Molecular phylogeny of the wood-boring beetle family Buprestidae and insights into the evolution of host use

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Abstract

The family Buprestidae, also known as jewel beetles or metallic wood-boring beetles, contains nearly 15,000 species in 522 genera. Together with the small family Schizopodidae (7 species, 3 genera), they form the superfamily Buprestoidea. Adult Buprestoidea feed on flowers or foliage, whereas larvae are mostly internal feeders, boring in roots or stems, or mining the leaves of woody or herbaceous plants. The subfamilial and tribal classification of Buprestoidea remains unsettled, with substantially different schemes proposed by different workers based on morphology. Here we report the first large-scale molecular phylogenetic study of the superfamily Buprestoidea based on data from 4 genes for 141 ingroup species. We used these data to reconstruct higher-level relationships, and to assess the current classification and the origins of the larval leaf-mining habit within Buprestidae. In our analyses, the monophyly of Buprestoidea was strongly supported, as was the monophyly of Schizopodidae and its placement sister to Buprestidae. Our results are largely consistent with the generally accepted major lineages of buprestoids, including clearly-defined agrilines, buprestines-chrysochroines, and early-branching julodines-polycestines. In addition to the Schizopodidae, three of the six subfamilies were monophyletic in our study: Agrilinae, Julodinae, and the monogeneric Galbellinae (*Galbella*). Polycestinae was monophyletic with the exception of the enigmatic Haplostethini. Chrysochroinae and Buprestinae were recovered together in a large mixed clade along with

Galbella. The interrelationships of Chrysochroinae and Buprestinae were not well resolved; however they were clearly polyphyletic, with chrysochroine genera falling into several different well-supported clades otherwise comprised of buprestine genera. All of Agrilinae was contained in a single strongly supported clade. Our results confirm multiple origins of larval leaf mining within Buprestidae. The leaf mining genus *Paratrachys* (Paratracheini) was recovered within the Acmaeoderioid clade, confirming the independent origins of leaf mining within Polycestinae and Agrilinae. Additionally, our results strongly suggest that the leaf mining agriline tribe Tracheini is polyphyletic, as are several of its constituent subtribes. External root feeding was likely the ancestral habit. The evolutionary transitions to internal feeding allowed access to a variety of additional plant tissues, including leaves, potentially spurring adaptive radiations of the diverse and highly specialized leaf mining buprestids.

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1. Introduction

Buprestidae, commonly known as jewel or metallic wood-boring beetles, is a large family of phytophagous elateriform beetles containing nearly 15,000 extant species in 522 genera (Bellamy, 2008). Together, Buprestidae and the small family Schizopodidae (7 species in 3 genera) comprise the supra-familial group Buprestoidea. Adult Buprestidae visit flowers or foliage, whereas larvae are mostly internal feeders, boring in roots and stems of various woody or herbaceous plants. Many wood-boring larval Buprestidae favor dead or dying branches on otherwise healthy trees, but some species are known to attack healthy living tissues – certain of these are serious pests. The larvae of some Buprestidae are leafminers, but this habit is confined to a few genera in the agriline tribes Tracheini and Aphanisticini (*sensu* Bellamy, 2003) and the enigmatic genus *Paratrachys* (Polycestinae).

The subfamilial and tribal classification of Buprestoidea remains unsettled, with substantially different schemes proposed by different workers over the past several decades (Cobos, 1980, 1986; Toyama, 1987; Holyński, 1988, 1993; Bellamy, 2003). These classifications differ in the numbers and composition of families, subfamilies and tribes. The most recent higher-level classification of Buprestidae (Bellamy, 2003) divides the family into six subfamilies (Julodinae, Polycestinae, Galbellinae, Chrysochroinae, Buprestinae, and Agrilinae) and maintains the family-level status of Schizopodidae, as originally proposed by LeConte (1859) and advocated by Nelson and Bellamy (1991). Bellamy (2003) also incorporates most of the groupings outlined by Volkovitsh (2001) based on his detailed study of buprestid antennal morphology. Volkovitsh's (2001) "complexes" form the basis for the subfamilial structure in Bellamy (2003), condensing some former subfamilies into the six in the current classification. The Bellamy (2003) higher classification, including the Volkovitsh "lineages" and other groupings, are used throughout this paper, except where indicated otherwise. We also follow subsequent nomenclatural changes, as reflected in Bellamy's (2008) world catalogue of Buprestidae.

Previously, Cobos (1980) recognized thirteen buprestid subfamilies: Julodinae, Schizopodinae, Thrincopiginae, Mastogeninae, Acmaeoderinae, Polycestinae, Chalcophorinae, Chrysobothrinae, Buprestinae, Sphenopterinae, Agrilinae, Trachyinae, and Cylindromorphinae. He later added a fourteenth (Galbellinae; Cobos 1986). Holyński (1993) proposed a radical restructuring of the classification, dividing the family into just four subfamilies (Schizopodinae, Julodinae, Buprestinae, and Agrilinae) with some tribes equivalent to Cobos' (1980) subfamilies, and grouping the polycestine, chalcophorine and buprestine lineages into a single subfamily. He also significantly reorganized the classification of Polycestinae (*sensu* Bellamy, 2003), concluding that the group is paraphyletic, and dividing it into several new tribes, even placing *Polycestina* within Buprestini. Holyński's (1993) classification scheme was met with much skepticism due to his omission of important morphological characters, including wing venation and larval characters, as well as a lack of testable hypotheses (Kolibac, 2000; Volkovitsh, 2001; Bellamy & Nelson, 2002); nevertheless several of his suggested relationships were incorporated into Bellamy's (2003) higher classification.

Although there have been a few phylogenetic studies of Buprestidae based on morphological characters, there have been no broadly taxon-sampled molecular phylogenetic studies of the family to date. The existing morphological cladistic analyses have reached conflicting conclusions and have not resolved the relationships among the major buprestid lineages. Nelson and Bellamy (1991) included a morphological cladistic analysis of several buprestoid genera in their revision of Schizopodidae, which re-established the family-level status of that group. In their analysis, Julodinae were sister to the remaining Buprestidae, with the relationships among the other subfamilies unresolved, partly due to limited taxon sampling. A more recent phylogeny based on morphological characters (Kolibac, 2000) placed Polycestinae sister to the remaining Buprestidae, with Julodinae branching off next. Kolibac's (2000) analysis also found support for an additional subfamily, Sphenopterinae, sister to Galbellinae and Agrilinae; he grouped these three subfamilies into an "Agriline lineage" and proposed a "Buprestine lineage" comprised of Buprestinae and Julodinae. Many of the conclusions in Kolibac (2000) directly contradicted Holyński's (1993) proposed classification. His phylogeny is also largely incompatible with the views of Volkovitsh (2001) and Bellamy (2003), particularly with respect to the relative placement of Polycestinae and Julodinae (see also the discussion of Polycestinae and *Galbella* below).

The only molecular phylogenetic study of Buprestidae to date (Bernhard *et al.*, 2005) focused on a small sample of Agrilinae (the *Agrilus viridis* species complex and a few genera of Holarctic leaf mining taxa) and did not address relationships among the subfamilies. Relationships among the major lineages of buprestids therefore remain controversial. Recent large-scale phylogenetic studies of beetles have also been inconclusive with regard to relationships within Buprestidae. Hunt *et al.* (2007) sampled representatives of five buprestid subfamilies, but the relationships recovered were not well supported and were inconsistent with existing hypotheses of buprestid relationships. Bocak *et al.* (2014) included 24 exemplar buprestids, but the interrelationships of the subfamilies were not discussed and do not appear well-resolved. In that study, Buprestidae was recovered within Byrrhoidea, sister to Heteroceridae, contradicting previous studies that placed Buprestidae sister to Byrrhoidea (e.g., Hunt *et al.*, 2007). However, the authors acknowledged that relatively limited taxon sampling of

Buprestidae to date (including the relatively little data available for Buprestidae in GenBank) has contributed to the difficulty of determining their placement (Bocak *et al.*, 2014).

The phylogenetic placement of several groups of Buprestidae remains particularly unstable. As noted above, there has been debate in the literature about whether Julodinae, or alternatively, Polycestinae, are sister to the remainder of Buprestidae. Julodinae have several characters often treated as "primitive," that distinguish them from the remaining buprestids, including antennal (Volkovitsh, 2001), larval (Volkovitsh?; Bílý, 1983?) and genital (Bílý, 1983?). For example, they lack a larval pronotal groove and have a short prosternum, both of which are shared with Schizopodidae. Most workers have considered Julodinae a lineage separate from the remaining buprestid lineages (Nelson and Bellamy, 1991; Holynski, 1993; Volkovitsh, 2001; Bellamy and Nelson, 2002); however the morphology-based phylogeny of Kolibac (2000) suggests they are a derived lineage within Buprestinae.

Polycestinae have similarly been described as "primitive" relative to other buprestids by some authors (Kolibac, 2000; Volkovitsh, 2001). As noted above, evidence for a monophyletic Polycestinae has been called into question by Holyński (1993), but studies of larval morphology (Volkovitsh and Hawkeswood, 1999), antennal morphology (Volkovitsh, 2001), and other adult morphological characters support their treatmen as an independent lineage.

The monophyly of Chrysochroinae and Buprestinae (*sensu* Bellamy, 2003) is also questionable, with some workers considering them a single subfamily due to a lack of consistent morphological characters distinguishing the two groups (Toyama, 1987; Holyński, 1993; Kolibac, 2000). However, Volkovitsh (2001) supported the maintenance of Chrysochroineae as a separate subfamily based on sufficiently distinct antennal characters.

Placement of the apomorphic genus *Galbella* has been especially unstable. It is currently placed in its own monogeneric subfamily (Galbellinae; Bellamy, 2003; Reitter, 1911; see also Cobos, 1986), but has frequently been grouped with Tracheini (Obenberger, 1937; Holyński, 1993). Kolibac (2000) proposed a subfamily Galbellinae comprised of several of the tracheines (*sensu* Bellamy, 2003) and the similarly enigmatic genus *Mastogenius* (currently placed in the subfamily Polycestinae); however that study relied on morphological and life history characters that may be shared due to similar constraints (e.g. small size) or adaptive pressures (e.g. feeding habit). Volkovitsh and Bílý (2001) argued that *Galbella* is more closely allied with *Mastogenius* and other Polycestines and that it should be placed within their "Buprestoid complex" rather than with "Agriloids."

Buprestoids are known for their often-brilliant metallic coloring and distinctive 'bullet' shape (Figure 1). Adults lay eggs in the soil or on plants, and the larvae of most species are endophagous, developing inside plant tissues. Most buprestids are woodborers, with larvae using dead or live wood of trees or shrubs, or mining just under the bark of trees or in twigs. However, buprestoids also exhibit a variety of other habits. Larval Julodinae and Schizopodidae are (to the extent known) external feeders that live in the soil and feed on roots (Holm and Bellamy, 1985; Nelson & Bellamy, 1991); recent work has confirmed a similar feeding habit in *Julodimorpha* (Buprestinae; Bílý *et al.*, 2013). Larval Polycestinae are borers mainly in dead wood, though some Acmaeoderini bore in the stems of herbaceous plants, and *Paratrachys* are leafminers. Chrysochroinae are wood borers, with the exception of some Sphenopterini that form galls in living plant tissues or bore in herbaceous plants. The larvae of Buprestinae are wood borers, generally attacking freshly killed or dead wood.

Figure 1. Superfamily Buprestoidea. (A.) fossil *Psiloptera weigelti,* (Buprestidae; photo: copyright Senckenberg, Messel Germany), middle Eocene of Messel, Germany, (B.) *Agrilus fuscipennis* (photo credit: T. C. MacRae, used by permission), C.) female *Schizopus laetus* (photo credit: C. Bellamy), (D.) *Acmaeodera macra* (photo credit: T. C. MacRae, used by permission), (E.) *Julodis onopordi* (photo credit: Siga; http://tinyurl.com/oyqcbxo), (F.) *Chrysobothris viridiceps* (photo credit: T. C. MacRae, used by permission), (G.) *Euchroma gigantea* (photo credit: S. Snäll, used by permission), (H.) *Pachyschelus* sp. (photo credit: S. Snäll, used by permission), and (I.) *Chrysochroa fulgidissima* (photo credit: Public domain).

Many Agrilinae are borers in dead wood, while others attack living tissue. Some of the latter are significant plant pests, including the invasive species *Agrilus planipennis* (emerald ash borer) and *Agrilus auroguttatus* (goldspotted oak borer) in the United States. Emerald ash borer has become one of the most destructive forest pests in the United States since its introduction in the 1990s, devastating ash forests and causing billions of dollars of damage (Herms and McCullough, 2014). Larvae of Cylindromorphina mine the stems of monocots. The larvae of several genera within Agrilinae are leafminers. Tracheini mine leaves on a variety of plants, including tree ferns (Tracheina), monocots (some Brachina), and dicots (Brachina, Leiopleurina, Pachyschelina). Aphanisticini include the leaf and stalk mining Aphanisticina, which mine the leaf blades and stalks of grasses, and Germaricina, which are thought to be leafminers in *Casuarina* (Casuarinaceae) (Bellamy, 1992).

The leaf mining genera of Agrilinae are unusually diverse, with over 2,000 described species, and account for a large proportion of the buprestid species in certain faunas. For example, Hespenheide (1991) reported that 73% of the buprestids collected at the Organization for Tropical Studies La Selva Biological Station in Costa Rica were leaf-mining species. The interrelationships among these leaf mining groups remain unclear. The genera have been variously grouped together in successive classifications, largely based on adult morphology, including body shape (generally sub-cylindrical in Aphanisticini versus broad and flattened in Tracheini) and other characters that may be associated with small size or feeding habit, such as prosternal grooves to receive the antennae or legs in adults.

The leaf mining genera (except for the polycestine *Paratrachys*) were previously placed together in a separate subfamily, Trachyinae *sensu* Cobos (1979). Cobos (1979) revised Trachyinae, including the current Tracheini, the leaf mining Aphanisticina and Germaricina, and

Cylindromorphoidina. *Galbella* was also included in Trachyinae, though Cobos discussed the possibility of separate subfamilial status for Galbellini, as previously established by Reitter (1911); Galbellinae was subsequently reinstated by Bellamy (1985). Cylindromorphinae, consisting only of the grass stalk mining Cylindromorphini, was accorded subfamilial rank by Cobos (1980) and maintained by Bellamy (1985). Bellamy (1992) reduced Cylindromorphinae to a tribe within Agrilinae and dismantled Trachyinae, placing each of the constituent tribes separately within Agrilinae.

Holyński (1993) significantly rearranged the classification of the leaf and stem mining genera. He established two tribes within Agrilinae: Aphanisticini, which included the former Cylindromorphini along with Germaricina, Aphanisticina, and Cylindromorphoidina (formerly Trachyinae). His Trachyini included the leaf mining Tracheina, Brachina, Leiopleurina, and Pachyschelina, and he transferred Galbellina back to the trachyines. The current classification (Bellamy 2003, 2008) maintains these groupings with the exception of *Galbella*, which has been returned to subfamilial status. Despite their traditional placement in one or two closely related tribes, some have questioned the close affiliation of the leaf-mining agrilines and suggested that some of their morphological similarities are due to convergent evolution (Bellamy & Williams, 1995; Kolibac, 2000; H. Hespenheide, pers. comm.). A better understanding of the interrelationships among the leaf mining genera and their proposed close relatives is critical for insight into the origins of the leaf mining habit in Buprestidae, and our taxon sample (specifically of Agrilinae) was assembled with this in mind.

Here we report the first large-scale molecular phylogenetic study of the superfamily Buprestoidea. Our study is based on DNA sequence data from 4 genes for 141 buprestoids and 5 outgroups. We use these data to address the aforementioned persistent questions about the composition and interrelationships of the major lineages of Buprestoidea. Specifically, we: (1) reconstructed the evolutionary relationships of the major groups of buprestoids and assessed the current classification, with particular attention to the family-level status of Schizopodidae, the relationships of Julodinae and Polycestinae to the rest of the buprestids, and the monophyly of Chrysochroinae and Buprestinae; and (2) investigated the location and number of origins of the larval leaf mining habit within Agrilinae.

2. Materials and Methods

Taxon sampling

We included DNA sequences from 146 exemplars (141 buprestoids and 5 outgroups) in this study. This included 137 individuals from 97 genera of Buprestidae, representing 42 of the 51 extant tribes (*sensu* Bellamy 2003, 2008). We also included four exemplars/species of Schizopodidae (of the 7 extant), representing all three extant genera. Leaf-mining genera in the agriline tribe Tracheini were more deeply sampled (more exemplars) than other groups because of our interest in the evolution of the leaf mining habit of larval Tracheini. Polyphagan beetles in five other closely related elateriform families (Byrridae, Callirhipidae, Dascillidae, Elateridae and Elmidae) were included as outgroups. Table 1 contains the taxonomic status, country of origin, and collection and Genbank accession numbers (KM364042-KM364525) for specimens included in this study. Voucher specimens are deposited at the Harvard University Museum of Comparative Zoology (MCZ) in Cambridge, Massachusetts, USA. All DNA sequences are reported here for the first time, except for the COI sequence of *Callirhipis* (Byrrhidae), which was obtained from GenBank (EF209549).

Table 1. Taxonomic status, DNA code(s), and DDBJ/EMBL/GenBank accession numbers for each specimen sequenced. The taxonomy used here for Buprestoidea follows Bellamy (2003), with subsequent changes following Bellamy (2008) and Bouchard *et al.* (2011), the latter of which is the source for outgroups. (L) indicates larval specimen(s) used.

Genus	Species	Family	Subfamily	Tribe	Subtribe	Country	Coll. no.	28S	18S	CAD	COI
Agrilus	*AO232*	Buprestidae	Agrilinae	Agrilini	Agrilina	Costa Rica	BUP0178	KM364165	KM364043	KM364424	no
Agrilus	*arbuti*	Buprestidae	Agrilinae	Agrilini	Agrilina	USA	BUP0041	KM364166	KM364044	KM364425	KM364308
Agrilus	*arizonus*	Buprestidae	Agrilinae	Agrilini	Agrilina	USA	BUP0067	KM364167	KM364045	no	KM364309
Agrilus	*decoloratus*	Buprestidae	Agrilinae	Agrilini	Agrilina		BUP0136	KM364168	KM364046	KM364426	KM364310
Agrilus	*latifrons*	Buprestidae	Agrilinae	Agrilini	Agrilina	Mexico	BUP0163	no	KM364157	KM364518	KM364420
Agrilus	*xanthonotus*	Buprestidae	Agrilinae	Agrilini	Agrilina	Costa Rica	BUP0177	KM364169	KM364047	no	no
Lepismadora	*algodones*	Buprestidae	Agrilinae	Agrilini	*incert. sed.*	USA	BUP0270	KM364188	KM364065	KM364441	no
Paragrilus	*aeraticollis*	Buprestidae	Agrilinae	Agrilini	Rhaeboscelidina	Costa Rica	BUP0179	KM364199	KM364075	KM364449	KM364333
Paragrilus	*sp.*	Buprestidae	Agrilinae	Agrilini	Rhaeboscelidina	Mexico	BUP0164	KM364200	KM364076	KM364450	KM364334
Aphanisticus	*sp.*	Buprestidae	Agrilinae	Aphanisticini	Aphanisticina		BUP0277	KM364171	KM364048	KM364427	KM364311
Cylindromorphus	*filum*	Buprestidae	Agrilinae	Aphanisticini	Cylindromorphina	Europe	BUP0286	KM364176	KM364053	KM364431	KM364316
Paracylindromorphus	*n. sp.*	Buprestidae	Agrilinae	Aphanisticini	Cylindromorphina		BUP03XX	KM364196	KM364073	KM364447	KM364331
Paracylindromorphus	*spinipennis carmenae*	Buprestidae	Agrilinae	Aphanisticini	Cylindromorphina	Spain	BUP0279	KM364197	no	no	no
Paracylindromorphus	*subuliformis*	Buprestidae	Agrilinae	Aphanisticini	Cylindromorphina	Europe	BUP0285	KM364198	KM364074	KM364448	KM364332
Germarica	*sp.*	Buprestidae	Agrilinae	Aphanisticini	Germaricina	Australia	BUP0003	KM364179	KM364056	KM364433	KM364317
Germarica	*sp.*	Buprestidae	Agrilinae	Aphanisticini	Germaricina	Australia	BUP0214	KM364180	KM364057	KM364434	KM364318
Diphucrania (=Cisseis)	*albosparsa*	Buprestidae	Agrilinae	Coraebini	Cisseina	Australia	BUP0016	KM364175	KM364052	KM364430	KM364315
Pachycisseis	*bicolor*	Buprestidae	Agrilinae	Coraebini	Cisseina	Australia	BUP0018	KM364193	KM364070	KM364445	no
Trypantius	*infrequens*	Buprestidae	Agrilinae	Coraebini	Dismorphina	Mexico	BUP0292	KM364207	no	no	KM364341
Geralius	*sp.*	Buprestidae	Agrilinae	Coraebini	Geraliina	Bolivia	BUP0297	KM364178	KM364055	no	no
Aaaba	*fossicollis*	Buprestidae	Agrilinae	Coraebini	*incert. sed.*	Australia	BUP0210	KM364164	KM364042	KM364423	KM364307
Meliboeithon	*intermedia*	Buprestidae	Agrilinae	Coraebini	*incert. sed.*	Australia	BUP0015	KM364190	KM364067	KM364442	KM364326
Meliboeus	*sp.*	Buprestidae	Agrilinae	Coraebini	Meliboeina	Armenia	BUP0175	KM364191	KM364068	KM364443	KM364327
Synechocera	*elongata*	Buprestidae	Agrilinae	Coraebini	Synechocerina	Australia	BUP0007	KM364202	KM364077	KM364452	KM364336
Anadora	*cupriventris*	Buprestidae	Agrilinae	Coraebini	Toxoscelina	S. Africa	BUP0247	KM364170	no	no	no

Table 1 (Continued).

Genus	Species	Family	Subfamily	Tribe	Subtribe	Country	Coll. no.	28S	18S	CAD	COI
Discoderoides	*immunitus*	Buprestidae	Agrilinae	Coraebini	Toxoscelina	S. Africa	BUP0242	KM364177	KM364054	KM364432	no
Pseudophlocteis	*vidua*	Buprestidae	Agrilinae	Coraebini	Toxoscelina	S. Africa	BUP0240	KM364201	no	KM364451	KM364335
Brachys	*kleinei*	Buprestidae	Agrilinae	Tracheini	Brachina	Costa Rica	BUP0201	KM364172	KM364049	no	KM364312
Lius	*mexicanus*	Buprestidae	Agrilinae	Tracheini	Brachina	Belize	BUP0323	KM364189	KM364066	no	KM364325
Taphrocerus	*depilis*	Buprestidae	Agrilinae	Tracheini	Brachina	Costa Rica	BUP0186	KM364203	KM364078	KM364453	KM364337
Taphrocerus	*fasciatus*	Buprestidae	Agrilinae	Tracheini	Brachina	Costa Rica	BUP0187	KM364204	KM364079	KM364454	KM364338
Taphrocerus	*fasciatus*	Buprestidae	Agrilinae	Tracheini	Brachina	Costa Rica	BUP0188	KM364205	KM364080	KM364455	KM364339
Taphrocerus	*shannoni*	Buprestidae	Agrilinae	Tracheini	Brachina	Costa Rica	BUP0190	KM364206	KM364081	KM364456	KM364340
Callimicra	*hoschecki*	Buprestidae	Agrilinae	Tracheini	Leiopleurina	Costa Rica	BUP0180	KM364173	KM364050	KM364428	KM364313
Callimicra	*pinguis*	Buprestidae	Agrilinae	Tracheini	Leiopleurina	Costa Rica	BUP0183	KM364174	KM364051	KM364429	KM364314
Leiopleura	*L040*	Buprestidae	Agrilinae	Tracheini	Leiopleurina	Costa Rica	BUP0193	KM364186	KM364063	KM364439	KM364323
Leiopleura	*sp.*	Buprestidae	Agrilinae	Tracheini	Leiopleurina	Costa Rica	BUP0197	KM364187	KM364064	KM364440	KM364324
Hylaeogena	*H037*	Buprestidae	Agrilinae	Tracheini	Pachyschelina	Costa Rica	BUP0200	KM364184	KM364061	KM364437	KM364321
Hylaeogena	*H039*	Buprestidae	Agrilinae	Tracheini	Pachyschelina	Costa Rica	BUP0199	KM364185	KM364062	KM364438	KM364322
Pachyschelus	*cupricauda*	Buprestidae	Agrilinae	Tracheini	Pachyschelina	Costa Rica	BUP0202	KM364194	KM364071	KM364446	KM364329
Pachyschelus	*undulatus*	Buprestidae	Agrilinae	Tracheini	Pachyschelina	Costa Rica	BUP0203	KM364195	KM364072	no	KM364330
Habroloma	*sp.*	Buprestidae	Agrilinae	Tracheini	Tracheina	Australia	BUP0013	KM364181	KM364058	KM364435	no
Habroloma	*sp.*	Buprestidae	Agrilinae	Tracheini	Tracheina	Indonesia	BUP0228	KM364182	KM364059	no	KM364319
Habroloma	*sp.*	Buprestidae	Agrilinae	Tracheini	Tracheina		BUP0282	KM364183	KM364060	KM364436	KM364320
Neotrachys	*estebana*	Buprestidae	Agrilinae	Tracheini	Tracheina	Costa Rica	BUP0184	KM364192	KM364069	KM364444	KM364328
Actenodes	*calcarata*	Buprestidae	Buprestinae	Actenodini		Mexico	BUP0161	KM364208	KM364082	no	KM364342
Agrilaxia	*flavimana*	Buprestidae	Buprestinae	Anthaxiini	Anthaxiina	USA	BUP0063	KM364211	KM364085	KM364458	KM364345
Romanophora	*verecunda*	Buprestidae	Buprestinae	Curidini	Curidina	Chile	BUP0147	KM364217	KM364090	KM364463	KM364351
Anthaxia (Haplanthaxia)	*melancholica*	Buprestidae	Buprestinae	Anthaxiini	Anthaxiina	S. Africa	BUP0087	KM364215	KM364088	no	KM364349
Anthaxia	*sp.*	Buprestidae	Buprestinae	Anthaxiini	Anthaxiina	S. Africa	BUP0088	KM364216	KM364089	KM364462	KM364350
Anthaxia (Haplanthaxia)	*sp.*	Buprestidae	Buprestinae	Anthaxiini	Anthaxiina	S. Africa	BUP0140	KM364214	no	KM364461	KM364348
Bilyaxia	*cordillerae*	Buprestidae	Buprestinae	Anthaxiini	Anthaxiina	Chile	BUP0148	KM364213	KM364087	KM364460	KM364347

Table 1 (Continued).

Genus	Species	Family	Subfamily	Tribe	Subtribe	Country	Coll. no.	28S	18S	CAD	COI
Brachelytrium	*holmi*	Buprestidae	Buprestinae	Anthaxiini	Anthaxiina	S. Africa	BUP0243	no	KM364158	KM364519	no
Chalcogenia	*sp.*	Buprestidae	Buprestinae	Anthaxiini	Anthaxiina	S. Africa	BUP0241	KM364222	KM364095	KM364467	KM364356
Euryspilus	*chalcodes*	Buprestidae	Buprestinae	Bubastini		Australia	BUP0261	KM364231	KM364101	no	no
Agaeocera	*gigas*	Buprestidae	Buprestinae	Buprestini	Agaeocerina	Mexico	BUP0158	KM364210	KM364084	no	KM364344
Buprestis	*laeviventris*	Buprestidae	Buprestinae	Buprestini	Buprestina	USA	BUP0055	KM364219	KM364092	no	KM364353
Buprestis (Yamina)	*sanguinea*	Buprestidae	Buprestinae	Buprestini	Buprestina	Spain	BUP0258	KM364253	KM364121	KM364488	KM364382
Pseudhyperantha	*jucunda*	Buprestidae	Buprestinae	Buprestini	*incert. sed.*	Malaysia	BUP0300	KM364266	KM364129	KM364494	KM364391
Trachykele	*blondeli*	Buprestidae	Buprestinae	Buprestini	Trachykelina	USA	BUP0143	KM364249	KM364117	KM364484	KM364379
Trachykele	*nimbosa or hartmanni*	Buprestidae	Buprestinae	Buprestini	Trachykelina		BUP0280	KM364250	KM364118	KM364485	KM364380
Chrysobothris	*calida*	Buprestidae	Buprestinae	Chrysobothrini		USA	BUP0039	KM364223	KM364096	KM364468	KM364357
Chrysobothris	*inaequalis*	Buprestidae	Buprestinae	Chrysobothrini		Mexico	BUP0166	KM364224	KM364097	KM364469	KM364358
Chrysobothris	*merkelii*	Buprestidae	Buprestinae	Chrysobothrini		USA	BUP0069	KM364225	KM364098	KM364470	KM364359
Coomaniella	*purpurascens*	Buprestidae	Buprestinae	Coomaniellini		Thailand	BUP0290	KM364226	KM364099	KM364471	KM364360
Anilara	*sp.*	Buprestidae	Buprestinae	Curidini	Anilarina	Australia	BUP0209	KM364212	KM364086	KM364459	KM364346
Selagis	*caloptera*	Buprestidae	Buprestinae	Curidini	Curidina	Australia	BUP0289	KM364243	KM364111	KM364480	KM364373
Selagis	*intercribrata*	Buprestidae	Buprestinae	Curidini	Curidina	Australia	BUP0022	KM364244	KM364112	KM364481	KM364374
Neocuris	*sp.*	Buprestidae	Buprestinae	Curidini	Neocurina	Australia	BUP0019	KM364240	KM364109	no	KM364371
Torresita	*cuprifera*	Buprestidae	Buprestinae	Curidini	*Torresita* g.g.	Australia	BUP0010	KM364248	KM364116	no	KM364378
Diadoxus	*juengi*	Buprestidae	Buprestinae	Epistomentini		Australia	BUP0002	KM364229	no	KM364473	KM364363
Epistomentis	*pictus*	Buprestidae	Buprestinae	Epistomentini		Chile	BUP0146	KM364230	no	KM364474	KM364364
Julodimorpha	*bakewelli*	Buprestidae	Buprestinae	Julodimorphini		Australia	BUP0257	KM364233	KM364103	KM364475	KM364365
Maoraxia	*littoralis*	Buprestidae	Buprestinae	Maoraxiini		Australia	BUP0017	KM364234	KM364104	KM364476	KM364366
Melanophila	*consputa*	Buprestidae	Buprestinae	Melanophilini	*Melanophila* g.g.	USA	BUP0056	KM364235	KM364105	KM364477	KM364367
Xenomelanophila	*miranda*	Buprestidae	Buprestinae	Melanophilini	*Melanophila* g.g.	USA	BUP0273	KM364252	KM364120	KM364487	no
Merimna	*atrata*	Buprestidae	Buprestinae	Melanophilini	*Merimna* g.g.	Australia	BUP0153	KM364237	no	KM364479	KM364369
Merimna	*atrata*	Buprestidae	Buprestinae	Melanophilini	*Merimna* g.g.	Australia	BUP0287	KM364238	KM364107	no	KM364370
Melobasis	*vittatus*	Buprestidae	Buprestinae	Melobasini		Australia	BUP0001	KM364236	KM364106	KM364478	KM364368
Nascio	*vetusta*	Buprestidae	Buprestinae	Nascionini		Australia	BUP0207	KM364239	KM364108	no	no

Table 1 (Continued).

Genus	Species	Family	Subfamily	Tribe	Subtribe	Country	Coll. no.	28S	18S	CAD	COI
Spectralia	*gracilipes*	Buprestidae	Buprestinae	*incert. sed.*	*Spectralia* g.g.	USA	BUP0206	KM364245	KM364113	KM364482	KM364375
Pterobothris	*corruscus*	Buprestidae	Buprestinae	Pterobothrini		Chile	BUP0294	KM364242	KM364110	no	no
Castiarina	*simulata*	Buprestidae	Buprestinae	Stigmoderini		Australia	BUP0006	KM364221	KM364094	KM364466	KM364355
Lasionota	*bivittata*	Buprestidae	Buprestinae	Stigmoderini		Chile	BUP0254	KM364227	KM364100	KM364472	KM364361
Lasionota	*minor*	Buprestidae	Buprestinae	Stigmoderini		Chile	BUP0151	no	no	KM364520	KM364421
Lasionota	*conjuncta stenoloma*	Buprestidae	Buprestinae	Stigmoderini		Chile	BUP0252	KM364228	no	no	KM364362
Stigmodera	*macularia*	Buprestidae	Buprestinae	Stigmoderini		Australia	BUP0021	KM364246	KM364114	no	KM364376
Oaxacanthaxia	*nigroaenea*	Buprestidae	Buprestinae	Thomassetiini	*incert. sed.*	Mexico	BUP0168	KM364241	no	no	KM364372
Augrabies	*schotiaphaga*	Buprestidae	Buprestinae	Thomassetiini		S. Africa	BUP0073	KM364218	KM364091	KM364464	KM364352
Thomasettia	*crassa*	Buprestidae	Buprestinae	Thomassetiini		S. Africa	BUP0086	KM364247	KM364115	KM364483	KM364377
Trigonogenium	*angulosum*	Buprestidae	Buprestinae	Trigonogenini		Chile	BUP0250	KM364251	KM364119	KM364486	KM364381
Hesperorhipis	*mirabilis*	Buprestidae	Buprestinae	Xenorhipidini	Xenorhipidina	USA	BUP0295	KM364232	KM364102	no	no
Chalcophora	*angulicollis*	Buprestidae	Chrysochroinae	Chrysochroini	Chalcophorina	USA	BUP0054	KM364255	KM364122	KM364489	KM364384
Pseudotaenia	*quadrifoveolata*	Buprestidae	Chrysochroinae	Chrysochroini	Chalcophorina	Australia	BUP0245	KM364267	KM364130	KM364495	no
Chrysochroa	*kaupi*	Buprestidae	Chrysochroinae	Chrysochroini	Chrysochroina	Indonesia	BUP0229	KM364256	no	no	no
Epidelus	*ceramensis*	Buprestidae	Chrysochroinae	Chrysochroini	Eucallopisticina	Indonesia	BUP0230	KM364260	KM364125	no	no
Cyphosoma	*lawsoniae*	Buprestidae	Chrysochroinae	Dicercini	Dicercina	Spain	BUP0264	KM364257	KM364123	KM364490	KM364385
Ectinogonia	*intermedia*	Buprestidae	Chrysochroinae	Dicercini	Dicercina	Chile	BUP0251	KM364258	KM364124	KM364491	KM364386
Haplotrinchus	*sp. (near inaequalis)*	Buprestidae	Chrysochroinae	Dicercini	Haplotrinchina	Indonesia	BUP0234	KM364264	no	no	no
Gyascutus	*dianae*	Buprestidae	Chrysochroinae	Dicercini	Hippomelanina	USA	BUP0032	KM364263	no	no	no
Evides	*gambiensis*	Buprestidae	Chrysochroinae	Evidini		S. Africa	BUP0139	KM364262	KM364127	KM364492	KM364389
Euchroma	*gigantea*	Buprestidae	Chrysochroinae	Paraleptodemini	Euchromatina	Ecuador	BUP0269	KM364261	KM364126	no	KM364388
Embrikillium	*patricium*	Buprestidae	Chrysochroinae	*incert. sed.*	*Chalcoplia* g.g.	S.Africa	BUP0092	KM364259	no	no	KM364387
Ampheremus	*cylindricollis*	Buprestidae	Chrysochroinae	*incert. sed.*	*Nanularia* g.g.	USA	BUP0023	KM364254	no	no	KM364383
Nanularia	*brunneata*	Buprestidae	Chrysochroinae	*incert. sed.*	*Nanularia* g.g.	USA	BUP0027	KM364265	KM364128	KM364493	KM364390
Sphenoptera (Hoplistura)	*sp.*	Buprestidae	Chrysochroinae	Sphenopterini		S. Africa	BUP0141	KM364268	KM364131	KM364496	KM364392
Galbella	*sp.*	Buprestidae	Galbellinae	Galbellini		S. Africa	BUP0127	KM364270	no	KM364498	KM364394
Galbella (Xenogalbella)	*hantamensis*	Buprestidae	Galbellinae	Galbellini		S. Africa	BUP0074	KM364269	KM364132	KM364497	KM364393

Table 1 (Continued).

Genus	Species	Family	Subfamily	Tribe	Subtribe	Country	Coll. no.	28S	18S	CAD	COI
Julodis	*cirrosa*	Buprestidae	Julodinae	Julodini		S. Africa	BUP0089	KM364271	no	KM364499	KM364395
Julodis	*recenta*	Buprestidae	Julodinae	Julodini		S. Africa	BUP0075	KM364272	KM364133	KM364500	KM364396
Julodis	*sulcicollis*	Buprestidae	Julodinae	Julodini		S. Africa	BUP0076	KM364273	no	KM364501	KM364397
Neojulodis	*sp.*	Buprestidae	Julodinae	Julodini		S. Africa	BUP0078	KM364275	KM364135	no	KM364399
Neojulodis	*sp.*	Buprestidae	Julodinae	Julodini		S. Africa	BUP0077	KM364274	KM364134	KM364502	KM364398
Neojulodis	*sp.*	Buprestidae	Julodinae	Julodini		S. Africa	BUP0079	KM364276	KM364136	no	KM364400
Acmaeodera	*digna*	Buprestidae	Polycestinae	Acmaeoderini	Acmaeoderina	Mexico	BUP0170	KM364277	no	no	KM364401
Acmaeodera (Paracmaeodera)	*fascigera*	Buprestidae	Polycestinae	Acmaeoderini	Acmaeoderina	S. Africa	BUP0133	KM364278	KM364137	KM364503	KM364402
Acmaeodera	*gibbula*	Buprestidae	Polycestinae	Acmaeoderini	Acmaeoderina	USA	BUP0061	KM364279	no	no	no
Acmaeodera	*oaxacae*	Buprestidae	Polycestinae	Acmaeoderini	Acmaeoderina	Mexico	BUP0155	KM364280	no	no	no
Acmaeodera	*sp.*	Buprestidae	Polycestinae	Acmaeoderini	Acmaeoderina	S. Africa	BUP0093	KM364281	KM364138	KM364504	KM364403
Nothomorpha	*pauperata*	Buprestidae	Polycestinae	Acmaeoderini	Nothomorphina	S. Africa	BUP0096	KM364289	KM364145	no	KM364409
Acmaeoderoides	*straminea*	Buprestidae	Polycestinae	Acmaeoderini	Acmaeoderoidina	USA	BUP0037	KM364282	no	no	no
Astraeus	*irregularis*	Buprestidae	Polycestinae	Astraeini		Australia	BUP0008	KM364283	KM364139	no	KM364404
Helferella	*manningensis*	Buprestidae	Polycestinae	Haplostethini		Australia	BUP0012	KM364285	KM364141	no	KM364406
Mastogenius	*n. sp.*	Buprestidae	Polycestinae	Haplostethini		Mexico	BUP0160	KM364286	KM364142	KM364506	KM364407
Mastogenius	*parallelus*	Buprestidae	Polycestinae	Haplostethini		Chile	BUP0255	KM364220	KM364093	KM364465	KM364354
Mastogenius	*robustus*	Buprestidae	Polycestinae	Haplostethini		USA	BUP0064	KM364287	KM364143	KM364507	KM364408
Paratrachys	*australius*	Buprestidae	Polycestinae	Paratracheini		Australia	BUP0014	KM364290	KM364146	KM364509	KM364410
Polycesta	*aruensis*	Buprestidae	Polycestinae	Polycestini	Polycestina	USA	BUP0060	KM364293	KM364149	KM364512	KM364413
Ptosima	*gibbicollis*	Buprestidae	Polycestinae	Ptosimini		USA	BUP0205	KM364294	KM364150	KM364513	KM364414
Chrysophana	*placida*	Buprestidae	Polycestinae	*incert. sed.*	*Chrysophana* g.g.	USA	BUP0058	KM364284	KM364140	KM364505	KM364405
Thrincopyge	*ambiens*	Buprestidae	Polycestinae	Thrincopygini		USA	BUP0024	KM364295	KM364151	KM364514	KM364415
Mimicoclytrina	*sp.*	Buprestidae	Polycestinae	Tyndarini	Mimicoclytrinina	Mexico	BUP0291	KM364288	KM364144	KM364508	no
Paratyndaris	*n. sp.*	Buprestidae	Polycestinae	Tyndarini	Tyndarina	Mexico	BUP0171	KM364291	KM364147	KM364510	KM364411
Paratyndaris	*olneyae*	Buprestidae	Polycestinae	Tyndarini	Tyndarina	USA	BUP0029	KM364292	KM364148	KM364511	KM364412
Tyndaris	*marginata*	Buprestidae	Polycestinae	Tyndarini	Tyndarina	Chile	BUP0248	KM364296	KM364152	KM364515	no
Tyndaris	*planata*	Buprestidae	Polycestinae	Tyndarini	Tyndarina	Chile	BUP0249	KM364297	KM364153	KM364516	no

Table 1 (Continued).

Genus	Species	Family	Subfamily	Tribe	Subtribe	Country	Coll. no.	28S	18S	CAD	COI
Xyroscelis	*crocata*	Buprestidae	Polycestinae	Xyroscelidini		Australia	BUP0004	KM364298	KM364154	KM364517	KM364416
Dystaxia	*elegans*	Schizopodidae	Schizopodinae	Dystaxiini		USA	BUP0025	KM364299	KM364155	no	KM364417
Glyptoscelimorpha	*marmorata*	Schizopodidae	Schizopodinae	Dystaxiini		USA	BUP0298	KM364300	KM364156	no	KM364418
Schizopus	*laetus*	Schizopodidae	Schizopodinae	Schizopodini		USA	BUP0272	KM364209	KM364083	KM364457	KM364343
Schizopus	*sallei*	Schizopodidae	Schizopodinae	Schizopodini		USA	BUP0299	KM364301	no	no	KM364419
Simplocaria	*semistriata*	Byrrhidae	Byrrhinae			Unknown	BT0017	KM364303	KM364160	KM364522	no
Callirhipis	*sp.*	Callirhipidae				Unknown	CO129	KM364305	KM364162	KM364524	EF209549[1]
Anorus	*piceus*	Dascillidae	Karumiinae			USA	MSC1281, BT0127	KM364302	KM364159	KM364521	KM364422
Selonodon	*floridensis*	Elateridae	Cebrioninae			USA	DDM0602	KM364306	KM364163	KM364525	no
Lara	*avara*	Elmidae	Larainae			USA	MSC1288	KM364304	KM364161	KM364523	no

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[1] Obtained from GenBank

DNA extraction, amplification and sequencing

Nearly all of the specimens used in this study were collected as adults and preserved in 95% EtOH. However, *Hesperorhipis mirabilis* and *Thrincopyge ambiens* were collected as larvae. Total genomic DNA was isolated from the abdomen, legs, or entire body of specimens using the QIAquick DNeasy Tissue Kit (Qiagen), following the manufacturer's protocol.

PCR amplification was carried out in 25 ul reactions, typically containing 11.6 ul HPLC water, 5 ul 5X buffer (Qiagen), 0.2 ul 10mM dNTPs (Qiagen), 1.5 ul $MgCl_2$, 0.2 ul Taq DNA Polymerase (Qiagen) and 1 ul of each 10mM primer. Five ul of Q solution (Qiagen) was added to each PCR reaction when the results of an initial PCR were poor, and in some cases improved yield. For each specimen, we targeted a total of 6756 bp of double-stranded DNA sequence data, including data from one mitochondrial and three nuclear markers: an approximately 1300 bp fragment of the protein-coding mitochondrial marker Cytochrome Oxidase I (COI); approximately 1300 bp of the nuclear protein-coding gene carbamoyl-phosphate synthase domain (CAD); approximately 1800 bp of the nuclear ribosomal gene 28S; and approximately 2000 bp of the nuclear ribosomal gene 18S. The primers used, including internal primers used in sequencing reactions, are listed in Table 2.

COI amplifications were carried out with the following temperature profile: a two minute denaturation step at 94°C followed by 40 cycles of 60 s at 94°C denaturation, 30 s at 50°C annealing, and 90 s at 72°C extension. The typical temperature profile for amplification of the nuclear markers consisted of a two-minute denaturation step at 94°C followed by 30 cycles of 60 s at 94°C denaturation, 60 s at 53-58°C annealing (temperature varied depending on primers), and 60 s at 72°C extension. For CAD we sometimes used a "touchdown" profile with annealing

Table 2. PCR and sequencing primers used in this study.

Locus	Primer Name	Use	Sequence	Citation
COI	s1718	amp/seq	GGAGGATTTGGAAATTGATTAGTTCC	Simon *et al.*, 1994
COI	a2771	amp/seq	GGATARTCAGARTAACGTCGWGGTATWC	Simon *et al.*, 1994
COI	LCO1490	amp/seq	GGTCAACAAATCATAAAGATATTGG	Folmer *et al.*, 1994
COI	HCO2198	amp/seq	TAAACTTCAGGGTGACCAAAAAATCA	Folmer *et al.*, 1994
COI	Jerry	amp/seq	GCTACTACATAATAKGTATCATG	Simon *et al.,* 1994
COI	Ben	amp/seq	CAACATTTATTTTGATTTTTTGG	Simon *et al.,* 1994
CAD	CD439F	amp/seq	TTCAGTGTACARTTYCAYCCHGARCAYAC	Wild & Maddison, 2008
CAD	CD630F	amp/seq	TCTCTTGGAGGTTTRGGNTCDGGDTTYGC	Wild & Maddison, 2008
CAD	CD688R	amp/seq	TGTATACCTAGAGGATCDACRTTYTCCATRTTRCA	Wild & Maddison, 2008
CAD	CD851R	amp/seq	GGATCGAAGCCATTAACATTYTCRTCHACCAT	Wild & Maddison, 2008
CAD	CD1098R2	nested amp	GCTATGTTGTTNGGNAGYTGDCCNCCCAT	Wild & Maddison, 2008
28S	Mo6	amp/seq	CCCCCTGAATTTAAGCATAT	Schmitz & Moritz, 1994
28S	D2B	seq	GTCGGGTTGCTTGAGAGTGC	Saux *et al.,* 2004
28S	D2B-r	seq	GCACTCTCAAGCAACCCGAC	Saux *et al.,* 2004
28S	D3A	seq	GACCCGTCTTGAAACACGGA	Saux *et al.,* 2004
28S	D3A-r	seq	TCCGTGTTCAAGACGGGTC	Saux *et al.,* 2004
28S	D3B	amp/seq	TCGGAAGGAACCAGCTACTA	Saux *et al.,* 2004
28S	ZX1	amp/seq	ACCCGCTGAATTTAAGCATAT	Van der Auwera *et al.,* 1994
28S	rD4.2b	amp/seq	CCTTGGTCCGTGTTTCAAGACGG	Whiting, 2002
28S	ZR1	seq	GTCTTGAAACACGGACCAAGGAGTCT	Mallat & Sullivan, 1998
28S	rD5b	amp/seq	CCACAGCGCCAGTTCTGCTTAC	Whiting, 2002
18S	18e-s	amp/seq	CTGGTTGATCCTGCCAGT	Halaynch *et al.,* 1998
18S	18p-c	amp/seq	TAATGATCCTTCCGCAGGTTCACCT	Halaynch *et al.,* 1998
18S	f1094	amp/seq	GGATCGTCGCAAGACGGACAGAAG	Sequeira *et al.,* 2000
18S	r1138	amp/seq	GTTAGAGGTTCGAAGGCG	Sequeira *et al.,* 2000

temperatures decreasing from 60-50°C in 2°C increments over 35 cycles. All reactions were subjected to a final extension step of 5 min at 72°C. PCR products were purified using shrimp alkaline phosphatase and exonuclease I (GE Healthcare) or a QIAquick PCR Purification Kit (Qiagen); or were gel purified using a QIAquick Gel Purification Kit (Qiagen).

Purified PCR products were sequenced in both directions using ABI PRISM BigDye Terminator Cycle Sequencing Kits (version 3.0 or 3.1; Applied Biosystems). Sequencing primers were identical to amplification primers, with the exception that additional internal primers were used to sequence long PCR fragments (Table 2). Cycle sequencing reactions were carried out on

ABI 3100 and 3730 automated sequencers (Applied Biosystems) at Harvard University.

Alignment

Sequences were assembled and edited using Sequencher version 4.7 (Gene Codes). The non-coding nuclear ribosomal DNA sequences (18S and 28S) were initially aligned with MUSCLE version 3.7 (Edgar, 2004) accessed via the CIPRES Science Gateway (Miller *et al.*, 2010) with default alignment parameters. Alignment of the protein-coding sequences (COI and CAD) was unambiguous. All aligned data were visualized and manually adjusted in Mesquite version 2.74 (Maddison & Maddison, 2011). Not all genes were obtained for all specimens (16.9% missing; Table 1).

Data partitions and model selection

We used PartitionFinder (Lanfear *et al.*, 2012) to select an appropriate partitioning scheme and to identify the best-fitting substitution model for each partition under the Bayesian information criterion (Posada & Crandall, 2001). A partitioning scheme with eight unlinked partitions (28S, 18S, CAD 1st positions, CAD 2nd positions, CAD 3rd positions, COI 1st positions, COI 2nd positions, COI 3rd positions) was identified as the optimal scheme and was used in all analyses. PartitionFinder was also used to compare the 24 substitution models implemented in MrBayes, identifying GTR+I+G as the best-fitting model for all partitions except CAD 1st positions, for which SYM+I+G was optimal.

Phylogenetic analyses

A maximum likelihood analysis of the combined data set was performed using RAxML version 7.3.0 (Stamatakis, 2006; Stamatakis *et al.*, 2008) via the CIPRES Science Gateway (Miller *et al.*, 2010). The GTRCAT model parameters were assessed separately for the eight partitions defined above. Maximum likelihood bootstrap support was assessed with the rapid bootstrap algorithm implemented in RAxML (Stamatakis *et al.*, 2008), and using the bootstop feature, which automatically stopped the bootstrap analysis after 300 replicates. We conducted parallel Bayesian MCMC analyses (Bayesian inference; BI) (Yang & Rannala, 1997) on the Odyssey supercomputing cluster at Harvard University, using MrBayes version 3.2 (Ronquist *et al.*, 2012) and applying the eight data partitions and models defined above, with default priors. Four concurrent paired runs, each with 32 chains, and together occupying 256 nodes on Odyssey, were run for 10^8 generations (estimated base frequencies; four gamma categories; default heating; trees sampled every 10^3 generations).

Ancestral states reconstruction

Ancestral states reconstruction was performed using Mesquite v. 2.75 (Maddison & Maddison, 2011). Ingroup taxa were coded as either leaf mining or non-leaf mining. Leaf mining taxa are listed in Table 3. Maximum likelihood character optimization was then performed on the maximum likelihood phylogram using the Markov k-state one-parameter model. Pie charts were used to indicate the estimated proportional likelihoods of each of the two possible character states at each node.

Table 3. Leaf mining taxa. *indicates multiple exemplars included.

Taxon	Subfamily	Tribe	Subtribe
Paratrachys australius	Polycestinae	Paratracheini	
Brachys kleinei	Agrilinae	Tracheini	Brachina
Lius mexicanus	Agrilinae	Tracheini	Brachina
Taphrocerus depilis	Agrilinae	Tracheini	Brachina
Taphrocerus fasciatus*	Agrilinae	Tracheini	Brachina
Taphrocerus shannoni	Agrilinae	Tracheini	Brachina
Callimicra hoschecki	Agrilinae	Tracheini	Leiopleurina
Callimicra pinguis	Agrilinae	Tracheini	Leiopleurina
Leiopleura L040*	Agrilinae	Tracheini	Leiopleurina
Leiopleura sp.	Agrilinae	Tracheini	Leiopleurina
Hylaeogena sp. 1	Agrilinae	Tracheini	Pachyschelina
Hylaeogena sp. 2	Agrilinae	Tracheini	Pachyschelina
Hylaeogena sp. 3	Agrilinae	Tracheini	Pachyschelina
Pachyschelus cupricauda	Agrilinae	Tracheini	Pachyschelina
Pachyschelus undulatus	Agrilinae	Tracheini	Pachyschelina
Habroloma sp.*	Agrilinae	Tracheini	Tracheina
Neotrachys estebana	Agrilinae	Tracheini	Tracheina

3. Results

The superfamily Buprestoidea was monophyletic under both maximum likelihood inference (MLI; Figure 2, branch lengths reported in Figure 3) and Bayesian inference (BI; Figure 4) [maximum likelihood bootstrap support (MLB) = 100%; Bayesian posterior probability support (BPP) = 1.0]. Within Buprestoidea, the monophyly of Schizopodidae received maximal support (MLB = 100%; BPP = 1.0), as did the placement of Schizopodidae as the sister group of Buprestidae (= all remaining Buprestoidea) (MLB = 99%; BPP = 1.0). Within the subfamily Julodinae (MLB = 100%; BPP = 1.0), the two representative genera, *Julodis* and *Neojulodis*, were maximally supported as monophyletic (MLB = 100%, BPP = 1.0; and MLB = 100%, BPP = 1.0, respectively). Julodinae and Polycestinae formed a clade, but nodal support was low (MLB < 50%, BPP = 0.78). Within this clade, Julodinae was recovered in a position sister to the enigmatic Haplostethini (*Helferella* and *Mastogenius*) under both MLI and BI (MLB = 100%; BPP = 1.0). While this placement received strong support under BI (BPP = 1.0), it was only weakly supported under MLI (MLB = 55%). The clade comprised of Julodinae + Haplostethini was sister (MLB < 50%; BPP = 0.78) to a maximally supported clade (MLB = 100%; BPP = 1.0) containing the remaining Polycestinae (the "Polycestinae clade").

The Polycestinae clade contained two maximally supported subclades: one comprising the Acmaeoderioid lineage *sensu* Volkovitsh (2001) (with the exception of Haplostethini, as mentioned above) (MLB = 100%; BPP = 1.0) and another containing the Polycestioid and Polyctesioid lineages *sensu* Volkovitsh (2001) (MLB = 99%; BPP = 1.0). Relationships within

Figure 2. (location in phylogeny indicated by inset) Maximum likelihood (ML) phylogenetic tree for 137 species of Buprestoidea including representatives of both families, all subfamilies and 42/51 extant tribes (*sensu* Bellamy 2003, 2008), plus five outgroups (from five elateriform families), resulting from an unconstrained partitioned analysis of concatenated DNA sequence data from four genes (COI, 18S, 28S and CAD), implemented in Garli (see Figure 3 for branch lengths). ML bootstrap support ≥50% from partitioned analyses in RAxML and posterior probabilities from the Bayesian analyses are indicated along branches. Green squares indicate taxa known to have leaf-mining larvae. Exemplar images are not to scale.

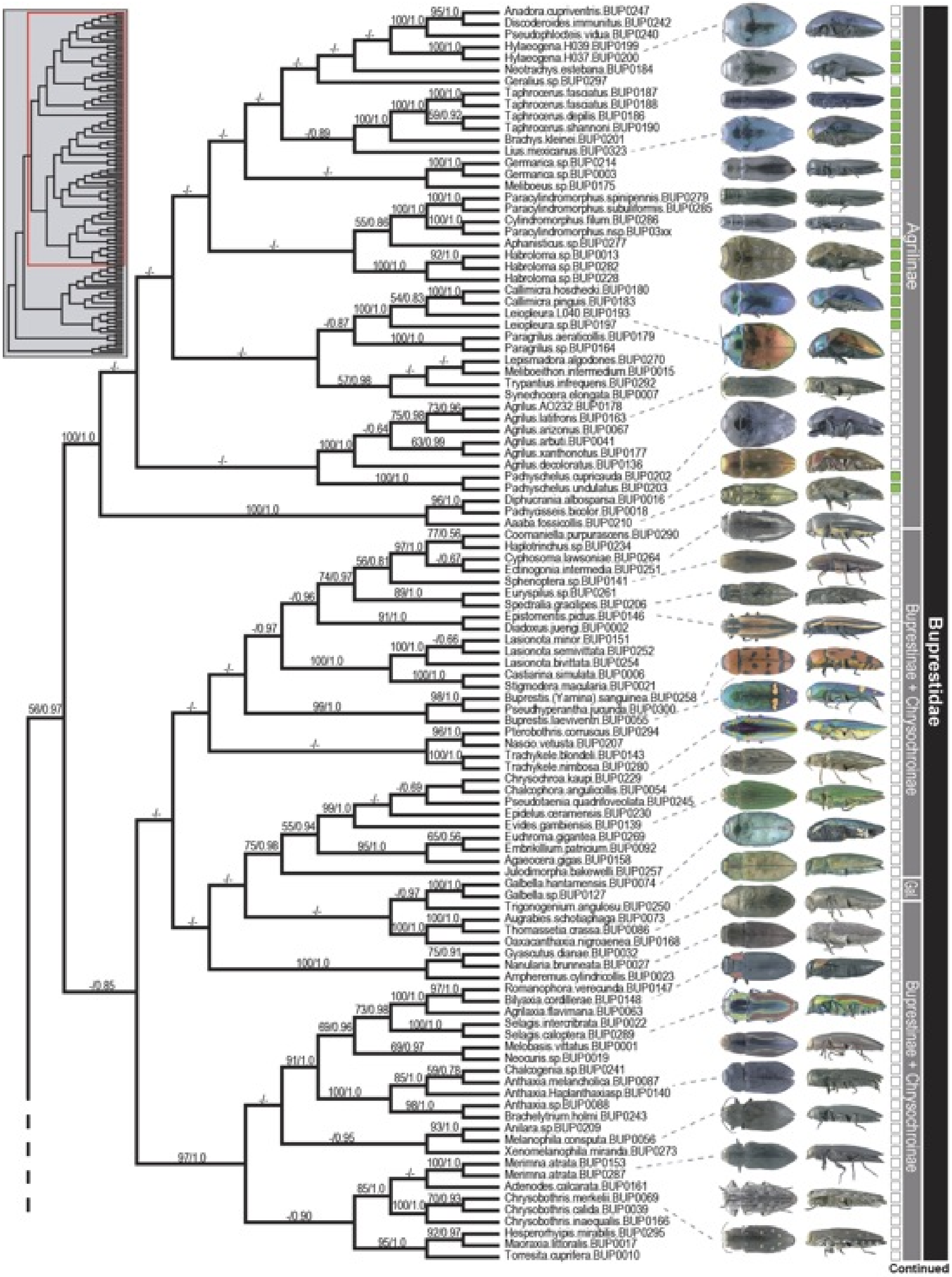

Figure 2 (Continued).

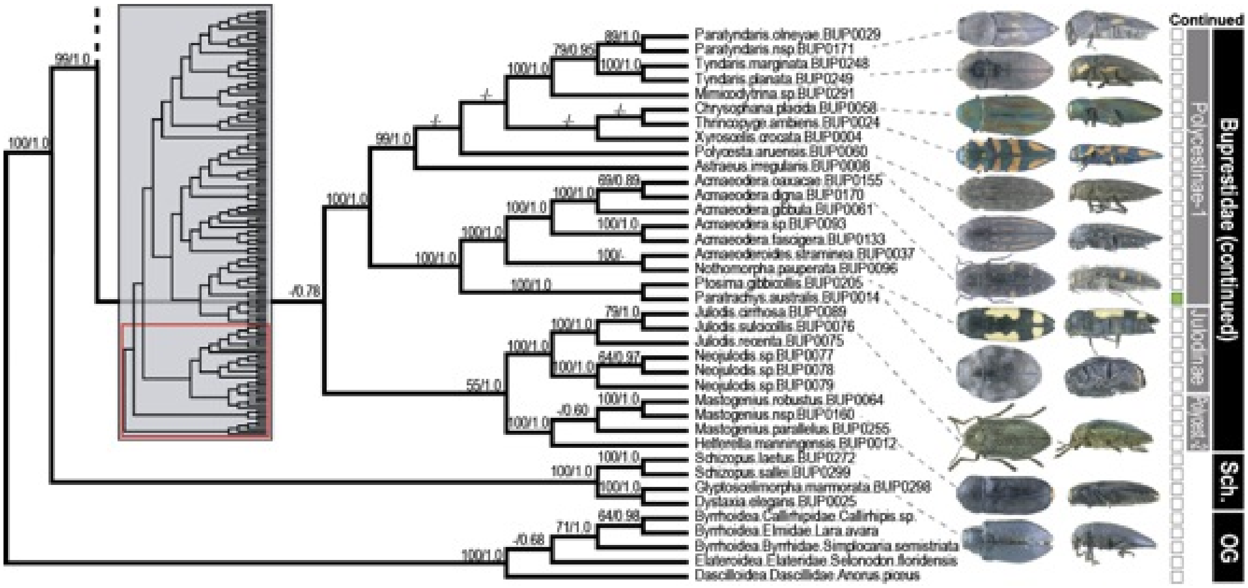

Figure 2 (Continued).

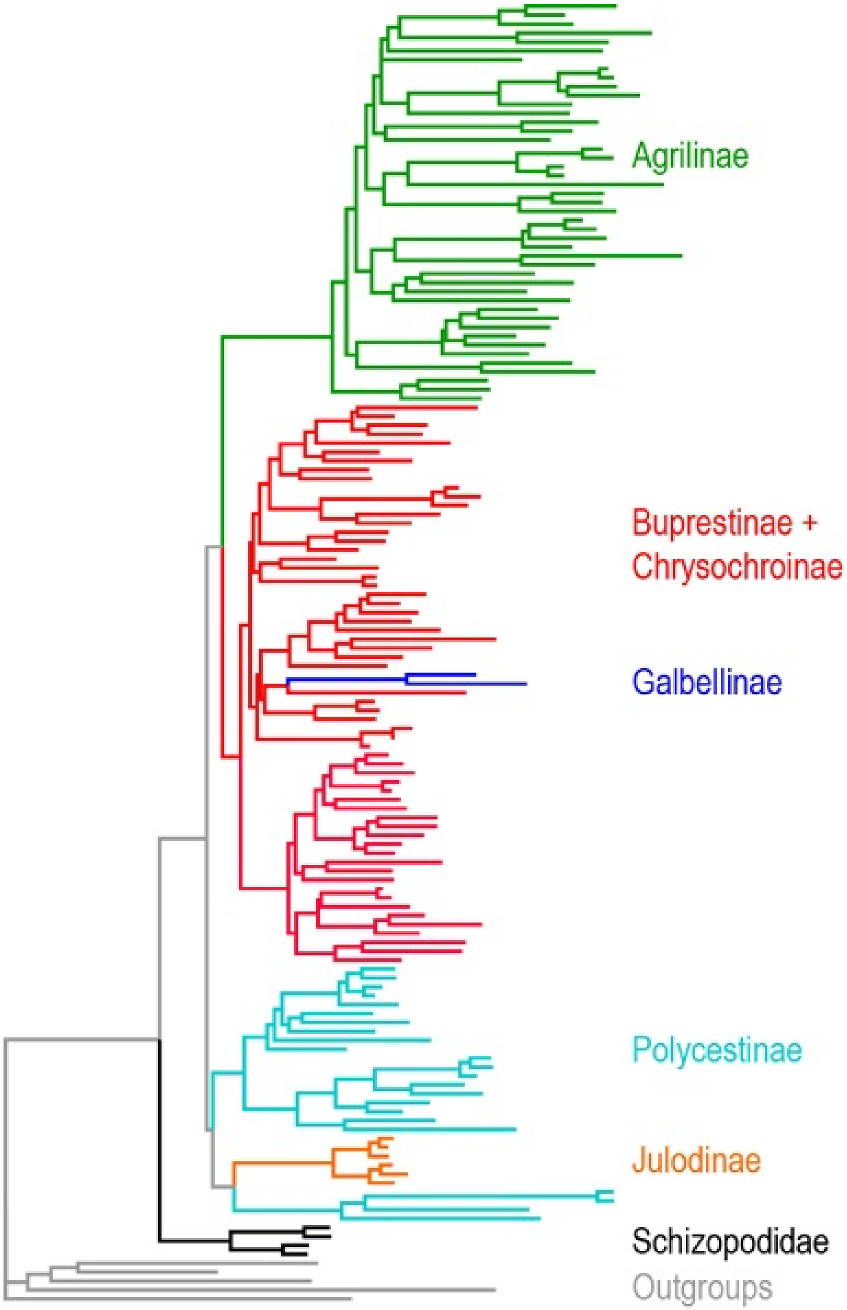

Figure 3. Maximum likelihood tree (phylogram) with branch lengths proportional to the number of nucleotide substitutions (also see Fig. 2)

Figure 4. Bayesian maximum clade credibility tree for 137 species of Buprestoidea including representatives of both families, all subfamilies and 42/51 extant tribes (*sensu* Bellamy 2003, 2008), plus outgroups (five species representing five elateriform families), resulting from an unconstrained partitioned analysis of concatenated DNA sequence data from four genes (COI, 18S, 28S and CAD), implemented in MrBayes. Bayesian posterior probabilities ≥0.50, are indicated along branches.

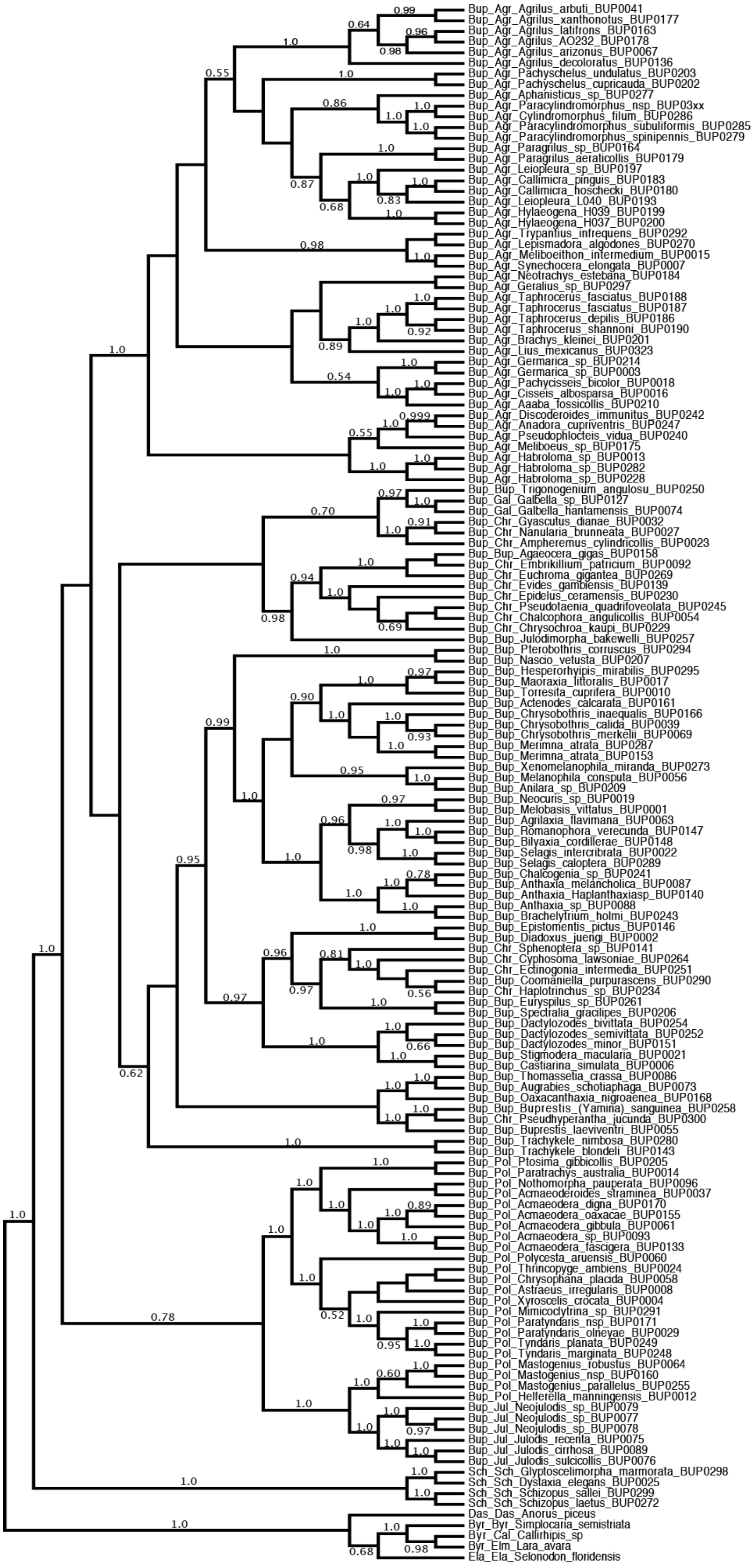

Figure 4 (Continued).

the clade of acmaeoderioids were strongly supported. There was maximal support (MLB = 100%; BPP = 1.0) for a sister relationship between *Paratrachys* (Paratracheini) and *Ptosima* (Ptosimini). These were sister (MLB = 100%; BPP = 1.0) to a clade containing Acmaeoderini, with *Acmaeodera* monophyletic (MLB = 100%; BPP = 1.0; 5 species sampled) and sister (MLB = 100%; BPP = 1.0) to a clade containing *Acmaeoderoides* (Acmaeoderoidina) and *Nothomorpha* (Nothomorphina). Within *Acmaeodera* there was maximal support for separate monophyletic Old World (MLB = 100%; BPP = 1.0, three representatives) and New World (MLB = 100%; BPP = 1.0, two representatives) lineages.

Relationships within the Polycestioid + Polyctesioid clade lacked nodal support, with the exception of Tyndarini, which was maximally supported as monophyletic (MLB = 100%; BPP = 1.0, five representatives). *Mimicoclytrina* (Mimicoclytrinina) was sister to a monophyletic Tyndarina (*Tyndaris* + *Paratyndaris*; MLB = 79%; BPP = 0.95). The representatives of the Gondwanian Prospherioid lineage *sensu* Volkovitsh (2008b) (*Astraeus* and *Xyroscelis*) were not recovered together. *Xyroscelis* (Xyroscelidini) was recovered sister to the Polyctesioid lineage *sensu* Volkovitsh (2001) (represented by *Chrysophana* and *Thrincopyge*), though without strong nodal support (MLB <50%; BPP <0.5). *Astraeus* was recovered sister to the Polycestioid + Polyctesioid clade, but that relationship also lacked strong nodal support.

A large clade was recovered containing the remaining Buprestidae (Chrysochroinae, Buprestinae, Galbellinae and Agrilinae), with strong support under BI (BPP = 0.97), but low support under MLI (MLB = 56%). Agrilinae was recovered within this large clade with maximal nodal support (MLB = 100%; BPP = 1.0) along with a clade containing Chrysochroinae, Buprestinae, and Galbellinae, which was recovered under both MLI (MLB <50%) and BI (BPP = 0.85). Neither Buprestinae nor Chrysochroinae were monophyletic in our analyses. There was strong support for a monophyletic *Galbella* (MLB = 100%; BPP = 1.0; two representatives), which was recovered in a position sister to the buprestine *Trigonogenium* under both MLI and BI. Nodal support for this placement was strong under BI (BPP = 0.97), but low under MLI (MLB < 50%).

A strongly-supported clade containing most of the buprestine Anthaxioid and Chrysobothrioid lineages *sensu* Volkovitsh (2001) (the "Chrysobothrioid-Anthaxioid clade"; MLB = 97%; BPP = 1.0) was recovered within the large clade comprised of Buprestinae + Chrysochroinae. This clade was sister to a large clade (lacking nodal support) that contained Chrysochroinae, Galbellinae, and the remaining Buprestinae, including the Buprestioid lineage *sensu* Volkovitsh (2001) and the Thomasettioid branch of the Anthaxioid lineage *sensu*

Volkovitsh (2001) (the "Buprestioid-Chrysochroine clade").The Chrysobothrioid-Anthaxioid clade contained a strongly supported clade containing most of the anthaxioids, including Anthaxiini, Curidini, and Melobasini (MLB = 91%; BPP = 1.0). All nodes within the anthaxioid clade had moderate to high nodal support; however, neither Anthaxiini, the genus *Anthaxia*, nor Curidini were monophyletic. The three representatives of *Anthaxia*, including two members of the subgenus *Haplanthaxia*, were recovered with the anthaxiines *Brachelytrium* and *Chalcogenia* in a maximally supported clade (MLB = 100%; BPP = 1.0). whereas the anthaxiines *Agrilaxia* and *Bilyaxia* were recovered in a maximally supported clade with *Romanophora* (Curidini; MLB = 100%; BPP = 1.0), sister to *Selagis* (Curidini; MLB = 73%; BPP = 0.98). Also within this clade, *Neocuris* [Curidini (*sensu* Bellamy, 2008)] was recovered sister to *Melobasis* (Melobasini) (MLB = 69%; BPP = 0.97).

Melanophilini (Chrysobothrioid lineage *sensu* Volkovitsh, 2001) were recovered in a clade with *Anilara* (MLB = <50%; BPP = 0.95), with strong support for *Anilara* sister to

Melanophila (MLB = 93%; BPP = 1.0). *Chrysobothris* (Chrysobothrini) was monophyletic (MLB = 100%; BPP = 1.0) and contained in a clade (MLB = 85%; BPP = 1.0) with the Chrysobothrioids *Merimna* (*Merimna* generic group *sensu* Volkovitsh 2001) and *Actenodes* (Actenodini). *Maoraxia*, which was placed outside of the major buprestine lineages *sensu* Volkovitsh (2001), was recovered sister to *Hesperorhipis* (MLB = 92%; BPP = 0.97) in a strongly-supported clade also containing *Torresita* (MLB = 95%; BPP = 1.0). The deeper relationships among the Anthaxioid and Chrysobothrioid clades lacked strong nodal support.

The remaining Buprestinae, mainly representatives of the Buprestioid lineage *sensu* Volkovitsh (2001), were recovered in the Buprestioid-Chrysochroine clade with Chrysochroinae and Galbellinae. The interrelationships of Chrysochroinae and Buprestinae were not well resolved; however the two subfamilies as currently defined were clearly polyphyletic, with chrysochroine genera falling into several different well-supported clades otherwise comprised of buprestine genera. The Buprestioid-Chrysochroine clade was further divided into two subclades (neither with strong nodal support). The first contained mainly representatives of the Buprestioid lineage with some chrysochroines interspersed. For example, the buprestine *Coomaniella* was recovered in a clade with the three representatives of the chrysochroine Dicercini (MLB = 97%; BPP = 1.0). Sister to that clade was *Sphenoptera,* another representative of the Dicercioid lineage *sensu* Bellamy (2003) (Sphenopterini + Dicercini) (MLB = 56%; BPP = 0.81). The buprestioids *Euryspilus* (Bubastini) and *Spectralia* [*Spectralia* generic group *sensu* Volkovitsh (2001)] were recovered together (MLB = 89%; BPP = 1.0), sister to the dicercioids and *Coomaniella* (MLB = 74%; BPP = 0.97). Epistomentini (Buprestioid lineage) was monophyletic (MLB = 91%; BPP = 1.0), as was Stigmoderini (MLB = 100%; BPP = 1.0). *Trachykele* (Buprestini) (monophyletic; MLB = 100%; BPP = 1.0) was recovered sister to *Nascio* (*Nascio* generic group) + *Pterobothris*

(Pterobothrini) (MLB = 96%; BPP = 1.0). The Chrysochroine *Pseudhyperantha* was recovered with the two representatives of *Buprestis* (Buprestini; including a representative of the subgenus *Yamina*) in a strongly supported clade (MLB = 99%; BPP = 1.0; sister to *Buprestis (Yamina)*: MLB = 98%; BPP = 1.0). Deeper nodes within this clade lacked strong nodal support.

Within the second large Buprestioid-Chrysochroinae clade was a clade containing most of the representatives of the Chrysochroioid lineage *sensu* Volkovitsh (2001) along with the buprestines *Julodimorpha* and *Agaeocera* (MLB = 74%; BPP = 0.98). Within that clade, Chrysochroini was monophyletic, though without strong nodal support (MLB = <50%; BPP = <0.5). A monophyletic Chrysochroini + Evidini was strongly supported (MLB = 99%; BPP = 1.0). Another clade contained several members of the Anthaxioid lineage *sensu* Volkovitsh (2001) along with Galbellinae. The Thomassetiini were monophyletic (MLB = 100%; BPP = 1.0) and sister to *Oaxacanthaxia* (MLB = 100; BPP = 1.0). That clade was recovered sister to the clade containing *Galbella* (Galbellinae) and the buprestine *Trigonogenium* (Trigonogeniini), though that node lacked strong nodal support. A third clade (MLB = 100%; BPP = 1.0) contained only the chrysochroines *Gyascutus* (Dicercini) and *Nanularia* and *Ampheremus* (*Nanularia* generic group (Bellamy, 2003)).

All Agrilinae were contained in a single strongly supported clade (MLB = 100%; BPP = 1.0). The deeper nodes within this clade generally received strong support under BI, but lacked strong MLB support. Coraebini were dispersed throughout Agrilinae, with strong nodal support for several clades representing coraebine subtribes. The three representatives of Cisseina formed a maximally supported clade (MLB = 100%; BPP = 1.0), including *Aaaba*. Toxoscelina was also monophyletic (MLB = 100%; BPP = 1.0). There was support, though weak under MLI (MLB = 57%; BPP = 0.98), for a clade containing the coraebines *Meliboeithon* (Clemina), *Trypantius*

(Dismorphina), and *Synochocera* (Synechocerina) along with *Lepismadora*. *Meliboeithon* and *Synechocera* were sister taxa in 77% of MLB replicates but that relationship was not found in the ML tree. *Geralius* (Geraliina) and *Meliboeus* (Meliboeina) were also included but the interrelationships among these and the other coraebine tribes lacked strong MLB and BPP support. Agrilini were represented by six species of *Agrilus* (Agrilina) and two species of *Paragrilus* (Rhaeboscelidina). Both genera were monophyletic in all bootstrap replicates (with maximal MLB and BPP support), but there was no support for a monophyletic Agrilini. Two of the three subtribes of Aphanisticini were monophyletic and together formed a clade (Aphanisticina + Cylindromorphina) with weak nodal support (MLB = 55%; BPP = 0.86). The genus *Germarica* (Germaricina) was excluded from that clade (recovered along with *Meliboeus* in an unsupported clade). Tracheini were similarly spread throughout the agriline clade. There was maximum support for the monophyly of Leiopleurina (*Leiopleura* + *Callimicra*) (MLB = 100%; BPP = 1.0). Bracheina was monophyletic but there was nodal support only for the clade containing *Brachys* + *Taphrocerus* (MLB = 100%; BPP = 1.0), with *Lius* sister to that clade but without strong nodal support (MLB < 50%; BPP = 0.89). Pachyschelina was not monophyletic under MLI or BI.

The several genera of leaf mining agrilines do not form a monophyletic group in the trees resulting from the MLI and BI analyses, and the reconstructed evolutionary history of larval feeding habits in Buprestoidea (either leaf mining, or non-leaf mining) revealed at least 3 origins of the leaf mining trait in Agrilinae and one origin in Polycestinae (*Paratrachys*) (Figure 5).

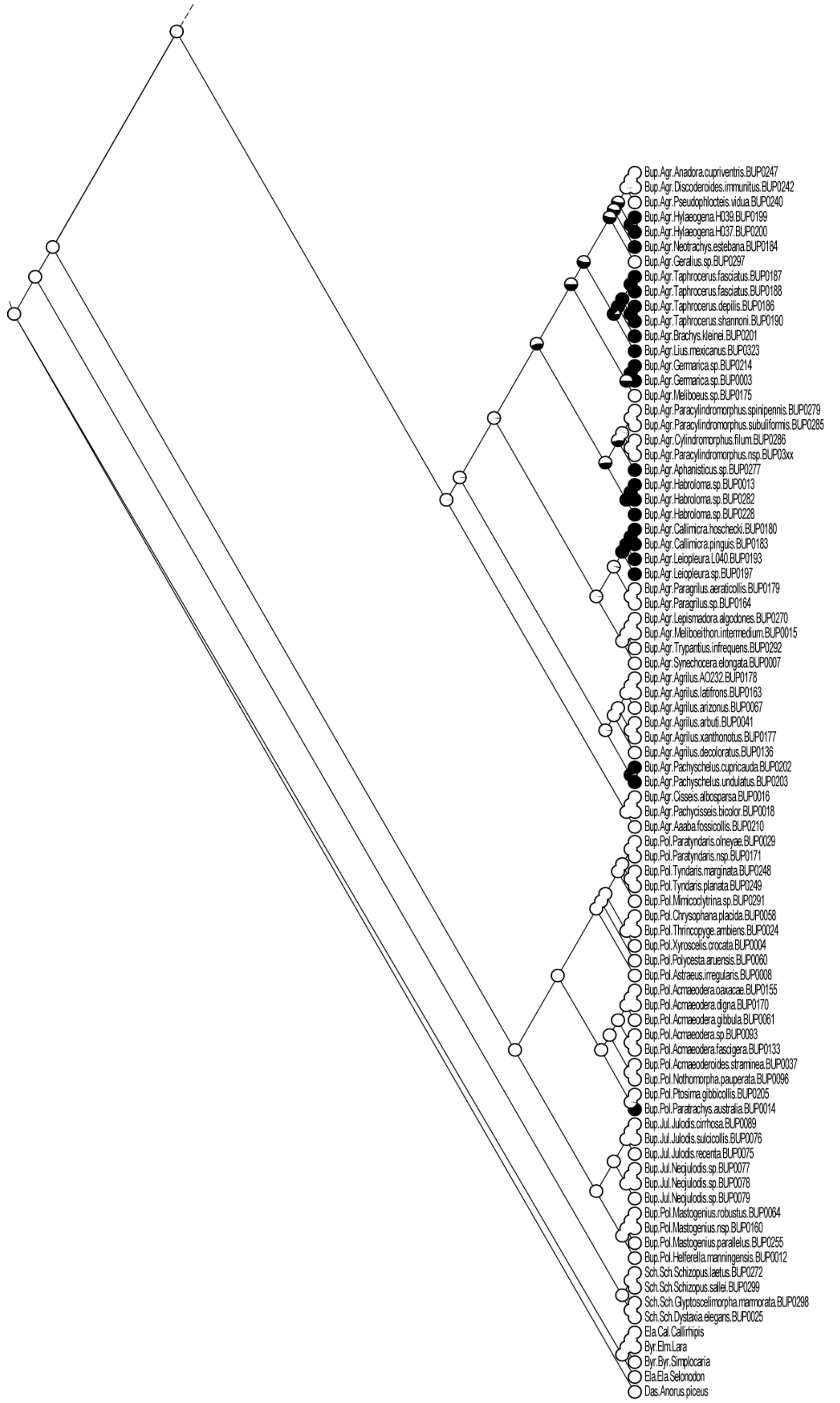

Figure 5. Reconstructed evolution of larval leaf-mining in Buprestoidea. We reconstructed ancestral states of larval feeding habit (leaf mining or non-leaf mining) in Buprestoidea on the maximum likelihood (ML) phylogenetic tree (see Fig. 2) using ML character optimization in Mesquite v. 2.75 (Maddison & Maddison, 2011). Optimization was performed using the Markov k-state one-parameter model. Pie charts indicate the estimated proportional likelihoods of each of the two possible character states (black = leaf mining, white = non-leaf mining).

4. Discussion

Our results provide extensive new insights into higher-level relationships within the superfamily Buprestoidea. The monophyly of Buprestidae, with Schizopodidae sister, was strongly supported and consistent with the separation of the two families originally proposed by LeConte (1859) and reestablished by Nelson and Bellamy (1991). There was insufficient nodal support at deeper levels to conclusively determine the interrelationships among all of the subfamilies of Buprestidae, but we can draw some important conclusions. Our results were largely consistent with the generally accepted major lineages of buprestoids, including clearly-defined agrilines, buprestines-chrysochroines, and early-branching julodines-polycestines. In addition to the Schizopodidae, two of the five non-monogeneric subfamilies recognized by Bellamy (2003, 2008)—Agrilinae and Julodinae—were monophyletic in the context of our taxon sampling. The monogeneric Galbellinae was also monophyletic but was nested within a non-monophyletic Buprestinae + Chrysochroinae. Polycestinae was monophyletic with the exception of the enigmatic Haplostethini.

Workers have variously proposed Polycestinae or Julodinae as the “primitive” branches of Buprestidae based on morphological characters, including, among other features, antennal sensory structures (e.g., Volkovitsh, 2001). We recovered a close relationship between Polycestinae and Julodinae, which together formed a clade (but with low nodal support) sister to the remaining Buprestidae. Rather than one of the two subfamilies branching off first, in our analysis Polycestinae and Julodinae together form a lineage separate from the remaining Buprestidae. Our results are consistent with the prevailing view of these two subfamilies as substantially distinct from the the other buprestid subfamilies, with characteristics (morphological and other) frequently interpreted as primitive among Buprestidae, and do not support proposals to merge Julodinae (e.g. Kolibac, 2000) or Polycestinae (e.g. Holynski, 1993) with the Buprestinae. Polycestinae was found to exclude the enigmatic Haplostethini, though nodal support was low for Haplostethini + Julodinae. Additional sampling within the Polycestinae in future studies should further illuminate the relationships among these lineages.

Within Polycestinae, our results correspond relatively closely to the lineages defined by Volkovitsh (2001) and adopted by Bellamy (2003, 2008), with the Polyctesioid and Polycestioid lineages closely related and sister to the Acmaeoderioid lineage. Volkovitsh (2008b) merged the Polyctesioid lineage with the Polycestioid lineage and proposed a new Gondawanian Prospherioid lineage. We found no support for the Prospherioid lineage *sensu* Volkovitsh (2008b), though nodal support within the Polycestioid clade was insufficient to rule out that hypothesis. A recent study of polycestine larval morphology (subsequent to publication of this study) similarly finds a lack of evidence for a separate Prospherioid lineage (Volkovitsh and Bílý, 2015). We found no support for the scheme laid out in Holyński (1993), which merged Polycestinae and Buprestinae, nor for placing *Polycesta* within his Buprestini.

The leaf mining genus *Paratrachys* (Paratracheini) was recovered sister to *Ptosima* (Ptosimini) within the Acmaeoderioid clade, which is consistent with the current classification (Bellamy, 2003) and the lineages established by Volkovitsh (2001, 2008a). This confirms the independent origin of leaf mining within Polycestinae. Though *Paratrachys* was placed with the agriline leafminers in some earlier classifications (Kerremans, 1903; Toyama, 1987), most recent work has treated *Paratrachys* as a member of the Polycestinae (following Cobos, 1979).

Old World and New World *Acmaeodera* were recovered in sister clades with maximal nodal support. This result is consistent with separate geographically defined evolutionary lineages: the assemblage "*Acmaeodera incisae*" *sensu* Kerremans (1906), which includes all

Afrotropical *Acmaeodera* except for two species of subgenus *Acmaeodera s. str.* and is restricted to the Old World (Volkovitsh, 1979), and the New World *Acmaeodera* (as well as the two African *Acmaeodera s.str.*).

Haplostethini (= Mastogenini) were traditionally treated as a separate subfamily (Mastogeninae *sensu* Bellamy, 1985). There has been substantial disagreement regarding their relationship to other Buprestidae, with various workers positing that they represent a "primitive" lineage, perhaps even outside of Buprestidae (see discussion in Bellamy, 1987a, 1991). *Mastogenius* and related genera were previously placed close to Agrilinae (Obenberger, 1937). Cobos (1980, 1981) and Holyński (1984) suggested that they are more closely related to Polycestinae, based on genital and other morphological characters. More recently, Holyński (1993) reduced Mastogeniini to a subtribe of his Thrincopygini, which contains a subset of the Polycestinae *sensu* Bellamy (2003), and suggested that Mastogeniini may represent the "ancestral" form of Thrincopygini. Volkovitsh (2001) elevated Mastogenini to tribal rank within his "Polycestoid complex" but placed them with the acmaeoderoids rather than close to Thrincopygini, based on antennal morphology. This placement was maintained in the most recent higher classification (Haplostethini *sensu* Bellamy, 2003, 2008). Our results show that Haplostethini belong within Buprestidae, in the clade comprised of Julodinae and Polycestinae, and specifically, suggest that they are most closely related to Julodinae. However, nodal support for this placement is weak. Our results are nonetheless consistent with the more traditional recognition of a separate subfamily Haplostethinae.

Our analyses recovered a large clade containing all Chrysochroinae and Buprestinae *sensu* Bellamy (2008). Several notable relationships are contained within this large clade. First, we found no evidence to support Chrysochroinae as it is currently defined (Bellamy, 2003), instead finding several chrysochroine clades interspersed with Buprestinae. This is in contrast to both the modern and traditional classification of Buprestidae, though there has been substantial flux in the classification of the groups which comprise the two subfamilies. Some authors have recently supported merging Chrysochroinae with Buprestinae due to the lack of clearly defined morphological differences (Toyama, 1987; Holyński, 1993). Volkovitsh (2001) maintained the two as separate "complexes" based on sensory organs (antennae), and these groupings are reflected in the current classification. While our topology does not correspond to the Chrysochroinae and Buprestinae *sensu* Bellamy (2003), it strongly contradicts the scheme proposed by Holyński (1993) at both the tribal and subfamily levels. A clearer picture of the interrelationships of these groups will require additional study. Nonetheless, further reassessment of the current classification is clearly appropriate.

The phylogeny (Figure 2) is broadly consistent with the evolutionary lineages within Buprestinae as laid out by Volkovitsh (2001), with several interesting exceptions. Within the large Buprestinae-Chrysochroinae clade, one clade contained all of the buprestine Anthaxioid and Chrysobothrioid lineages, while a second large clade contained the Buprestioid lineage along with Chrysochroinae. Melanophilini were recovered within the Anthaxioid-Chrysobothrioid clade; this placement is consistent with the close relationship between Melanophilini and Chrysobothrini first proposed by Holyński (1993) and supported by antennal morphology (Volkovitsh, 2001). Several relationships within the Anthaxioid-Chrysobothrioid clade were initially surprising, but turned out to have been anticipated in the taxonomic literature. The enigmatic genus *Maoraxia* was previously thought closely related to *Mastogenius* (Obenberger, 1937), though Cobos (1957) did not include the genus in his key to the Mastogeninae. Holyński (1984) transferred *Maoraxia* to its own tribe (Maoraxiini) and Bellamy and Williams (1985) transferred it to the subtribe Anthaxiae (later Anthaxiini; see Bellamy 1986), citing differences from *Mastogenius* in both larval and adult morphology. Volkovitsh (2001) placed Maoraxiini outside of his major buprestine lineages, citing conflicting characteristics that demonstrated similarity to both the Buprestioid and Anthaxioid lineages. More recently, Bílý and Volkovitsh (2005) concluded that *Maoraxia* belongs within the Anthaxioid lineage based on larval morphology. In the topology presented here, *Maoraxia* is most closely related to the anthaxioids *Torresita* and *Hesperorhipis*, in the sister clade to the Chrysobothrioid lineage *sensu* Volkovitsh (2001). There is some precedent for the relationship to *Torresita,* as both were previously included in the Australian Anthaxiae (Bellamy & Williams, 1985). The inclusion of the North American *Hesperorhipis* in this clade is nonetheless surprising.

The clade containing Chrysobothrini, Actenodini, and *Merimna* [currently in Melanophilini *sensu* Bellamy (2003)] is equivalent to Chrysobothrini *sensu* Holyński (1993) and is supported by morphological data, including the dorsally convergent eyes and specialized antennal morphology of Actenodini and Chrysobothrini (Volkovitsh, 2001). *Merimna* is an enigmatic Australian genus that was originally described as a *Chrysobothris* and has variously been placed with Melanophilini (Kerremans, 1903; Cobos, 1986; Bellamy, 2003) or Actenodini (Bellamy, 1985; Holyński, 1993); our results clearly support its inclusion with Actenodini and Chrysobothrini. Like *Melanophila*, *Merimna* uses infrared receptors to locate forest fires in order to mate and lay eggs in freshly burned wood; however, the infrared organs in the two genera differ both in their structure and mechanism (Schmitz *et al.*, 1997; Schmitz *et al.*, 2000).

We found strong support for a clade containing the majority of the anthaxioid taxa (including Anthaxiini, Curidini, and Melobasini), but neither the Anthaxiini nor *Anthaxia* were monophyletic. Our results shed light on the relationships within the Anthaxioid lineage, which have been recently debated (Holyński, 1988, 1989, 1993; Volkovitsh, 2001; Bílý & Volkovitsh, 2005, 2007). Notably, we found strong support for separate Old World and Australian/New World lineages within the Anthaxioid clade. The anthaxiines *Bilyaxia* (Chile) and *Agrilaxia* (USA) were recovered within a maximally-supported clade with the neotropical curidine *Romanophora,* within a strongly-supported larger clade containing Australian Curidini and Melobasini; this clade was sister to a clade containing the remaining Anthaxiini, all from the Old World. This result is consistent with the hypothesis of independent geographic lineages presented by Bílý and Volkovitsh (2005, 2007) and contradicts Holyński's (1988, 1993) subtribal scheme.

Several of the unexpected relationships we recovered involved Australian taxa whose classification has been unstable: these include the anthaxioid genera *Torresita* and *Maoraxia,* discussed previously, as well as *Anilara*, *Neocuris,* and *Melobasis*. All of these genera are currently placed close together in various small tribes within the Anthaxioid lineage *sensu* Volkovitsh (2001), but their relationships to each other and other Buprestinae have long been uncertain. Volkovitsh (2001) noted the morphological similarities of the latter three genera to both Anthaxiini and Melanophilini. We found support for a close relationship between *Anilara* and the Melanophilini, sister to the main Anthaxioid clade. *Neocuris* and *Melobasis* were recovered together within the Anthaxioid clade, near *Selagis*, and not closely related to *Anilara*.

Galbella was recovered with the buprestine *Trigonogenium* [Trigonogeniini in the Anthaxiinioid branch *sensu* Volkovitsh (2001)]. While the clade lacks nodal support, it is clear that *Galbella* does not fall within Agrilinae, as proposed by Holyński (1993), nor is it closely related to *Mastogenius*, as suggested by Kolibac (2000). Its placement within the Buprestinae-Chrysochroinae clade casts doubt on the status of Galbellinae as a separate family. The interesting morphological similarities between *Galbella* and some Tracheini, including ventral grooves to accept folded legs and antennae, are likely a product of convergent evolution related to small size or adult habit.

The clade sister to *Galbella* and *Trigonogenium* contains representatives of Thomasettiini along with *Oaxacanthaxia*, whose placement within the Thomasettiinioid branch *sensu* Volkovitsh (2001) was considered uncertain (Bellamy, 2003). These two clades in turn form the sister group to the previously discussed Chrysochroioid clade. Although Volkovitsh (2001) did not predict the inclusion of *Galbella* here, his analysis of antennal sensory structures did support a close relationship between *Trigonogenium* and Thomasettini, and he also discussed the similarity of *Trigonogenium* to some Buprestioids, which is consistent with their placement within the Buprestioid-Chrysochroine clade rather than with the Anthaxioid lineage. Bílý and Volkovitsh (2007) maintained *Trigonogenium* within the Anthaxioid lineage based on its previously unknown larval morphology, noting its many plesiomorphic characteristics. Further morphological and molecular sampling is needed to fully understand the relationship of *Trigonogenium* to other Buprestinae.

Chrysochroinae are currently divided into two major lineages (the Dicercioid and Chrysochroioid lineages *sensu* Bellamy, 2003). Although some relationships within these lineages have been well-defined and stable, there are many genera whose placement within Chyrsochroinae is uncertain. Representatives of Chrysochroinae [*sensu* Bellamy (2003)] were recovered mainly in three separate clades within the larger Buprestioid-Chrysochroinae clade, indicating that they do not represent a single lineage, but rather multiple lineages within the buprestine grade. Several authors have supported merging Chrysochroinae with Buprestinae, suggesting that there is no clear delineation between the two subfamilies (Toyama, 1987;

Holyński, 1988, 1993). The topology presented here is consistent with this view; however, Chrysochroinae do fall into strongly supported clades that are substantially consistent with the major morphology-based lineages within the subfamily (Volkovitsh, 2001). This affirms the utility of antennal characters for assessing relationships among some buprestids, but also underscores their insufficiency for fully resolving the relationships within and among lineages.

Most of the representatives of the Chrysochroioid lineage (*sensu* Bellamy, 2003) were recovered in a single clade along with the buprestine *Agaeocera*, and sister to *Julodimorpha.* The strongly supported monophyly of Chrysochroini and its placement sister to Evidini are unsurprising given their morphological similarities (see Volkovitsh, 2001). Recovered sister to that clade was a clade containing *Euchroma* (Paraleptodemini) and *Embrikillium* (*Chalcoplia* generic group, *sensu* Volkovitsh, 2001), which are among the several chrysochroine genera whose relationships have been unclear (Volkovitsh, 2001). *Embrikillium* has previously been placed in both Dicercini (Psilopterini *sensu* Bellamy, 1985) and Chrysochroini (Bellamy, 1988). Volkovitsh (2001) discussed its unusual antennal structures and placed it in a "generic group" (equivalent to tribe) within the Chrysochroioid lineage with other similar Afrotropical genera. Our results suggest that *Embrikillium* is closely related to *Euchroma* and other members of Paraleptodemini, which have also been separated from the Dicercini and Chrysochroini based on unusual antennal morphology (Volkovitsh, 2001).

The inclusion of the enigmatic genus *Agaeocera* with *Euchroma* and *Embrikillium* was unexpected. The placement of *Agaeocera* has been the subject of considerable debate. It was originally placed in Chalcophorinae (Chrysochroinae *sensu* Bellamy (2003)), but has more recently been placed in Buprestinae, either in its own tribe (Agaeocerini; Nelson, 1982) or within Buprestini. In Holyński's (1993) major reorganization of the classification, Agaeocerina was placed within Thrincopygini (with members of the current Polycestinae). Bellamy (2003, 2008) returned Agaeocerina to its more traditional placement in Buprestini, following Westcott (2000) and Volkovitsh (2001). Our results are consistent with the original placement of *Agaeocera* with members of the Chrysochroinae, close to Paraleptodemini or Poecilonotini (both within the Chrysochroioid lineage).

Similarly, the placement of *Julodimorpha* has been uncertain. In the present analysis, there is moderate support for its placement sister to the Chrysochroioid clade. Historically, *Julodimorpha* has variously been placed in Stigmoderinae (Saunders, 1871; Théry, 1929); Polycestini (Kerremans, 1902, 1904); Chalcophorinae (Holm, 1979; Bellamy, 1986); Buprestinae *sensu* Holyński (1993); Julodinae (Kolibac, 2000); or Buprestinae (Buprestioid lineage *sensu* Volkovitsh (2001)) (Volkovitsh, 2001; Bellamy, 2003, 2008). Most recently, Bílý *et al.* (2013) studied *Julodimorpha* larval morphology and found similarities to both chrysochroines and buprestines. Based on the topology presented here, *Julodimorpha* is clearly part of the Buprestinae-Chrysochroinae clade, and likely closely related to the Chrysochroioid lineage *sensu* Bellamy (2003). The superficial morphological similarities with Julodinae are thus likely a result of convergence due to the shared larval habit of external root feeding.

The genera *Nanularia* and *Ampheremus* are currently placed in a tribal-level *"Nanularia* generic group" based on Volkovitsh's (2001) study of antennal morphology (Bellamy, 2003); however, in the present study these genera are recovered with *Gyascutus* (Dicercini, Hippomelanina), sister to the rest of the Buprestioid-Chrysochroine clade, which is consistent with their traditional placement in Hippomelanina (Bellamy, 1987b; Holyński, 1993; Nelson and Bellamy, 1996). Indeed, *Nanularia* was previously considered a subgenus of either *Gyascutus* or the closely related *Hippomelas*.

A clade containing Dicercini and Sphenopterini was recovered within the Buprestioid lineage, well removed from the main Chrysochroioid clade. Dicercini has generally been regarded as closely related to Chrysochroini, with Holyński (1993) arguing that the characters used to delimit the two groups are unreliable. However, the two tribes differ markedly in larval characters and antennal structures (Volkovitsh, 2001), and our results instead suggest a close relationship between the Dicercioid lineage and the Buprestioid lineage of Buprestinae. The placement of *Sphenoptera* sister to the Dicercini is in accordance with Volkovitsh's (2001) placement of Sphenopterini within his Psilopteroid lineage, (Dicercioid lineage *sensu* Bellamy, 2003) based on antennal and larval characters. Sphenopterini are morphologically distinct and were previously considered a separate subfamily, placed between Chrysochroinae and Buprestinae (Cobos, 1980; Bellamy, 1985), though they were placed within Anthaxiini by Holyński (1988, 1993).

The enigmatic genus *Coomaniella* was also recovered in the Dicercini clade, sister to *Haplotrinchus*. This was not expected, but its current placement within the buprestine Anthaxiioid lineage *sensu* Volkovitsh (2001) (Bellamy, 2003) was uncertain due to its unusual morphology (see Volkovitsh, 2001). *Coomaniella* has generally been placed with Anthaxiini, though Bílý (1974) proposed a close relationship to Melanophilini. Volkovitsh (2001) notes that it differs significantly from both groups in its antennal sensory structures. Our results strongly suggest that *Coomaniella* is closely related to the Dicercini and the Buprestioid lineage *sensu* Volkovitsh (2001). The relationship between *Coomaniella* and Dicercini is further supported by larval characters (Bílý and Volkovitsh, 2015).

Most of the representatives of the Buprestioid lineage were recovered in this clade, with the Dicercioids nested within it. The evolutionary "branches" within the Buprestioid lineage laid out by Volkovitsh (2001) were not supported, but nodal support was low at the deeper nodes within this Buprestioid clade. Stigmoderini was monophyletic but was not recovered with *Euryspilus* (Bubastini), which Volkovitsh (2001) placed close to that tribe in his "Stigmoderioid branch." Epistomentini, which was placed with Chrysochroinae until its transfer to Buprestinae by Volkovitsh (2001), was recovered close to *Euryspilus* and *Spectralia,* which were sister to the Dicercioids. Additional taxon sampling would help tease apart the relationships among these genera and others in the Buprestioid and Dicercioid lineages.

A single chrysochroine taxon (*Pseudhyperantha*) was recovered apart from all other chrysochroines in a buprestine clade, with *Buprestis*. Our results support the placement of *Pseudhyperantha* within Buprestini, and suggest that it may be closely affiliated with the *Buprestis* subgenus *Yamina*. *Pseudhyperantha* was placed in Dicercini (Chrysochroinae) in Bellamy (2003). Bílý *et al.* (2009) suggested that *Pseudhyperantha* belongs in Buprestini based on antennal, ovipositor, and wing venation characters, agreeing with Toyama (1989). Holyński (2008, 2011) argued that the genus should be placed in Stigmoderini, but this is not supported by the morphological evidence presented in Bílý *et al.* (2009) nor the results of our analyses.

Trachykele, which is currently placed in Buprestini, was recovered with *Nascio* and *Pterobothris,* though that relationship lacked nodal support. *Nascio* was previously placed in Buprestini, and is currently placed in its own tribal level "*Nascio* generic group" close to Buprestini (Volkovitsh, 2001; Bellamy, 2003). *Pterobothris* is an enigmatic genus with similarities to both agrilines and buprestines and is currently placed in its own tribe based on its combination of larval and antennal characters (Volkovitsh, 2001). The close relationship of *Nascio* and *Pterobothris* was anticipated by Holyński (1988, 1993), who placed the two genera together in a subtribe within his Anthaxiini; however, our results instead support their placement with the other Buprestioids rather than Anthaxioids. A study of larval characters also found support for a close relationship between *Nascio* and *Pterobothris* (Bílý & Volkovitsh, 2007). The close relationship of *Nascio* and *Trachykele* was also previously suggested (Kurosawa, 1988). Our results suggest that these three genera are closely related and that they branched off early in the Buprestioid lineage.

Agrilinae was clearly monophyletic; however, we found little nodal support for the traditionally recognized tribes within Agrilinae. Coraebini and Tracheini were dispersed throughout the agriline clade, and though many of the recovered relationships lacked nodal support, our results certainly cast doubt on the monophyly of the agriline tribes as currently defined. The current tribal and subtribal classification of Agrilinae is based on a morphological phylogenetic study by Kubáň *et al.* (2000). Previously, the distinction between Agrilini and Coraebini had been based mainly on two characters: presence of a hypomeral carina and brush-like setae on the ovipositor, the importance of which were called into question by Kubáň *et al.* (2000), particularly in the case of ovipositor type, which may be adapted to ovipositing on narrow smooth plant surfaces such as stems. Our molecular phylogeny further challenges the accepted division of Agrilinae into the large tribes Coraebini and Agrilini. We found support for several coraebine subtribes, including Toxoscelina (sister to the tracheine *Hylaeogena*) and Cisseina [including *Aaaba*, which had been tentatively placed in the subtribe by Bellamy (2003)], which was recovered sister to all remaining Agrilinae. The agriline genus *Lepismadora* was recovered in an otherwise Coraebine clade, which is consistent with its former placement with Coraebini prior to its transfer to Agrilini by Kubáň *et al.* (2000). Given the lack of nodal support at deeper nodes within the Agriline clade, more work is needed to clarify the relationships among these subtribes; nonetheless, our results provide insights into the agriline taxa and relationships that should be revisited in future studies.

The monophyly of Cylindromorphina and Aphanisticina is consistent with Holyński's (1993) merging of the two into a single tribe (Aphanisticini), although Germaricina was recovered separate from that clade, and the neotropical Cylindromorphoidina were not included in our analysis.

Despite the lack of nodal support at deeper nodes within Agrilinae, our results strongly suggest that the leaf mining Tracheini is polyphyletic, as are several of its constituent subtribes. In some cases, the phylogenetic results appear to reflect biogeographical groupings that differ from the accepted tracheine subtribes. For example, of the Tracheina, the Neotropical genus *Neotrachys* does not appear to be closely related to the Old World *Habroloma*, which was instead recovered in a clade with the Old World Cylindromorphini. *Neotrachys* was recovered close to the Neotropical *Hylaeogena* (Pachyschelina) under MLI. *Pachyschelus,* on the other hand, was recovered sister to *Agrilus* (Agrilini). Bracheina was monophyletic, with monocot-mining *Taphrocerus* and dicot-mining *Brachys* sister to dicot-mining *Lius*. The New World Leiopleurina was also monophyletic, and recovered with *Paragrilus* (Agrilini), also from the New World. *Paragrilus* are not leafminers, and this likely indicates an independent origin of leaf mining, though support for the relationship is not strong. Under BI, *Hylaeogena* was also recovered in that clade. Bellamy and Hespenheide (1988) discussed the presence of "ring setae" in adults of three monocot-feeding genera (*Taphrocerus*: Bracheina), *Paracylindromorphus*, and *Aphanisticus* (both Aphanisticini). *Taphrocerus* was not recovered close to the two aphanisticines, which suggests that these setae may have evolved convergently in association with monocot-feeding. The leaf mining habit has not been confirmed in *Callimicra*

(Leiopleurina), and the subcylindrical morphology of this group may indicate a stem mining habit (H. Hespenheide, pers. comm.).

Close relatives of the Buprestoidea feed externally, including the outgroups used in the present study, with the exception of some dead wood boring Callirhipidae and Elmidae. Feeding in dead wood has generally been assumed to be the ancestral habit in Buprestidae (Kolibac, 2000); however our results indicate that external root feeding was likely the ancestral larval feeding habit in Buprestoidea given the presence of the habit in both the Schizopodiae and the early-branching Julodinae. Julodinae, which are external feeders, may have retained this ancestral habit, or they may represent a secondary origin of external feeding. The apparent evolutionary transitions to internal feeding allowed access to a variety of additional plant tissues, including leaves. Many groups within Buprestidae feed on dead or dying wood, including all but a few Polycestinae. Several specialized genera, such as *Melanophila* and *Merimna*, have evolved mechanisms for detecting forest fires (e.g., Schmitz *et al.*, 1997). Other groups are able to feed on living plant tissues. These have diversified to exploit a variety of plant tissues, including living wood, woody stems and roots, pine cones, grasses, and leaves. Several groups within Agrilinae (e.g. *Cylindromorpha*) have evolved to feed within twigs or stems, and have a corresponding subcylindrical form. The transition to feeding on living tissue not surprisingly appears to be associated with a greater degree of host plant specialization, particularly in Agrilinae, of which several species groups feed on members of a single genus (Bellamy and Nelson, 2002).

The leaf mining agrilines do not form a monophyletic group in the trees resulting from the MLI and BI analyses and almost certainly represent multiple origins of the leaf mining habit. Many of these genera are diverse and highly specialized, possibly indicating adaptive radiations.

Although nodal support is insufficient to confidently pinpoint shifts in feeding habits within the Agrilinae, our maximum likelihood reconstruction of the evolution of leaf mining in Buprestoidea (Figure 5) suggests that the larval leaf mining habit arose at least three times within Agrilinae, and once within Polycestinae (*Paratrachys*). Recent studies of antennal sensory structure morphology provide additional support for the non-monophyly of Tracheini and multiple origins of leaf mining within Agrilinae (Volkovitsh, unpublished). More complete sampling of Agrilini and Coraebini, and more definitive resolution of relationships within subfamily Agrilinae, are nonetheless needed to fully resolve and interpret the origins of the leaf mining habit in Agrilinae. A more fully resolved phylogeny of Agrilinae would facilitate comparative studies of leaf mining and wood boring groups of Buprestoidea, which could shed light on the effects of shifts to leaf mining on rates of diversification and disparity of host use, as well as the apparent morphological convergence of leaf mining taxa.

Larval feeding habits (e.g. internal or external feeding, stem or leaf feeding) likely have important macroevolutionary consequences, but relatively few studies have explicitly addressed this (Farrell & Sequeira, 2004; Marvaldi *et al.,* 2002; Nyman *et al.,* 2006, 2010; Leppänen *et al.,* 2012). Host tissue preference is often evolutionarily conservative (Farrell & Sequeira, 2004; McKenna & Farrell, 2005, 2006), but when shifts in tissue use occur, they may influence rates of host shifts and thus the likelihood of host plant-mediated ecological speciation (Leppänen *et al.,* 2012). For instance, because different feeding habits expose insects to differing levels of pressure from natural enemies (Connor & Taverner, 1997; Hawkins, 1994), and competition (Denno *et al.,* 1995), they may affect the frequency and magnitude of host shifts. Interpreting the evolution of host use within Buprestoidea will require a more detailed understanding of host use, including larval habit and host plant data. Nonetheless, recent studies, e.g., by Bílý *et al.* (2009, 2013) and Hawkeswood

(2002, 2007a, b), provide new and important insights into the morphology and natural history of Buprestidae at all life stages, helping set the stage for future such studies.

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