Testing the Taxonomy of Amphidorini Leconte (Coleoptera: Tenebrionidae): A Molecular Phylogeny Leveraging Museum Sequencing

Authors: Johnston, M. Andrew, Smith, Aaron D., Kanda, Kojun, Kamiński, Marcin J., Naverette, Priscila, et al.

Source: Annales Zoologici, 72(1) : 49-68

Published By: Museum and Institute of Zoology, Polish Academy of Sciences

URL: https://doi.org/10.3161/00034541ANZ2022.72.1.003

ANNALES ZOOLOGICI (Warszawa), 2022, 72(1): 49-68

TESTING THE TAXONOMY OF AMPHIDORINI LECONTE (COLEOPTERA: TENEBRIONIDAE): A MOLECULAR PHYLOGENY LEVERAGING MUSEUM SEQUENCING

M. ANDREW JOHNSTON[1,*], AARON D. SMITH[2,3], KOJUN KANDA[4],
MARCIN J. KAMIŃSKI[2,5], PRISCILA NAVERETTE[3],
LUCIO A. SANCHEZ[3], ROLF L. AALBU[6], KELLY B. MILLER[7],
QUENTIN D. WHEELER[8], and NICO M. FRANZ[1]

[1]Biodiversity Knowledge Integration Center, Arizona State University, Tempe, USA
ORCID: 0000-0002-0166-6985
[2]Purdue University, Department of Entomology, West Lafayette, USA
ORCID: 0000-0002-1286-950X
[3]Northern Arizona University, Department of Biological Sciences, Flagstaff, USA
[4]USDA Systematic Entomology Laboratory, c/o Smithsonian Institution, National Museum of Natural History, Washington, DC, USA / ORCID: 0000-0001-5561-8471
[5]Zoological Museum, Museum and Institute of Zoology, Polish Academy of Sciences, Warszawa, Poland / ORCID: 0000-0002-2915-0614
[6]Department of Entomology, California Academy of Sciences, 55 Music Concourse Dr., San Francisco, California, USA
[7]Department of Biology and Museum of Southwestern Biology, University of New Mexico, Albuquerque, USA
[8]State University of New York, College of Environmental Science and Forestry, Syracuse, USA / ORCID: 0000-0002-9621-1480
**corresponding author: e-mail: ajohnston@asu.edu*

Citation: Johnston, M.A., Smith, A.D., Kanda, K., Kamiński, M.J., Naverette, P., Sanchez, L.A., Aalbu, R.L., Miller, K.B., Wheeler, Q.D., Franz, N.M. 2022. Testing the taxonomy of Amphidorini LeConte (Coleoptera: Tenebrionidae): a molecular phylogeny leveraging museum sequencing. Annales Zoologici, 72: 49–68. doi: 10.3161/00034541ANZ2022.72.1.003

Received: 7 October, 2021 **Accepted**: 20 January, 2022 **Printed**: 30 March 2022

⁂

Abstract.— The tribe Amphidorini LeConte, 1862, commonly known as the Desert Stink Beetles, is a species-rich group of flightless darkling beetles in the subfamily Blaptinae Leach, 1815 distributed throughout the Western Hemisphere and contains 252 valid species-group taxa within seven genera. In this study we provide molecular phylogenetic analyses based on seven loci to assess both the tribal monophyly and composition of Amphidorini as well as the genus and species-group relationships within the tribe. We find strong support for the exclusion of the South American genus *Nycterinus* Eschscholtz, 1829 from the rest of the otherwise North American tribe. *Nycterinus* is recovered in a distantly related clade comprising several Western Hemisphere tribes of Tenebrioninae Latrielle, 1802 and is placed as incertae sedis within that subfamily. Within the remaining 23 genera and subgenera within Amphidorini, 11 were recovered as distinct lineages. The composition and relationships of genera and subgenera of the large genus *Eleodes* Eschscholtz, 1829 are discussed along with the need for an overhaul in genus-group classification which will likely require the elevation to genus of many current constituent lineages of *Eleodes*.

⁂

Key words.— Desert Stink Beetles, *Eleodes*, False Wire Worms, Museomics, *Nycterinus*

PL ISSN 0003-4541 © Museum and Institute of Zoology PAS
doi: 10.3161/00034541ANZ2022.72.1.003

INTRODUCTION

The Desert Stink Beetles of the tribe Amphidorini LeConte, 1862 comprise a conspicuous and diverse element of the Western Hemisphere darkling beetle fauna (Aalbu *et al*. 2002, Bousquet *et al*. 2018). The tribe currently contains 252 valid species-group taxa accommodated in seven genera (Peña 1971, Bousquet *et al*. 2018, Johnston 2019): *Eleodes* Eschscholtz, 1829 (208 species, 24 subspecies), *Eleodimorpha* Blaisdell, 1909 (1 sp.), *Embaphion* Say, 1824 (8 sp., 3 ssp.), *Lariversius* Blaisdell, 1947 (1 sp.), *Neobaphion* Blaisdell, 1925 (4 sp.), *Nycterinus* Eschscholtz, 1829 (20 sp., 2ssp.), and *Trogloderus* LeConte, 1879 (10 sp.). The tribe is distributed in the Western Hemisphere with the vast majority of the diversity generally occurring in the arid western regions of North America (Bousquet *et al*. 2018) and the single genus *Nycterinus* inhabiting southwestern South America (Peña 1971). Species, generic, and tribal concepts within the group have experienced a complex systematic history (Johnston *et al*. 2015) resulting in over 430 available names within its largest genus *Eleodes* (Thomas 2005, Bousquet *et al*. 2018).

Members of Amphidorini are also important economically and as model study systems, especially for insect physiology in arid environments. The larvae, called 'false wireworms,' have been recorded as moderate to severe pests of wheat and other crops in the great plains (Parks 1918, Wade and St. George 1923, Calkins and Kirk 1975, Rogers *et al*. 1988, Quiroga-Murcia *et al*. 2016). Amphidorines, along with other tenebrionids generally, maintain a critical balance between retaining water in their arid habitats and the water needs for the production of defensive chemicals (Hadley 1970, 1972, 1977, Kramm and Kramm 1972, Slobodchikoff 1983, Cooper 1983, 1993). Many studies have relied on this group to investigate thermal and desiccation tolerance (Ahearn and Hadley 1969, Ahearn 1970, Bohm and Hadley 1977, Kenagy and Stevenson 1982, Cooper 1983, 1993), chemical ecology of cuticular and defensive secretions (Roth and Eisner 1962, Tschinkel 1975a,1975b, 1975c, Hadley 1977), and invertebrate distribution patterns across desert ecosystems (Richman *et al*. 1982, Tanner and Packham 1965, Rickard 1971, Thomas 1983, 1984, Quinn *et al*. 1990).

The last comprehensive treatment of Amphidorini was completed by Blaisdell (1909) with recent workers generally reviewing subgenera or regional faunas (Triplehorn 1996, Triplehorn *et al*. 2009, 2015, Aalbu *et al*. 2012, Triplehorn and Thomas 2012, 2015, Johnston *et al*. 2015, Johnston 2015, 2016, 2019). In his revision, Blaisdell (1909) included evolutionary trees summarizing his hypotheses of species relationships – the first such use known to us throughout the systematic entomological literature. Three subsequent works included limited formal phylogenetic assessments: a phenetic morphological analysis of several components of the *Eleodes* subgenus *Blapylis* Horn, 1870 (Somerby 1972), a cladistic analysis of larval morphology for 14 species of *Eleodes* (Smith *et al*. 2014), and a targeted molecular phylogeny of *Trogloderus* (Johnston 2019). Amphidorini exemplars have been included in multiple recent phylogenetic efforts which culminated in its inclusion in the newly resurrected and redefined subfamily Blaptinae Leach, 1815 (Kanda 2017, Kamiński *et al*. 2018, 2021, Lumen *et al*. 2020); however, the very limited taxon sampling in these studies were not adequate to fully test the monophyly of the tribe or any relationships within. Nevertheless, preliminary phylogenetic evidence suggests that the genera and subgenera of Amphidorini as currently circumscribed are not monophyletic (Johnston 2018, 2019, Kamiński *et al*. 2021). The current taxonomic status of the tribe is best represented by the catalogs of North American tenebrionids (Bousquet *et al*. 2018) and global tenebrionid genus-group names (Bouchard *et al*. 2021).

The present study was conducted to provide the first comprehensive assessment of Amphidorini in over 100 years and the first robust phylogeny to be used as a framework to synthesize existing and future component revisions. The goal of this study is to produce a densely sampled species-level phylogeny to test the monophyly of the Amphidorini at the subgeneric, generic and tribal levels.

MATERIAL AND METHODS

Taxon sampling and vouchers

The two above-mentioned goals of this paper were realized by analyzing two separate molecular datasets (Appendix 1 and 2). This approach was selected in order to avoid potential biases caused by the unevenness of taxon sampling between Amphidorini and the remaining tribes in the analysis, which tested the monophyly of the tribe.

Species identifications were performed by MAJ and ADS using recent treatments (Aalbu *et al*. 2012, Triplehorn and Thomas 2012, 2015, Triplehorn *et al*. 2015, Johnston *et al*. 2015, Johnston 2019) in conjunction with examination of primary types representing over 350 nominal taxa within Amphidorini. Species concepts largely follow the recent treatments (cited above) and those of Johnston (2018).

Monophyly of Amphidorini. In order to test the constitution of the tribe, 21 selected representatives of all currently recognised genera were subjected to an analysis together with 23 outgroups from a wide spectrum of other tenebrionid tribes representing Blaptinae and Tenebrioninae (Appendix 1), Namely: two species of Blaptini Leach, 1815, two of Platynotini Mulsant & Rey, 1853, five of Opatrini Brullé, 1832 all representing Blaptinae; two of Amarygmini Gistel, 1848, one of Centronopini Doyen 1989, two of Cerenopini Horn 1870, two of Eulabini, Horn 1870, one of Melanimonini Seidlitz, 1894, two of Scotobiini Solier, 1838, and three of Tenebrionini Latreille, 1802 all classified within Tenebrioninae. The above-mentioned tribes were selected based on historical aspects (taxa treated as closely related by previous authors, e.g. Kamiński *et al*. 2021), general morphological similarity of some of the representatives, and similar distributional patterns. The focus on assessing Amphidorini monophyly limits the use of this dataset for examining broader relationships among Tenebrionidae as a whole and instead tests clustering within the Blaptinae versus with other groups of tenebrionidae. The tribe Melanimonini was used to root these analyses as it has been recovered amongst the earliest diverging members of Tenebrioninae (see Kergoat *et al*. 2014, Kanda 2017).

Relations among Amphidorini. To test the generic and species group relationships within Amphidorini, three outgroups (*Notibius puberulus* LeConte, 1851 and *Blapstinus fortis* LeConte, 1878 of Opatrini and *Blaps mucronata* Latreille, 1804 of Blaptini) were chosen based on the tribal phylogenetic results in order to root a tree including 159 Amphidorini OTUs (Appendix 2). All currently recognized genera were included in the OTU sampling, namely: one *Eleodimorpha*, seven *Embaphion*, three *Lariversius*, three *Neobaphion*, and two *Trogloderus*. A total of 143 OTUs were used for the large genus *Eleodes* which span all currently recognized subgenera: three of *Amphidora* Eschscholtz, 1829; two of *Ardeleodes* Blaisdell, 1937; five of *Blapylis* Horn, 1870; eight of *Caverneleodes* Triplehorn, 1975; one of *Chaseleodes* Thomas, 2015; one of *Cratidus* LeConte, 1862; two of *Discogenia* LeConte, 1866; 29 of *Eleodes* s.s.; one of *Heteropromus* Blaisdell, 1909; nine of *Litheleodes* Blaisdell, 1909; eight of *Melaneleodes* Blaisdell, 1909; four of *Metablapylis* Blaisdell, 1909; five of *Omegeleodes* Triplehorn and Thomas, 2012; 15 of *Promus* LeConte, 1862; seven of *Pseudeleodes* Blaisdell, 1909; 28 of *Steneleodes* Blaisdell, 1909; three of *Tricheleodes* Blaisdell, 1909; and 12 of *incertae sedis* species.

DNA extraction and sequencing for ethanol-preserved specimens

Molecular protocols for extraction and sequencing of DNA follow recent studies of related darkling beetles (Kanda 2017, Kamiński *et al*. 2018, Johnston 2019, Lumen *et al*. 2020), and are summarized below. Genomic DNA was extracted from head capsules or thoracic musculature dissected from beetles, previously stored in ethanol at -20°C, using a DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany,). No cuticle was ground during the extraction process. Seven gene fragments were amplified: 12s mitochondrial ribosomal RNA (12s, 356bp from the open reading frame corresponding to the small subunit ribosomal RNA), 28s ribosomal RNA (28s, 1041bp from the open reading frame corresponding to the large subunit ribosomal RNA in the regions of D1-D3), CAD/rudimentary [CAD2 sensu Moulton and Wiegmann (2004), 750bp of a nuclear protein coding gene), cytochrome c oxidase subunit II (COII, 699bp of a mitochondrial protein coding gene), cytochrome c oxidase subunit I (COI, 822bp of a mitochondrial protein coding gene), histone 3 (H3, 348bp of a nuclear protein coding gene), and wingless (wg, 477bp of a nuclear protein coding gene). The seven loci produced a total alignment length of 4493bp. PCRs were performed using ExTaq (Takara) with primers and thermocycler protocols given in Wild and Maddison (2008), Kanda *et al.* (2015), and Lumen *et al*. (2020). PCR clean-up, quantification, and sequencing were performed by the Genetics Core Facility of the University of Arizona. Sequencing was performed on an Applied Biosystems 3730XL DNA Analyzer.

Museum sequencing

DNA from museum specimens representing five Amphidorini species *Eleodes* (*Caverneleodes*) *easterlai* Triplehorn, 1975, *Eleodes* (*Caverneleodes*) *sprousei* Triplehorn and Reddel, 1991, *Eleodes* (*Pseudeleodes*) *granosa* LeConte, 1866, *Eleodimorpha bolcan* Blaisdell, 1909, and *Neobaphion alleni* Triplehorn, 1989) was extracted under a UV-sterilized laminar flow hood with dedicated equipment using QIAamp DNA Micro kits (Qiagen) following the manufacturer's protocol with the addition of carrier RNA (for details see Kanda *et al*. 2015). Extractions were made from abdomens, without grinding the cuticle, to minimize damage to specimens. As the obtained DNA was already fragmented, no further fragmentation was required before library preparation. Libraries were built using the NEBNext Ultra II DNA Library Prep kits (New England BioLabs), following the manufacturer's protocols and barcoded using NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1). Libraries were sequenced using a HiSeq3000 (Illumina) maintained by Oregon State University Center for Genomic Research and Biocomputing (CGRB). Each sample was given roughly a tenth of a 150 bp end lane.

Phylogenetic inference methods

All loci were aligned using MAFFT v. 7.130b (Katoh and Standley 2013) as implemented through Mesquite v. 3.11 (Maddison and Maddison 2018), visually inspected for accuracy, and placed into coding frame for protein coding genes to inspect for stop codons or possible paralogs and to allow for testing partition strategies discussed below (Kanda *et al*. 2015, Kanda 2017). Alignments were further verified by generating gene trees for each locus to identify and remove any highly anomalous branches potentially corresponding to sequencing errors or paralogs. The final alignments made across all taxa were then used to create two datasets to test Amphidorini tribal composition along with diverse tenebrionid outgroups and intra-tribal generic and species relationships, respectively.

Monophyly of Amphidorini. This dataset was separated into two partition schemes using the best scheme recovered by PartitionFinder (version 2, Lanfear *et al*. 2017) under both linked and unlinked branch-length constraints. Phylogenetic inference was performed using both maximum likelihood and Bayesian inference on each of the two partition schemes. RAxML 8.2 (Stamatakis 2014) was used to perform 10 replicates to find the best-scoring tree and to generate 1000 rapid bootstrap replicates, with the bootstrap scores mapped to each branch of the best tree. MrBayes 3.2 (Ronquist and Huelsenbeck 2003) was run using four chains for 10 million generations and sampled every 1000 generations with the following parameters unlinked across partitions: transition/transversion rate, substitution rates, character state frequencies, proportion of invariable sites, and gamma shape. The first 25% of trees were discarded as burnin. The inferred tribal relationships were identical for both schemes but the four-partition unlinked branch-length scheme is preferred and presented below to be consistent with the within-Amphidorini analyses discussed below.

Relationships among Amphidorini. This dataset was similarly analyzed using PartitionFinder under linked and unlinked branch-length constraints and using both resultant schemes for both RAxML and MrBayes analyses as outlined above for the first dataset.

Data availability

Sequence alignments, partition analysis, and phylogenetic trees from all inferences are available for download as a published dataset (Johnston 2022). All newly generated sequence data are available through NCBI's GenBank (OM745750–OM745894, OM746966–OM747533).

RESULTS

Amphidorini monophyly and composition

Maximum likelihood and Bayesian analyses recovered highly congruent topologies for the tribal monophyly dataset (Fig. 1) across partitioning schemes (see Johnston 2022). Amphidorini was recovered in two distinct clades. First, the North American components formed an expected monophyletic lineage sister to the palearctic Blaptini within the reinstated concept of Blaptinae (Kamiński *et al*. 2021). *Nycterinus* was recovered in a strongly supported clade, which we here refer to as the 'scotobiine clade', including the tribes Scotobiini, Eulabini, Cerenopini, and Tenebrionini (in part).

Amphidorini generic composition and relationships

Congruent topologies were similarly recovered between maximum likelihood and Bayesian analysis for generic and species relationships within the tribe.

Analyses were sensitive to partitioning scheme, particularly in regard to the branching pattern of the deepest nodes within Amphidorini. The general trend is one of numerous generally well-supported clades which more or less correspond to current genera and subgenera whose relationships to each other are represented by shorter branches with poor support. Topology incongruence primarily revolved around the unsupported early-diverging relationships. Here, and for the purpose of the discussion, we present the topology inferred from the unlinked branch length partition scheme (Figs. 2–4). Four higher level groupings, highlighted in Figure 2, broadly correspond to clades recovered from phylotranscriptomic analyses (Johnston 2018). These are the "embaphion clade," "blapylis clade," "metablapylis group," and "core-eleodes clade." Among these, we recover very strong support for the monophyly of the core-eleodes clade, which contains the type species of *Eleodes* and the bulk of the species-level diversity currently placed within the genus, and for the embaphion clade, which strongly supports the monophyly of the genera *Embaphion* and *Neobaphion*. The monophyly of the blapylis clade and the metablapylis group is more equivocal and the relationships between all four of these clades are not supported and vary based on partitioning scheme (see published dataset Johnston 2022). In particular, some members of the metablapylis group were recovered as early diverging lineages in a grade leading to the rest of the tribe; however, since neither of the topologies are supported for the placement of these taxa we refer to them as a group instead of a clade in recognition that

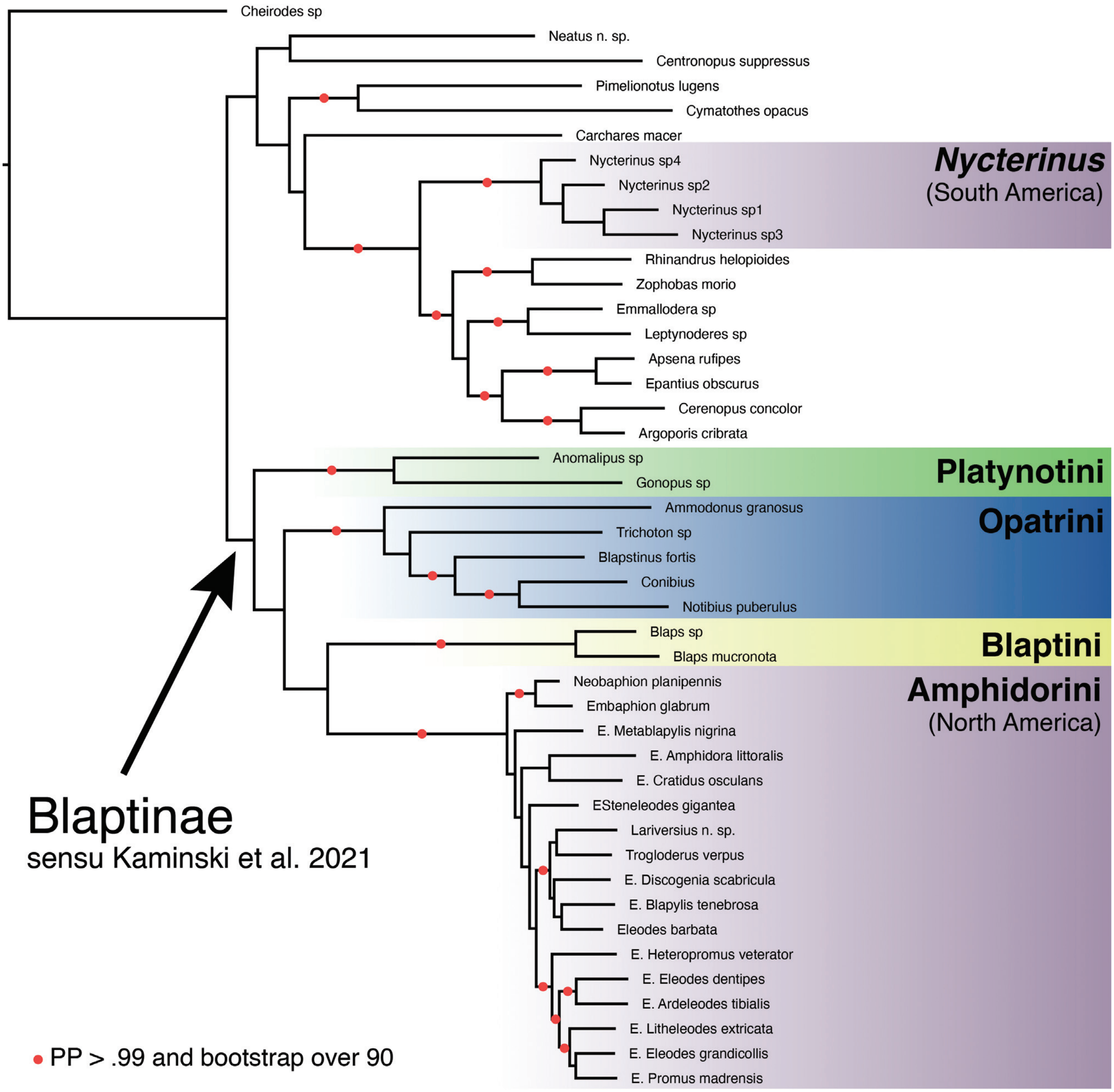

Figure 1. Recovered phylogeny for the Monophyly of Amphidorini dataset. Topology shown corresponds to the maximum likelihood reconstruction highlighting the two unrelated clades of Amphidorini and the three other Blaptinae tribes sampled. Branches marked with a red dot were recovered in both Bayesian inference and maximum likelihood analyses with at least .99 posterior probabilities and 90 bootstrap values, respectively.

their monophyly is dubious. Table 1 summarizes the topologies recovered by each of the analyses.

Out of the 19 non-monotypic genus-group taxa, as defined by Bousquet *et al.* (2018), only seven were recovered as monophyletic: the genera *Embaphion* and *Trogloderus* and the *Eleodes* subgenera *Blapylis*, *Discogenia*, *Melaneleodes*, *Pseudeleodes*, and *Steneleodes*. Relationships and composition of the constituent genera and subgenera are discussed in more detail below.

DISCUSSION

Placement of *Nycterinus*

Nycterinus is hereby placed as *incertae sedis* within the subfamily Tenebrioninae. Taxon sampling across Tenebrionidae was not dense enough to draw major conclusions about extralimital tribal and subfamilial composition, but several relationships are

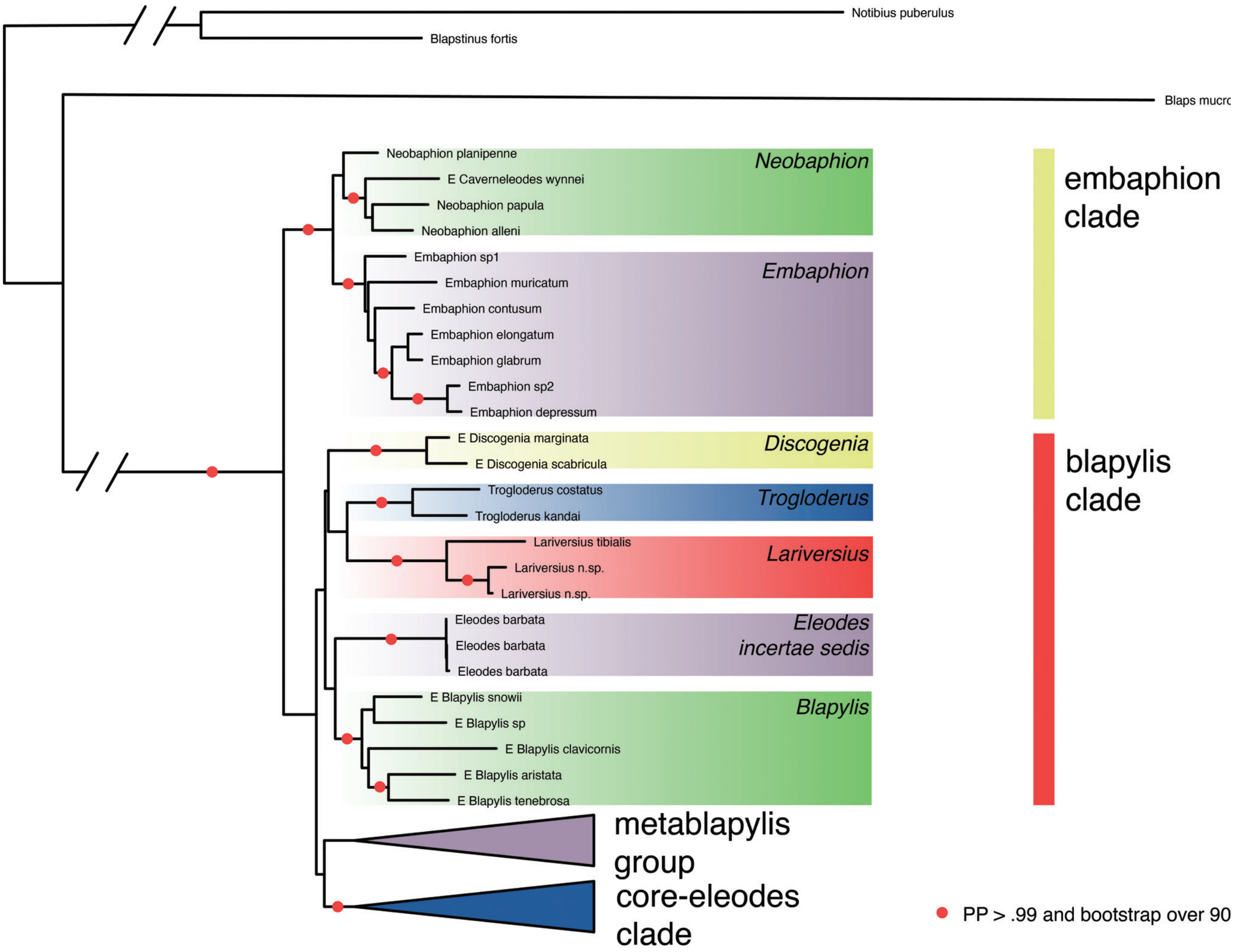

Figure 2. Among-Amphidorini Phylogeny. Topology shown corresponds to the maximum likelihood reconstruction showing the four major clades recovered within Amphidorini. Branches marked with a red dot were recovered in both Bayesian inference and maximum likelihood analyses with at least .99 posterior probabilities and 90 bootstrap values, respectively.

significant to point out within the scotobiine clade. First, the representatives of Tenebrionini did not form a monophyletic lineage (Fig. 1), and indeed the widespread Western Hemisphere genera *Rhinandrus* LeConte, 1866 and *Zophobas* Dejean, 1834 show affinities to *Nycterinus* along with the Western Hemisphere tribes Cerenopini, Eulabini, and Scotobiini. This is somewhat unsurprising given ambiguous definitions of many Western Hemisphere lineages that have not been reviewed recently (Johnston *et al*. 2020) and because *Nycterinus* was recovered near a composite 'Tenebrionini' taxon based on morphological cladistic and phenetic analyses (Doyen and Tschinkel 1982).

The clade containing *Nycterinus* can generally be recognized in the Western Hemisphere externally by the generally elongate head capsule and compound antennal sensoria and internally by possessing pleated defensive glands and lacking a spherical spermatheca (Berry 1973, Doyen and Tschinkel 1982, Silvestro *et al*. 2015, Johnston *et al*. 2020). This group has historically been associated with the Eastern-Hemisphere Scaurini Billberg, 1820 or the global Tenebrionini. More data are needed to understand the exact nature of the relationships within this group, but the defensive glands and antennal sensoria clearly exclude them from Blaptinae and place them near more derived groups of the Tenebrioninae and Stenochiinae Kirby, 1837 (Kanda 2017, Johnston *et al*. 2020).

Genus-group composition

The lack of branch support at the base of Amphidorini complicates the delimitation of genera based on molecular data alone. The current broad concept of

Table 1. Summary of major topological elements by analysis. Major groups are annotated as monophyletic or not and the earliest diverging clade sister to the rest of the tribe is indicated.

Analysis	embaphion clade	blapylis clade	metablapylis group	core eleodes clade	Earliest diverging
Unlinked, 2 partitions					
RAxML - ML	Yes	Yes	Yes	Yes	embaphion clade
Majority Rule Bootstrap Consensus	Yes	Yes		Yes	n/a
MrBayes	Yes	Yes	all but *Tricheleodes*	Yes	embaphion clade
Linked, 9 partitions					
RAxML - ML	Yes	Yes		Yes	Amphidora
Majority Rule Bootstrap Consensus	Yes	Yes		Yes	n/a
MrBayes	Yes	Yes	all but *Tricheleodes*	Yes	embaphion clade

Eleodes spans three of the four groups defined above (Fig. 2), with the embaphion clade being the only exception, and if maintained would require the inclusion of the genera *Trogloderus*, *Lariversius*, and *Eleodimorpha*. However, there is no unequivocal support for the sister relationship of the embaphion clade to the remainder of the Amphidorini. This has strong nomenclatural ramifications because *Embaphion* is the oldest available genus-group name within the tribe and would take priority over *Eleodes* were an even broader concept of that genus to be adopted. It is clear that the currently recognized six genera do not sufficiently convey the evolutionary history of the group, though taxonomic changes will require significant morphological investigation (Johnston and Smith, in prep.). Each of the four main groups are further discussed with regards to their main subcomponents.

The embaphion clade (Fig. 2) comprises two well-supported lineages. The monophyletic *Embaphion* is consistent with the historically recognized group which is diagnosable by its upturned pronotal margins and ovipositor morphology (Blaisdell 1909, Johnston *et al*. 2015). Sister to the latter genus is *Neobaphion*, which was erected for species with ovipositor morphology similar to *Embaphion* but the body habitus resembling *Eleodes* (Blaisdell 1925, Triplehorn and Aalbu 1985, Johnston *et al*. 2015). Notably, the species *Eleodes wynnei* Aalbu, Smith and Triplehorn, 2012 is recovered in this clade with strong support. The latter species was described in the subgenus *Caverneleodes* which is herein recovered as polyphyletic and discussed in more detail below. Museum sequencing of *Neobaphion alleni*, currently known only from the five specimens of the type series, confirmed the generic placement of this rare species and provides insight for future revisionary studies on a genus where most species are relatively uncommon in collections.

The blapylis clade (Fig. 2) comprises five lineages which range from the Rocky mountains to the Pacific coast, not ranging into the Great Plains or arid reaches of continental Mexico. *Trogloderus*, *Lariverisus*, and *Eleodes barbata* Wickham, 1918 are all strongly associated with sandy substrates and dunes in the Intermountain Region (Johnston 2019). The latter species was recently placed as *incertae sedis* within *Eleodes* due to a lack of morphological similarity with any other known subgenera (Johnston 2016). The subgenera *Blapylis* and *Discogenia* are each recovered as well-supported monophyletic lineages which are consistent with their historical recognition (Blaisdell 1909, Somerby 1972, Bousquet *et al*. 2018). This clade was recovered in every analysis though with variable statistical support. Female ovipositor morphology and integument sculpturing seem to further unite these taxa morphologically.

The heterogeneous metablapylis group (Fig. 3) consists of six well-supported lineages which span most of the geographic range of the tribe, though its monophyly is not well supported and different topologies are found across different analyses (see dataset in Johnston 2022). This group is in part morphologically supported by most members bearing a wide and evenly tapered elytral epipleuron which is not found in any of the other clades defined here. The largest component lineage corresponds to the *Eleodes* subgenus *Steneleodes* which is well established based on female ovipositor morphology and is particularly diverse in central Mexico (Blaisdell 1909, Bousquet *et al*. 2018). The *Eleodes* subgenus *Metablapylis* is recovered in a strongly supported clade intermixed with most sampled members of the subgenus *Caverneleodes*. Museum sequencing of the latter's type species, *Eleodes easterlai* Triplehorn, crucially places it within this clade; hence, a detailed morphological reexamination

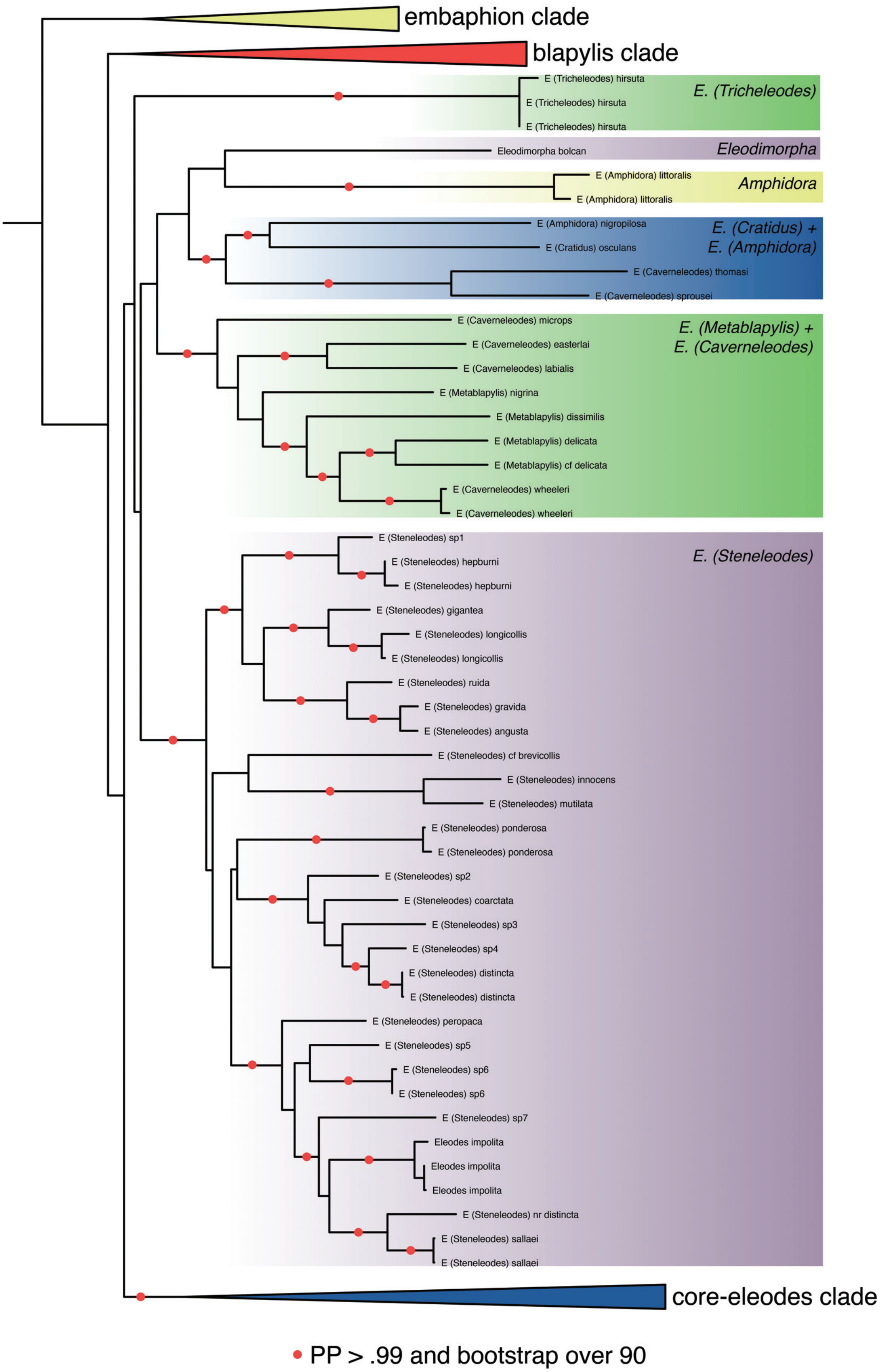

Figure 3. Among-Amphidorini phylogeny continued. Topology shown is the same as Fig. 2 with the metablapylis group expanded. Branches marked with a red dot were recovered in both Bayesian inference and maximum likelihood analyses with at least .99 posterior probabilities and 90 bootstrap values, respectively.

and characterization is recommended for these taxonomic groups. Museum sequencing further confirmed the distinct monotypic lineage *Eleodimorpha*, which was recovered sister to a monotypic lineage of the type species of the current *Eleodes* subgenus and tribal basionym *Amphidora*, both of which are restricted to California. Placement of the monotypic *Eleodes* subgenus *Tricheleodes* was unstable across all analyses and its inclusion in the metablapylis group is dubious. However, the morphological circumscription by Johnston (2016) is supported. The final lineage in this group consists of the *Eleodes* subgenus *Cratidus*, including part of the current subgenus *Amphidora* as a sister lineage to two current members of the subgenus *Caverneleodes*, including the museum-sequenced *E.* (*Caverneleodes*) *sprousei* along with *E.* (*Caverneleodes*) *thomasi* Aalbu, Smith & Triplehorn 2012. This clade is well supported in the analyses but has not been recognized in previous taxonomic treatments within Amphidorini and warrants further examination to potentially identify morphological synapomorphies shared between the species.

The core-eleodes clade (Fig. 4) encompasses the bulk of what is currently treated within the genus *Eleodes*, including all sampled members of nine subgenera. The monophyly of this clade is very strongly supported, as are many of its constituent component clades, yet we again see a lack of support for many of the relationships between these components. In addition to the monotypic subgenera *Ardeleodes* and *Heteropromus*, the recently reviewed subgenera *Pseudeleodes* (see Johnston 2016) and *Melaneleodes* (see Triplehorn and Thomas 2012) were the only previously recognized groups recovered as monophyletic. The nominate subgenus *Eleodes* was recovered as polyphyletic, with the California-restricted type species *Eleodes dentipes* Eschscholtz, 1829 recovered in a small clade with several species which range southward into Baja California. The remainder of the nominate subgenus was recovered as a paraphyletic grade which includes components of the subgenera *Promus* and *Litheleodes*. The latter subgenera were recovered as polyphyletic, with *Litheleodes*, as historically defined and recently revised by Triplehorn and Thomas (2015), largely nested within *Promus* except for its most atypical member *Eleodes arcuata* Casey, 1884 (Triplehorn and Thomas 2015) representing an early-diverging lineage within the core-eleodes clade. *Promus* was defined for a number of species distributed in the United States (Blaisdell 1909, Johnston 2015) and was expanded to include a large number of species from Mexico in a worldwide catalog for the family (Gebien 1938). The species from the United States, including the type *Eleodes opaca* (Say, 1824) were recovered with the bulk of *Litheleodes*, whereas a large portion of the later included Mexican species were recovered in a strongly supported clade that also includes the currently monotypic *Omegeleodes*.

Evolutionary insights

The phylogeny presented here may provide some insights to the origins and biogeographic trends within Amphidorini, though phylogenetic dating and historical biogeographic analyses are complicated by the lack of reliable fossil calibrations. The exclusion of *Nycterinus* and sister relationship between the western North American Amphidorini and Palearctic Blaptini suggests the origin of Amphidorini was due to either a vicariance event across Laurasia or a subsequent dispersal event to the Nearctic, perhaps through a land bridge. A broader phylogenetic study within the family is necessary in order to infer lineage ages required for testing either of these hypotheses. In addition, Kamiński *et al*. (2021) recovered Amphidorini sister to (Blaptini + Platyscelidini Lacordaire, 1859), the latter being a Palearctic lineage not included in our analyses which would be critical to include for understanding the origins of these groups. A dispersal event from the Palearctic may be further supported by most of Amphidorini's lineages with a center of diversity found in the far western regions of the United States from the California Floristic province and Intermountain Region, i.e. embaphion clade: *Neobaphion*, *Embaphion*; blapylis clade: *Trogloderus*, *Lariversius*, *E. barbata*, *Blapylis*; metablapylis clade: *Tricheleodes*, *Eleodimorpha*, *Amphidora*, *Cratidus*, *Metablapylis*; core-eleodes clade: *Litheleodes*, *Eleodes* (*Eleodes*) (Bousquet *et al*. 2018, Johnston 2019). The lineages that inhabit the eastern and southern range of Amphidorini are primarily more derived groups within the core-eleodes clade that also have some species present in the more western regions, i.e. the *Eleodes* subgenera *Melaneleodes*, *Omegeleodes*, and *Promus*.

Morphological character systems historically used to classify Amphidorini groups need to be reevaluated in the context of this phylogeny. The primary system for generic and subgeneric placement used by Blaisdell (1909) and carried on through recent works is the female ovipositor. Species placements based on ovipositor morphology were largely supported across our phylogeny, but placements based upon external characters (e.g., cavernicoly and long legs in *Caverneleodes* see Triplehorn 1975 and Aalbu *et al*. 2012) and via cataloguing efforts instead of revisionary works (e.g., *Promus* see Johnston 2015) were not. Previously used external diagnostic characters may yet provide some utility in identifications, but are

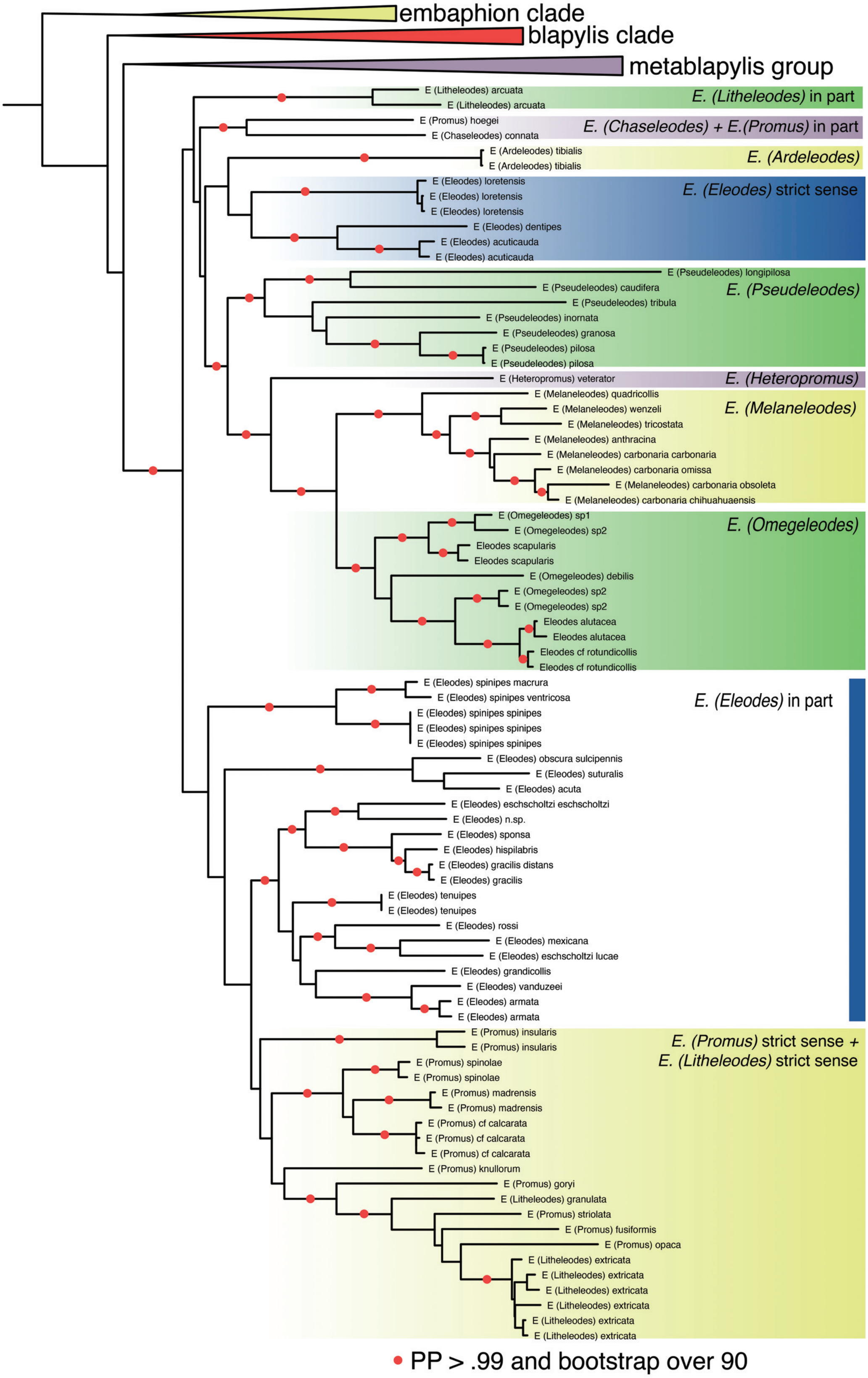

Figure 4. Among-Amphidorini phylogeny continued. Topology shown is the same as Fig. 2 with the core-eleodes clade expanded. Branches marked with a red dot were recovered in both Bayesian inference and maximum likelihood analyses with at least .99 posterior probabilities and 90 bootstrap values, respectively.

nearly all homoplasious across the tree, including males with profemoral spines (found in e.g., *Cratidus*, *Trogloderus*, *Eleodes* (*Eleodes*), some *Eleodes* (*Promus*)), all femora with spines (found in e.g., *E. armata* LeConte, 1851 and *E. loretensis* Blaisdell, 1923), and dense protarsal setal pads (found in e.g., *Eleodes* subgenera *Amphidora*, *Blapylis*, and *Promus*).

The phylogenetic hypotheses presented in this paper are strongly divergent from current taxonomy. To illuminate this phylogeny/classification gap (Franz 2005), Table 2 summarizes the current taxonomic classification and the likely changes needed to address the inferences discussed above. We look forward to future studies that reconcile this molecular phylogenetic scaffold with the rich legacy of Amphidorini morphology to robustly circum-scribe and diagnose composite genera and subgenera.

Table 2. Summary of current Amphidorini classification following the catalog of Bousquet *et al.* (2018) according to the molecular phylogenetic reconstruction presented here.

Current classification (Bousquet *et al.* 2018)			Phylogenetic assessment		
Genus	Subgenus	Valid species	Major clade	Monophyly	Remarks
Eleodes		208	–	–	
	Amphidora	3	metablapylis group	–	type species is unrelated to all others
	Ardeleodes	1	core eleodes clade	monophyletic	
	Blapylis	51	blapylis clade	monophyletic	
	Caverneleodes	12	–	–	widely polyphyletic, type species belongs in *Metablapylis*
	Chaseleodes	2	core eleodes clade	paraphyletic	likely must accommodate more species
	Cratidus	2	metablapylis group	–	likely includes most *Amphidora* species
	Discogenia	2	blapylis clade	monophyletic	
	Eleodes	30	core eleodes clade	–	type species is unrelated to most others
	Heteropromus	1	core eleodes clade	monophyletic	
	Litheleodes	9	core eleodes clade	–	type and most species belong within *Promus*
	Melaneleodes	12	core eleodes clade	monophyletic	
	Metablapylis	6	metablapylis group	paraphyletic	includes type and most species of *Caverneleodes*
	Omegeleodes	1	core eleodes clade	'paraphyletic'	this monotypic subgenus must be expanded greatly
	Promus	21	core eleodes clade	paraphyletic	type and most species form a clade that includes *Litheleodes*
	Pseudeleodes	8	core eleodes clade	monophyletic	
	Steneleodes	28	metablapylis group	monophyletic	
	Tricheleodes	1	metablapylis group	monophyletic	
	incertae sedis	18	–	–	
Eleodimorpha		1	metablapylis group	monophyletic	
Embaphion		8	embaphion clade	monophyletic	
Lariversius		1	blapylis clade	monophyletic	
Neobaphion		4	embaphion clade	paraphyletic	includes one *Caverneleodes* species
Trogloderus		10	blapylis clade	monophyletic	

ACKNOWLEDGEMENTS

We are thankful for the support of all the curators and collections managers who have helped us during museum visits to see type specimens and develop taxon concepts for the species identified in this study. Two anonymous reviewers gave very helpful feedback and improved this manuscript greatly. This research was supported by the National Sciences Foundation (DEB–1258154/ 1523605/ 1612282) and by the National Science Centre, Poland (OPUS 19 #2020/37/B/NZ8/ 02496 project).

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture (USDA). The USDA is an equal opportunity provider and employer.

REFERENCES

Aalbu, R.L., A.D. Smith, and C.A. Triplehorn. 2012. A revision of the *Eleodes* (subgenus *Caverneleodes*) with new species and notes on cave breeding *Eleodes* (Tenebrionidae: Amphidorini). Annales Zoologici (Warszawa), 62: 199–216. https://doi.org/10.3161/000345412X652729.

Aalbu, R.L., C.A. Triplehorn, J.M. Campbell, K.W. Brown, R.E. Somerby, and D.B. Thomas. 2002. 106. Tenebrionidae Latreille 1802. *In*: Arnett R.H., Thomas M.C., Skelley P.E., Frank J.H. (Eds). American beetles. Volume 2. Polyphaga: Scarabaeoidea through Curculionoidea. CRC Press, Boca Raton, 463–509.

Ahearn, G.A. 1970. The control of water loss in desert tenebrionid beetles. Journal of Experimental Biology, 53: 573–595.

Ahearn, G.A. and N.F. Hadley. 1969. The effects of temperature and humidity on water loss in two desert tenebrionid beetles *Eleodes armata* and *Cryptoglossa verrucosa*. Comparative Biochemistry and Physiology, 30: 739–749.

Berry, R.L. 1973. The Cerenopini and Eulabini, two tribes previously included in the Scaurini (Coleoptera: Tenebrionidae). Annals of the Entomological Society of America, 66(1): 70–77.

Blaisdell, F.E. 1909. A monographic revision of the Coleoptera belonging to the tenebrionide tribe Eleodiini inhabiting the United States, Lower California, and adjacent islands. Bulletin of the United States Museum, No. 63., vi + 524 pp. (+ 13 pls). https://doi.org/10.5962/bhl.title.48543.

Blaisdell, F.E. 1925. Studies in the Tenebrionidae, No. 2 (Coleoptera). Proceedings of the California Academy of Sciences, 14(16): 369–390.

Bohm, B.C. and N.F. Hadley. 1977. Tritium- determined water flux in the free-roaming desert tenebrionid beetle, *Eleodes armata*. Ecology, 58: 407–414.

Bouchard, P.B., Y. Bousquet, R.L. Aalbu, M.A. Alonso-Zarazaga, O. Merkel, and A.E. Davies. 2021. Review of the genus-group names in the family Tenebrionidae (Insecta, Coleoptera). ZooKeys, 1050: 1–633. https://doi.org/10.3897/zookeys.1050.64217.

Bousquet, Y., D.B. Thomas, P. Bouchard, A.D. Smith, R.L. Aalbu, M.A. Johnston, W.E. Steiner Jr. 2018. Catalogue of Tenebrionidae (Coleoptera) of North America. ZooKeys, 728: 1–455. https://doi.org/10.3897/zookeys.728.20602.

Calkins, C.O. and V.M. Kirk. 1975. False wireworms bear watching: a repeat performance? South Dakota Farm & Home Research, 26: 1–15.

Cooper, P.D. 1983. Components of evaporative water loss in desert tenebrionid beetles *Eleodes armata* and *Cryptoglossa verrucosa*. Physiological Zoology, 56: 47–55.

Cooper, P.D. 1993. Field metabolic rate and cost of activity in two tenebrionid beetles from the Mojave Desert of North America. Journal of Arid Environments, 24: 165–175.

Doyen, J.T. and W.R. Tschinkel. 1982. Phenetic and cladistics relationships among tenebrionid beetles (Coleoptera). Systematic Entomology, 7(2): 127–183. https://doi.org/10.1111/j.1365-3113.1982.tb00129.x.

Franz, N.M. 2005. On the lack of good scientific reasons for the growing phylogeny/classification gap. Cladistics, 21(5): 495–500. https://doi.org/10.1111/j.1096-0031.2005.00080.x.

Gebien, H. 1938. Katalog der Tenebrioniden. Teil II. Mitteilungen der Münchener Entomologischen Gesellschaft, 28: 49–80, 283–428.

Hadley, N.F. 1970. Micrometeorology and energy exchange in two desert arthropods. Ecology, 51: 434–444.

Hadley, N.F. 1972. Desert species and adaptation. American Scientist, 60: 338–347.

Hadley, N.F. 1977. Epicuticular lipids of the desert tenebrionid beetle, *Eleodes armata*: seasonal and acclimatory effects on composition. Insect Biochemistry, 7: 277–283.

Johnston, M.A. 2015. A checklist and new species of *Eleodes* Eschscholtz (Coleoptera: Tenebrionidae) pertaining to the Subgenus *Promus* Leconte, with a key to United States species. The Coleopterists Bulletin, 69: 11–19. https://doi.org/10.1649/0010-065X-69.1.11.

Johnston, M.A. 2016. Redefinition of the *Eleodes* Eschscholtz subgenera *Tricheleodes* Blaisdell and *Pseudeleodes* Blaisdell, with the description of a new species (Coleoptera: Tenebrionidae). Annales Zoologici (Warszawa), 66(4): 665–679. https://doi.org/10.3161/00034541ANZ2016.66.4.018.

Johnston, M.A. 2018. Diversity and distribution of the desert stink beetles: Systematics of the Amphidorini LeConte, 1862 (Coleoptera: Tenebrionidae). PhD Thesis, Arizona State University, 226 pp. https://repository.asu.edu/items/51646.

Johnston, M.A. 2019. Phylogenetic revision of the psammophilic *Trolgoderus* LeConte (Coleoptera: Tenebrionidae), with biogeographic implications for the Intermountain Region. Peerj 7:e8039 https://doi.org/10.7717/peerj.8039.

Johnston, M.A. 2022. Molecular Phylogeny of Amphidorini (Coleoptera: Tenebrionidae). https://doi.org/10.5281/zenodo.5829502.

Johnston, M.A., D. Fleming, N.M. Franz, and A.D. Smith. 2015. Amphidorini Leconte (Coleoptera: Tenebrionidae) of Arizona: Keys and species accounts. The Coleopterists Bulletin, 69: 27–54. https://doi.org/10.1649/0010-065x-69.mo4.27.

Johnston, M.A., A.D. Smith, K. Matsumoto, and M.J. Kamiński. 2020. On the taxonomic placement of *Penichrus* Champion, 1885 and a synopsis of North American Opatrini (Coleoptera: Tenebrionidae: Blaptinae). Annales Zoologici

(Warszawa), 70(4): 765–774. https://doi.org/10.3161/00034541ANZ2020.70.4.017.

Kamiński, M.J., K. Kanda, R. Lumen, A.D. Smith, and D. Iwan. 2018. Molecular phylogeny of Pedinini (Coleoptera: Tenebrionidae) and its implications for higher-level classification. Zoological Journal of the Linnean Society, zly033. https://doi.org/10.1093/zoolinnean/zly033.

Kamiński, M. J., Lumen, R., Kanda, K., Iwan, D., Johnston, M. A., Kergoat, G., Bouchard, P., Bai, X-L., Li, X.-M., Ren, G.-D., and A. D. Smith. 2021. Reevaluation of Blapimorpha and Opatrinae: addressing a major phylogeny-classification gap in darkling beetles (Coleoptera: Tenebrionidae: Blaptinae). Systematic Entomology, 46(1): 140–156. https://doi.org/10.1111/syen.12453.

Kanda, K. 2017. Phylogenetic studies in Tenebrionidae (Coleoptera) and related families. Ph.D. thesis, Oregon State University, 265 pp. https://ir.library.oregonstate.edu/concern/graduate_thesis_or_dissertations/qj72pd34k.

Kanda, K., J.M. Pflug, J.S. Sproul, M.A. Dasenko, and D.R. Maddison. 2015. Successful Recovery of Nuclear Protein-Coding Genes from Small Insects in Museums Using Illumina Sequencing. PLoS ONE, 10(12): e0143929. https://doi.org/10.1371/journal.pone.0143929.

Katoh, K. and D.M. Standley. 2013. MAFFT Multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution, 30(4): 772–780. https://doi.org/10.1093/molbev/mst010.

Kenagy, G.J. and R.D. Stevenson. 1982. Role of body temperature in the seasonality of daily activity in tenebrionid beetles of eastern Washington. Ecology, 63: 1491–1503.

Kergoat, G.J., L. Soldati, A-L., Clamens, H. Jourdan, R. Jabbour-Zahab, G. Genson, P. Bouchard, and F.L. Condamine. 2014. Higher level molecular phylogeny of darkling beetles (Coleoptera: Tenebrionidae). Systematic Entomology, 39(3): 486–499. https://doi.org/10.1111/syen.12065.

Kramm, R.A. and K.R. Kramm. 1972. Activities of certain species of *Eleodes* in relation to temperature, season and time of day at Joshua Tree National Monument (Coleoptera: Tenebrionidae). Southwestern Naturalist, 16: 42–47.

Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T., and B. Calcott. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Molecular biology and evolution, 34(3): 772–773. https://doi.org/10.1093/molbev/msw260.

Lumen, L., K. Kanda, D. Iwan, A.D. Smith, and M.J. Kamiński. 2020. Molecular insights into the phylogeny of Blapstinina (Coleoptera: Tenebrionidae: Opatrini). Systematic Entomology, 45(2): 337–348. https://doi.org/10.1111/syen.12398.

Maddison, W. P. and D.R. Maddison. 2018. Mesquite: a modular system for evolutionary analysis. Version 3.61, http://www.mesquiteproject.org.

Moulton, J.K. and B.M. Wiegmann. 2004. Evolution and phylogenetic utility of CAD (rudimentary) among Mesozoic-aged Eremoneuran Diptera (Insecta). Molecular Phylogenetics and Evolution, 31: 363–378.

Parks, H.B. 1918. Notes on *Eleodes tricostata* Say. Journal of Economic Entomology, 11: 388.

Peña, L.E. 1971. Revisión del género *Nycterinus* Eschscholtz 1829 (Coleoptera – Tenebrionidae. Boletín de Museo Nacional de Historia Natural (Chile), 32: 159–172.

Quinn, M.A., R.L. Kepner, D.D. Walgenbach, R.N. Foster, R.A. Bohls, P.D. Pooler, K.C. Reuter, and J.L. Swain. 1990. Effect of habitat and perturbation on populations and community structure of darkling beetles (Coleoptera: Tenebrionidae) on mixed-grass rangeland. Environmental Entomology, 19: 1746–1755.

Quiroga-Murcia, D. E., I. Zenne de Polanía, and F.J. Posada-Flórez. 2016. Preliminary evaluation of pathogens affecting *Eleodes longicollis punctigerus* Blaisdell (Coleoptera: Tenebrionidae). Revista U.D.C.A Actualidad & Divulgacion Cientifica, 19(1): 37–43.

Rickard, W.H. 1971. Observations on the distribution of *Eleodes hispilabris* (Say) (Coleoptera: Tenebrionidae) in relation to elevation and temperature in the Rattlesnake Hills. American Midland Naturalist, 85: 521–526.

Richman, D.B., E.W. Huddleston, and M. Ortiz. 1982. Seasonal activity of tenebrionid beetles in New Mexico mesquite dunes. Southwestern Naturalist, 27: 305–308.

Rogers, L.E., N.E. Woodley, J.K. Sheldon, and P.A. Beedlow. 1988. Diets of darkling beetles (Coleoptera: Tenebrionidae) within a shrub- steppe ecosystem. Annals of the Entomological Society of America, 81: 782–791.

Ronquist, F. and J.P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19(12): 1572–1574. https://doi.org/10.1093/bioinformatics/btg180.

Roth, L.M. and T. Eisner. 1962. Chemical defenses of Arthropods. Annual Review of Entomology, 7: 107–136.

Slobodchikoff, C.N. 1983. Water balance and temperature preferences, and their role in regulating activity times of tenebrionid beetles. Oikos, 40: 113–119.

Smith, A.D., R. Dornburg, and Q.D. Wheeler. 2014. Larvae of the genus *Eleodes* (Coleoptera, Tenebrionidae): matrix-based descriptions, cladistic analysis, and key to late instars. ZooKeys, 415: 217–268. Doi 10.3897/zookeys.415.5887.

Somerby, R.E. 1972. Systematics of *Eleodes* (*Blapylis*) with a revision of the caseyi group using taximetric methods (Coleoptera: Tenebrionidae). Ph.D. Thesis, University of California, Riverside, xxv + 441 pp.

Silvestro, V.A., A.E. Giraldo Mendoza, and G.E. Flores. 2015. Pumiliofossorum: a new genus of Scotobiini (Coleoptera: Tenebrionidae) with two new species from Peru, and a revised key for the genera of the tribe. Zootaxa, 3986(4): 461–471. http://dx.doi.org/10.11646/zootaxa.3986.4.5.

Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analyses of large phylogenies. Bioinformatics, 30(9): 1312–1313. https://doi.org/10.1093/bioinformatics/btu033.

Tanner, V.M. and W.A. Packham. 1965. Tenebrionidae beetles of the Nevada test site. Brigham Young University Science Bulletin (Biological Series), 6(1): 1–44.

Thomas, D.B. 1983. Tenebrionid beetle diversity and habitat complexity in the Eastern Mojave Desert. The Coleopterists Bulletin, 37(2): 135–147. http://www.jstor.org/stable/4008003.

Thomas, D.B. 1984. The life history and ecology of the pinacate beetle, *Eleodes armatus* LeConte (Tenebrionidae). Coleopterists Bulletin, 38: 150–159.

Thomas, D.B. 2005. Blaisdell's formae and homonyms in the genus *Eleodes* Eschscholtz (Coleoptera: Tenebrionidae:

Embaphionini). Annales Zoologici (Warszawa), 55: 549–560.

Triplehorn, C.A. 1975. A new subgenus of *Eleodes*, with three new cave-inhabiting species (Coleoptera: Tenebrionidae). The Coleopterists Bulletin, 29: 39–43.

Triplehorn, C.A. 1996. *Eleodes* of Baja California (Coleoptera: Tenebrionidae). Ohio Biological Survey Bulletin (New Series), 10(2), vi + 39 pp.

Triplehorn, C.A. and R.L. Aalbu. 1985. A review of the genus *Neobaphion* Blaisdell with description of a new species from Nevada (Coleoptera: Tenebrionidae: Eleodini). Proceedings of the Entomological Society of Washington, 87(3): 587–592.

Triplehorn, C.A. and D.B. Thomas. 2012. Studies in the genus *Eleodes* Eschscholtz with a revision of the subgenus *Melaneleodes* Blaisdell and *Omegeleodes*, new subgenus (Coleoptera: Tenebrionidae: Eleodini). Transactions of the American Entomological Society, 137 [2011]: 251–281. https://www.jstor.org/stable/41550034.

Triplehorn, C.A. and D.B. Thomas. 2015. A revision of *Eleodes* subgenus *Litheleodes* Blaisdell (Coleoptera: Tenebrionidae). The Coleopterists Society Monograph, 14: 11–21. https://doi.org/10.1649/0010-065X-69.mo4.11.

Triplehorn, C.A., D.B. Thomas, and E.G. Riley. 2009. The genus *Eleodes* Eschscholtz (Coleoptera: Tenebrionidae) in Texas. The Coleopterists Bulletin, 63(4): 413–437. https://doi.org/10.1649/1177.1.

Triplehorn, C.A., D.B. Thomas, and A.D. Smith. 2015. A revision of *Eleodes* subgenus *Eleodes* Eschscholtz (Coleoptera: Tenebrionidae). Transactions of the American Entomological Society, 141: 156–196. https://doi.org/10.3157/061.141.0111.

Tschinkel, W.R. 1975a. A comparative study of the chemical defensive system of tenebrionid beetles. Chemistry of the secretions. Journal of Insect Physiology, 21: 753–783.

Tschinkel, W.R. 1975b. A comparative study of the chemical defensive system of tenebrionid beetles. Defensive behavior and ancillary features. Annals of the Entomological Society of America, 68: 439–453.

Tschinkel, W.R. 1975c. Unusual occurrence of aldehydes and ketones in the defensive secretion of the tenebrionid beetle *Eleodes beameri*. Journal of Insect Physiology, 21: 659–671.

Wade, J.S. and R.A. St. George. 1923. Biology of the false wireworm, *Eleodes suturalis*. Journal of Agricultural Research, 36: 547–566.

Wild, A.L. and D.R. Maddison. 2008. Evaluating nuclear protein-coding genes for phylogenetic utility in beetles. Molecular Phylogenetics and Evolution, 48(3): 877–891. https://doi.org/10.1016/j.ympev.2008.05.023.

Appendix 1. The following table lists each OTU used in the Tribal monophyly analyses, showing the taxonomic name, the current tribal placement, and the specimen voucher code. Voucher codes that start with KKDNA and TB are deposited with A.D. Smith collection housed at Purdue University and have specimen data available through http://tenebrionidbase.org/. Voucher codes starting with E are either deposited with the latter (most E1-E60) or placed in the M. Andrew Johnston collection housed at Arizona State University and have specimen data available online though https://serv.biokic.asu.edu/ecdysis/collections/misc/collprofiles.php?collid=4.

Taxon	Voucher Code	Tribe	Has sequence data for given locus						
			12S	28S	CAD	COII	WG	H3	COI
Ammodonus granosus Fall, 1912	E129	Opatrini	Yes	Yes	Yes	Yes	Yes	No Data	Yes
Anomalipus sp.	TB14734	Platynotini	Yes	Yes	Yes	Yes	Yes	No Data	Yes
Apsena rufipes (Eschscholtz, 1829)	TB15409	Eulabini	Yes	No Data	Yes	Yes	Yes	Yes	Yes
Argoporis cribrata (LeConte, 1861)	E56	Cerenopini	Yes	Yes	No Data	Yes	No Data	Yes	Yes
Blaps mucronota Latreille, 1804	TB15113	Blaptini	Yes	Yes	Yes	Yes	Yes	No Data	No Data
Blaps sp2	TB17180	Blaptini	Yes	Yes	Yes	Yes	Yes	No Data	No Data
Blapstinus fortis LeConte, 1878	E69	Opatrini	Yes	Yes	Yes	Yes	Yes	No Data	Yes
Carchares macer Pascoe, 1887	E149	Scaurini	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Centronopus suppressus (Say, 1835)	E71	Centronopini	Yes	Yes	Yes	Yes	Yes	No Data	No Data
Cerenopus concolor LeConte, 1851	TB15484	Cerenopini	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Cheirodes sp.	E148	Melanimonini	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Conibius sp.	E139	Opatrini	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Cymatothes opacus Solier, 1848	E72	Amarygmini	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Appendix 1. Continued.

Taxon	Voucher Code	Tribe	Has sequence data for given locus						
			12S	28S	CAD	COII	WG	H3	COI
Emmallodera sp	TB15191	Scotobiini	Yes	Yes	No Data	No Data	Yes	No Data	Yes
Epantius obscurus LeConte, 1851	TB15187	Eulabini	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Gonopus sp	TB14732	Platynotini	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Leptynoderes sp	TB15192	Scotobiini	No Data	Yes	Yes	Yes	Yes	Yes	Yes
Neatus n.sp.	TB15296	Tenebrionini	Yes	Yes	Yes	Yes	Yes	No Data	Yes
Notibius puberulus LeConte, 1851	E138	Opatrini	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Pimelionotus lugens (Fahreus, 1870)	TB14731	Amarygmini	Yes	Yes	Yes	Yes	Yes	No Data	Yes
Rhinandrus helopioides (Kraatz, 1880)	E65	Tenebrionini	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Trichoton sp	TB13471	Opatrini	Yes	Yes	No Data	Yes	Yes	No Data	Yes
Zophobas morio (Fabricius, 1777)	TB15298	Tenebrionini	Yes	Yes	Yes	Yes	No Data	No Data	Yes
Eleodes (*Amphidora*) *littoralis* (Eschscholtz, 1829)	TB15447	Amphidorini	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Ardeleodes*) *tibialis* Blaisdell, 1909	E145	Amphidorini	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Blapylis*) *tenebrosa* Horn, 1870	E47	Amphidorini	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Cratidus*) *osculans* (LeConte, 1851)	E36	Amphidorini	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Discogenia*) *scabricula* LeConte, 1858	E67	Amphidorini	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (Eleodes) *dentipes* Eschscholtz, 1829	TB15297	Amphidorini	Yes	Yes	Yes	Yes	Yes	No Data	Yes
Eleodes (*Eleodes*) *grandicollis* Mannerheim, 1843	TB13402	Amphidorini	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes barbata Wickham, 1918	TB15989	Amphidorini	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Heteropromus*) *veterator* Horn, 1874	E74	Amphidorini	Yes	No Data	Yes	Yes	Yes	Yes	Yes
Eleodes (*Litheleodes*) *extricata* (Say, 1824)	E63	Amphidorini	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Metablapylis*) *nigrina* LeConte, 1858	E38	Amphidorini	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Promus*) *madrensis* Johnston, 2015	E128	Amphidorini	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Steneleodes*) *gigantea* Mannerheim, 1843	E20	Amphidorini	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Embaphion glabrum Blaisdell, 1909	E50	Amphidorini	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Lariversius n.sp.	TB15302	Amphidorini	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Neobaphion planipenne (LeConte, 1866)	E43	Amphidorini	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Nycterinus sp1	TB15411	Amphidorini	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Nycterinus sp2	E75	Amphidorini	Yes	Yes	No Data	Yes	Yes	Yes	Yes
Nycterinus sp3	ElFaro	Amphidorini	Yes	No Data	No Data	Yes	Yes	Yes	Yes
Nycterinus sp4	TB15448	Amphidorini	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Trogloderus costatus LeConte, 1862	E60	Amphidorini	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Appendix 2. The following table lists each OTU used in the Amphidorini genus and species group relationships analyses, showing the taxonomic name including the current subgeneric placement and the specimen voucher code. Voucher codes that start with KKDNA and TB are deposited with A.D. Smith collection housed at Purdue University and have specimen data available through http://tenebrionidbase.org/. Voucher codes starting with E are either deposited with the latter (most E1-E60) or placed in the M. Andrew Johnston collection housed at Arizona State University and have specimen data available online though https://serv.biokic.asu.edu/ecdysis/collections/misc/collprofiles.php?collid=4.

Taxon	Voucher Code	Has sequence data for given locus						
		12S	28S	CAD	COII	WG	H3	COI
Blaps mucronota Latreille, 1804	TB15113	Yes	Yes	Yes	Yes	–	No Data	No Data
Blapstinus fortis LeConte, 1878	E69	Yes	Yes	Yes	Yes	Yes	No Data	No Data
Notibius puberulus LeConte, 1851	E138	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodimorpha bolcan Blaisdell, 1909	KKDNA0407	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Embaphion contusum LeConte, 1858	E46	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Embaphion depressum (LeConte, 1851)	E73	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Embaphion elongatum Horn, 1870	TB15300	Yes	Yes	Yes	Yes	No Data	Yes	Yes
Embaphion glabrum Blaisdell, 1909	E50	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Embaphion muricatum (Say, 1824)	E51	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Embaphion sp1 Carlsbad, NM	TB15451	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Embaphion sp2 New Mexico	TB17171	No Data	Yes	Yes	Yes	Yes	Yes	Yes
Lariversius n.sp.	Eureka	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Lariversius n.sp.	TB13435	Yes	Yes	Yes	Yes	Yes	Yes	No Data
Lariversius tibialis Blaisdell, 1947	E70	Yes	No Data	No Data	Yes	No Data	Yes	Yes
Neobaphion alleni Triplehorn, 1989	KKDNA0426	Yes	Yes	No Data	Yes	No Data	No Data	Yes
Neobaphion papula Triplehorn and Aalbu, 1985	E157	Yes	No Data	No Data	Yes	No Data	No Data	Yes
Neobaphion planipenne (LeConte, 1866)	E43	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Trogloderus costatus LeConte, 1879	E176	Yes	Yes	Yes	Yes	Yes	Yes	No Data
Trogloderus kandai Johnston, 2019	E81	Yes	Yes	Yes	Yes	Yes	Yes	No Data
Eleodes (*Amphidora*) *littoralis* Eschscholtz, 1829	TB15446	Yes	Yes	No Data	Yes	Yes	Yes	Yes
Eleodes (*Amphidora*) *littoralis* Eschscholtz, 1829	TB15447	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Amphidora*) *nigropilosa* (LeConte, 1851)	E90	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Ardeleodes*) *tibialis* Blaisdell, 1909	E145	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Ardeleodes*) *tibialis* Blaisdell, 1909	TB15112	Yes	Yes	Yes	Yes	No Data	Yes	Yes
Eleodes (*Blapylis*) *aristata* Somerby, 1977	TB13389	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Blapylis*) *clavicornis* Eschscholtz, 1829	E83	No Data	Yes	No Data	Yes	Yes	Yes	Yes
Eleodes (*Blapylis*) *snowii* Blaisdell, 1909	E31	Yes	Yes	Yes	Yes	Yes	Yes	No Data
E *Blapylis* sp	E142	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Blapylis*) *tenebrosa* Horn, 1870	E47	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Caverneleodes*) *easterlai* Triplehorn, 1975	KKDNA0425	Yes	Yes	No Data	Yes	No Data	Yes	Yes
Eleodes (*Caverneleodes*) *labialis* Triplehorn, 1975	TB15486	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Caverneleodes*) *microps* Aalbu, Smith, and Triplehorn, 2012	E76	Yes	No Data	Yes	No Data	Yes	Yes	Yes
Eleodes (*Caverneleodes*) *sprousei* Triplehorn and Reddell, 1991	KKDNA0410	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Caverneleodes*) *thomasi* Aalbu, Smith, and Triplehorn, 2012	E77	No Data	Yes	No Data	Yes	Yes	Yes	Yes

Appendix 2. Continued.

Taxon	Voucher Code	Has sequence data for given locus						
		12S	28S	CAD	COII	WG	H3	COI
Eleodes (*Caverneleodes*) *wheeleri* Aalbu, Smith, and Triplehorn, 2012	E25	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Caverneleodes*) *wheeleri* Aalbu, Smith, and Triplehorn, 2012	E7	Yes	Yes	Yes	Yes	Yes	Yes	No Data
Eleodes (*Caverneleodes*) *wynnei* Aalbu, Smith, and Triplehorn, 2012	E26	Yes	No Data	No Data	Yes	No Data	No Data	Yes
Eleodes (*Chaseleodes*) *connata* Solier, 1848	E58	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Cratidus*) *osculans* (LeConte, 1851)	E36	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Discogenia*) *marginata* Eschscholtz, 1829	TB15890	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Discogenia*) *scabricula* LeConte, 1858	E67	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Eleodes*) *acuta* (Say, 1824)	TB17182	Yes	No Data	No Data	Yes	Yes	Yes	Yes
Eleodes (*Eleodes*) *acuticauda* LeConte, 1851	E34	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Eleodes*) *acuticauda* LeConte, 1851	TB15444	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Eleodes*) *armata* LeConte, 1851	E13	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Eleodes*) *armata* LeConte, 1851	TB13139	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Eleodes*) *dentipes* Eschscholtz, 1829	TB15297	Yes	Yes	Yes	Yes	Yes	No Data	Yes
E (*Eleodes*) *eschscholtzi eschscholtzi* Solier, 1848	E23	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Eleodes*) *eschscholtzi lucae* LeConte, 1866	E21	Yes	No Data	Yes	Yes	Yes	Yes	Yes
Eleodes (*Eleodes*) *gracilis distans* Blaisdell, 1909	TB15474	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Eleodes*) *gracilis* LeConte, 1858	E28	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Eleodes*) *grandicollis* Mannerheim, 1843	TB13402	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Eleodes*) *hispilabris* (Say, 1824)	E37	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Eleodes*) *loretensis* Blaisdell, 1923	TB15485	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Eleodes*) *loretensis* Blaisdell, 1923	TB15982	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Eleodes*) *loretensis* Blaisdell, 1923	TB15983	Yes	Yes	No Data	Yes	Yes	Yes	Yes
Eleodes (*Eleodes*) *mexicana* Blaisdell, 1943	TB15483	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Eleodes*) n.sp. Baja California Sur	TB13388	Yes	No Data	Yes	Yes	Yes	Yes	No Data
Eleodes (*Eleodes*) *obscura sulcipennis* Mannerheim, 1843	E11	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Eleodes*) *rossi* Blaisdell, 1943	E35	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Eleodes*) *spinipes macrura* Champion, 1892	TB15473	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Eleodes*) *spinipes spinipes* Solier, 1848	E155	Yes	Yes	No Data	Yes	Yes	Yes	Yes
Eleodes (*Eleodes*) *spinipes spinipes* Solier, 1848	TB15076	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Eleodes*) *spinipes spinipes* Solier, 1848	TB15077	Yes	No Data	No Data	Yes	No Data	Yes	Yes
Eleodes (*Eleodes*) *spinipes ventricosa* LeConte, 1858	E18	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Eleodes*) *sponsa* LeConte, 1858)	E39	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Eleodes*) *suturalis* (Say, 1824)	E19	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Eleodes*) *tenuipes* Casey, 1890	E17	Yes	Yes	Yes	Yes	Yes	Yes	No Data

Appendix 2. Continued.

Taxon	Voucher Code	Has sequence data for given locus						
		12S	28S	CAD	COII	WG	H3	COI
Eleodes (*Eleodes*) *tenuipes* Casey, 1890	TB15993	Yes	Yes	Yes	Yes	No Data	Yes	No Data
Eleodes (*Eleodes*) *vanduzeei* Blaisdell, 1923	TB15674	Yes	No Data	Yes	Yes	Yes	Yes	No Data
Eleodes (*Heteropromus*) *veterator* Horn, 1874	E74	Yes	No Data	Yes	Yes	Yes	Yes	Yes
Eleodes (*Litheleodes*) *arcuata* Casey, 1884	E64	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Litheleodes*) *arcuata* Casey, 1884	TB13393	Yes	Yes	Yes	No Data	No Data	Yes	No Data
Eleodes (*Litheleodes*) *extricata* (Say, 1824)	E140	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Litheleodes*) *extricata* (Say, 1824)	E63	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Litheleodes*) *extricata* (Say, 1824)	E62	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Litheleodes*) *extricata* (Say, 1824)	E55	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Litheleodes*) *extricata* (Say, 1824)	E52	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Litheleodes*) *extricata* (Say, 1824)	E48	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Litheleodes*) *granulata* LeConte, 1857	TB15994	Yes	Yes	No Data	Yes	Yes	Yes	Yes
Eleodes (*Melaneleodes*) *anthracina* Blaisdell, 1909	E173	Yes	Yes	No Data	Yes	No Data	No Data	Yes
Eleodes (*Melaneleodes*) *carbonaria carbonaria* (Say, 1824)	E14	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Melaneleodes*) *carbonaria chihuahuaensis* Champion, 1884	E136	Yes	Yes	Yes	Yes	Yes	No Data	Yes
Eleodes (*Melaneleodes*) *carbonaria obsoleta* (Say, 1824)	E42	Yes	No Data	Yes	Yes	No Data	No Data	Yes
Eleodes (*Melaneleodes*) *carbonaria omissa* LeConte, 1858	E12	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Melaneleodes*) *quadricollis* Eschscholtz, 1829	TB15293	Yes	Yes	Yes	Yes	Yes	No Data	Yes
Eleodes (*Melaneleodes*) *tricostata* (Say, 1824)	E68	Yes	Yes	No Data	Yes	Yes	No Data	Yes
Eleodes (*Melaneleodes*) *wenzeli* Blaisdell, 1925	E15	Yes	Yes	Yes	Yes	Yes	No Data	Yes
Eleodes (*Metablapylis*) cf. *delicata* Blaisdell, 1929	TB15710	Yes	No Data	Yes	Yes	Yes	Yes	Yes
Eleodes (*Metablapylis*) *delicata* Blaisdell, 1929	E141	Yes	Yes	No Data	Yes	Yes	Yes	Yes
Eleodes (*Metablapylis*) *dissimilis* Blaisdell, 1909	TB15478	Yes	Yes	No Data	Yes	Yes	Yes	Yes
Eleodes (*Metablapylis*) *nigrina* LeConte, 1858	E38	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Omegeleodes*) *debilis* LeConte, 1858	TB15988	Yes	Yes	No Data	Yes	No Data	Yes	Yes
Eleodes (*Omegeleodes*) sp. Oaxaca, Mexico	E146	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Omegeleodes*) sp. Oaxaca, Mexico	E159	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Omegeleodes*) sp. Oaxaca, Mexico	E161	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Omegeleodes*) sp. Tlaxcala, Mexico	E132	Yes	No Data	Yes	Yes	Yes	Yes	Yes
Eleodes (*Promus*) cf *calcarata* Champion, 1884	E163	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Promus*) cf *calcarata* Champion, 1884	E32	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Promus*) cf *calcarata* Champion, 1885	E165	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Promus*) *fusiformis* LeConte, 1858	E44	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Appendix 2. Continued.

Taxon	Voucher Code	Has sequence data for given locus						
		12S	28S	CAD	COII	WG	H3	COI
Eleodes (*Promus*) *goryi* Solier, 1848	E27	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Promus*) *hoegei* Champion, 1885	E40	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Promus*) *insularis* Linell, 1899	E54	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Promus*) *insularis* Linell, 1899	TB15981	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Promus*) *knullorum* Triplehorn, 1971	E24	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Promus*) *madrensis* Johnston, 2015	E10	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Promus*) *madrensis* Johnston, 2015	E128	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Promus*) *opaca* (Say, 1824)	TB17183	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Promus*) *spinolae* Solier, 1848	TB15080	Yes	No Data	No Data	Yes	No Data	No Data	Yes
Eleodes (*Promus*) *spinolae* Solier, 1848	TB15082	Yes	Yes	No Data	Yes	Yes	Yes	Yes
Eleodes (*Promus*) *striolata* LeConte, 1858	E80	Yes	Yes	Yes	Yes	Yes	No Data	No Data
Eleodes (*Pseudeleodes*) *caudifera* LeConte, 1858	E29	Yes	Yes	No Data	Yes	Yes	Yes	Yes
Eleodes (*Pseudeleodes*) *granosa* LeConte, 1866	KKDNA0392	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Pseudeleodes*) *inornata* Johnston, 2016	E175	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Pseudeleodes*) *longipilosa* Horn, 1891	TB15304	Yes	No Data	No Data	Yes	No Data	Yes	No Data
Eleodes (*Pseudeleodes*) *pilosa* Horn, 1870	E45	No Data	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Pseudeleodes*) *pilosa* Horn, 1870	TB15449	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Pseudeleodes*) *tribula* Thomas, 2005	E61	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Steneleodes*) *angusta* Eschscholtz, 1829	E168	Yes	Yes	No Data	Yes	No Data	No Data	Yes
Eleodes (*Steneleodes*) *brevicollis* Gemminger, 1870	E174	Yes	Yes	No Data	Yes	No Data	Yes	Yes
Eleodes (*Steneleodes*) *coarctata* Champion, 1885	E172	No Data	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Steneleodes*) *distincta* Solier, 1848	E154	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Steneleodes*) *distincta* Solier, 1848	TB15079	Yes	No Data	No Data	Yes	No Data	Yes	Yes
Eleodes (*Steneleodes*) *gigantea* Mannerheim, 1843	E20	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Steneleodes*) *gravida* (Eschscholtz, 1829)	E30	Yes	Yes	No Data	Yes	Yes	Yes	Yes
Eleodes (*Steneleodes*) *hepburni* Champion, 1884	E8	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Steneleodes*) *hepburni* Champion, 1884	TB15475	Yes	No Data	No Data	Yes	Yes	Yes	No Data
Eleodes (*Steneleodes*) *innocens* LeConte, 1866	E41	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Steneleodes*) *longicollis* LeConte, 1851	E16	Yes	No Data	Yes	Yes	Yes	Yes	Yes
Eleodes (*Steneleodes*) *longicollis* LeConte, 1851	TB15295	Yes	No Data	No Data	Yes	Yes	Yes	Yes
Eleodes (*Steneleodes*) *mutilata* Blaisdell, 1921	E9	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Steneleodes*) nr *distincta* Solier, 1848	E144	No Data	Yes	No Data	Yes	No Data	Yes	Yes
Eleodes (*Steneleodes*) *peropaca* Champion, 1892	TB15476	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Appendix 2. Continued.

Taxon	Voucher Code	Has sequence data for given locus						
		12S	28S	CAD	COII	WG	H3	COI
Eleodes (*Steneleodes*) *ponderosa* Champion, 1884	E22	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Steneleodes*) *ponderosa* Champion, 1884	TB15078	Yes	Yes	Yes	Yes	Yes	No Data	Yes
Eleodes (*Steneleodes*) *ruida* (Say, 1835)	E33	No Data	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Steneleodes*) *sallaei* Champion, 1885	E169	Yes	Yes	No Data	Yes	No Data	Yes	Yes
Eleodes (*Steneleodes*) *sallaei* Champion, 1885	TB15075	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Steneleodes*) sp Aguascalientes, Mexico	TB15114	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Steneleodes*) sp Hidalgo, Mexico	E160	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Steneleodes*) sp Federal District, Mexico	E170	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Steneleodes*) sp Puebla, Mexico	E162	Yes	Yes	No Data	Yes	Yes	Yes	Yes
Eleodes (*Steneleodes*) sp Puebla, Mexico	TB15083	Yes	Yes	Yes	Yes	No Data	Yes	Yes
Eleodes (*Steneleodes*) sp Puebla, Mexico	TB15084	Yes	Yes	No Data	Yes	Yes	Yes	Yes
Eleodes (*Steneleodes*) sp Queretaro, Mexico	TB15481	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Steneleodes*) sp	TB15303	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes (*Tricheleodes*) *hirsuta* LeConte, 1861	E164	No Data	Yes	Yes	Yes	Yes	Yes	No Data
Eleodes (*Tricheleodes*) *hirsuta* LeConte, 1861	TB15305	Yes	Yes	Yes	Yes	Yes	Yes	No Data
Eleodes (*Tricheleodes*) *hirsuta* LeConte, 1861	TB15479	Yes	No Data	No Data	Yes	No Data	Yes	Yes
Eleodes alutacea Solier, 1848	E171	Yes	Yes	No Data	Yes	Yes	Yes	Yes
Eleodes alutacea Solier, 1848	E49	No Data	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes barbata Wickham, 1918	E59	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes barbata Wickham, 1918	TB15989	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes barbata Wickham, 1918	TB15992	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes cf *rotundicollis* (Eschscholtz, 1829)	E167	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes cf *rotundicollis* (Eschscholtz, 1829)	TB15085	Yes	Yes	Yes	Yes	No Data	Yes	Yes
Eleodes impolita (Say, 1835)	E166	No Data	Yes	No Data	Yes	No Data	Yes	Yes
Eleodes impolita (Say, 1835)	E53	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes impolita (Say, 1835)	TB15074	Yes	Yes	Yes	Yes	No Data	Yes	Yes
Eleodes scapularis Champion, 1884	E158	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eleodes scapularis Champion, 1884	TB15081	Yes	Yes	Yes	Yes	No Data	Yes	Yes