Osphyinae (Melandryidae)

The classification of Eustrophinae has been debated and it has been shifted back and forth between Tetratomidae and Melandryidae over the years (Crowson, 1955, 1966) and is discussed in both families in the Lawrence et al. (2010) but Bouchard et al. (2011) list it belonging to the Tetratomidae. In all analyses *Mycetoma suturale* (Eustrophinae) always forms a supported relationship with *Conopalpus testaceus* (Osphyinae) (BS = 100, PP = 0.64), which is recovered as a supported sister group (BS = 78, PP = 0.64) to a monophyletic Scraptiidae. Scraptiidae is divided into two main lineages, Anaspidinae and Scraptiinae, which are supported as sister lineages in our analyses (BS = 62, PP = 0.85). Originally anaspidines were classified within the Melandryidae and scraptiines were included in the Mordellidae until Crowson (1955) proposed the link between the two subfamilies and creating the family. Beutel and Friedrich (2005) proposed a link between Anthicidae + Aderidae (Euglenidae)+ Scraptiidae on the basis of larval characters, however our results reject this hypothesis. Hunt et al. (2007) also recover a supported relationship between scraptiids and Osphyinae but the Scraptiidae is not recovered as a monophyletic lineage.

4.3. Future directions

Molecular phylogenetic analysis has, and will continue to have a prominent role in systematic research on beetles. The phylogeny of Tenebrionoidea presented here demonstrates that the standard set of mitochondrial and ribosomal genes currently used to examine phylogenetic relationships within the order Coleoptera, do not contain sufficient phylogenetic signal to reconstruct deeper level divergences within this superfamily. Although a few interfamilial relationships were recovered, very little support was recovered for higher level divergences. Removing rogue taxa improved the support values within the trees, however conflicting phylogenetic signal was still apparent within the alignment. More slowly evolving and suitable genes should be sequenced to improve phylogenetic signal. Given the observed conflict in signal within all genes it is unlikely that increased taxon sampling will significantly improve the recovery of deeper level divergences. Well-supported resolution at lower taxonomic levels (subfamily and below) however mean that these genes can be used to define sets of monophyletic taxa from which exemplars can be chosen for more intensive sequencing/morphological analysis. Only once we have a robust phylogeny of lineage should any attempt to investigate evolutionary hypotheses such as divergence dating and character evolution to avoid any premature conclusions. We encourage critical investigation into the strength of a data set, in particularly transparency in data quality and completeness. It is important to accept that we cannot resolve all relationships within a phylogeny and publishing unresolved phylogenies is of as much interest that of entirely supported ones. The role of phylogenies extends past confirming all relationships but should highlight new areas for investigation. The inability to resolve relationships between most families within the Tenebrionoidea in all past morphological and molecular phylogenies raises interesting questions into diversification of the superfamily and maybe an ideal candidate to identify new genes for phylogenetic reconstruction.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2014.05.028.

References

Abdullah, M., 1965. *Protomeloe argentinensis*, a new genus and species of Meloidae (Coleoptera), with remarks on the significance of cantharidin and the phylogeny of the families Pyrochroidae, Anthicidae, Meloidae and Cephaloidae. Ann. Mag. Nat. Hist. (13th Series), 247–254.

Abdullah, M., 1967. Some phylogenetic conclusions on the Eurygeniinae (Coleoptera: Anthicidae), with a review of the North American species of *Eurygenius* including the description of a new species (*E. darlingtoni*) from Texas. Entomol. News. 78, 180–188.

Aberer, A.J., Krompaß, D., Stamatakis, A., 2011. RogueNaRok: An Efficient and Exact Algorithm for Rogue Taxon Identification. Heidelberg Institute for Theoretical Studies: Exelixis-RRDR-2011-10, November 2011. <http://sco.h-its.org/exelixis/publications.html>.

Aberer, A.J., Krompaß, D., Stamatakis, A., 2013. Pruning rogue taxa improves phylogenetic accuracy: an efficient algorithm and webservice. Syst. Biol. 62, 162–166.

Beutel, R.G., Friedrich, F., 2005. Comparative study of larvae of Tenebrionoidea (Coleoptera: Cucujiformia). Eur. J. Entomol. 102, 241–264.

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

Bouchard, P., Bousquet, Y., Davies, A.E., Alonso-Zarazaga, M.A., Lawrence, J.F., Lyal, C.H.C., Newton, A.F., Reid, C.A.M., Schmitt, M., Ślipiński, S.A., Smith, A.B.T., 2011. Family-group names in Coleoptera (Insecta). ZooKeys 88, 1–972.

Crowson, R.A., 1955. The Natural Classification of the Families of Coleoptera. Nathaniel Lloyd, London, 187pp.

Crowson, R.A., 1960. The phylogeny of Coleoptera. Ann. Rev. Entomol. 5, 111–134.

Crowson, R.A., 1966. Observations on the constitution and sub- families of the family Melandryidae (Coleoptera). Eos 41, 507–513.

Crowson, R.A., 1981. The Biology of the Coleoptera. Academic Press, London, pp. 694–698.

Danforth, B.N., Lin, C.P., Fang, J., 2005. How do insect nuclear ribosomal genes compare to protein-coding genes in phylogenetic utility and DNA substitution patterns? Syst. Entomol. 30, 549–562.

Drummond, A.J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Duran, C., Field, M., Heled, J., Kearse, M., Markowitz, S., Moir, R., Stones-Havas, S., Sturrock, S., Thierer, T., Wilson, A., 2012. Geneious v5.6. http://www.geneious.com.

Falin, Z.H., 2003. Phylogenetic Analysis and Revision of the Genera and Subfamilies of the Ripiphoridae (Coleoptera). PhD.

Gunter, N.L., Leavengood, J.M., Bartlett, J.S., Chapman, E.G., Cameron, S.L., 2013. A molecular phylogeny of the checkered beetles and a description of Epiclininae a new subfamily (Coleoptera: Cleroidea: Cleridae). Syst. Entomol. 38, 626–636.

Haran, J., Timmermans, M.J., Vogler, A.P., 2013. Mitogenome sequences stabilize the phylogenetics of weevils (Curculionoidea) and establish the monophyly of larval ectophagy. Mol. Phylogenet. Evol. 67, 156–166.

Heath, T.A., Hedtke, S.M., Hillis, D.M., 2008. Taxon sampling and the accuracy of phylogenetic analyses. J. Syst. Evol. 46, 239–257.

Hemp, C., Dettner, K., 1997. Morphology and chemistry of mesothoracic glands in anthicid beetles (Coleoptera: Anthicidae). Entomol. Gen. 22, 97–108.

Hunt, T., Bergsten, J., Levkanicova, Z., Papadopoulou, A., St. John, O., Wild, R., Hammond, P.M., Ahrens, D., Balke, M., Caterino, M.S., Gómez-Zurita, J., Ribera, I., Barraclough, T.G., Bocakova, M., Bocak, L., Vogler, A., 2007. A comprehensive phylogeny of beetles reveals the evolutionary origins of a superradiation. Science 318, 1913–1916.

Huson, D., 1998. SplitsTree: a program for analyzing and visualizing evolutionary data. Bioinformatics 14, 68–73.

Huson, D.H., Bryant, D., 2006. Application of phylogenetic networks in evolutionary studies. Mol. Biol. Evol. 23, 254–267.

Lacordaire, T., 1859. Histoire Naturelle des Insectes. Genera des Coléoptères. Tome 5. pls. 48–60. Libraire Encyclopédique de Roret, Paris, 750pp.

Lanfear, R., Calcott, B., Ho, S.Y.M., Guindon, S., 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29, 1695–1701.

Lawrence, J.F., 1977. The family Pterogeniidae, with notes on the phylogeny of the Heteromera. Coleopt. Bull. 31, 25–56.

Lawrence, J.F., 1991. Order Coleoptera; Mycetophagidae; Archeocrypticidae; Pterogeniidae; Ciidae; Tetratomidae; Melandryidae; Mordellidae; Colydiidae; Monommidae; Zopheridae; Perimylopidae; Chalcodryidae; Tenebrionidae; Cephaloidae; Oedemeridae; Mycteridae; Trictenotomidae (Tenebrionoidea). In: Stehr, F.W. (Ed.), Immature Insects, vol. 2. Springer, Dubuque, Iowa, 144–184; 498–500; 500–501; 501–502; 502–504; 504–505; 505–508; 508–509; 512–514; 514–515; 518–519; 520; 520–528; 529; 534–535; 535–537; 539.

Lawrence, J.F., 1994. The larva of *Sirrhas variegata*, sp. nov., with notes on the Perimylopidae, Ulodidae (stat. nov.), Zopheridae and Chalcodryidae (Coleoptera: Tenebrionoidea). Invertebr. Taxon. 8, 329–349.

Lawrence, J.F., Newton Jr., A.F., 1982. Evolution and classification of beetles. Annu. Rev. Ecol. Syst. 13, 261–290.

Lawrence, J.F., Newton Jr., A.F., 1995. Families and subfamilies of Coleoptera (with selected genera, notes, references and data on family-group names). In: Pakaluk, J., Slipinski, S.A. (Eds.), Biology, Phylogeny, and Classification of Coleoptera: Papers Celebrating the 80th Birthday of Roy A. Crowson. Museum i Instytut Zoologii PAN, Warszawa, pp. 779–1006.

Lawrence, J.F., Pollock, D.A., Ślipiński, A., 2010. 11. Tenebrionoidea. Introduction, phylogeny. In: Leschen, R.A.B., Beutel, R.G., Lawrence, J.F. (volume Eds.), Coleoptera, beetles, vol. 2. Morphology and systematics (Elateroidea, Bostrichiformia, Cucujiformia partim). In: Kristensen, N.P., Beutel, R.G. (Eds.), Handbook of Zoology. A Natural History of the Phyla of the Animal Kingdom, vol. IV. Arthropoda: Insecta. Part 38, Walter de Gruyter, Berlin, New York, pp. 487–491.

Lawrence, J.F., Slipinski, A., Seago, A.E., Thayer, M.K., Newton, A.F., Marvaldi, A.E., 2011. Phylogeny of the Coleoptera based on morphological characters of adults and larvae. Ann. Zool. 61, 1–217.

Levkanicova, Z., 2009. Molecular Phylogeny of the Superfamily Tenebrionoidea (Coleoptera: Cucujiformia).Thesis (2009), Palacky University, Olomouc, Czech Republic.

Lin, C.P., Danforth, B.N., 2004. How do insect nuclear and mitochondrial gene substitution patterns differ? Insights from Bayesian analyses of combined data sets. Mol. Phylogenet. Evol. 30, 686–702.

McKenna, D.D., Sequeira, A.S., Marvaldi, A.E., Farrell, B.D., 2009. Temporal lag and overlap in the diversification of weevils and flowering plants. PNAS 106, 7083–7088.

Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), 14 November 2010, New Orleans, LA, pp 1–8.

Miller, M.A., Holder, M.T., Vos, R., Midford, P.E., Liebowitz, T. Chan, L., Hoover, P., Warnow, T., 2013. The CIPRES Portals. CIPRES. <http://www.phylo.org/sub_sections/portal>.

Rambaut, A., Drummond, A.J., 2007. Tracer v1.5. <http://beast.bio.ed.ac.uk/Tracer>.

Rosenberg, N.A., 2007. Statistical tests for taxonomic distinctiveness from observations of monophyly. Evolution 61, 317–323.

Sanderson, M.J., Shaffer, H.B., 2002. Troubleshooting molecular phylogenetic analysis. Ann. Rev. Ecol. Syst. 33, 49–72.

Schunger, I., Beutel, R.G., Britz, R., 2003. Morphology of immature stages of *Prostomis mandibularis* (Prostomidae, Tenebrionoidea, Coleoptera). Eur. J. Entomol. 100, 357–370.

Sheffield, N.C., Song, H., Cameron, S.L., Whiting, M.F., 2009. Nonstationary evolution and compositional heterogeneity in beetle mitochondrial phylogenomics. Syst. Biol. 58, 381–394.

Slipinski, S.A., Leschen, R.A.B., Lawrence, J.F., 2011. Order Coleoptera Linnaeus, 1758. In: Zhang, Z.-Q. (Ed.), Animal Biodiversity: An Outline of Higher-level Classification and Survey of Taxonomic Richness. Zootaxa, vol. 3148, pp. 203–208.

Song, H., Sheffield, N.C., Cameron, S.L., Miller, K.B., Whiting, M.F., 2010. What happens when the phylogenetic assumptions are violated? The effect of base compositional heterogeneity and among-site rate heterogeneity in beetle mitochondrial phylogenomics. Syst. Entomol. 35, 429–448.

Timmermans, M.J.T.N., Dodsworth, S., Culverwell, C.L., Bocak, L., Ahrens, D., Littlewood, D.T., Pons, J., Vogler, A.P., 2010. Why barcode? High-throughput multiplex sequencing of mitochondrial genomes for molecular systematics. Nucleic Acids Res. 38, e197.

Wägele, J.W., Mayer, C., 2007. Visualizing differences in phylogenetic information content of alignments and distinction of three classes of long-branch effects. BMC Evol. Biol. 7, 147.

Wild, A.L., Maddison, D.R., 2008. Evaluating nuclear protein-coding genes for phylogenetic utility in beetles. Mol. Phylogenet. Evol. 48, 877–891.

Wilkinson, M., 1996. Majority-rule reduced consensus trees and their use in bootstrapping. Mol. Biol. Evol. 13, 437–444.

Wheeler, Q.D., 1986. Revision of the genera of Lymexylidae (Coleoptera: Cucujiformia). Bull. Am. Mus. Nat. Hist. 183, 113–210.

Xia, X., Xie, Z., 2001. DAMBE: data analysis in molecular biology and evolution. J. Hered. 92, 371–373.

Zwickl, D.J., Hillis, D.M., 2002. Increased taxon sampling greatly reduces phylogenetic error. Syst. Biol. 51, 588–598.