

Article

Phylogenetic Placement of the Plesioclytini (Coleoptera: Cerambycidae: Cerambycinae)

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Abstract: The tribe Plesioclytini was recently erected for a single genus of cerambycine longhorn beetle. The group was diagnosed from a proposed sister lineage, the diverse Clytini; however, a formal phylogenetic analysis was not performed due to limitations in data availability. Here, we present a phylogenetic reconstruction from five loci, that Plesioclytini is not sister to Clytini, but is instead only distantly related. Subsequent morphological investigations provide additional support for this placement.

Keywords: longhorn beetles; cerambycine; classification



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1. Introduction

Cerambycidae Latreille, 1802, longhorn beetles, is a large family with 38,368 species to date [1–3]. Linsley (1961) [4] split Cerambycidae into eight subfamilies: Cerambycinae Latreille, 1825 (12,423 species), Dorcasominae Lacordaire, 1869 (38 species), Lamiinae Latreille, 1825 (21,679 species), Lepturinae Latreille, 1825 (1831 species), Parandrinae Blanchard, 1845 (128 species), Prioninae Latreille, 1802 (1242 species), Necydalinae Latreille, 1825 (73 species), and Spondylidinae Audinet-Serville, 1832 (4 species) [1,3,5,6]. Švácha and Danilevsky (1987) also recognized eight subfamilies, but instead of Dorcasominae and Spondylidinae, they proposed Apatophyseinae and Aseminae [7]. Disteniidae and Oxypeltidae have also been considered subfamilies at times [8]. Napp (1994) [8] and Bouchard et al. (2011) [5] proposed adding the subfamily Anoplodermatinae, but Švácha and Lawrence [3] argue against this, and today, the eight subfamilies that Linsley (1961) [4] proposed are recognized [9]. The phylogenetic relationships at the family and subfamily levels remain uncertain due to the diversity within each family and limited data availability [9,10]. While the relationships between the subfamilies are unstable, the monophyly of the majority of them is not in question.

Cerambycinae is the second largest subfamily with 1844 genera split across 111 tribes around the world and display a wide variety of characteristics and behaviors [1,11–13]. Clytini Mulsant, 1839, is one of the most diverse and abundant tribes with 1844 species divided among 84 genera [1,14]. They are commonly found throughout North America, are usually yellow, white, or gray patterned, and are easily recognizable [15]. This tribe has many beneficial and pest species that cover a wide range of larval host plants, feeding behaviors, and climates, etc., which helps to explain the current diversity [4]. The genus *Plesioclytus* was proposed by Giesbert (1993) [16] and was assumed to be closely related to *Clytus* Laicharting, 1784, and was originally placed in the Clytini.

The species *Plesioclytus relictus* Giesbert (1993) was described from five specimens collected in relict sand dunes in the southern portion of Lake Wales Ridge, Florida. Later, another species in this genus, *Plesioclytus morrissi* (Wappes and Skelley (2015)), was collected and described from a relict sand dune system near the Ochoopee River of central Georgia [14]. While *P. morrissi* had been observed on *Chrysoma pauciflosculosa* (Michx.) (woody goldenrod) and *Licania michauxii* Prance (gopher apple), both *P. morrissi* and *P. relictus* were found feeding on *Polygonum polygamum* (Vent.) as larvae [14,17]. Both species feed on the roots of *P. polygamum*, which grow in deep, sandy soil [17]. It is unclear why these species do not infest the stems—it was possibly due to sun and heat exposure from the bare sand. The roots of *P. polygamum* are capable of swelling to accommodate the maturing larvae; however, the roots can become fragile due to annual infestations, often from multiple larvae infesting the plant at the same time.

Wappes and Skelley (2015) [14] erected the tribe Plesioclytini for the genus *Plesioclytus* based on several morphological differences from Clytini. They compared *Plesioclytus* to 28 of the 35 New World genera Clytini and found that, if included there, the definition of the tribe would need significant modification. They used five character comparisons to justify erecting the Plesioclytini. First, the procoxal cavities are closed or nearly closed compared with the obviously open procoxal cavities in Clytini. Second, the elytra of Plesioclytini are abbreviated and somewhat dehiscent, and diverge from the base to the apex, exposing the underlying wings, whereas, in Clytini, the elytra are long, cover the abdomen, and are contiguous from the base to the apex. Third, the elytral apices of Plesioclytini are obviously rounded and lack spines or spicules, but in Clytini, the external angle is always spined. Fourth, the metafemora of Plesioclytini are short and end at less than three-fourths of the length of the elytra, whereas, the metafemora in Clytini are longer and nearly reach the elytral apices. Fifth, the Plesioclytini lack metafemoral spines, but these are visible and long in some Clytini. It is possible that additional species currently placed in the Clytini, or even other cerambycine tribes, might be better placed in the Plesioclytini. Recent specimens of *Plesioclytus* have been collected, allowing for DNA analysis to be performed, and a formal phylogenetic analysis utilizing several molecular markers to be carried out.

2. Materials and Methods

2.1. Taxon Sampling

Fresh specimens of *Plesioclytus* were collected by KES as part of a natural history study [17]. Two specimens of each of the two described species were able to be sequenced. Our sampling scheme was heavily guided by the Bezark photographic catalog [11] but showed some similarity to Lee and Lee (2020) [18]. Ingroup sampling included 105 species, grouped into 25 of the 111 tribes of Cerambycinae (Supplementary Table S1), downloaded from NCBI Genbank [11,18–25]. Both Old World and New World lineages were heavily targeted due to past geographic limitations when discussing placement of the group. Outgroups included representatives from the Lamiinae, Lepturinae, Spondylidinae, and Prioninae [18,21,25–27].

2.2. Specimens Examined

Specimens were examined with a Leica S6D microscope. Habitus photographs were taken on a Leica Z16 APO microscope using a JVC KY-F75U digital camera and stacked with Syncroscopy Automontage software, version 5.01.005. Images were compiled into plates using GIMP 2 software (version 2.10.12).

2.3. Gene Sampling

Adult beetle specimens were first identified, then extracted and vouchered. Remains were deposited in the BYU genomics collection, Provo, UT. Genomic DNA was extracted from thoracic muscle tissue with a Qiagen Blood and Tissue Kit (Valencia, CA, USA), following manufacturer's protocols. Quantification of DNA extracted was completed using a ThermoScientific NanoDrop 2000 spectrophotometer. Three loci were amplified,

totaling 2095 base pairs (bp) of sequence data for each specimen, as follows: mitochondrial large subunit rRNA '16S' (527 bp), mitochondrial large subunit rRNA '12S' (854 bp), and cytochrome c oxidase subunit I 'CO1' (714 bp). Polymerase chain reactions (PCR) were performed using a BioRad C1000 thermocycler. Primers were adapted from Powell et al. (2020) [28] and synthesized by Eurofins (Louisville, KY, USA); PCR conditions, including annealing temperature ranges, are also given. Resulting PCR products were visualized using gel electrophoresis and a 1% agarose gel, stained with Ethidium Bromide. PCR products were cleaned with Exosap, purified with Sephadex, and sequenced at the BYU sequencing center.

2.4. Alignment and Tree Reconstruction

Sequences were aligned using MAFFT v.7.45 [29] implemented within Geneious v.11.0.3 [30]. Alignments were concatenated and exported for analysis. Best fit DNA substitution models were identified with ModelFinder [31]. A maximum likelihood (ML) analysis was performed in IQ-Tree v.1.6.11 (10,000 ultrafast bootstrap replicates) [32,33]. The resulting topology was edited and annotated with iTOL v.3 [34].

3. Results

The dataset gathered for a maximum likelihood phylogenetic reconstruction was based on 628 bp of COI, 614 bp of 18S rRNA, 694 bp of 16S rRNA, 561 bp of 28S rRNA, and 429 bp of wingless, for a total of 2926 bp of concatenated nucleotide sequence. The best fit model, recovered by ModelFinder and based on BIC, is GTR+F+I+G4 (Figure 1).

The outgroups (Lamiinae, Lepturinae, Prioninae, and Spondylidinae) formed monophyletic grouping with high nodal support. Lepturinae is reconstructed as the sister group to Cerambycinae (BS 100). The analysis recovered Cerambycinae as monophyletic with maximal support (BS 100) with only 12 of the 25 tribes included as monophyletic. Five tribes are represented by a single taxon (Heteropsini, Hylotrupini, Obriini, Oemini, and Xystrocerini). Heteropsini is sister to Eburiini (BS 49), Hylotrupini is sister to Compsocerini (BS95), Obriini is nested within Stenopterini (BS 56), and Xystrocerini is sister to Callichromatini (BS 100). Plesioclytini is recovered as sister to Oemini (BS 97), with the two tribes in turn being sister to a clade with the remaining 13 tribes sampled, including the Clytini.

4. Discussion

Our phylogeny largely agrees with previous molecular analyses for the group, the most recent being Lee and Lee (2020) [18]. Both trees resulted in two distinct Cerambycinae clades with a similar tribal composition. One of the two clades in our phylogeny contains Callichromatini, Cerambycini, Cleomenini, Molorchini, Oabriini, Pyrestini, Stenhomalini, Stenopterini, Thraniini, and Xystrocerini. Lee and Lee (2020) [18] found a similar clade but with differences in relationships among the tribes, and does not include Phoracanthini, which we recovered as monophyletic and basal to the rest of the Cerambycinae. Both recovered Molorchini sister to Cerambycini, Thraniini sister to Cleomenini, Pyrestini sister to Cerambycini, and Xystrocerini sister to Callichromatini. A major difference is Oabriini being nested within Stenopterini instead of being sister to Stenhomalini.

The second clade we recovered included Anaglyptini, Callidiini, Callidiopini, Cleomenini, Compsocerini, Clytini, Eburiini, Elaphidiini, Hesperophanini, Heteropsini, Hylotruperini, Oemini, Plesioclytini, Tillomorphini, and Trachyderini. While we did not include Dichophyiini as Lee and Lee (2020) [18] did, we were able to include Plesioclytini, Heteropsini, and Eburiini. With the addition of these tribes, a few of the relationships stayed the same, but the majority were altered. A couple of the relationships that stayed the same include *Teratoclytus* falling outside of the Clytini, Cleomenini nested within Callidiopini, and Hylotruperini being sister to Compsocerini. While Callidiini is split into two clades in both trees, the placement is different potentially due to the addition of Eburiini and Plesioclytini. The clade represented by *Phymatodes* is now sister to a clade composed of Heteropsini and Eburiini; whereas, in Lee and Lee (2020) [18], it was sister to a clade containing Compsocerini and Hylotruperini. Oemini, which was originally sister to the other Callidiini clade in Lee and Lee (2020) [18], is now sister to Plesioclytini.

Plesioclytini was proposed as a tribe, based on the morphological characteristics that separated it from Clytini, but their relationship had never been formally discussed. The findings here support the idea that Plesioclytini is in fact its own tribe and clearly not related to Clytini. Taxon sampling was limited by available data. The phylogeny could be strengthened by additional tribes and representatives of tribes that have already been included. There are several diverse tribes (e.g., Hexoplonini, Neoibidionini, and Piezocerini) with little to no publicly available data, which could potentially alter the results. The tribes with only one species included (Heteropsini, Hylotruperini, Oabriini, Oemini, and Xystrocerini) could benefit from more representation. Additional representatives of Oemini could help support the placement of Plesioclytini.

Wappes and Skelley (2015) [14] distinguished Plesioclytini from the Clytini but did not attempt to provide tribal relationships. While preliminary, our molecular results place the Plesioclytini as sister to the Oemini and near to the Tillomorphini. There are several characters that each lineage both share and vary in (Figure 2). The Plesioclytini and Tillomorphini both have finely faceted eyes; procoxal cavities closed or narrowly open behind; procoxal cavities generally not angulate externally and not exposing trochantin; prosternal process broadly expanded behind procoxae; femora clavate, basally narrow and expanded toward the apex; and the antennae variously modified. These two tribes differ, in that the elytra are sclerotized and cover the abdomen and the metafemur attains the elytral apex in the Tillomorphini, while in the Plesioclytini, the elytra are reduced and dehiscent and the hind femur is clearly shorter than the elytral apex. The tribes Oemini and Plesioclytini share a lightly sclerotized and somewhat dehiscent elytra and have the metafemur clearly shorter than the elytral apex. In contrast to the Plesioclytini, the Oemini have coarsely faceted eyes; procoxal cavities widely open behind, with a narrow to extremely reduced prosternal process that is never expanded behind; procoxal cavities angulated externally, exposing trochantin; unmodified femorae; and unmodified antennae.

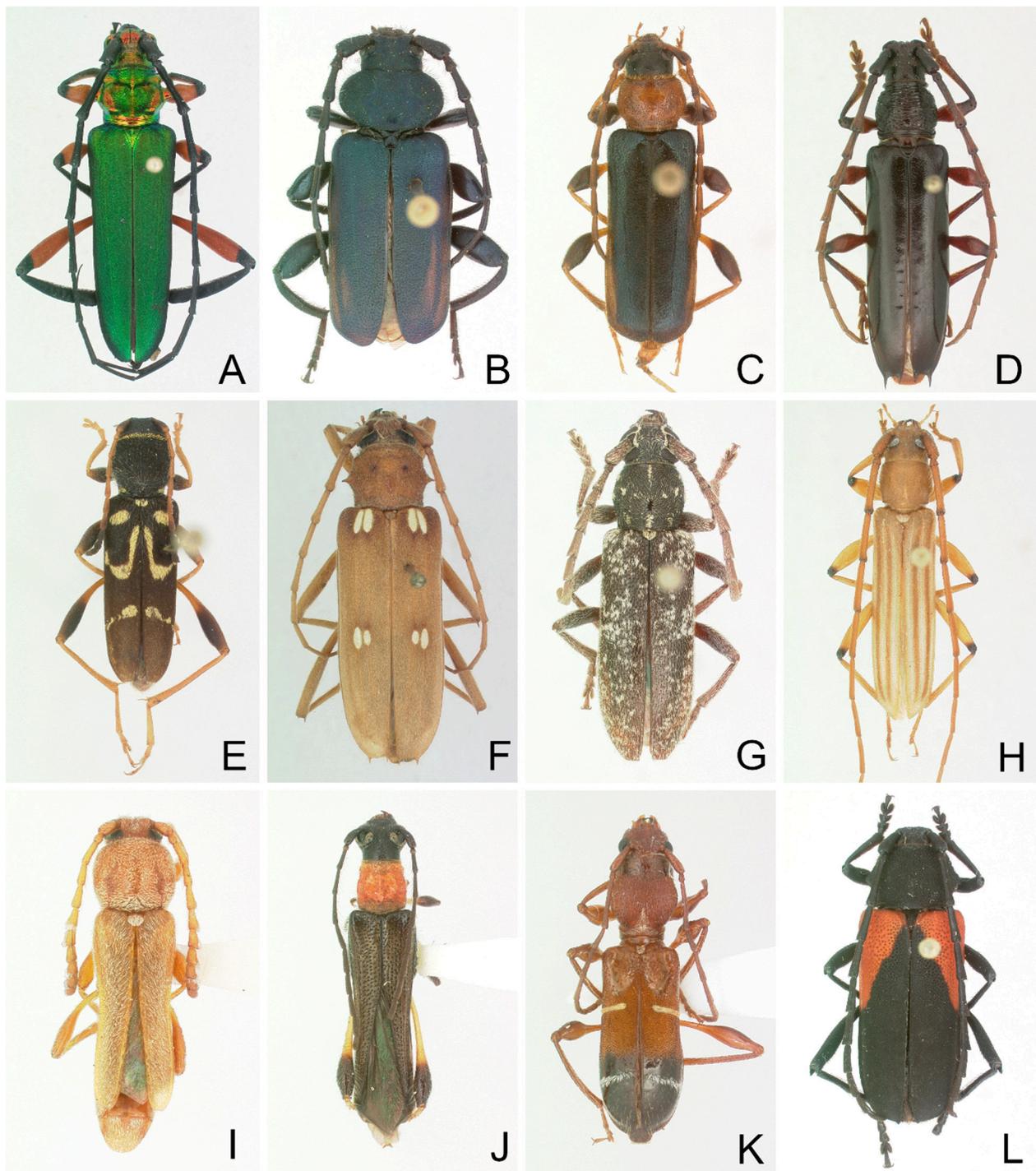


Figure 2. Dorsal habitus of select tribes represented in the phylogeny. (A) Callichromatini—*Plinthocoelium suaveolens* (Linnaeus). (B) Callidiini—*Callidium antennatum* (Newman). (C) Callidiini—*Phymatodes testaceus* (Linnaeus). (D) Cerambycini—*Coleoxestia femorata* (Gounelle). (E) Clytini—*Clytus ruricola* (Olivier). (F) Eburini—*Eburia quadrigemina* (Say). (G) Elaphidiini—*Anelaphus inermis* (Newman). (H) Oemini—*Malacopterus tenellus* (Fabricius). (I) Plesioclytini—*Plesioclytus morrisoni* (Wappes and Skelley). (J) Stenopterini—*Callimoxys ocellaris* (Hammond and Williams). (K) Tillomorphini—*Eudercus pini* (Olivier). (L) Trachyderini—*Purpuricenus humeralis* (Fabricius).

5. Conclusions

Our analyses support the tribal status of both Plesioclytini and Clytini, but do not support a sister group relationship. Further testing with additional tribal and species representation may elucidate higher level relationships.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/d13110597/s1>, Table S1: NCBI Genbank accession numbers for taxa included in the phylogenetic analysis.

Author Contributions: K.E.S. collected specimens that L.N.S. and G.S.P. used to generate sequence data. L.N.S. ran analyses and wrote original manuscript. G.S.P. and K.E.S. conceptualized and designed the project, while S.M.B. supervised. G.S.P., K.E.S., and S.M.B. also wrote, edited, and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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