

# A molecular phylogenetic study of Cucujidae s.l. (Coleoptera: Cucujoidea)

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**Abstract.** Of all the superfamilies within the megadiverse order Coleoptera (Insecta), Cucujoidea (Cucujiformia) is arguably the most problematic taxonomically. The families comprising Cucujidae s.l. (Silvanidae, Laemophloeidae, Passandridae and Cucujidae s.s. represent a large portion of cucujoid diversity. Herein we present the results of a rigorous molecular phylogenetic analysis of Cucujidae s.l. using maximum-likelihood and Bayesian analyses of seven genes. Representatives of over half of the families of Cucujoidea (excluding the cerylonid series), as well as a broad sampling of Silvanidae and Laemophloeidae, were analysed. The monophyly of Cucujidae s.l. is rejected but a subgrouping of taxa that may form the core of a natural cucujoid lineage is recovered. This clade consists of two large monophyletic groups including several families each. Relationships among these smaller cucujoid groups are discussed, including several novel phylogenetic hypotheses, whereas morphological characters considered significant for classification in Cucujidae s.l. are evaluated in light of these phylogenetic hypotheses. Silvaninae, Telephanini, Brontini and Brontinae are recovered as monophyletic in the Bayesian analysis, but the former two are recovered as paraphyletic in the maximum-likelihood analysis. Our results support the placement of *Psammoecus* Latreille within Telephanini and also recover a paraphyletic *Telephanus* Erichson. Silvaninae is divided into three lineages, each representing a potential tribal lineage. Laemophloeidae is rendered paraphyletic in all analyses by Propalticidae and the latter is herein formally transferred to Laemophloeidae **stat.n.** Several suprageneric laemophloeid clades are recovered and discussed as potential higher-level groups. *Laemophloeus* Dejean is not recovered as monophyletic.

## Introduction

Coleoptera (Insecta) represents one of the largest radiations of diversity on Earth. Four suborders, 24 superfamilies, 211 families and over 350 000 described beetle species (Bouchard *et al.*, 2011) reflect an astounding breadth of phenotypic diversity, and the order presents numerous taxonomic and classification challenges. One superfamily that retains a disproportionate amount

of these problems is Cucujoidea, which currently includes 36 families (Lord *et al.*, 2010; Leschen & Ślipiński, 2010). Crowson (1955) originally defined this superfamily as ‘Clavicornia’ using the following features: antennae usually clubbed, front coxae not projecting, tarsi not 5-5-4 in both sexes, ventrites not connate, aedeagus of the ring-type, larvae with distinct prostheca and not obviously belonging to any other well-defined groups of beetles. Today, Cucujoidea is regarded as a taxonomic ‘dumping ground’ of mostly little brown beetles and has repeatedly been shown to be an artificial group in phylogenetic studies (Robertson *et al.*, 2004, 2008; Hunt *et al.*, 2007; Lawrence

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et al., 2011; Bocak et al., 2014). In light of these findings, efforts have been made to recognize natural subgroupings within Cucujoidea that could form the basis for a new, more natural classification for this clade. Several recent phylogenetic studies have recovered one large, well-supported clade within Cucujoidea, the cerylonid series (Crowson, 1955), which includes nine recognized families (Robertson et al., 2008; Lord et al., 2010). Thus far, the cerylonid series is the only well-supported, large subgrouping of Cucujoidea to emerge; no other large clades have been well supported in phylogenetic studies.

The informal names 'lower Cucujoidea' (*sensu* Leschen, 1996) and 'basal Cucujoidea' (Leschen et al., 2005), were coined for cucujoid families that did not belong to the cerylonid series (Appendix S1). This grouping, although not formal, has been widely used among the systematics community but there is no consensus as to its membership or monophyly. Several smaller groups within the remaining cucujoids have been proposed, with varying degrees of support, including among others, the nitidulid (Audisio, 1993; Leschen, 1996; Cline et al., 2014), erotylid, silvanid and cucujid-series (Hunt et al., 2007; Bocak et al., 2014), but there is a need for further evaluation of nearly every proposed group. Numerous taxa remain uncertainly allied with well-supported groups and even families generally thought to be closely related, have received conflicting support depending on the data used.

One such grouping of taxa is 'Cucujidae s.l.' (Fig. 1), comprising members of what was once the single family (Appendix S1). This historically broader family concept contained an heterogeneous assortment of beetles united by a subcortical lifestyle and a flattened body shape, although there has been debate over its membership (Appendix S1). However, recent efforts have resulted in the elevation of several subgroups of this larger concept into separate families, including Silvanidae (Crowson, 1955), Laemophloeidae (Thomas, 1993) and Passandridae (Crowson, 1955). Cucujidae s.s. (Crowson & Sen Gupta, 1969) retains comparatively few genera. In addition, it is increasingly evident that other families are allied with this group of cucujoids, such as Phalacridae (Thomas, 1984a, 1993; Gimmel, 2013) and Propalticidae (Thomas, 1984a). The taxonomic history of these families is complicated so a brief summary is provided for the current families in Appendix S1.

Silvanidae (silvanid flat bark beetles) (Fig. 1A–C) are thought to form a monophyletic assemblage that includes 58 genera and nearly 500 species worldwide (Thomas & Leschen, 2010c). The family is currently split into two subfamilies: Brontinae Lawrence & Newton with 20 genera and Silvaninae Grouvelle with 38 genera. Brontinae is further split into two tribes, Telephanini LeConte and Brontini Erichson, each with ten genera. This tribal split is based mainly on characters of the aedeagus and procoxal cavities (Thomas, 2003; Thomas & Nearn, 2008). Telephanini are generally associated with plants and plant debris, whereas Brontini are most often encountered subcortically. Both tribes are thought to be fungivorous. Silvaninae has not been split into tribes, yet a wide variety of lifestyles is represented in the subfamily, including subcortical fungus feeding, leaf litter dwelling, ant inquilinity, facultative predation and seed feeding (Thomas & Leschen, 2010c). The latter ecological

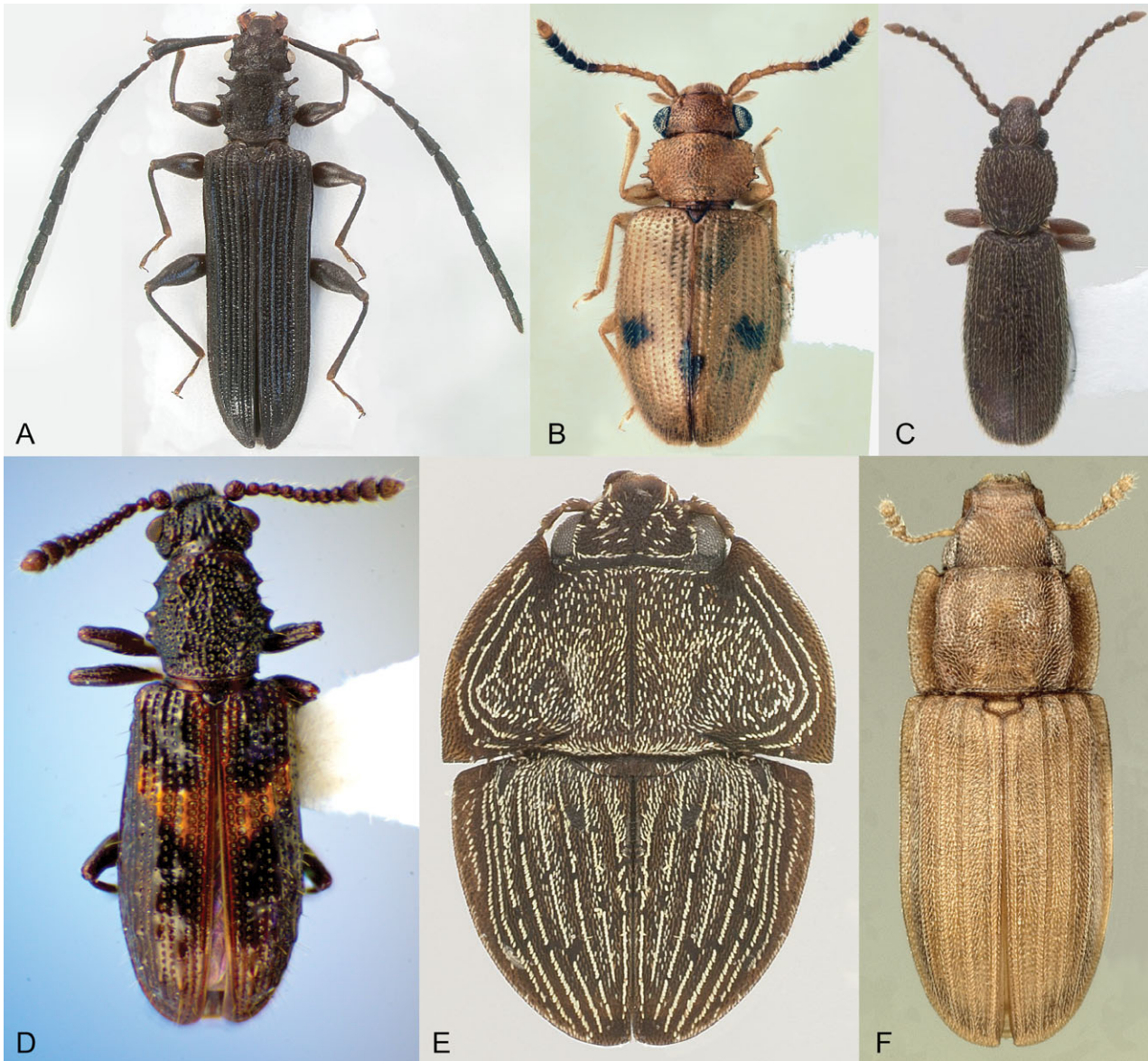
group (e.g. *Oryzaephilus* Ganglbauer, *Nausibius* Redtenbacher, *Cathartus* Reiche and *Ahasverus* Gozis) has received the most taxonomic attention due to their economic importance as pests of stored grains (Thomas & Leschen, 2010c). The sister group of Silvanidae remains uncertain. Phylogenetic analyses have suggested Cucujidae s.s. (Leschen et al., 2005; Bocak et al., 2014), Passandridae (Robertson et al., 2008), *Hymaea* Pascoe (Hymaeninae: Phloeostichidae) (Lawrence et al., 2011) and even the entire superfamily Curculionoidea (Hunt et al., 2007).

Laemophloeidae (lined flat bark beetles) (Fig. 1F) are a family of approximately 430 species arranged in 37 genera (Thomas & Leschen, 2010b). The family is widespread in forested regions of the world. Most members are subcortical and are thought to feed on fungi although, some genera are likely predaceous on bark beetles (Curculionidae: Scolytinae) and others on scale insects (Coccoidea) (Thomas & Leschen, 2010b). Some members of *Cryptolestes* Ganglbauer are important pests of stored grain. No suprageneric classification has been proposed because a phylogenetic analysis of the family is lacking (Thomas & Leschen, 2010b), but a few informal genus-groups have been suggested (Thomas, 1984a, d; 1988). A close affinity among Laemophloeidae, Phalacridae, Propalticidae and Passandridae was suggested by Thomas (1984a) and subsequent studies have generally supported this hypothesis (e.g. Leschen et al., 2005; Robertson et al., 2008; Lawrence et al., 2011; Bocak et al., 2014), although poor taxon sampling or insufficient data have produced inconsistencies (Leschen et al., 2005; Robertson et al., 2008; Lawrence et al., 2011; Bocak et al., 2014).

Passandridae (parasitic flat bark beetles) form a small family of 109 species in nine genera (Burckhardt & Ślipiński, 2010). The few known larvae are ectoparasitic on wood-boring beetles or parasitic wasps. Most species are restricted in distribution, although a few are widespread (some through human activities) (Burckhardt & Ślipiński, 2010). Due to their unique biology and resulting morphology, the family is widely regarded as monophyletic, an assumption that is supported by a cladistic analysis of morphological data by Burckhardt & Ślipiński (2003).

Cucujidae s.s. (flat bark beetles) retain only 4 genera and 48 species (Thomas & Leschen, 2010a). *Pediacus* Shuckard and *Cucujus* Fabricius are primarily Holarctic. The remaining two genera, *Palaestes* Perty and *Platysus* Erichson, are restricted to the Neotropics and Australian areas, respectively. Cucujid beetles are usually encountered subcortically. A few species have been reported to be predaceous, but the biology remains unknown for most species (Thomas & Leschen, 2010a).

Phalacridae (shining mould beetles) comprise 34 genera and 635 species (Gimmel, 2013) and have a generally convex body shape that is superficially dissimilar to most other cucujid-type taxa. Many species feed on fungi associated with rotting plants (e.g. smuts, stem rusts, ergots) (Lawrence et al., 2010). Several genera contain palynophagous (pollen feeding) species (Gimmel, 2013). Also fungivorous, Cyclaxyridae form a small group (two species) of sooty mould feeders endemic to New Zealand that only recently received familial status (Gimmel et al., 2009). Its affinities are currently unclear. Although historically they were placed within Phalacridae (Gimmel et al., 2009), they have



**Fig. 1.** Examples showing the diversity of the Cucujidae s.l., all dorsal habitus images created by focus stacking. Images not to scale. (A) *Macrohyliota spinicollis* (Brontinae: Silvanidae) from Malaysia (body length: ~11.0 mm). (B) *Psammoecus trimaculatus* (Brontinae: Silvanidae) from Brazil (body length ~3.5 mm). (C) *Airaphilus* sp. (Silvaninae: Silvanidae) from the USA (body length: ~3.1 mm). (D) *Hymaea magna* (Hymaeinae: Phloeostichidae) from Australia (body length: ~4.0 mm). (E) *Slipinskogenia* sp. (Propalticidae) from Central African Republic (body length: ~2.5 mm). (F) *Carinophloeus raffrayi* (Laemphloeidae) from Malawi (body length: ~2.2 mm). Figure 1D by Thomas McElrath, all others by Michael Thomas.

only been recovered as sister to Phalacridae in a recent morphological phylogeny using a limited number of outgroups and this relationship received only weak support (Gimmel, 2013). Rather equivocally, *Cyclaxyra* Broun have been placed as the sister taxon to *Lamingtonium* Sen Gupta & Crowson (Lawrence *et al.*, 2011), *Tasmosalpingus* Lea (Leschen *et al.*, 2005) and *Pediacus* (Bocak *et al.*, 2014).

Agapythidae, Priasilphidae, Phloeostichidae (Fig. 1D), Myraboliiidae and Tasmosalpingidae are infrequently collected, primarily austral taxa that were until recently united in a single

family Phloeostichidae s.l. Recent analyses, however, suggest that several of these lineages are of independent origin (Leschen *et al.*, 2005; Lawrence *et al.*, 2011). The sister taxa for most of these families are uncertain and few have been included in molecular phylogenetic studies (Bocak *et al.*, 2014). Some species have been shown to be mould- or fungus-feeders, although information about host and feeding preferences is scarce (Leschen *et al.*, 2005).

The family Propalticidae (Fig. 1E), comprising only two Old World genera (*Propalticus* Sharp and *Slipinskogenia* Gimmel)

and 43 species (Gimmel, 2011), has a convoluted taxonomic history, although recent analyses have consistently placed it as either the sister taxon to (Leschen *et al.*, 2005; Hunt *et al.*, 2007; Lawrence *et al.*, 2011), or within, (Bocak *et al.*, 2014) Laemophloeidae. The former relationship is consistent with some historical concepts (Thomas, 1984a; Lawrence & Newton, 1995). Propalticids are thought to feed on lichens or fungi on the surface of living trees, where adults are commonly found (Gimmel, 2011).

Cryptophagidae (silken fungus beetles) include about 600 species in 60 genera (Leschen, 2010) which historically have been allied with Languriini (Erotylidae) and several taxa have moved back and forth between the two families due to superficial resemblances (Leschen, 1996). Thus, historical affinities of Cryptophagidae were perhaps erroneously thought to be with Erotylidae, but recent phylogenetic analyses have recovered the family as a close relative of several Cucujidae s.l. taxa (Hunt *et al.*, 2007; Lawrence *et al.*, 2011; Bocak *et al.*, 2014). Leschen (1996) suggested a close relationship between Cryptophagidae and either Hobartiidae or Cavognathidae, with more distant affinities to Cucujidae s.l. Although generally fungivorous, the biology of this family is quite diverse, with numerous inquilinous species and plant associates (Leschen, 1996).

Silvanidae, Laemophloeidae, Passandridae and Cucujidae s.s. form the core of Cucujidae s.l., but the other families mentioned above may be closely related. Several of the sister-group relationships proposed have been based on morphological characters (Crowson & Sen Gupta, 1969; Thomas, 1984a, 1993; Leschen *et al.*, 2005; Lawrence *et al.*, 2011; Gimmel, 2013). The most widely used include the state of the procoxal and meso-coxal cavities, the inversion of the aedeagus and the relative lengths of the protibial spurs. Each has been used for phylogenetic inference from the interfamilial to the generic levels within these families, but the polarity of the adjacent character states has never been independently tested.

Given the conflicting phylogenetic estimates frequently based on insufficient gene sampling (Hunt *et al.*, 2007; Robertson *et al.*, 2008; Bocak *et al.*, 2014) and historical taxonomic confusion surrounding the Cucujidae s.l., a rigorous molecular phylogenetic analysis of Cucujidae s.l. was conducted to test the familial and intrafamilial relationships of this heretofore poorly-sampled group. In addition, the above four anatomical characters proposed as important to the classification of these groups were independently evaluated in light of the phylogenetic hypothesis generated from the molecular data.

## Materials and methods

### *Taxon sampling and DNA sequencing*

Taxon sampling focused primarily on Silvanidae (22 species) and Laemophloeidae (25 species). This sampling broadly covers both silvanid subfamilies (Brontinae & Silvaninae) as well as both tribes within Brontinae (Telephanini & Brontini). No other families of Cucujidae s.l. have a formally proposed suprageneric classification. Within Laemophloeidae, exemplars

from numerous hypothesized genus-groups (Thomas 1984a, d; 1988) were selected to represent a broad range of geographical and behavioural diversity. For numerous species-poor groups, only a single exemplar was used to test interfamilial relationships. This was the case for Cucujidae, Myraboliidae, Propalticidae, Phloeostichidae, Agapythidae, Priasilphidae, Monotomidae, Cyclaxyridae and other cucujoid outgroups. For more species-rich or supposed sister-lineages (e.g. Cryptophagidae, Phalacridae, Passandridae and Nitidulidae) multiple exemplars were included. Finally, for more distant outgroups, the following exemplars were included: four cerylonid series taxa (three Coccinellidae, one Latridiidae), four Tenebrionoidea (two Tenebrionidae, two Salpingidae) and one Cleroidea (Cleridae) as the most distant outgroup. In total, 81 taxa were included in the analysis (Appendix S2). Specimens used in this study were collected into 100% EtOH and stored at  $-80^{\circ}\text{C}$ . Techniques and protocols associated with specimen dissection, clearing and vouchering follow Robertson *et al.* (2004, 2008, 2013). Genomic DNA was extracted using the Qiagen DNeasy tissue kit (Qiagen, Valencia, CA, U.S.A.). Voucher specimens are deposited in the University of Georgia Collection of Arthropods (UGCA) Coleoptera Tissue Collection, Athens, GA (most) or in the Brigham Young University Insect Genomics Collection (BYU IGC). We sampled seven genes in this study: nuclear 18S rRNA, 28S rRNA, *histone subunit 3 (H3)* and mitochondrial 12S rRNA, 16S rRNA, *cytochrome-c oxidase subunit I (COI)* and *subunit II (COII)*. Primers and conditions used follow those detailed in Robertson *et al.* (2013). Product yield, specificity and potential contamination were monitored using agarose gel electrophoresis and UV-light visual verification. PCR products were transferred to GeneMate vacuum plates and subjected to  $-25$  psi vacuum for 10 min to remove PCR residues. Reaction products were cleaned using Sephadex matrix micro beads and sequenced using BigDye Terminator v3.1 (Applied Biosystems, Foster City, CA, U.S.A.) on an ABI 3730 DNA Analyser (ABI, Foster City, CA, U.S.A.). Assembly and editing of resulting contig sequences was performed in Sequencher 4.2.2 (Genecodes, 1999). All resulting nucleotide sequences were cross-referenced using a BLAST search of the GenBank nucleotide database prior to use in this study to check for contamination. Some sequences were downloaded from GenBank or provided by collaborators (Appendix S2).

### *Sequence alignment and partitioning*

The *COI*, *COII* and *H3* gene regions required no further adjustment or alignment (since they were length invariant and contained no indels) and were not subjected to the following alignment protocols. Ribosomal markers (12S, 16S, 18S, 28S) were aligned in MAFFT (Katoh & Standley, 2013) and refined in Muscle (Edgar, 2004). Alignments were visually inspected in Geneious 6.1.4 and corrected for obvious alignment errors. Even after rigorous alignment, these genes included regions of extreme length variation, thus Gblocks 0.91b was used to remove ambiguously aligned regions under the least stringent conditions (Castresana, 2000; Talavera & Castresana,

2007). Alignments of individual markers were concatenated in Sequence Matrix (Vaidya *et al.*, 2011). Partition Finder 1.0.1 was used to select among 13 different partitions (by gene & by codon position for protein-coding genes) using the AICc selection criterion, unlinked branch lengths and a greedy search algorithm (Lanfear *et al.*, 2012). The partitioned dataset was subjected to heuristic maximum likelihood analysis using the program RAxML (Stamatakis, 2006) hosted on the Cipres Science Gateway (Miller *et al.*, 2010) ([www.phylo.org](http://www.phylo.org)) with rapid bootstrap replicates (terminated by the RAxML algorithm) using a GTR+G model. Two independent RAxML analyses were performed to ensure convergence of runs. The topology with the best likelihood score is discussed below when it differed from the Bayesian analysis (Figure S1). The same dataset was subjected to a mixed model Bayesian analysis in MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) hosted on the CIPRES Science Gateway. The partitioned Bayesian analysis consisted of four independent runs of 30 million generations, flat priors, unlinked partitions, four chains (one cold, three hot) and trees sampled every 1000 generations. Tracer 1.5 (Rambaut & Drummond, 2009) was used to graphically determine stationarity, burn-in and convergence of runs. Trees sampled after the burn-in were used to create a 50% majority-rule consensus tree (Fig. 2). Each gene was also subjected to an individual RAxML analysis using similar conditions and visually inspected to assess general contributions of each gene to the dataset. Branch lengths proportionally adjusted relative to the gene with the longest branch lengths (*H3*) and compared (Figure S2). Individual gene trees are available as supporting information (Figures S3–S9).

### Morphological characters

Four morphological characters (Appendix S3) widely used for classification of cucujid group taxa (Thomas, 1984a, c, d, 1988, 1993, 2009; Leschen, 1996; Leschen & Ślipiński, 2010) were mapped onto a cladogram of the 50% majority-rule Bayesian tree (Figs 3, 4) using maximum-likelihood probabilistic ancestral character state reconstruction models in the StotchChar package (Maddison & Maddison, 2006) of Mesquite 2.75 (Maddison & Maddison, 2011) using the default one-parameter Markov k-state model. Pie charts were used to show the estimated probability of the likelihood of each state at each node. Ancestral State reconstructions were as follows:

*Aedeagus uninverted vs. aedeagus inverted*: This character state was scored entirely from the literature, which is described in different ways depending on the source, but the concept used herein is a combination of Lawrence *et al.* (2011) and Thomas (2003). In general, it refers to the orientation of the tegmen relative to the proctiger (segment IX of Lawrence *et al.*, 2011) of the male genitalia, which in the inverted condition is rotated 180°, such that the parameres are located on the ventral aspect of the median lobe (Thomas, 2003).

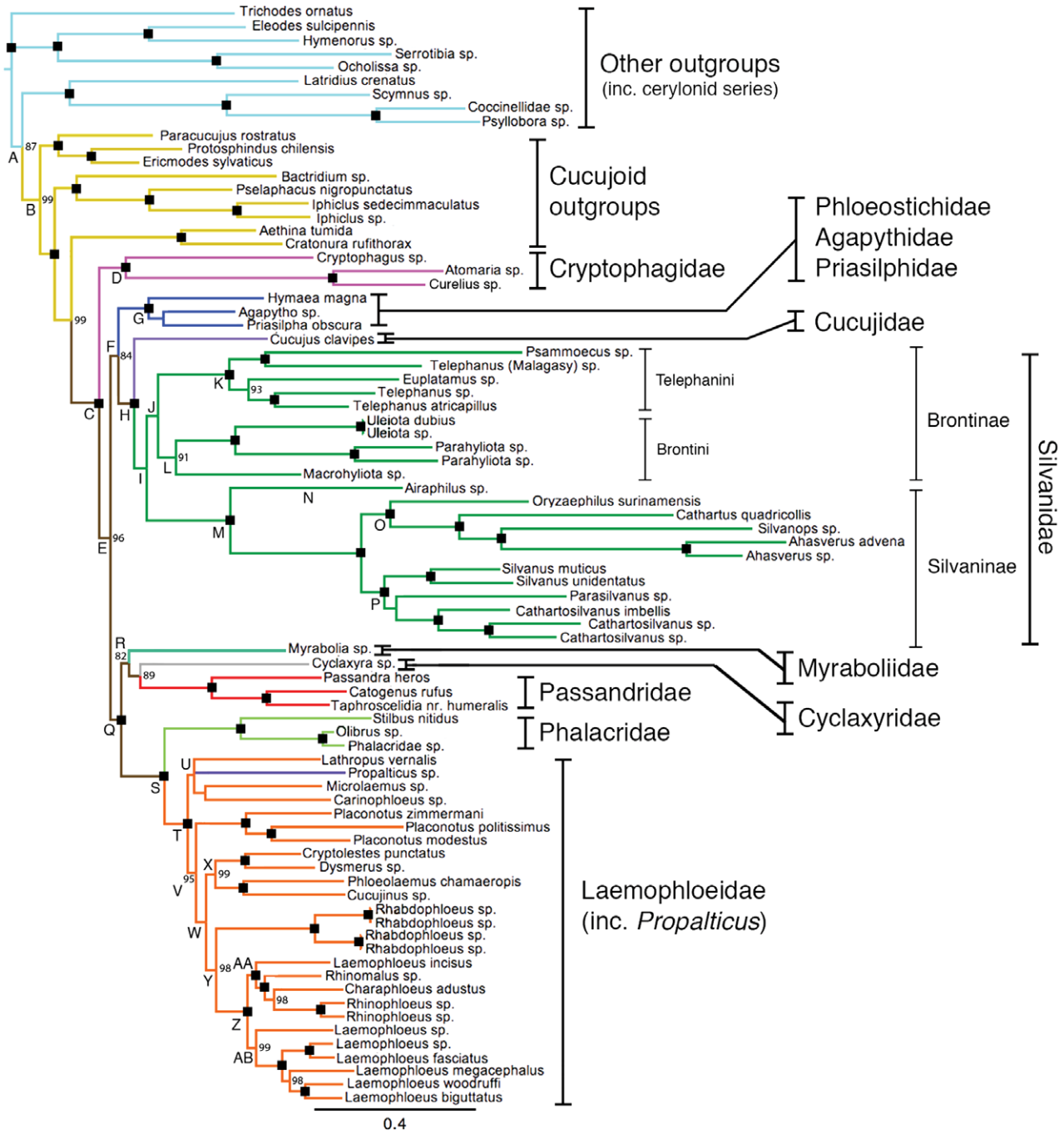
*Protibial spurs equal vs. protibial spurs unequal*: This character state was scored entirely from the literature (see above) and simply refers to the relative lengths of the two spurs at the end of the protibia. If one is distinctly longer (and sometimes wider) than the other then the character was coded as unequal. Thus the unequal character state may refer to cases in which one of the spurs is not present or highly reduced, or it may refer to cases where it is merely of shorter length.

*Procoxal cavities closed vs. procoxal cavities open*: This character state was scored entirely from the literature and in this case does not distinguish between narrowly or broadly closed or open. In addition, this feature was only scored for the external condition of the cavity, not the internal condition, which is often scored as a completely separate character. For the purposes of this study, when any extension of the prosternal and/or postcoxal process completely separated the procoxal cavity from the mesoventrites, the character was scored as closed. If this division was not complete, then the character was scored as open.

*Mesocoxal cavities closed vs. mesocoxal cavities open*: This character state refers to the lateral closure of the mesocoxal cavity (Lawrence *et al.*, 2011). If only the mesoventrite and metaventrite were involved in such a lateral closure then the character was scored as mesocoxal cavities closed. If any other sclerites were involved (e.g. mesepimeron, metepimeron), then the character was scored as mesocoxal cavities open. For this character, the condition reported in the literature (Lefkovitch, 1962) was confirmed by the authors for *Microlaemus* Lefkovitch, *Propalticus*, *Carinophloeus* Lefkovitch and *Lathropus* Erichson. All other taxa were scored entirely from literature.

### Results

Complete gene coverage was not obtained for all 81 terminals in this study, but was as follows: *18S*: 81/81; *28S*: 80/81, *H3*: 54/81, *12S*: 77/81; *16S*: 75/81; *COI*: 77/81; *COII*: 72/81. Sequences generated from this study are deposited on GenBank under the accession numbers KP133861–KP134301 (Appendix S2). The final combined nucleotide dataset comprised 6984 characters, only 543 of which were invariant. Graphical analysis of the trace plots from the Bayesian analyses determined that in two of the four runs convergence and stationarity was reached after 3 million generations, which were discarded as burn-in and the remainder of the sampled trees from the posterior distribution were used to construct the 50% majority rule consensus tree. The two runs that did not converge were discarded. Examination of individual gene trees (Figure S2) showed that *H3* had the longest branch lengths (most nucleotide substitutions/site) of all genes sampled in this study and *18S* had the least. No individual gene tree recovered all the relationships found in the combined analysis, an outcome that is not surprising given that individual genes (especially from different regions) have different histories and evolve at different rates (Liu *et al.*, 2009). The results of the

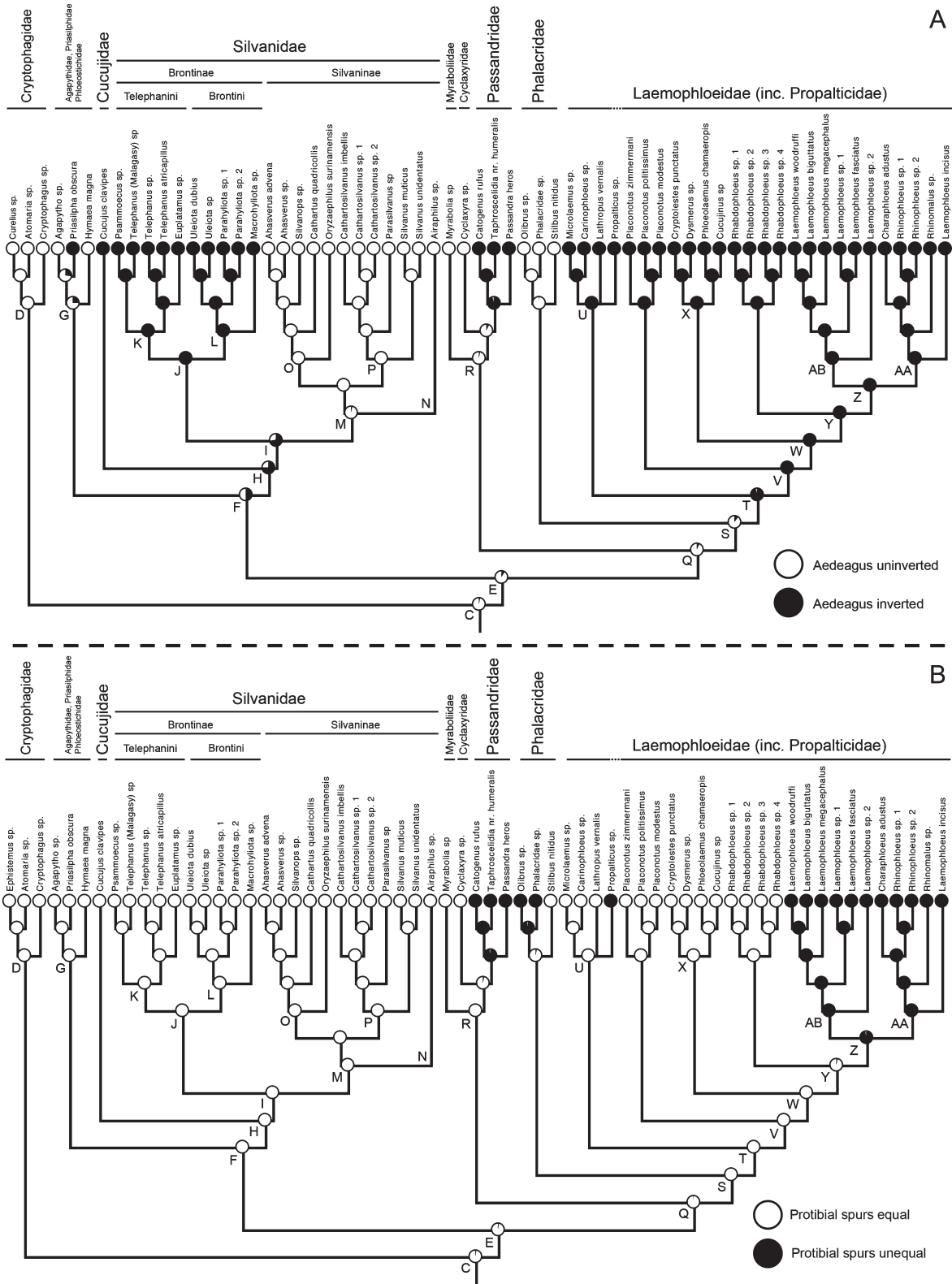


**Fig. 2.** Bayesian 50% majority rule consensus tree. Nodes marked with a black square indicate a 100% posterior probability. Support values for nodes of less than 75% posterior probability are not indicated. Branch colours mark family-level clades, unless otherwise indicated. Brown indicates internal branches of uncertain taxonomic affinity.

Bayesian (Fig. 2) and RaxML (Figure S1) analyses were largely concordant with few exceptions, which are noted when present. Otherwise all relationships discussed were recovered in both analyses. Important nodes discussed in the text are referenced with a letter corresponding to the nodes in Fig. 2. Posterior probabilities and bootstrap support values are provided in this

format: (Node letter in Fig. 2: Posterior probability, Bootstrap support), for example, (C: 100, 100).

The cerylonid series (100, 90) was recovered as monophyletic with strong support and was weakly supported as sister to the remaining Cucujoidea (A: 87, 56). Several small monophyletic groups were recovered that sequentially form sister



**Fig. 3.** Ancestral maximum-likelihood character state reconstruction and optimization of characters coded from literature into a matrix and optimized as proportional likelihoods using Mesquite under the Mk1 model, onto the Bayesian 50% majority rule consensus tree. (A) ‘Aedeagus uninverted (white)’ or ‘aedeagus inverted (black)’. (B) ‘Protibial spurs equal (white)’ or ‘protibial spurs unequal (black)’.

groups to the remaining cucujoid families, including the following: Boganiidae + (*Ericmodes* Reitter + *Protosphindus* Sen Gupta & Crowson); Monotomidae + monophyletic Erotylidae; monophyletic Nitidulidae; and monophyletic Cryptophagidae. Cryptophagidae (D: 100, 90) were recovered as sister (C: 100, 100) to a clade (E: 96, <75) comprising two large subclades, one containing Silvanidae and a few small families (referred to below as the silvanid clade) and the other containing Laemophloeidae, Phalacridae and a few small families (referred to below as the laemophloeid clade). The silvanid clade (F: 84, <75) consisted of a moderately supported clade (G: 100, 92) of (Phloeostichidae: *Hymaea* + (Agapythidae + Priasilphidae) which was then sister to a well-supported grouping of Cucujidae + monophyletic Silvanidae (H: 100, 93). The laemophloeid clade (Q: 100, 78) consisted of Myrabolidae + (Cyclaxyridae + monophyletic Passandridae) (R: 82, 58) and formed the sister group to a strongly supported clade (S: 100, 100) comprising Phalacridae + paraphyletic Laemophloeidae. Phalacridae (U: 100, 100) were recovered as the sister group to Laemophloeidae. *Propalticus* was recovered within Laemophloeidae (see below). Silvanidae were monophyletic with strong support in both analyses (I: 100, 91). The same was true for Silvaninae (M: 100, 100) and Telephanini (K: 100, 100). In the Bayesian analysis, the subfamily Brontinae (J: PP = 55) and the tribe Brontini (L: PP = 91) were each recovered as monophyletic, but in the ML analysis (Figure S1), the tribe Brontini was rendered paraphyletic because *Macrohyliota* Thomas was recovered with very weak support (BS < 50) as sister to Telephanini in a clade that is sister to the remaining Brontini + Silvaninae (BS = 91). In the ML analysis (Figure S1), a monophyletic *Placonotus* Macleay was recovered as sister to the remaining Laemophloeidae (inc. *Propalticus*), with strong support (BS = 99). The next diverging laemophloeid lineage was a poorly supported branch (BS = 33) comprising two clades: (*Lathropus* + *Microlaemus*) and (*Propalticus* + *Carinophloeus*). These relationships are reversed in the Bayesian analysis, which recovered a polytomy comprising the latter clade (U: PP = 56) that was the sister (T: PP = 100) to a monophyletic *Placonotus* + all remaining laemophloeids (V: PP = 95). *Dysmerus* Casey, *Cryptolestes*, *Phloeolaemus* Casey and *Cucujinus* Arrow were recovered as a strongly supported (X: 99, 85) genus-group clade. *Rhabdophloeus* Sharp was recovered as monophyletic (100, 100) and sister (Y: 98, 60) to a clade (Z: 100, 100) containing various species of *Laemophloeus* Dejean, which was rendered paraphyletic with respect to a clade containing *Rhinomalus* Gemminger, *Charaphloeus* Casey and *Rhinophloeus* Sharp (100, 75).

## Discussion

### Early diverging cucujoid lineages

The apparent monophyly of Cucujoidea (A: 87, 56) (Fig. 2) and the non-cerylonid series cucujoids (B: 99, 99) (Fig. 2) recovered in the present study may seem surprising given the findings of recent studies (e.g. Robertson *et al.*, 2004, 2008;

Hunt *et al.*, 2007; Lawrence *et al.*, 2011; Bocak *et al.*, 2014; Kergoat *et al.*, 2014). Our findings should be regarded with caution, however, because the taxon sampling for the current study was not designed to investigate those higher-level relationships. This study unequivocally rejects the monophyly of Cucujidae s.l. (Silvanidae + Passandridae + Laemophloeidae + Cucujidae s.s.), which was not recovered as a monophyletic group in any analysis, supporting the current recognition of each as a separate family and corroborates numerous morphological analyses and treatments that suggested they should be classified separately (Crowson, 1955; Thomas, 1984a; Leschen *et al.*, 2005; Lawrence *et al.*, 2011). Multiple other families were intermixed among the core of Cucujidae s.l. Support for this expanded, multi-family clade was mixed (node E: 96, <75) (Fig. 2) and may include other cucujoid families for which DNA sequences are presently unavailable, such as Cavognathidae, Tasmosalpingidae, Lamingtoniidae and Hobartiidae.

### Cryptophagidae

The family Cryptophagidae, represented in the present study by *Cryptophagus* Herbst, *Atomaria* Stephens and *Curelius* Casey, was strongly supported as monophyletic in both analyses (D: 100, 90) and was recovered as the sister group to the remaining Cucujoidea s.l. (C: 100, 100). It has been placed close to silvanids and cucujids in morphological analyses (Leschen *et al.*, 2005; Lawrence *et al.*, 2011), whereas previous molecular analyses have recovered it near Laemophloeidae + Propalticidae (Hunt *et al.*, 2007; Bocak *et al.*, 2014). Leschen (1996) considered both Hobartiidae and Cavognathidae to be closely related to Cryptophagidae, yet neither was recovered near Cryptophagidae in the analysis of Leschen *et al.* (2005); these families should be included in future molecular analyses of the Cucujoidea, as they could clarify the placement of Cryptophagidae within Cucujoidea.

### Silvanid clade

The silvanid clade (F: 84, 52) contained at least the families Silvanidae, Cucujidae, Phloeostichidae (*Hymaea*) (Fig. 1D), Agapythidae (*Agapytho* Broun) and Priasilphidae (*Priasilpha* Broun). The placement of the latter three families close to Cucujidae + Silvanidae is consistent with the morphological analysis of Lawrence *et al.* (2011), but this sister grouping was not strongly supported in the present study (F: 84, 52). The clade comprising Phloeostichidae, Agapythidae and Priasilphidae was well supported (G: 100, 92). The taxon sampling within this clade was sparse and exemplars representing other presumed closely related taxa (e.g. *Priastichus* Crowson, *Phloeostichus* Redtenbacher and *Tasmosalpingus*) are needed. The grouping of Cucujidae and Silvanidae as sister taxa was well supported (H: 100, 93) in this analysis as well as several morphological analyses (e.g. Leschen *et al.*, 2005; Lawrence *et al.*, 2011). Characters supporting this sister grouping include antennal insertions being concealed by a frontal ridge, procoxae with



a long, concealed lateral extension, meso-metaventral junction simple, bases of frontal arms contiguous in larva and larval spiracles annular (Leschen *et al.*, 2005).

### Silvanidae

Silvanidae formed a well-supported monophyletic group in these analyses (I: 100, 91) and are also supported by the combination of several well-defined adult and larval morphological characters (Leschen *et al.*, 2005), including procoxal cavities internally closed, galea at least 2.5× as wide as lacinia, scutellary striole absent, parameres fused to phallobase, larval pretarsus unisetose and larval abdominal tergum simple (Leschen *et al.*, 2005; Thomas & Leschen, 2010c). However, the currently defined suprageneric classification of Silvanidae with two subfamilies, Brontinae (Brontini + Telephanini) and Silvaninae (Thomas, 2003; Thomas & Nearn, 2008), was only supported by the Bayesian analysis. In the RaxML analysis, *Macrohyliota* (Fig. 1A), a member of the tribe Brontini, was recovered as the sister group to a monophyletic Telephanini, albeit with poor support (BS = <50). In addition, Brontini in part (*Uleiota* Latreille + *Parahyliota* Thomas) were recovered as sister to the subfamily Silvaninae (BS = <50). These relationships rendered both Brontinae and Brontini paraphyletic with respect to Silvaninae. In contrast, the Bayesian analysis was largely concordant with the current internal classification of Silvanidae, although it should be noted that the monophyly of Brontinae was poorly supported (PP = 55). Brontinae, unlike Silvaninae, have an inverted aedeagus (a possibly plesiomorphy shared with Cucujidae) (Fig. 3A). Brontini also have open procoxal cavities, unlike Telephanini and Silvaninae, which have the closed condition (Fig. 4A), a condition that seems remarkably stable within the family, especially when compared to the number of transitions within Laemophloeidae (see discussion below). Given the weak support for the monophyly of Brontinae and the questionable monophyly of the tribe Brontini recovered here and in the morphological analysis of Thomas & Nearn (2008), it is clear that more attention is needed to clarify the relationships within subfamily. The potential paraphyly of Brontini as observed is caused by the unstable position of *Macrohyliota*. Interestingly, *Macrohyliota* is the only representative of this tribe in the current analyses that has *Dendrophagus*-type tarsi (Thomas, 2003).

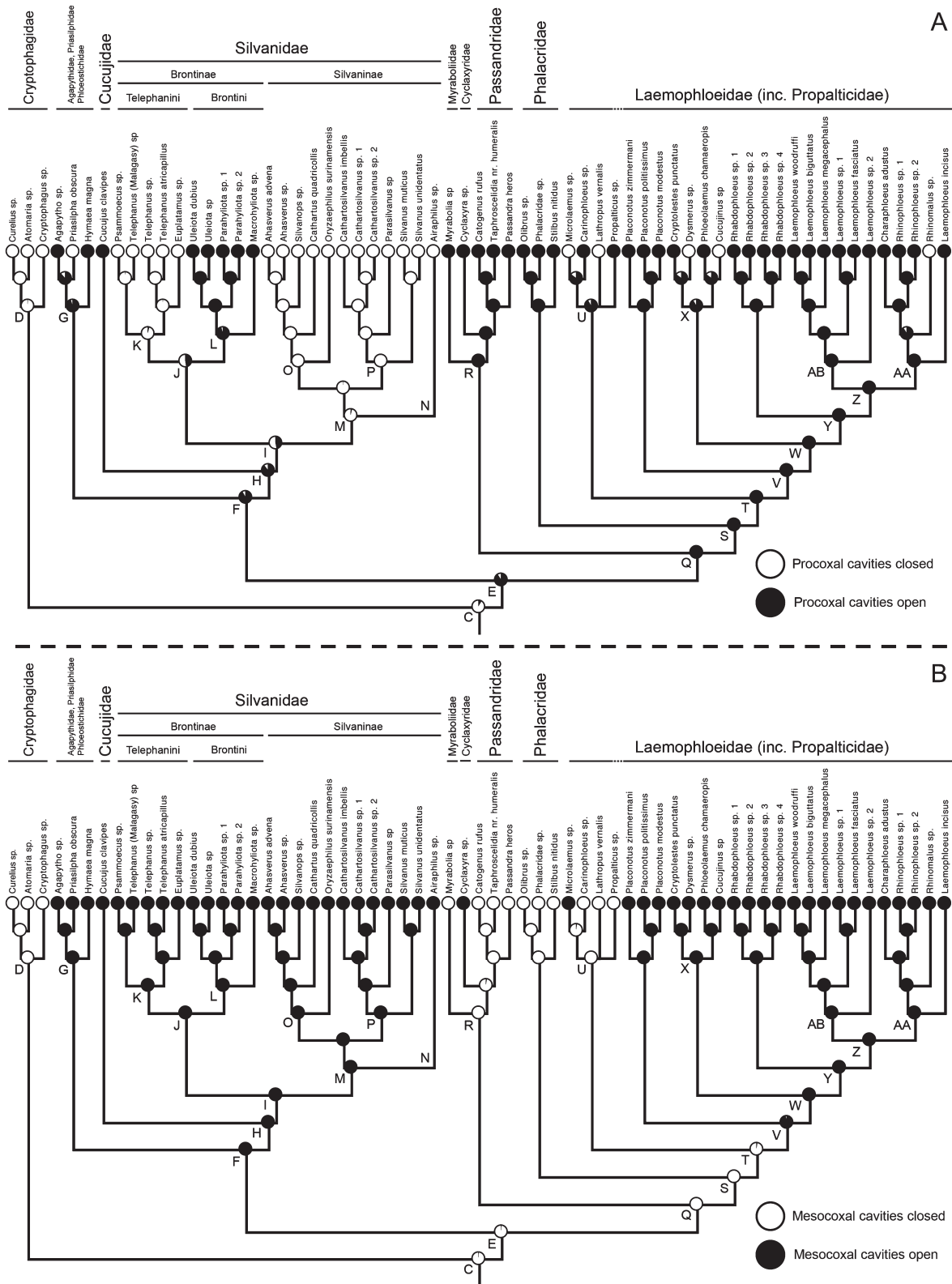
Our results corroborate the monophyly of Telephanini (K: 100, 100), but the generic limits within the tribe may be artificial. *Psammoecus* Latreille (Fig. 1B), historically restricted to the Old World, was considered by Pal *et al.* (1984) to be unique enough to warrant it being treated as a monotypic subfamily Psammoeicinae Pal. Thomas (1984b) disagreed and this analysis supports the latter hypothesis, as it is consistently recovered embedded within the clade with other telephanines. *Telephanus* Erichson, a mostly Neotropical group with some Old World representatives, was recovered in two clades, thus rendered paraphyletic with respect to *Psammoecus* and *Euplatamus* Sharp. The first was an Old World clade containing the aforementioned *Psammoecus* and a Malagasy species of *Telephanus* that differs from other congeners in having a scutellary striole. The second clade

included two typical *Telephanus* (not possessing a scutellary striole) from the New World that are sister to *Euplatamus*. The Malagasy *Telephanus* likely represents a distinct group of telephanines that could possibly be recognized separately pending a thorough revision of the genus.

Silvaninae were strongly supported as monophyletic (M: 100, 100) and comprised three main lineages (Fig. 2: N, O, P). *Airaphilus* Redtenbacher (N) (Fig. 1C) formed the sister group to the remaining Silvaninae, which are subtended by a notably longer branch (Figure S2). Indeed, the long branch separating *Airaphilus* from the remaining silvanines indicates the significant molecular distance underlying this bifurcation. This genus is unique from other Silvaninae in possessing a well-developed dorsal mandibular mycangium. The remaining Silvaninae were split into two well-supported clades, one comprising *Oryzaephilus*, *Cathartus*, *Silvanops* Grouvelle and *Ahasverus* (O: 100, 94) and the other comprising *Silvanus* Latreille, *Parasilvanus* Grouvelle and *Cathartosilvanus* Grouvelle (P: 100, 85). The mandibles in both groups have only a weakly incised line and no dorsal mycangium is present. All genera within this subfamily with multiple exemplars (*Cathartosilvanus*, *Silvanus* and *Ahasverus*) were recovered as monophyletic with strong support (100, 100). Except for *Oryzaephilus*, the three genera in the former clade (O) possess lobed or incrassate tarsal segments. The three genera in the latter clade (P) (as well as several others not sampled in this study) were regarded by Halstead (1973) as being closely related, citing the form of the tarsi, finely denticulate prothoracic sides and a three-segmented club of a particular form. Further investigation of the potential synapomorphies uniting these genera seems warranted, as each clade represents a potential tribe within Silvaninae.

### Laemophloeid clade

The laemophloeid clade formed the sister group to the silvanid clade and comprised Myraboliidae, Cyclaxyridae, Passandridae, Phalacridae, Laemophloeidae and Propalticidae (Q: 100, 78). There remains uncertainty regarding the exact relationships of these families due to the moderate to poor support recovered for several branches. The laemophloeid clade had a basal split that divides the group into two main lineages. The first clade was only weakly to moderately supported (R: 82, <75) and included Myraboliidae (*Myrabolia* Reitter) as sister to Cyclaxyridae (*Cyclaxyra*) + Passandridae (89, 66). Passandridae were recovered as monophyletic with strong support (100, 100). In preliminary analyses (not including *Cyclaxyra*), Passandridae often formed the sister group to the clade comprising Phalacridae, Laemophloeidae and Propalticidae. Support for this alternative resolution was likely adding to the instability at the base of the laemophloeid clade. Interestingly, Hunt *et al.* (2007) recovered Passandridae as sister to Cucujidae, whereas in Bocak *et al.* (2014), Passandridae were nested within Nitidulidae. In the morphological analysis of Leschen *et al.* (2005), Myraboliidae, Passandridae, Phalacridae and Laemophloeidae + Propalticidae were interspersed among several cucujoid taxa either not included in the present study



**Fig. 4.** Ancestral maximum-likelihood character state reconstruction and optimization of characters coded from literature into a matrix and optimized as proportional likelihoods using Mesquite under the Mk1 model, onto the Bayesian 50% majority rule consensus tree. (A) ‘Procoxal cavities closed (white)’ or ‘procoxal cavities open (black)’. (B) ‘Mesocoxal cavities closed (white)’ or ‘mesocoxal cavities open (black)’.

(e.g. Lamingtoniidae, Cavognathidae, Tasmosalpingidae and Smicripidae) or far-removed in the tree (e.g. Nitidulidae). No previous analyses have recovered Cyclaxyridae as the sister group to Passandridae and thus our findings provide putative support for a transition from mycophagy to parasitism in the evolutionary history of Passandridae.

The placement of Myrabioliidae as the sister group to *Cyclaxyra* + Passandridae was a novel finding. Previously, Leschen *et al.* (2005) recovered Myrabioliidae as the sister to a much larger clade of ten cucujoid families including Passandridae and *Cyclaxyra*, but also including more distantly related cucujoid families such as Nitidulidae and Smicripidae. Leschen *et al.* (2005) included *Cavognatha* Crowson and *Lamingtonium* in their study, which were recovered closer to Passandridae than *Myrabilia*. Lawrence *et al.* (2011) found *Myrabilia* to be sister to *Cavognatha*. Further analyses should include these families, whose placement within the larger context of Cucujoidea remains elusive and whose addition may resolve some of the weak support for the deeper nodes in this area of the tree. In addition, further inquiry into the feeding habits of *Myrabilia* (which are currently unclear) will shed light on the evolution of parasitic habits within this clade. If *Myrabilia* is shown to be mycophagous, this would support a hypothetical mycophagous passandrid ancestor.

The second major clade in the laemophloeid series comprised Phalacridae + Laemophloeidae (inc. Propalticidae) with high support (S: 100, 100), corroborating previous studies suggesting that these families are closely related (Thomas, 1984a; Leschen *et al.*, 2005; Hunt *et al.*, 2007; Robertson *et al.*, 2008; Bocak *et al.*, 2014). The monophyly of Phalacridae was also supported (100, 100) but broader taxon sampling is needed within this diverse family to address the current suprageneric classification. Thomas (1984a, 1993) suggested that Phalacridae, Laemophloeidae, Propalticidae (Fig. 1E) and Passandridae form a natural lineage based on a number of morphological features including unequal protibial spurs (Fig. 3B), structural affinities of the male genitalia (Fig. 3A) and the presence of pronotal lines and elytral cells. The present study confirmed the close affiliation of Phalacridae, Laemophloeidae and Propalticidae. However, when the tibial spur (Fig. 3B) and male genitalic characters (Fig. 3A) were viewed in light of the phylogenetic findings, the topology suggested that these features may represent convergences, especially the unequal protibial spurs (Fig. 3B). In *Propalticus*, this character may be involved with its unique ability to jump using its forelegs. The inverted aedeagus also seems to be a convergent feature (Fig. 3A), having evolved twice within the laemophloeid series (Passandridae & Laemophloeidae). A similar pattern seems to have occurred within the silvanid series, with at least two independent evolutions of the inverted aedeagus (Cucujidae + Silvanidae & Priasilphidae), although the Silvaninae may have secondarily lost the inversion. This character may be involved in having an end-to-end mating position or a subcortical habitat (Thomas, 1984a).

The monophyly of Laemophloeidae was not supported by either analysis due to the nested placement of *Propalticus* within the family. These results were consistent with the molecular

analysis of Bocak *et al.* (2014). The clade of Laemophloeidae (including *Propalticus*) received nearly maximum support (T: 100, 99) in both analyses, strongly suggesting that the family Propalticidae (including *Propalticus* and *Slipinskogenia*) (Fig. 1E) should be subsumed within Laemophloeidae. Although *Slipinskogenia* was not included in this study, it shares unequivocal synapomorphies with *Propalticus* including a strengthened medial prothoracic endocarina and forelegs modified for saltation (Gimmel, 2011). Based on the findings of the present study, *Propalticus* Sharp and *Slipinskogenia* Gimmel are formally transferred to the family Laemophloeidae **stat.n.** Numerous analyses have previously suggested a sister grouping of Propalticidae and Laemophloeidae (Leschen *et al.*, 2005; Hunt *et al.*, 2007; Lawrence *et al.*, 2011), but because of the limited sampling strategy, the nested position of Propalticidae within Laemophloeidae remained undetected, or poorly supported (Bocak *et al.*, 2014). *Lathropus*, *Microlaemus* and *Carinophloeus* (Fig. 1F) are each anatomically odd among laemophloeids (Crowson & Sen Gupta, 1969; Thomas, 2010) and thus their phylogenetic position has been historically elusive (Thomas, 1984a,d; Thomas & Leschen, 2010b). When compared to most laemophloeid genera all three are atypical in having a more obvious antennal club and denser setation on the body. In addition, some lack the obvious constriction behind the head and the closure of the procoxal and mesocoxal cavities varies (Fig. 4). Within Laemophloeidae, *Propalticus* grouped consistently with these three genera, albeit with poor support (<75, <75), but it shares many of the unusual characters exhibited by them. Similarly, *Propalticus* and *Slipinskogenia* possess ample setation and a more pronounced (wider) antennal club. *Lathropus*, *Propalticus* and *Carinophloeus* also possess closed mesocoxal cavities (Fig. 4B), a character shared with Phalacridae, albeit equivocally, indicating its plesiomorphic nature, since the rest of Laemophloeidae have open mesocoxal cavities (Fig. 4B). It is also noteworthy that the mesocoxal cavities in *Microlaemus* are only narrowly open, whereas they are widely open in the remaining laemophloeids (not including *Lathropus* & *Carinophloeus*) (Fig. 4B). *Carinophloeus* and *Propalticus* also retain open procoxal cavities (Fig. 4A) whereas *Microlaemus* and *Lathropus* have closed procoxal cavities. These morphological features, especially the conditions of the mesocoxal cavities, suggest that this group could represent the earliest diverging lineage of the extant laemophloeids (Thomas, 1984a,d) because its likely sister group, Phalacridae, shares these character states (Thomas & Leschen, 2010b). Now that *Propalticus* and *Slipinskogenia* are grouped within Laemophloeidae, a search for derived morphological characters, not just plesiomorphies or secondary losses, should be undertaken. However, it is certainly worth noting that although this hypothesis was supported in the Bayesian analysis, with *Propalticus*, *Lathropus*, *Microlaemus* and *Carinophloeus* forming the sister group to the remaining Laemophloeidae, it was not supported as such in the RaxML analysis, where the clade (BS = <75) was recovered one node higher on the laemophloeid tree, with a monophyletic *Placonotus* (100, 100) forming the sister group to the remaining Laemophloeidae. Nonetheless, *Placonotus* retains none of the characters discussed above and has been

hypothesized previously to be more closely related to genera such as *Gannes* Lefkovich, *Parandrita* LeConte & Horn or *Laemophloeus* (Thomas 1984c).

A clade comprising *Dysmerus* + *Cryptolestes* (100, 100) as sister to the clade *Phloeolaemus* + *Cucujinus* (100, 99) was supported by the current analysis. This larger clade, which was moderately well supported (X: 99, 85) here, is also supported morphologically based on the structure of male genitalia, body shape and modifications to the male antennal scape, which is expanded in both *Dysmerus* and *Cryptolestes* (Thomas, 1988, 2009). Thomas (1988b) also included *Leptophloeus* Casey in this informal group. *Dysmerus* and *Cryptolestes* are two of the few laemophloeid genera with predaceous members [feeding on scolytines and scale insects, respectively (Thomas & Leschen, 2010b)]. *Phloeolaemus* is also commonly collected in areas where scolytine beetles may be found, although no definitive associations have been made.

Our analyses recovered a clade of monophyletic *Rhabdophloeus* Sharp (100, 100) as sister (Y: 98, <75) to a well-supported clade (Z: 100, 100) comprising the *Laemophloeus* group of genera (see below), which is contrary to previous hypotheses that suggest a close relationship of *Rhabdophloeus* with the *Carinophloeus*-group of genera (Thomas, 1984d). However, a close relationship with *Odontophloeus* Thomas has also been suggested (Thomas, 1984d) and upon further sampling of that family, as well as other Neotropical genera, it is possible that this clade could be expanded to include these. All share a lengthened terminal antennomere and undulating pronotal margins with several teeth. *Odontophloeus dives* (Sharp) is strikingly similar to members of *Rhabdophloeus* (Thomas, 1984b, 1993). Little is known of their biology to suggest that they might share similar habitats or associations.

*Laemophloeus* was not recovered as monophyletic in our analyses, as it was rendered paraphyletic by the *Rhinomalus* genus-group; instead, a North American clade (100, 100) of five *Laemophloeus* species was recovered as the sister to a South American representative of the genus (AB: 99, <75). The Nearctic members of this genus are currently being revised (M.C. Thomas, in preparation) and morphological characters may be discovered that support the unique nature of Nearctic *Laemophloeus* as recovered in this analysis. *Laemophloeus incisus*, *Charaphloeus*, *Rhinomalus* and *Rhinophloeus* were recovered in a well-supported clade (AB: 100, 100). The latter three taxa share a suite of derived characters, including the loss of one or more (of the three) elytral cells, absence of lateral elytral carina and tarsomere one being longer or subequal to the penultimate tarsomere (Thomas, 1984a). All of these genera, including *Laemophloeus*, possess an acuminate abdominal intercoxal process (Thomas, 2013). *Laemophloeus incisus* and its hypothesized sister species, *L. mathani* Grouvelle, are unique among congeners in lacking a pronotal antebasal denticle and in possessing atypical genitalia (Thomas, 2014). Further examination of these two species seems warranted based on these results.

Further investigations into food preferences and life histories within cucujoids should reveal insights about the apparently numerous historical transitions between parasitism, predation,

phytophagy, palynophagy and mycophagy within them. Unfortunately, such information is usually poorly documented or unconfirmed for many Cucujoidea. For most genera of Laemophloeidae, for example, food preferences are completely unknown or represent assumptions that are simply based on associations with a particular habitat. The remarkable diversity of lifestyles shown by the laemophloeid and silvanid series, as well as the remaining Cucujoidea, despite low numbers of species relative to other Coleoptera superfamilies, is an interesting evolutionary phenomenon that merits further attention. This group of cucujoids is also well suited for addressing biogeographic patterns, because there are numerous groups with relictual Gondwanan distributions (e.g. Phloeostichidae, Myraboliidae). Expanded taxon sampling to include more members of such families, as well as additional genera of Laemophloeidae and Silvanidae, and focusing on poorly sampled biogeographic regions with unique and endemic faunas would allow more accurate inferences about such questions. For example, sampling of the remaining genera of Cucujidae and Phloeostichidae (of mostly Austral, Oriental and South American distribution) would allow for the development of a more robust hypothesis regarding the origins of the silvanid clade. Strikingly, the placement of the only Malagasy specimen included in this analysis suggests that a genus needs revision; undoubtedly further sampling from this region would reveal numerous important insights. Poor sampling of taxa from these areas remains a problem in modern molecular phylogenetic analyses (Bocak *et al.*, 2014).

## Supporting Information

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

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**Figure S1.** RaxML most likely tree. Nodes marked with a black square indicate bootstrap support of 100%. Support values for nodes of less than 75% bootstrap are not indicated. Branch colours mark family-level clades, unless otherwise indicated. Brown indicates internal branches of uncertain taxonomic affinity.

**Figure S2.** Scaled RaxML most likely gene trees for each gene. Scale bar = 0.4 nucleotide substitutions/site. Coloured branches correspond to the coloured groups in Fig. 2 and Figure S1.

**Figure S3.** RaxML most likely gene tree for the *12S* gene. Scale bar = nucleotide substitutions/site. Coloured branches correspond to the coloured groups in Fig. 2 and Figure S1.

**Figure S4.** RaxML most likely gene tree for the *16S* gene. Scale bar = nucleotide substitutions/site. Coloured branches correspond to the coloured groups in Fig. 2 and Figure S1.

**Figure S5.** RaxML most likely gene tree for the *18S* gene. Scale bar = nucleotide substitutions/site. Coloured branches correspond to the coloured groups in Fig. 2 and Figure S1.

**Figure S6.** RaxML most likely gene tree for the 28S gene. Scale bar = nucleotide substitutions/site. Coloured branches correspond to the coloured groups in Fig. 2 and Figure S1.

**Figure S7.** RaxML most likely gene tree for the COI gene. Scale bar = nucleotide substitutions/site. Coloured branches correspond to the coloured groups in Fig. 2 and Figure S1.

**Figure S8.** RaxML most likely gene tree for the COII gene. Scale bar = nucleotide substitutions/site. Coloured branches correspond to the coloured groups in Fig. 2 and Figure S1.

**Figure S9.** RaxML most likely gene tree for the H3 gene. Scale bar = nucleotide substitutions/site. Coloured branches correspond to the coloured groups in Fig. 2 and Figure S1.

**Appendix S1.** Abbreviated history of relevant Cucujidae s.l. classification schemes.

**Appendix S2.** Taxa, associated genes amplified, and accession numbers in this study. Cells left blank were not successfully amplified.

**Appendix S3.** Nexus file of four morphological characters scored in text. Only taxa depicted in Figs 3, 4 were scored for these characters.

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