

**A Multi-locus Molecular Phylogeny of the Core Genera of Ellescini (Coleoptera:
Curculionidae: Curculioninae) with a Review of the Species of *Ellescus* in North America**

Jake Lewis

Thesis submitted to the University of Ottawa
in partial fulfillment of the requirements for the
Master of Science in Biology

Department of Biology
Faculty of Science
University of Ottawa

© Jake Lewis, Ottawa, Canada, 2022

ABSTRACT

A combination of morphological and molecular techniques were used to investigate the taxonomy of the species of *Ellescus* Dejean, 1821 in North America (Curculionidae: Curculioninae: Ellescini), as well as investigate the phylogenetic relationships amongst genera within the tribe Ellescini. Four valid species of *Ellescus* were documented from the Nearctic Region. These include the temperately distributed *E. michaeli* n. sp., the west coast endemic species *E. californicus* (Casey, 1885) (resurrected from synonymy with *E. ehippiatus* (Say, 1831)), the widespread, hypervariable *E. ehippiatus*, and the Holarctic *E. bipunctatus* (Linnaeus, 1758) (of which *E. borealis* (Carr, 1920) is found to be a junior synonym). The European species, *E. scanicus* (Paykull, 1792), is determined to have been erroneously reported from North America. The Maximum Likelihood and Bayesian Inference analyses based on CO1, CytB, 28S, and 16S sequence data found moderate to strong support for the monophyly of *Ellescus* and moderate to strong support for *Proctorus* LeConte, 1876 as a lineage nested within *Dorytomus* Germar, 1817. The genera *Proctorus* and *Rodotymus* Zumpt, 1932 are placed in synonymy under *Dorytomus*.

RESUMÉ

Une combinaison de techniques morphologiques et moléculaires a été utilisée pour étudier la taxonomie des espèces d'*Ellescus* Dejean, 1821 en Amérique du Nord (Curculionidae : Curculioninae : Ellescini), ainsi que pour étudier les relations phylogénétiques entre les genres au sein de la tribu Ellescini. Quatre espèces valides d'*Ellescus* ont été documentées pour la région néarctique. Il s'agit notamment d'*E. michaeli* n. sp., l'espèce endémique de la côte ouest *E. californicus* (Casey, 1885) (précédemment considéré comme synonyme plus récent d'*E. ephippiatus* (Say, 1831)), l'*E. ephippiatus* très répandu et hypervariable, et l'*E. bipunctatus* qui présente une répartition holarctique (Linnaeus, 1758) (dont *E. borealis* (Carr, 1920) est un synonyme plus récent). L'espèce européenne, *E. scanicus* (Paykull, 1792), aurait été signalée par erreur en Amérique du Nord. Les analyses de maximum de vraisemblance et bayésiennes basées sur les données de séquences CO1, CytB, 28S et 16S suggère un soutien modéré à fort pour la monophylie d'*Ellescus* et un soutien modéré à fort pour *Proctorus* LeConte, 1876 en tant que lignée nichée dans *Dorytomus* Germar, 1817. Les genres *Proctorus* et *Rodotymus* Zumpt, 1932 sont placés en synonymie sous *Dorytomus*.

ACKNOWLEDGEMENTS

First and foremost, I wish to express my sincerest gratitude to my advisors, Dr. Robert Anderson and Dr. Julian Starr, and committee members, Dr. Jeffrey Skevington and Dr. Patrice Bouchard, for their guidance and enthusiasm. This project required that I learn many skills that were entirely foreign to me when I began this project as a simple naturalist (e.g. PCR, phylogenetic theory and software), so I thank you all for the patience and support. I must also especially thank my primary advisor, Dr. Robert Anderson, with whom I worked with most extensively, for his kindness and understanding.

The Covid-19 pandemic made specimen acquisition a major challenge for this project, and I would have been unable to complete this thesis on-time if it was not for the support of collection managers, curators, and associates at institutions across North America, Europe and Japan. Thank you all! In particular, I am indebted to Dr. Michael Košťál for collecting and mailing weevil specimens from Slovakia, all of which generated fantastic sequence data for a dozen non-native taxa that otherwise would have been impossible to procure.

I must also express my thanks to Roger Bull and Anna Ginter for their unwavering support and truly abnormal degree of patience throughout my DNA lab training. The skills I learned in the Canadian Museum of Nature DNA lab were crucial for this project and will undoubtedly remain relevant in my future research endeavors.

I am indebted to my partner, Narumi, for her assistance in the 2020 field season and her continuing support of my “*mushitori-shounen*” entomological endeavors. Even on the 8 hour long field day where you only collected one ant, I still appreciated the moral support and lunch!

Lastly, I would like to thank my mentors from the high school and undergraduate eras, Dr. Donald McAlpine, Paul Brunelle, Dr. Reginald Webster, and Thomas Mueller. If it was not for you all, I likely never would have had the opportunity (or sense) to pursue an exciting career of discovery and adventure in entomology.

TABLE OF CONTENTS

ABSTRACT.....	II
RESUMÉ.....	III
ACKNOWLEDGEMENTS.....	IV
TABLE OF CONTENTS.....	V
LIST OF TABLES.....	VI-VII
LIST OF FIGURES.....	VIII-XI
 Chapter 1: General Introduction.....	1
1.1 An introduction to the weevils (Coleoptera: Curculionoidea).....	2
1.2 Thesis objectives and significance.....	5
 Chapter 2: A Review of the Species of <i>Ellescus</i> in North America.....	8
2.1 Introduction.....	9
2.2 Methods.....	13
2.2.1 Specimen acquisition.....	13
2.2.2 Dissection protocol, morphological analysis and imaging.....	15
2.2.3 DNA extraction protocols.....	15
2.2.4 Gene and primer selection, polymerase chain reaction (PCR) and sequencing reactions.....	16
2.2.5 Sequence editing, alignment and analysis.....	18
2.3 Results.....	19
2.3.1 Overview of results.....	19
2.3.2 Key to the species of <i>Ellescus</i> in North America (including <i>E. scanicus</i>).....	20
2.3.3 Species profiles.....	22
2.3.4 Barcoding (CO1) + ITS2.....	41
2.4 Discussion.....	41
 Chapter 3: A Multi-locus Molecular Phylogeny of the Core Genera of Ellescini.....	105
3.1 Introduction.....	106
3.2 Methods.....	109
3.2.1 Specimen acquisition and taxon sampling.....	109
3.2.2 Dissection protocol, morphological analysis and imaging.....	111
3.2.3 DNA extraction protocols.....	111
3.2.4 Gene and primer selection, polymerase chain reaction (PCR) and sequencing reactions.....	112
3.2.5 Sequence editing, alignment and phylogenetic analysis.....	114
3.3 Results.....	117
3.4 Discussion.....	118
 REFERENCES.....	133
 Appendix A: Supplementary Material for Chapter 3.....	157

LIST OF TABLES

Table 2.1	Updated status of species of <i>Elleuscus</i> (Coleoptera: Curculionidae) and their distributions based on O'Brien and Wibmer (1982), Bousquet et al. (2013), Alonso-Zarazaga et al. (2017) and the current study. The rightmost column reflects the updated distributional and taxonomic results of this study.....	44
Table 2.2	Notes on the type specimen repositories of the currently recognized species of <i>Elleuscus</i> (Coleoptera: Curculionidae) reported from North America (O'Brien and Wibmer 1982; Bousquet et al. 2013). Note that this includes <i>E. scanicus</i> (Paykull, 1792), a species erroneously reported from North America, and <i>E. californicus</i> (Casey, 1885), a species which is currently treated as either a junior synonym or subspecies of <i>E. ephippiatus</i> (Say, 1831) (O'Brien and Wibmer 1982; Poole and Gentili 1996).....	45
Table 2.3	List of COI and ITS2 primers used in the generic study of <i>Elleuscus</i> (Coleoptera: Curculionidae). Forward primers are listed first, followed by reverse primers....	46
Table 2.4	Collection data for specimens used in the neighbor-joining COI and ITS2 gene trees for <i>Elleuscus</i> (Coleoptera: Curculionidae). The identities of specimens associated with sequences downloaded from GenBank or BOLD were verified morphologically through institutional loans.....	47
Table 2.5	Percent identity of COI sequences for representatives of species of <i>Elleuscus</i> (Coleoptera: Curculionidae) (Aligned COI length: 702). Note that this measure specifies the percentage of sites (columns) in the DNA sequence alignment that are identical between sequences.....	50
Table 2.6	Percent identity of ITS2 sequences for representatives of species of <i>Elleuscus</i> (Coleoptera: Curculionidae) (Aligned ITS2 length: 795). Note that this measure specifies the percentage of sites (columns) in the DNA sequence alignment that are identical between sequences.....	59
Table 3.1	List of primers used in the phylogenetic study of Elleuscini (Coleoptera: Curculionidae). Forward primers are listed first, followed by reverse primers...	121
Table 3.2	List of PCR thermal cycling conditions and DNA polymerase used for each gene used in the phylogenetic study of Elleuscini (Coleoptera: Curculionidae).....	122

Table 3.3	A list of taxa and genes used in the phylogenetic analyses of Ellescini (Coleoptera: Curculionidae). The DNA extraction number is given for each gene (format: LEW_AAA). In some cases it was not possible to obtain all sequence data from one specimen; in those cases sequence data from another specimen of the same species was used. When a taxon lacks sequence data this is indicated by an “X” under the associated gene.....	123
Table 3.4	Associated collection data for specimens used in the phylogenetic analyses of Ellescini (Coleoptera: Curculionidae).....	124
Table 3.5	Statistics for genes used in the phylogenetic analysis of Ellescini (Coleoptera: Curculionidae). Note that pairwise identity (%) represents the average percent identity across the entire alignment.....	126

LIST OF FIGURES

Figure 1.1	A selection of weevils illustrating their morphological diversity: a) A flightless denizen of arid habitats, <i>Ophryastes turbinatus</i> (Champion, 1911) - Big Bend National Park, Texas, U.S.A. - 23 June 1982 - R.S. Anderson; b) A wetland species and strong swimmer, <i>Listronotus delumbis</i> (Gyllenhal, 1834) - Val-des-Monts, Quebec, Canada - 23 June 2020 - J.H. Lewis; c) A small, drab species, <i>Ellescus californicus</i> (Casey, 1885) - Alta Sierra, California, U.S.A. - 15 July 1992 - Henry and Anne Howden; d) An iridescent tropical weevil species, <i>Eurhinus</i> sp. - Huehuetenango, Guatemala - 15 October 2017 - E. Fuller; e) A possible mimic of bird droppings, <i>Cryptorhynchus lapathi</i> (Linnaeus, 1758) - Rockwood Park, New Brunswick, Canada - 7 July 2021 - J.H. Lewis; f) A large, cryptically patterned species that is easily overlooked when at rest on dark, rough-bark trees, <i>Ectatorhinus adamsii</i> Lacordaire, 1865 - Kounandai, Kanagawa Prefecture, Japan - 27 May 2019 - J.H. Lewis.....	7
Figure 2.1	Male <i>Ellescus bipunctatus</i> (Linnaeus, 1758) with conspicuous glabrous carina extending longitudinally over more than half of the last sternite.....	62
Figure 2.2	Male <i>Ellescus ephippiatus</i> (Say, 1831) with distinct swelling at tip of last sternite, not extending over more than half of the last sternite. Note that this is reduced or absent in some specimens.....	63
Figure 2.3	Dorsal photograph of the aedeagi of <i>Ellescus</i> : a) <i>E. scanicus</i> (Paykull, 1792), b) <i>E. bipunctatus</i> (Linnaeus, 1758), c) <i>Ellescus michaeli</i> 1, d) <i>E. californicus</i> (Casey, 1885) e) <i>E. ephippiatus</i> (Say, 1831).....	64
Figure 2.4	Metepisternum and lateral half of metasternum densely punctate and covered with appressed white-yellow scales, cuticle in those regions mostly concealed - <i>Ellescus scanicus</i> (Paykull, 1792).....	65
Figure 2.5	Metepisternum and lateral half of metasternum not densely punctate or covered with appressed white-yellow scales, with cuticle in those regions mostly concealed - <i>Ellescus bipunctatus</i> (Linnaeus, 1758).....	66
Figure 2.6	Lateral photograph of <i>Ellescus scanicus</i> (Paykull, 1792).....	67
Figure 2.7	Dorsal photograph of <i>Ellescus scanicus</i> (Paykull, 1792).....	68
Figure 2.8	Lateral photograph of <i>Ellescus bipunctatus</i> (Linnaeus, 1758) (specimen #1).....	69
Figure 2.9	Dorsal photograph of <i>Ellescus bipunctatus</i> (Linnaeus, 1758) (specimen #1).....	70
Figure 2.10	Lateral photograph of <i>Ellescus bipunctatus</i> (Linnaeus, 1758) (specimen #2).....	71

Figure 2.11	Dorsal photograph of <i>Ellescus bipunctatus</i> (Linnaeus, 1758) (specimen #2).....	72
Figure 2.12	Lateral photograph of <i>Ellescus bipunctatus</i> (Linnaeus, 1758) (specimen #3).....	73
Figure 2.13	Dorsal photograph of <i>Ellescus bipunctatus</i> (Linnaeus, 1758) (specimen #3).....	74
Figure 2.14	Lateral photograph of the <i>Ellescus borealis</i> (Carr, 1920) holotype (CNC 379701). Note that <i>E. borealis</i> is a junior synonym of <i>E. bipunctatus</i> (Linnaeus, 1758), as evidence by the lack of morphological characters separating these taxa.....	75
Figure 2.15	Dorsal photograph of the <i>Ellescus borealis</i> (Carr, 1920) holotype (CNC 379701). Note that <i>E. borealis</i> is a junior synonym of <i>E. bipunctatus</i> (Linnaeus, 1758), as evidence by the lack of morphological characters separating these taxa.....	76
Figure 2.16	Photograph of the 5 th sternite of the <i>Ellescus borealis</i> (Carr, 1920) holotype (CNC 379701). Note the carina running longitudinally along the center of the 5 th sternite, a character only present in males of <i>E. bipunctatus</i> and <i>E. scanicus</i> (Paykull, 1792).....	77
Figure 2.17	<i>Ellescus borealis</i> (Carr, 1920) holotype labels.....	78
Figure 2.18	Lateral photograph of <i>Ellescus californicus</i> (Casey, 1885).....	79
Figure 2.19	Dorsal photograph of <i>Ellescus californicus</i> (Casey, 1885).....	80
Figure 2.20	Lateral photograph of the <i>Ellescus californicus</i> (Casey, 1885) holotype (USNMENT 01448984 / TYPE USNM 36721). Note the discolored lateral bands on the pronotum, and contrast in cuticular coloration between the metasternum (black), legs (rufous), and rostrum (rufous). The collection locality (California) further substantiates this as the same distinct form described in this work.....	81
Figure 2.21	<i>Ellescus californicus</i> (Casey, 1885) holotype labels.....	82
Figure 2.22	Lateral photograph of <i>Ellescus michaeli</i> n. sp. (specimen #1).....	83
Figure 2.23	Dorsal photograph of <i>Ellescus michaeli</i> n. sp. (specimen #1).....	84
Figure 2.24	Lateral photograph of <i>Ellescus michaeli</i> n. sp. (specimen #2).....	85
Figure 2.25	Dorsal photograph of <i>Ellescus michaeli</i> n. sp. (specimen #2).....	86
Figure 2.26	Lateral photograph of <i>Ellescus ehippiatus</i> (Say, 1831) (specimen #1).....	87

Figure 2.27	Dorsal photograph of <i>Ellescus ehippiatus</i> (Say, 1831) (specimen #1).....	88
Figure 2.28	Lateral photograph of <i>Ellescus ehippiatus</i> (Say, 1831) (specimen #2).....	89
Figure 2.29	Dorsal photograph of <i>Ellescus ehippiatus</i> (Say, 1831) (specimen #2).....	90
Figure 2.30	Lateral photograph of <i>Ellescus ehippiatus</i> (Say, 1831) (specimen #3).....	91
Figure 2.31	Dorsal photograph of <i>Ellescus ehippiatus</i> (Say, 1831) (specimen #3).....	92
Figure 2.32	Lateral photograph of the <i>Ellescus ehippiatus</i> (Say, 1831) syntype (NHRS-JLKB000073656). Note the largely red body contrasting with the black metasternum and metepisternum (not found in <i>E. michaeli</i> , the only other similar species in the region).....	93
Figure 2.33	Dorsal photograph of the <i>Ellescus ehippiatus</i> (Say, 1831) syntype (NHRS-JLKB000073656). Note the weak lateral discolored pronotal bands, which are not found in <i>E. bipunctatus</i> (Linnaeus, 1758).....	94
Figure 2.34	<i>Ellescus ehippiatus</i> (Say, 1831) syntype labels.....	95
Figure 2.35	North American range map for <i>Ellescus bipunctatus</i> (Linnaeus, 1758). Provinces, territories, and states for which the species is recorded are shaded in gray. Maps were generated in SimpleMappr (Shorthouse 2010).....	96
Figure 2.36	North American range map for <i>Ellescus californicus</i> (Casey, 1885). Provinces, territories, and states for which the species is recorded are shaded in gray. Maps were generated in SimpleMappr (Shorthouse 2010).....	97
Figure 2.37	North American range map for <i>Ellescus michaeli</i> n. sp. Provinces, territories, and states for which the species is recorded are shaded in gray. Maps were generated in SimpleMappr (Shorthouse 2010).....	98
Figure 2.38	North American range map for <i>Ellescus ehippiatus</i> (Say, 1831). Provinces and states for which the species is recorded are shaded in gray. Maps were generated in SimpleMappr (Shorthouse 2010).....	99
Figure 2.39	A rough copy of specimens (with associated collection localities) that were mailed by Thomas Say to Carl Schönherr. Note that the collection locality (Connecticut) beside entry 117 (<i>Phyxelis rigidus</i>) may have been erroneously interpreted as applying to subsequent entries (including entry 121 - <i>E. ehippiatus</i>), which would explain the labelling of the <i>E. ehippiatus</i> syntype deposited in the Schönherr collection (NHRS). Document deposited in the Academy of Natural Science of Philadelphia (ANSP).....	100

Figure 2.40	Neighbor-joining barcode tree (CO1) for <i>Ellescus</i> species under a Jukes-Cantor substitution model (10000 bootstrap replicates, support threshold of 70%). Branch support values are represented by color (green > 90%, red-yellow 70-90%). Note that <i>Ellescus ephippiatus</i> and <i>Ellescus michaeli</i> n. sp. do not separate using CO1, but do when using the faster evolving marker ITS2.....	101
Figure 2.41	Neighbor-joining barcode tree (ITS2) for <i>Ellescus</i> species under a Jukes-Cantor substitution model (10000 bootstrap replicates, support threshold of 70%). Branch support values are represented by color (green > 90%, red-yellow 70-90%). Note that <i>Ellescus ephippiatus</i> and <i>Ellescus michaeli</i> n. sp. do not separate using CO1, but do when using the faster evolving marker ITS2.....	102
Figure 2.42	Lateral photograph of <i>Adorytomus</i> sp. (Japan, Aichi Prefecture, Mt. Ouyama, 25 March 1992, H. Kojima; HKC).....	103
Figure 2.43	<i>Adorytomus</i> sp., showing the laterally expanded region of the elytra over the anterior quarter of the metepisternum, which is covered in hairs ventrally (shown by white arrow).....	104
Figure 3.1	Lateral photograph of <i>Proctorus armatus</i> (LeConte, 1876). Note the ventral projections on the 5 th sternite.....	127
Figure 3.2	Oblique-angle photograph of the last sternite of <i>Proctorus armatus</i> (LeConte, 1876), illustrating the ventral projections.....	128
Figure 3.3	Lateral photograph of <i>Dorytomus dorsalis</i> (Linnaeus, 1758). Note the ventral projections on the 5 th sternite.....	129
Figure 3.4	Oblique-angle photograph of the last sternite of <i>Dorytomus dorsalis</i> (Linnaeus, 1758), illustrating the ventral projections.....	130
Figure 3.5	Maximum Likelihood tree of the core genera of Ellescini (Coleoptera: Curculionidae: Curculioninae) using CO1, CytB, 16S, and 28S sequence data with gene and codon partitioning (codon partitioning only in CO1/CytB). Branch support values at the nodes represent SH-aLRT values and ultrafast bootstrap (UFBoot) values, respectively.....	131
Figure 3.6	Majority rule consensus tree (Bayesian) of the core genera of Ellescini (Coleoptera: Curculionidae: Curculioninae) using CO1, CytB, 16S, and 28S sequence data with gene and codon partitioning (codon partitioning only in CO1/CytB). Branch support values at the nodes represent posterior probabilities.....	132

Chapter 1: General Introduction

1.1 An introduction to the weevils (Coleoptera: Curculionoidea)

Weevils (Coleoptera: Curculionoidea) represent one of the most diverse animal groups on the planet (Marshall 2006). Despite over 62,000 species having been described, one estimate asserts that this accounts for only 25% of the world's extant weevil fauna (Oberprieler et al. 2007). Although weevils clearly reach their greatest diversity in tropical zones (Oberprieler et al. 2014), in North America over 7068 weevil species in 843 genera are known to occur (O'Brien and Wibmer 1982) and new species continue to be discovered from the continent (Anderson 2016, 2018). Weevils are remarkably varied in habits and morphology; they can be strong fliers or flightless (Figure 1.1a) (Anderson 2002), desert specialists (Shelef et al. 2013) or strong swimmers occurring in wetland areas (Figure 1.1b) (O'Brien 1981; Morris 1995), canopy specialists (Sprick and Floren 2018) or eyeless troglobites (Howarth and Stone 1990; Paquin and Anderson 2009), small (< 4mm) or large (> 90mm) (Painting and Holwell 2013; Marshall 2018), nondescript (Figure 1.1c) or brightly colored (Figure 1.1d), mimics (Figure 1.1e) (see also Vanin and Guerra 2012), or cryptically patterned (Figure 1.1f) (see also Wilts et al. 2012).

The characteristic feature of weevils (excluding the subfamilies Scolytinae and Platypodinae) is the rostrum (Anderson 2002; Davis 2017): an extension of the head ventrally with the mouthparts situated at the tip. In some groups the rostrum is elongate and curved (Figure 1.1d, e, f), whereas in others it is short and broad (Figure 1.1a). Most weevils are phytophagous, and the larvae are remarkably diverse in natural history (Oberprieler et al. 2014). Indeed, for any given part of a plant there is likely a weevil associated with it at some developmental stage (Bright 1993; Anderson 2002; Oberprieler et al. 2014). Although greatly outnumbered by their phytophagous relatives, some weevils also feed on dung (Wassell 1966), fungi (Bright 1993), or

are insectivorous (Zwolfer and Bennett 1969). Recent phylogenomic work suggests that weevils co-evolved with angiosperms and reached their maximal rate of diversification during the mid-Cretaceous (McKenna et al. 2009). Anderson (1995) asserts that the evolution and use of the rostrum in oviposition site excavation at least partially explains the success and diversity of weevils within the Coleoptera as it allowed for oviposition deep in plant tissues, where eggs and larvae are further protected from predation, dry spells, and parasites. Given their associations with plants, it is unsurprising that weevils are also one of the most economically important insect pest groups (Bright 1976), and have been used extensively as biological control agents (Oberprieler et al. 2007). Some of the most well-known native pests in North America include *Pissodes strobi* (Peck, 1817) whose larvae develop under the bark of coniferous trees (King et al. 1997), and *Dendroctonus ponderosae* which attacks pine trees (Bentz 2008). Numerous weevil species were transported to North America from Europe on ornamental plants during the late 18th century (Anderson 2002). Many of these have become established widely in North America (Bright and Bouchard 2008; Ciegler 2010). In some cases, such as the accidental introduction of *Hypera postica* (Gyllenhal, 1813), the damage to the alfalfa farming industry resulted in the loss of tens of millions of U.S. dollars (Radcliffe and Flanders 1998). Arguably one of the most damaging introduced species, the black vine weevil *Otiorhynchus sulcatus* (Fabricius, 1775), has an especially broad host range and is a major pest in vineyards and strawberry farms (Masaki et al. 1984; Fisher and Bruck 2004). In Oregon alone, the damage caused by *O. sulcatus* was documented to have cost nurseries and fruit farms over 3 million U.S. dollars per year since its establishment (Fisher and Bruck 2004). Foreign weevil species continue to be reported from the Nearctic region (e.g. Sweeney et al. 2012; Anderson et al. 2013; Franklin et al. 2021).

Within Coleoptera, four suborders have been recognized: Polyphaga, Adephaga, Myxophaga, and Archostemata (Lawrence and Newton 1982; Zhang et al. 2018; McKenna et al. 2019). The relationships between the suborders was a subject of debate for decades, however recent phylogenomic work found high support for a Polyphaga (Adephaga (Archostemata, Myxophaga)) topology (Zhang et al. 2018; McKenna et al. 2019). The weevils (Curculionoidea) fall within the Polyphaga, a suborder characterized by a complete 1st sternite, lacking a notopleural suture, and by venational characters of the hind wings (Lawrence and Newton 1982; Marshall 2018). With over 300,000 described species, this is the largest suborder of beetles and also contains some of the most well-known beetle families, such as the longhorn beetles (Cerambycidae), lady beetles (Coccinellidae), and fireflies (Lampyridae) (Marshall 2018). Within the Polyphaga, the Curculionoidea have historically been considered sister to Chrysomeloidea (together forming the Phytophaga) as these superfamilies possess multiple morphological synapomorphies (Lawrence and Newton 1982); a relationship which is also strongly supported by recent phylogenomic work (Zhang et al. 2018; McKenna et al. 2019).

Phylogenomic studies of family level relations within Curculionoidea have found some support for purely morphology-based taxonomies (Oberprieler et al. 2014; Gunter et al. 2016; Shin et al. 2017), with families such as Anthribidae and Nemonychidae representing some of the oldest lineages. The phylogenetic placement of the most species-rich family, Curculionidae (>50000 described species), has been disputed (Anderson 1995; Shin et al. 2017). The recent phylogenomic work of Shin et al. (2017) however, found maximal support for Curculionidae (Brachycerinae, Dryophthorinae, Platypodinae, Bagoinae, Hyperinae, Entiminae, Cyclominae, Scolytinae, Curculioninae, Molytinae, Mesoptilinae, Cossoninae, Conoderinae) as the sister

group to Brentidae, from which it split during the genesis of angiosperms 132-180 million years ago. Despite this recent progress at higher taxonomic levels, low morphological phylogenetically informative signal below the sub-familial level has hindered the development of a complete, coherent taxonomic framework in the family Curculionidae (Bright and Bouchard 2008; Davis 2017; Shin et al. 2017).

1.2 Thesis objectives and significance

Here, in an effort to add to the subfamilial knowledge of Curculionidae, I investigate for the first time the molecular systematics of core genera within Ellescini Thompson, 1859 (Curculionidae: Curculioninae), a tribe which is distributed throughout the Holarctic region and whose members specialize on willows (Salicaceae). In particular, this work aims to substantiate the monophyly of the genus *Elleus* Dejean, 1821 and determine the phylogenetic placement of the related genus *Proctorus* LeConte, 1876. I also use morphological and molecular techniques to investigate the composition and taxonomy of the species of *Elleus* in North America, a natural clade for which species definitions have remained unclear and for which no identification keys currently exist. This work will add significantly to our understanding of an understudied weevil tribe, and will also serve as a basis for future taxonomic work. This research also has implications for conservation. Climate warming and anthropogenic disturbance are driving global insects declines and extinctions (Forister et al. 2019; Didham et al. 2020), and urbanization can result in significant decreases in willow-feeding weevil diversity (Su et al. 2011). A lack of clear, accurate taxonomy can hinder the recognition of decline or extinction when species are not separable from congeners. The implementation of targeted conservation

efforts is also not possible when multiple distinct species (possibly with different natural histories) and unresolved distributions are treated under the same name.

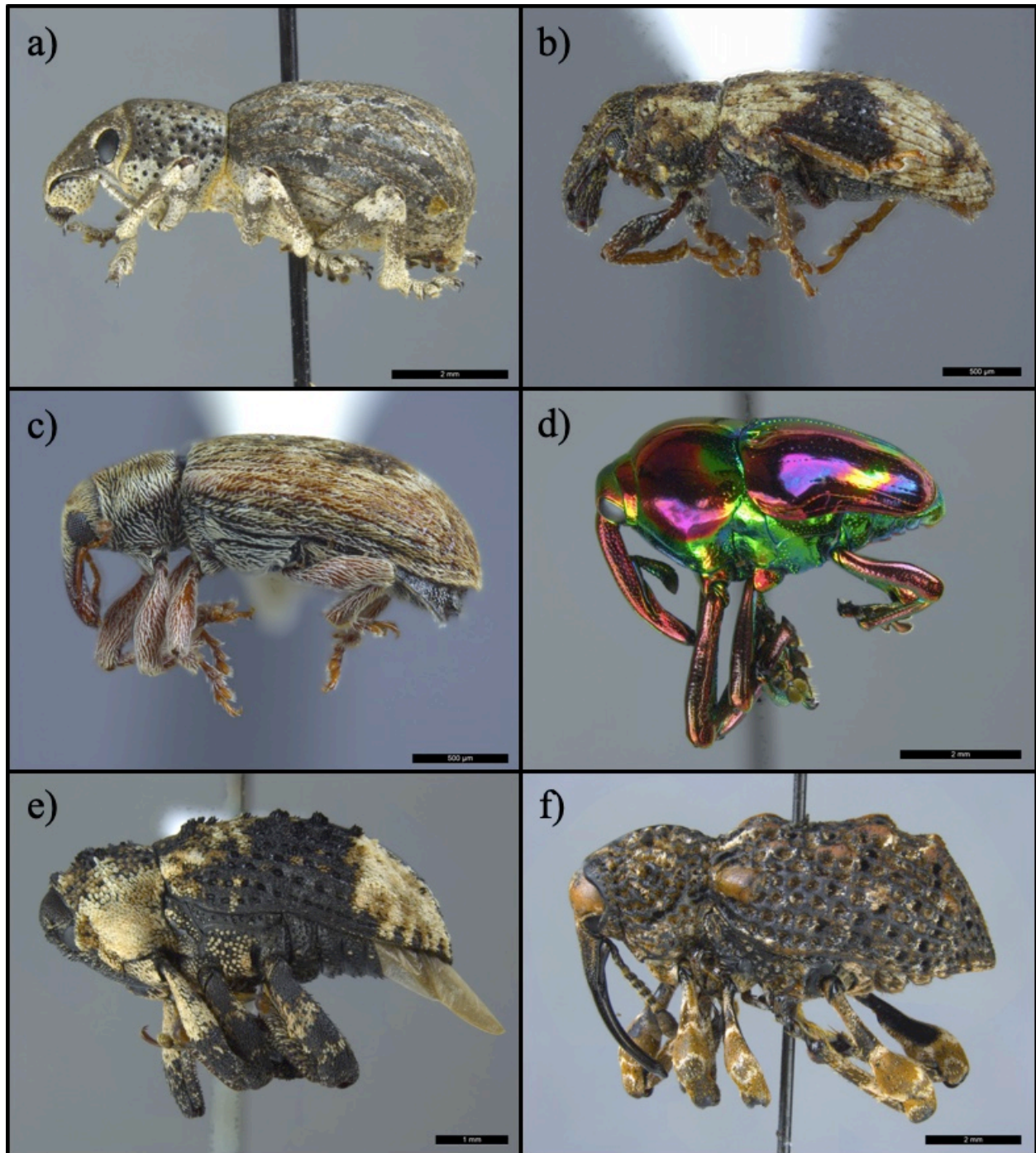


Figure 1.1 A selection of weevils illustrating their morphological diversity: a) A flightless denizen of arid habitats, *Ophryastes turbinatus* (Champion, 1911) - Big Bend National Park, Texas, U.S.A. - 23 June 1982 - R.S. Anderson; b) A wetland species and strong swimmer, *Listronotus delumbis* (Gyllenhal, 1834) - Val-des-Monts, Quebec, Canada - 23 June 2020 - J.H. Lewis; c) A small, drab species, *Ellescus californicus* (Casey, 1885) - Alta Sierra, California, U.S.A. - 15 July 1992 - Henry and Anne Howden; d) An iridescent tropical weevil species, *Eurhinus* sp. - Huehuetenago, Guatemala - 15 October 2017 - E. Fuller; e) A possible mimic of bird droppings, *Cryptorhynchus lapathi* (Linnaeus, 1758) - Rockwood Park, New Brunswick, Canada - 7 July 2021 - J.H. Lewis; f) A large, cryptically patterned species that is easily overlooked when at rest on dark, rough-bark trees, *Ectatorhinus adamsii* Lacordaire, 1865 - Kounandai, Kanagawa Prefecture, Japan - 27 May 2019 - J.H. Lewis.

Chapter 2: A Review of the Species of *Ellescus* in North America

Nomenclatural Disclaimer: Although this thesis contains phrases such as “new species” and “new synonymy”, it is not issued for the public and permanent scientific record or for the purposes of zoological nomenclature. Names will soon be published in a scientific journal, which will validate all of the nomenclatural acts presented within the current thesis.

2.1 Introduction

The weevil genus *Ellescus* Dejean, 1821 (Coleoptera: Curculionidae: Ellescini) currently includes nine recognized species worldwide (Table 2.1) (O’Brien and Wibmer 1982; Alonso-Zarazaga et al. 2017), which are distributed throughout the Holarctic region, including North America, across Europe, Russia, Japan (Alonso-Zarazaga and Lyal, 1999), Korea (Han and Park 2015), and China (Su et al. 2012). Members of the genus are associated with the closely related (Salicaceae) north temperate plant genera *Salix* Linnaeus and *Populus* Linnaeus (Anderson 2002), and the larvae are known to develop in female catkins (Scherf 1964). Pupation takes place in the soil, after the catkins have fallen to the ground (Hoffmann 1954; Scherf 1964). The first published appearance of the genus name appears in Dejean (1821) and included nine species, two of which (*E. scanicus*, *E. bipunctatus*) are still considered valid. Dieckmann (1970) reports that the genus name translates from Latinized Greek to “world-famous” [weltbekannt], a reference which may be referring to the broad geographic range of some species. Species of *Ellescus* commonly appear under the alternate spelling “*Elleschus*”, an unjustified emendation by Schoenherr (1836) (Dieckmann 1970; Alonso-Zarazaga and Lyal 1999; Alonso-Zarazaga et al. 2017). One species included in O’Brien and Wibmer (1982), *E. carludovicae* (Guenther, 1936), has been subsequently transferred to *Perelleschus* Wibmer & O’Brien on morphological grounds (Franz and O’Brien 2001). Furthermore, this species is distributed in Costa Rica and is associated with the plant genus *Carludovica* Ruiz & Pavon (Cyclanthaceae), which would misalign with the Salicaceae-associated, Holarctic habits of true *Ellescus* species. Additionally, it was previously noted in the literature that *Ellescus* requires revision in Japan (Takakuwa and Fujita 2010), and that the Japanese endemic species, namely, *E. bicoloripes* Voss and Chujo, 1960 and *E. pauper* Chujo and Voss, 1960, are associated with the plant genus *Rhododendron* L.

(Ericaceae) and actually belong to another genus (*Adorytomus* Voss) (pers. comm. Dr. Hiroaki Kojima, Tokyo University of Agriculture).

The genus *Ellescus* is currently placed in the tribe Ellescini along with *Dorytomus*, *Proctorus*, and *Rodotymus* (Alonso-Zarazaga and Lyal, 1999; Clark 2010; Caldara et al. 2014). Two genera which were previously included in Alonso-Zarazaga and Lyal (1999) under Ellescini, namely, *Minyrus* Schoenherr, 1835 and *Ontoctetorus* Faust, 1895 were recently moved to Anthonomini (Clark 2010). Furthermore, the Australian genus *Sellechus* Lea, 1908 is thought to belong to Storeini, and the placement of the Indomalaysian genera *Bathrorygma* Marshall, 1926 and *Minyrophilus* Voss, 1957 within Ellescini is dubious (Caldara et al. 2014). The east Asian genus *Adorytomus* Voss, 1953 occurs on members of Theaceae and Ericaceae (Kojima and Truong 2012; pers. comm. Dr. Hiroaki Kojima, Tokyo University of Agriculture), and the remaining four genera, namely, *Dorytomus*, *Ellescus*, *Proctorus*, and *Rodotymus* Zumpt, 1932, occur exclusively on Salicaceae (Alonso-Zarazaga and Lyal, 1999; Anderson 2002; Alonso-Zarazaga et al. 2017). The genera *Dorytomus* (100+ species) and *Ellescus* (7-9 species) are Holarctic in distribution, *Proctorus* (2 spp.) is endemic to North America, and *Rodotymus* (monotypic) occurs in Kazakhstan and its bordering countries (Alonso-Zarazaga and Lyal, 1999; Anderson 2002). Thus, based on geographic distribution, host preference, and recent taxonomic work, it is apparent that only *Ellescus*, *Proctorus*, *Dorytomus*, and *Rodotymus* are clearly related.

A key to the west European species of *Ellescus* is presented in Hoffmann (1954), who describes the genus as having a “...well developed rostrum, equal to the prothorax or shorter, slightly arched, cylindrical; linear, oblique scrobes, directed towards the lower part of the eye.

Scape reaching the eye; funicle of 7 articles, the first thick, elongated, obconical, much longer than the second which is of the same shape, those following short, transverse. Eyes large, oval. Prothorax weakly transverse, barely rounded laterally, subtruncated at the base. Elytra convex, oblong, with obtuse angular shoulders, almost straight sides, not widened at the back, rounded together at the top, and covering the pygidium. Femora thick. Short tarsi. Tarsal claws strongly divergent, toothed” (in French; Hoffmann 1954, pg. 1129). The sexes are easily differentiated as the first two sternites are medially concave in males (Hoffmann 1954). As in many other weevil groups the rostrum is longer in females (Hoffmann 1954; Anderson 2002), and the last two tergites are sclerotized in males (only last tergite in females) (as in Whitehead et al. 2018). Anderson (2002) separates *Ellescus* from the related genera *Dorytomus* and *Proctorus* based on basal teeth on the tarsal claws and the lack of a femoral tooth (*Dorytomus*: simple tarsal claws, with femoral tooth; *Proctorus*: basal teeth on the tarsal claws, with femoral tooth).

From North America, four species have been reported, namely, *E. bipunctatus* (Linnaeus, 1758), *E. borealis* (Carr, 1920), *E. ephippiatus* (Say, 1831), and *E. scanicus* (Paykull, 1792) (O’Brien and Wibmer 1982; Bousquet et al. 2013). Type specimen repositories for the currently recognized species of *Ellescus* reported from North America are presented in Table 2.2, which also includes *Ellescus californicus* (Casey, 1885), a form which is currently treated as either a junior synonym or subspecies of *E. ephippiatus* (O’Brien and Wibmer 1982; Poole and Gentili 1996). Two of these (*E. bipunctatus*, *E. scanicus*) were described from European specimens; however, it is unclear whether these were introduced or are native to the continent as reports vary (Hatch 1971; O’Brien and Wibmer 1982; Mattson et al. 1994; Bousquet et al. 2013). This is likely due to the lack of clear species definitions for species occurring in North America, as an

inability to properly identify specimens would hinder attempts to detect the arrival of adventive species and track their range expansion. Indeed, it has been noted that the genus requires revision in North America (Anderson 2002). Publicly available DNA barcode data (<https://www.boldsystems.org/>) for species of *Ellescus* suggests misidentification rates of up to 50%, and preliminary dissection work for this study also found similar misidentification rates in two large Canadian collections (Canadian Museum of Nature, Canadian National Collection of Insects). As the species of *Ellescus* were largely described based on differences in color, and no comprehensive study of the group has been conducted that takes male genitalia or molecular data into account, species level identification has remained difficult.

Here, I also use a combination of morphological and molecular techniques to investigate the composition and taxonomy of the species of *Ellescus* in North America, as species definitions have remained unclear for species on the continent. Methodology includes examination of external morphology, dissection of males (genitalia), and DNA barcoding (CO1, ITS2). I also review and develop a photographic key to the species of *Ellescus* in North America, that will allow users without access to a reference collection to identify specimens with relative ease. This work follows the Phylogenetic Species Concept (Wheeler and Platnick 2000), which defines a species as an irreducible lineage that is identified by a unique set of characters. Morphological, molecular (CO1, ITS2), ecological (e.g. host plant use), and biogeographical characters are all considered when deciding whether to synonymize, elevate or describe new taxa. It is worth noting that there is no way of objectively “weighting” characters or character sets in the species delineation process. However, it is assumed here that divergence in genital characters represent especially strong evidence of isolation (see Masly 2012). With regards to

molecular data, is unclear to what degree a sequence need differ from its neighbor to warrant the description of a new species. Some degree of molecular divergence in CO1 and ITS2 sequences does not necessarily imply isolation and sequence divergence rates vary between groups (Navajas et al. 1998; Skevington 2005; Elias et al. 2006; Meier et al. 2006; Whitworth et al. 2007). For this reason, while molecular sequence data is used to complement the morphological characters, it is not used in the absence of morphological evidence to substantiate taxonomic changes.

2.2 Methods

2.2.1 Specimen acquisition

Specimens were borrowed from museum and university insect collections in North America, Europe, and Japan. Institution names and associated acronyms used in this work are presented below:

AFCF : Atlantic Forestry Centre, Canadian Forest Service, Natural Resources Canada, Fredericton, New Brunswick, Canada

ASUIC : Arizona State University Insect Collection, Tempe, Arizona, U.S.A. (includes specimens from the Charles W. O'Brien collection)

CBG : Center for Biodiversity Genomics, Guelph, Ontario, Canada

CMNC : Canadian Museum of Nature, Ottawa, Ontario, Canada

CNCI : Canadian National Collection of Insects, Ottawa, Ontario, Canada

CUAC : Clemson University Arthropod Collection, Clemson, South Carolina, U.S.A.

DEBU : University of Guelph Insect Collection, Guelph, Ontario, Canada

HKC : Hiroaki Kojima Collection, Tokyo University of Agriculture, Tokyo, Japan

KSC : Kyle Schnepf Collection, Gainesville, Florida, U.S.A.

LSAM : Louisiana State Arthropod Museum, Baton Rouge, Louisiana, U.S.A.

MEM : Mississippi State Entomology Museum, Mississippi, Mississippi, U.S.A.

MIZ : Museum of the Institute of Zoology, Polish Academy of Sciences, Warsaw, Masovia, Poland

NBM : New Brunswick Museum insect collection, Saint John, New Brunswick, Canada

RBCM : Royal British Columbia Museum, Victoria, British Columbia, Canada

RWC : Reginald Webster Collection, Charters Settlement, New Brunswick, Canada

UAMIC : University of Alaska Museum Insect Collection, Fairbanks, Alaska, U.S.A.

ZMUO : University of Oulu Zoological Museum, Oulu, North Ostrobothnia, Finland

ZSM : Zoologische Staatssammlung München, München, Bavaria, Germany

Specimens were also procured through targeted collecting efforts in Quebec and Ontario, Canada, as well as by Dr. Michael Košťál in Slovakia. Collection efforts concentrated on *Salix* and *Populus* and consisted of beating branches and sifting leaf litter at the base of plants from mid-April to mid-July 2020/2021. For large trees a 7-meter extendable net was used to beat branches that would otherwise have been out of reach. Specimens collected in the field were placed in individual vials (i.e., 1 vial per specimen) with >95% ethanol and stored in a freezer at -23°C until DNA extraction. All specimens collected in the field were deposited in the Canadian Museum of Nature Collection (CMNC). In addition to examining the species covered in this work (including *E. scanicus*), specimens of the Old World species *E. infirmus* (Herbst, 1795), *E. languidus* (Faust, 1882), and *E. brevirostris* (Desbrochers des Loges, 1875) were also examined.

2.2.2 Dissection protocol, morphological analysis and imaging

A total of 56 male specimens and several female specimens were dissected using standard protocols and genitalia were cleared in a water + KOH solution (see Smith 1979). Dissected specimens were organized according to genitalic differences, and a search for delineating external characters was carried out using a 50X WILD M5A microscope (WILD Heerbrugg, Heerbrugg, Switzerland). Measurements of length and specimen imaging was completed using a Leica Z16 APOA camera and LAS image stacking software (Leica Microsystems, Wetzlar, Germany). Full body measurements presented in the species profiles (Section 2.3.2) were made from dorsal photographs, and were measured from the posterior-most tip of the elytra to the vertex of the head. Characters given in the diagnoses for each species (Section 2.3.2) are listed in order of importance (most important to least important). Range maps were created in SimpleMappr (Shorthouse 2010).

2.2.3 DNA extraction protocols

Specimens recently collected from the field and stored in 95% ethanol in a freezer at -23°C produced satisfactory sequence data using methods outlined in Ivanova et al. (2006). This technique requires removing 2-3 legs and crushing them into a powder form using a grinding medium (1 mm diameter silicon carbide beads). Dried museum specimens (pointed) however, did not produce adequate sequence data using the method of Ivanova et al. (2006). For this reason, the method of Santos et al. (2018) was used. In this latter method, rather than removing and grinding legs of specimens, entire specimens are placed in tubes with a digestion buffer (194 µl WID buffer (Whole Insect Digestion Buffer) + 6 µl Proteinase K) and incubated for 16 hours at 56°C. This allows for the extraction of all of the DNA contained within a specimen, which is

necessary with small, older weevil specimens as single legs do not contain enough high-quality, amplifiable DNA. Furthermore, the whole insect extraction technique is non-destructive and was also not found to alter the shape of the male genitalia, allowing for morphological study afterwards. All DNA tubes were labeled with unique identifiers (LEW_####) and stored in a fridge at 4°C throughout the course of this study.

2.2.4 Gene and primer selection, polymerase chain reaction (PCR) and sequencing reactions

The mitochondrial protein-coding “barcode gene” CO1 (Cytochrome c oxidase subunit I) has become widely used in taxonomic studies because it is short and easily amplified, structurally conserved, and taxonomically informative (Hebert et al. 2003; Rach et al. 2017). Since 2003, the gene has been used in thousands of taxonomic and faunistic studies (Cywinska et al. 2006; Rach et al. 2007; Rivera and Currie 2009; Hamada et al. 2010; Evangelista et al. 2013; Pauly et al. 2015; Pentinsaari et al. 2014, 2019; Lewis et al. 2019). While CO1 does delineate many insect taxa effectively, it does fail in some groups (Elias et al. 2006; Meier et al. 2006; Whitworth et al. 2007). Generally, COI-based barcoding has been shown to accurately delineate weevil species and has been widely applied (Riedel et al. 2009, 2013; Stepanovic et al. 2015; Grebennikov 2016; Pentinsaari et al. 2014, 2019). Barcode trees (i.e., CO1 gene trees) are commonly constructed using some measure of genetic distance and Neighbor-Joining (NJ) (Saitou and Nei 1987; Srivathsan and Meier 2012). The most frequently used distance measure is the Kimura-2-Parameter (K2P) (Kimura 1980); however, it has been suggested by some that the use of K2P is unjustified and does not provide any significant advantage over other measures (Collins et al. 2012; Srivathsan and Meier 2012). The widespread use of K2P is likely due to its

initial use by Hebert et al. (2003) and the fact that the measure is built into BOLD (Barcode of Life Data Systems) (Ratnasingham and Hebert 2007). In addition to CO1, the nrDNA ITS2 (internal transcribed spacer 2) has also been widely used in species delineation, often in combination with CO1 (Li et al. 2010; Yao et al. 2010; Yusseff-Vanegas and Agnarsson 2017). This spacer is particularly useful in cases where CO1 fails to delineate taxa, as it evolves faster (Navajas et al. 1998; Skevington 2005). Furthermore, ITS2 has been shown to accurately delineate species in taxonomic beetle studies (e.g., Fossen et al. 2016; Gallego and Galian 2001). Here, to complement the morphological analysis of *Ellescus*, both CO1 and ITS2 sequences were obtained according to the following procedures.

Except for the specific primers used, initial polymerase chain reaction (PCR) recipes (15µl volume reactions) were the same for both CO1 and ITS2: Per sample – DNA grade water (9.05µl), Q5 buffer (5X) (3µl) (<https://www.neb.ca/q5>), dNTPs (10mM) (0.3µl), primers (10µM) (0.75µl each) (Table 2.3), Q5 polymerase (2 U/µl) (0.15µl), and template DNA (1µl). PCR temperature profiles were the same for both marker: Initial (98°C / 30 seconds) - [Denaturing (98°C / 10 seconds) - Annealing (56°C / 30 seconds) - Extension (72°C / 30 seconds)] (34 cycles) - Final (72°C / 5 minutes). Following the methods of Astrin et al. (2012), it was found that a thermocycler program which combines “Touch Down” and “Step Up” procedures (see Chapter 3 - Table 3.2) was particularly effective at producing strong CO1 PCR products, and in some cases resulted in strong bands where a simple thermocycler program failed to produce any bands. This program was used in all subsequent CO1 PCRs. In instances where PCR products remained weak despite altering thermocycler programs, the amount of template DNA was increased to 2µl (DNA grade water decreased to 8.05µl), which occasionally produced

satisfactory sequence data. Additionally, it was found that a higher PCR volume (50µl) of DNA grade water (32.5µl), Q5 buffer (5X) (10µl), dNTPs (10mM) (1µl), primers (10µM) (2.5µl each), Q5 polymerase (2 U/µl) (0.5µl), and template DNA (1µl), was particularly effective at generating strong PCR products for samples taken from older, mounted specimens. PCR products were visualized using agarose gel electrophoresis with TAE buffer (Tris-acetate-EDTA buffer) and ethidium bromide stain. Strong products were selected for sequencing. Satisfactory PCR products were diluted and sequencing reactions were carried out using the following recipe: Per sample - DNA grade water (6.2µl), ABI buffer (5X) (1.8µl), primer (10µM concentration) (0.5µl), BigDye (0.5µl) (<https://www.thermofisher.com/ca/en/home.html>), and PCR product (1µl). The thermocycler program for sequencing reactions was as follows: Initial (95°C / 3 min.) – [Denaturing (96°C / 30 sec.) - Annealing (50°C / 20 sec.) - Extension (60°C / 4 min.)] (30 cycles). To ensure that unused dye-labelled ddNTPs are removed from the sequencing reactions purification was carried out through an ethanol/EDTA/sodium acetate precipitation process. Double stranded sequencing was performed on an ABI 3500XL sequencer (Applied Biosystems™, ThermoFisher Scientific, Waltham, Massachusetts). It should also be noted that mounted specimens procured through ZMUO and CBG were already barcoded (CO1), and thus it was only necessary to verify taxonomic identifications for those specimens.

2.2.6 Sequence editing, alignment and analysis

All sequence editing, alignments and analyses were performed in Geneious Prime ver. 2021.1.1 (<https://www.geneious.com>). Raw sequences were first trimmed using the built in “Trim Ends” function (error probability limit = 0.05; default settings), and visually scanned afterwards. When ambiguous regions still occurred near the ends despite the automatic trimming,

sequences were manually trimmed to remove these ambiguities. Low quality sequences (HQ < 50%) were removed from the analysis. As a precautionary step, all sequences were run through BLAST (Altschul et al. 1990) to ensure that non-target DNA had not been amplified. Alignment of sequences was straightforward for both genes and was performed in MUSCLE ver. 3.8.425 (Edgar 2004) using default settings. To visualize pairwise distances between sequences, a neighbor-joining tree with 10000 bootstrap replicates was constructed for both markers under a Jukes-Cantor substitution model and with a 70% support threshold. All specimen collection data associated with sequences used in the neighbor-joining trees are listed in Table 2.4. Tree stylization was enhanced in FigTree ver. 1.4.4 (Rambaut 2009).

2.3 Results

2.3.1 Overview of results

Four genitalic forms (males) were found in North America (Fig. 2.3b-e), which correspond to *E. bipunctatus*, *E. californicus* (resurrected status), *E. ephippiatus* and *E. michaeli* n. sp. A key to the four North American species and species profiles are presented in Sections 2.3.2 and 2.3.3, respectively. External morphology was found to be moderately useful for separating the species; however dissections of males is necessary to confidently identify species in some cases. Important external characters included the presence of structures on the 5th ventrite of males (Figures 2.1, 2.2), the density of scales on the metasternum and metepisternum (Figures 2.4, 2.5), the presence or absence of discolored pronotal bands (Figures 2.8-2.15, 2.18-2.20, 2.22, 2.23, 2.26-2.29, 2.33), and cuticular color (Figures 2.18, 2.20, 2.22, 2.23, 2.26, 2.28, 2.30, 2.32). DNA barcoding (CO1, ITS2) was useful for species delineation of *Ellescus*, and

those molecular data were fully congruent with morphology (see Section 2.3.4 for barcoding results).

2.3.2 Key to the species of *Ellescus* in North America (including *E. scanicus*)

*Unless otherwise stated, characters referred to in this key work for both sexes. As females lack an aedeagus as well as useful characters on the 5th ventrite, they are more difficult to separate. In some cases, associated males must be used to confirm identifications. The first couplet of the key provides characters for sexing specimens.

ia) First two sternites depressed medially. With shiny, medial carina extending longitudinally over last sternite, or with distinct medial swelling at tip of last sternite (occasionally very reduced or totally absent) (Figures 2.1 and 2.2, respectively). Last two tergites sclerotized (viewing this character often requires relaxing and manipulating specimens). Rostrum shorter.....[Male]

ib) First two sternites not distinctly depressed medially. Last sternite unmodified. Only last tergite sclerotized. Rostrum longer.....[Female]

1a) Males with glabrous carina extending longitudinally over more than half of last sternite; not prominent at apex of sternite [reduced in some specimens] (Figure 2.1). Metepisternum and lateral half of metasternum densely punctate and covered with appressed white-yellow scales (Figure 2.4), or with metepisternum and lateral half of metasternum not densely punctate or scaled, with cuticle in those regions mostly visible and with interspaces shining (Figure 2.5). Pronotum rarely with regions of discolored scales laterally (Figure 2.8-2.15). Fore femur with or without minute tooth or swelling. Apex of aedeagus not pointed, well rounded or subquadrate (Figure 2.3a, b). Aedeagus without fully protruding basal v-shaped structures (Figure 2.3a, b)2

1b) Males with prominent swelling at apex of last sternite, not extending over more than half of last sternite [reduced to absent in some smaller specimens] (Figure 2.2). Metepisternum and lateral half of metasternum not as densely punctate or scaled, cuticle in those regions mostly visible and with interspaces shining (Figure 2.5). Pronotum with regions of discolored scales laterally [faint in some specimens] [occasionally reduced or absent completely] (Figure 2.18-2.20, 2.22, 2.23, 2.26-2.29, 2.33). Fore femur without tooth or swelling. Apex of aedeagus ending in a well-rounded to sharp point (Figure 2.3c-e). Aedeagus with fully protruding basal v-shaped structures (Figure 2.3c-e)3

2a) Metepisternum and lateral half of metasternum densely punctate and covered with appressed white-yellow scales, cuticle in those regions mostly concealed (Figure 2.4). Fore femur occasionally with minute tooth or swelling ventrally. Apex of aedeagus subquadrate (Figure 2.3a). Aedeagus with distinct v-shaped endophallus (Figure 2.3a).....*E. scanicus* (Paykull, 1792) (European; not present in North America)

- 2b)** Metepisternum and lateral half of metasternum not as densely punctate or scaled, cuticle in those regions mostly visible and with interspaces shining (Figure 2.5). Fore femur rarely with swelling. Apex of aedeagus rounded (Figure 2.4b). Aedeagus lacking v-shaped endophallus (instead with hook-like structure and roughened musculature) (Figure 2.4b).....*E. bipunctatus* (Linnaeus, 1758) [Pg. 22]
- 3a)** Cuticle mixed in color (red, black, yellow, brown).....4
- 3b)** Cuticle entirely red or pale yellow.....6 [Dissection of males required]
- 4a)** Metasternum not contrasting with black femora, tibiae, and rostrum (Figure 2.22). Elytral coloration distinctive and highly contrasting: dark with transverse bright orange band in apical half (Figure 2.22, 2.23). Smaller (1.9-2.2 mm). Apex of aedeagus ending in broadly rounded tip; lateral edges of apex dorsoventrally flattened, with hairs distributed along surface (Figure 2.3c).....*Ellescus michaeli* n. sp. (in part) [Pg. 29]
- 4b)** Metasternum black and contrasting with reddish legs and apical half of rostrum (Figure 2.18, 2.20, 2.26, 2.28, 2.30, 2.32). Elytral coloration variable, but never as above (Figure 2.19, 2.27, 2.29, 2.31, 2.33). Many specimens larger (2.0-2.9 mm). Apex of aedeagus more pointed; apex with two prominent tufts of hairs (Figure 2.3d-e).....5
- 5a)** Apex of aedeagus quadrate and coming to a distinct, sharp point; lateral edges of aedeagus heavily sclerotized (Figure 2.3d). Restricted to California and southern Oregon (Figure 2.36).....*Ellescus californicus* (Casey, 1885) (in part) [Pg. 26]
- 5b)** Apex of aedeagus rounded, ending in a distinct point (Figure 2.3e). Widespread across North America (although apparently absent from California and southern Oregon) (Figure 2.38).....*Ellescus ephippiatus* (Say, 1831) (in part) [Pg. 31]
- 6a)** Apex of aedeagus ending in broadly rounded tip; lateral edges of apex dorsoventrally flattened, with hairs distributed along surface (Figure 2.3c). Smaller species (1.9-2.2 mm).....*Ellescus michaeli* n. sp. (in part) [Pg. 29]
- 6b)** Apex of aedeagus ending in sharp tip (Figure 2.3d-e). Most specimens larger (2.0-2.9 mm).....7
- 7a)** Apex of aedeagus quadrate and coming to a distinct, sharp point; lateral edges of aedeagus heavily sclerotized (Figure 2.3d). Restricted to California and southern Oregon (Figure 2.36).....*Ellescus californicus* (Casey, 1885) (in part) [Pg. 26]
- 7b)** Apex of aedeagus rounded, ending in a distinct point (Figure 2.3e). Widespread across North America (although apparently absent from California and southern Oregon) (Figure 2.38).....*Ellescus ephippiatus* (Say, 1831) (in part) [Pg. 31]

2.3.3 Species profiles

***Ellescus bipunctatus* (Linnaeus, 1758)**

Figures 2.1, 2.3b, 2.5, 2.8-2.17, 2.35

Curculio bipunctatus Linnaeus 1758: 380 (original description); Paykull 1792: 58 (description, comment).

Curculio unipunctatus Olivier 1791: 495 (description).

Orchestes ruficornis Zetterstedt 1837: 185 (description).

Mecinus erythrocerus Abeille de Perrin 1910: 83 (description).

Elleschus bipunctatus; Hubbard et al. 1878: 621 (taxonomic comment); Austin 1880: 49 (catalogue); Hamilton 1889: 156 (distribution); Slosson 1897: 239 (checklist); Blatchley and Leng 1916: 283 (identification, distribution); Hoffman 1954: 1131 (identification); O'Brien and Wibmer 1982: 116 (catalogue, distribution); Mattson et al. 1994: 10 (catalogue).

Elleschus bipustulatus; Hatch 1971: 342 (error; catalogue).

Elleschus borealis Carr 1920: 220 (description); Bousquet et al. 2013: 326 (catalogue, distribution); **New synonym.**

Ellescus bipunctatus - Bousquet et al. 2013: 326 (catalogue, distribution); Webster et al. 2016: 373-374 (distribution); Alonso-Zarazaga 2017: 196 (catalogue, distribution); Legalov 2020: 496 (catalogue, distribution).

Type locality and data: “Europa” – described from an unknown number of specimens.

Location of types not known. Here, I adopt the consensus definition of this species based on identified material in the MIZ, ZSM, and ZMUO, as well as the descriptions of Paykull (1792) and Hoffman (1954).

Diagnosis: Length (2.5-2.9 mm). Shiny carina or swelling running longitudinally along last sternite of males (absent in females). Scales on pronotum uniform in color (usually white) or only with central band of discoloration; lacking discolored lateral bands. Metasternum and metepisternum with scales relatively sparse not obscuring underlying cuticle. Apex of aedeagus

smoothly rounded (semi-circular); not subquadrate or distinctly pointed. Aedeagus with distinct sclerotized hook like structure and tuberculate internal sac; lacking any protruding basal structures. Femora lacking minute tooth, but occasionally with very small swelling. Cuticular coloration red and/or black in most specimens; rarely entirely pale yellow. Distributed across temperate and boreal North America (absent from the southwest and southeast).

Distribution in North America (Figure 2.35): This species is distributed widely across Canada, from New Brunswick to Northwest Territories, and is also known from several U.S. states, including Alaska, Indiana, Massachusetts, and Michigan.

Specimens examined: **AUSTRIA:** Lower Austria, Tullnerbach, A. Winkler (2, CMNC); Wendbach (1, MIZ), COL000400; **CANADA: Alberta:** Calgary, 14 Jun 1953 – 18 Jun 1959, B. & J. Carr (8, CNCI); Ghost Dam, 18 Jun 1959 – 22 May 1981, B. & J. Carr (7, CNCI); Edmonton, 19 Apr 1919, F.S. Carr (1, CNCI); Edmonton, 10 May 1916, A.C. Davis (4, CNCI); 24 miles south of Lloydminster, 30 May 1963, F.I.S., on *Populus tremuloides* (1, CNCI); McMurray, 4 June 1953, Brooks-Wallis (1, CNCI); Exshaw, 27 Jun 1954, B.F. & J.L. Carr (1, CNCI); **Manitoba:** Aweme (Criddle), 28 April 1903 (1, DEBU), DEBU01089104; Aweme (N. Criddle), 19 May 1922 (1, CNCI); “Manitoba” (1, RBCM), RBCM EENT991-111634; Gillam, 11 Jun – 17 Jul 1950, W.J. Brown & J.F. McAlpine (27, CNCI); Onanole, Riding Mountain National Park, 8 June 1937 – 15 June 1938, W.J. Brown (15, CNCI); Carberry, 9 May 1953, Brooks-Kelton (1, CNCI); Makinak (3, CNCI); **New Brunswick:** Queens Co., Cranberry Lake P.N.A. (46.1125°N, 65.6075°W), 3-25 May 2011, M. Roy & V. Webster, Red Oak forest Lindgren funnel trap (2, NBM), NBM-070113 - NBM-070115; Carleton Co., Belleville Meduxnekeag Valley Nature Preserve (46.1878°N, 67.6705°W), 18 August 2008, R.P. Webster (1, NBM), NBM-070105; **Northwest Territories (new territorial record):** Norman Wells, 9 Apr 1949 - 26 May 1953, S.D. Hicks & C.D. Bird (75, CNCI); **Ontario:** Prince Edward Co., 28 May 1919 - 1 Jun 1923, J.F. Brimley (11, CNCI); Rainy River District, 14 May 1924, J.F. Brimley (6, CNCI); Emo, 21 Jun 1924, J.F. Brimley (2, CNCI); Carleton Co., Constance Bay, 6

Aug 1959, H. Howden, on sweetfern (1, CNCI); Carleton Co., Constance Bay, 17 April 1968 - 29 April 1998, Henry & Anne Howden (60, CMNC); Carleton Co., Constance Bay, 1970, S. Peck (1, CNCI); Carleton Co., Constance Bay, 21 April 1977, M. Sanborne (2, CMNC); Carleton Co., Constance Bay, 26 Apr 1935, W.J. Brown (5, CNCI); Carleton Co., Constance Bay, 19 May 1933, W.J. Brown (22, CNCI); Prince Edward Co., 14 Aug 1964, J.F. Brimley (4, ASUIC), UAIC1072869; Ottawa Co., Ottawa, Nepean Twp., 22 April 1976, M. Sanborne, on willow (2, CMNC); Ottawa Co., Ottawa, 23 May 1965, A.T. Howden (1, CMNC); Ottawa Co., Ottawa, 6 May 1930, W.J. Brown (3, CNCI); Ottawa Co., Ottawa, 17 May 1961, Kelton (1, CNCI); Ottawa Co., Mer Bleue, 13 May 1932 – 10 May 1933, W.J. Brown (38, CNCI); Kenora District, Minnitaki, 12 May 1960, F.I.S., on female *Populus tremuloides* catkins (2, CNCI); Hartington, Eel Lake, South Frontenac (44.563°N, 76.5522°W), 6 September 2017, J. deWaard (1, CBG), BIOUG35446-H11, ELPCH211-17; Hartington, Eel Lake, South Frontenac (44.5645°N, 76.5516°W), 25 July - 8 August 2017, G. Blagoev (1, CBG), BIOUG35442-G10, ELPCG9470-17; **Quebec:** Gatineau, near Boucher Forest (45.427673°N, 75.823561°W), 2 May 2021, J.H. Lewis, beaten from *Populus tremuloides* (2, CMN), LEW_118; Gatineau, Mont Cascades (45.588793°N, 75.845822°W), 13 May 2021, J.H. Lewis, beaten from *Salix* (1, CMNC), LEW_90; Estrie Region, Scotstown, 14-21 May 2012, C. Levesque (2, CNCI); Kirks Ferry, 25 May 1950, B.P. Beirne, in light trap (1, CNCI); Laniel, 14 Aug 1982, W.J. Brown (1, CNCI); Gatineau Park, Harrington Lake, 31 May 1954, R. McCondochie (1, CNCI); Cartier, Mount Jacques, 9 Jul 1954, W.J. Brown (1, CNCI); Kazubazua, 25 May 1933, W.J. Brown (8, CNCI); Seven Islands, 8 Jun 1929, W.J. Brown (6, CNCI); **Saskatchewan (new provincial record):** Prince Albert, 3 July 1954, Brooks-Wallis (1, CNCI); Fish Creek, 20 May 1928, K.M. King (3, CNCI); Elbow, 23 June 1954, Brooks-Wallis (1, CNCI); **FINLAND:** Espoo, Vanttila (60.1829°N, 24.6157°E), 6 Aug 2012, E. Helve (1, ZMUO), ZMUO.004794, KJ962308; Kuusamo, Aroniemi (66.2741°N, 29.7033°E), 14 June 2014, Mikko Pentinsaari (2, ZMUO), ZMUO.016797, ZMUO.016798, COLFH362-14; COLFH363-14; **GERMANY:** Laucha an der Unstrut, C. Schenkling (4, CNCI); Bavaria, Holzkirchen, 19 May 1957 - 8 May 1959, K. Gaigl (4, ZSM); Bavaria, Dietramszell, 26 Apr 1959, K. Gaigl (1, ZSM); **POLAND:** Owl Mountains (Eulengeb) (2, MIZ), COL000397; Sudety-G, Stolowe, Zacisze, 15 May 1947, M. Wegrzecki (1, MIZ), COL000398; Sierakow, 15 August 1950, B. Burakowski (1, MIZ), COL000399; Schlesien (1, MIZ), COL000401; **RUSSIA:** 2011, B. Korotyeav (3, CMNC); **SLOVAKIA:** Čačín,

Bystřická vrchovina hills (48°40.3'N, 19°15.4'E), 6 July 2020, Michael Košťál (2, CMNC), LEW_95, LEW_97. **UNITED STATES OF AMERICA: Alaska (new state record):** Moose Creek (64.71119°N, 147.10143°W), 26 May - 9 June 2015, J. Hagelin, spruce, meadow, hanging Malaise (1, UAMIC), UAM100387793; **Indiana:** Morgan Co., 14 July - 7 August 2012, K.E. Schnepf, Malaise Trap (1, CMNC), LEW_57 / LEW_104; **Massachusetts (new state record):** Middlesex Co., Natick, 15 Jul 1928, C.A. Frost (1, CNCI); Middlesex Co., Natick, 18 April 1952, C.A. Frost, *Salix catkins* (1, CMNC); **Michigan:** Cheboygan Co., Cheboygan, 12 May 1921, S. Moore (1, ASUIC), UAIC1072870.

Natural history: This species is Holarctic in distribution and occurs in North America from New Brunswick to Alaska. It has been collected on *Populus tremuloides* (Michaux) and *Salix bebbiana* Sargent in North America (this study), and in Europe from *S. capraea* L., *S. viminalis* L., *S. cinerea* L., and *S. aurita* L. (Hoffmann 1954). Specimens of *E. bipunctatus* have also been collected in Lindgren funnel traps set in the canopy of *P. grandidentata* Michaux in New Brunswick (Webster et al. 2016). The species has also been collected along with *E. scanicus* in fermenting bait traps (beer/wine and sugars) in Russia (Ruchin et al. 2021).

Remarks: Molecular data suggest some level of differentiation between the European and North American populations of this species (see section 2.3.3); however, dissections and examinations of external morphology did not uncover any significant differences in forms warranting formal naming. Therefore, it is assumed in this work that these represent the same species. This has implications for the F.S. Carr described species *E. borealis* as this is simply an all-red form of *E. bipunctatus* (see below). It is unknown whether *E. bipunctatus* was introduced to North America, is naturally Holarctic, or whether both hold true. The limited CO1 and ITS2 data available (Figures 2.40, 2.41) do not suggest North American and European populations

mixing, although broader sampling could be conducted to further investigate the above possibilities.

Taxonomic notes: The holotype specimen of *E. borealis* Carr, 1920 (Figures 2.14-2.17) was examined and was determined to represent a male, all-red form of *E. bipunctatus*, on the basis of the following factors and morphological characters: (1) possessing a conspicuous, shiny carina extending longitudinally over more than half of the of the last sternite (Figure 2.16), (2) with the metepisternum and lateral half of the metasternum not densely punctate or scaled, and with the cuticle in those regions mostly visible (Figure 2.14), and (3) dissections of similar, all-red specimens from the Canadian Prairies are all *E. bipunctatus*. As such, *E. borealis* should be treated as a new junior synonym of *E. bipunctatus*. Label data for the holotype of *E. borealis* is as follows: CANADA, Alberta, Edmonton, 24 May 1919, F.S. Carr [Edmonton Alta 24.v 1919 F.S. Carr] (Figure 2.17). Type *Elleschus borealis* - No. 419. CNC 379701.

***Elleschus californicus* (Casey, 1885) resurrected status**
Figures 2.3d, 2.18-2.21, 2.36

Elleschus californicus Casey 1885: 193 (original description).

Elleschus ephippiatus var. *californicus*; O'Brien and Wibmer 1982: 116 (catalogue, distribution).

Elleschus ephippiatus; Poole and Gentili 1996: 215 (catalogue).

Type locality and data: This species was described from two specimens collected in California, U.S.A. The holotype (USNM) is labelled with one brown rectangular label that reads

“Cal.”, a second brown rectangular label that reads “californicus type. Casey.”, a third brown rectangular label that reads “CASEY bequest 1925”, a red rectangular label that reads “TYPE USNM 36721”, and an institutional ID label that reads “USNMENT 01448984”. Although the holotype was not examined, high-resolution lateral and ventral photographs of the holotype provided by USNM confirms that the assignment of names used here is accurate.

Diagnosis: Length (2.1-2.6 mm). Distinct swelling at tip of last sternite of males, not extending over more than half of last sternite. Scales on pronotum usually not uniform in color, with discolored central and lateral bands. Metasternum and metepisternum with scales relatively sparse not obscuring underlying cuticle. Apex of aedeagus quadrate and coming to a distinct, sharp point; heavily sclerotized. Aedeagus with protruding basal structures. Femora lacking minute tooth. Restricted to California and southern Oregon.

Distribution in North America (Figure 2.36): This species is restricted to California and southern Oregon. The apparent lack of overlap in the ranges of *E. californicus* and *E. ephippiatus* is notable and further sampling of both species in the region may reveal differences in host plant preferences.

Specimens examined: UNITED STATES OF AMERICA: California: Sutter Co., Nicolaus, 18 April 1971, Fred G. Andrews (1, CMNC); San Bernardino Co., Needles, 8 April 1989, W.F. Barr, on willow (2, CMNC); San Bernardino Co., Victorville, 16 May 1955, W.R.M. Mason (1, CNCI); Kern Co., Wofford Heights, 14-15 July 1992, Henry & Anne Howden (4, CMNC); Kern. Co., Alta Sierra, 15 July 1992, Henry and Anne Howden (3, CMNC); Modoc Co., Cedarville, 1 April 1946, W.F. Barr (1, CMNC); Los Angeles Co., Boquette, Boquet

Canyon, 11 April 1974, M.W. Hetz (1, ASUIC); Sonoma Co., 2 miles north of Cloverdale, 27 Mar 1964, C.W. O'Brien, on *Salix* (6, CNCI); **Oregon (new state record)**: Lake Co., 8 miles west of Adel, 24 May 1973, K.J. Goeden (4, CMNC); Klamath Co., Sprague River canyon, 5 miles east of Bly, 22 May 1958, Vertrees & Schuh, on *Salix* (1, CMNC).

Natural history: This species is restricted to California and southern Oregon, where it has been collected on *Salix* species.

Remarks: This species occupies a region known for high rates of animal and plant endemism (Harrison 2013), including several endemic *Salix* and *Populus* species (Little 1979). Further sampling and recording of host plant use should be undertaken, which might help explain mechanisms of speciation for this species. Although this species was previously synonymized with *E. ephippiatus*, the combination of unique male genitalia and biogeography substantiate *E. californicus* as a distinct species. Attempts to amplify the barcode region of CO1 and ITS2 from specimens of *E. californicus* failed. Nonetheless, this species is clearly closely related to *E. ephippiatus* and *Ellescus michaeli* n. sp., as it possesses (1) a swelling at the tip of the last sternite (males only), (2) discolored (scales) pronotal bands, and (3) a pointed aedeagus. In addition to the species' North American distribution, this species is virtually indistinguishable externally from some individuals of *E. ephippiatus*, which further supports its close relatedness to that species.

***Ellescus michaeli* new species**
Figures 2.3c, 2.22-2.25, 2.37

Specimens examined: Holotype male (CMNC); Canada, Ontario, Mer Bleue Bog, 45.3973°N, 75.5144°W, 13 May 2021, R.S. Anderson, beaten off Salix sp.

Paratypes; **CANADA: Alberta:** Edmonton, 10 May 1915 - 3 May 1918, F.S. Carr (2, ASUIC; 1 CNCI); Peace River (18 miles south), 2 Jun 1950, P. Rubtsoff (3, CNCI); Ghost Dam, 10 Jun 1980, B.F. & J.L. Carr (1, CNCI); Calgary, 21 May 1956, B.F. & J.L. Carr (1, CNCI); **Manitoba:** Riding Mountain National Park, 4-6 Jun 1938, W.J. Brown (3, CNCI); Carberry, 8 - 10 May 1953, Brooks – Kelton (5, CNCI); Makinak (4, CNCI); **Ontario:** Prince Edward Co., 10 May 1915 - 26 May 1955, Brimley (11, CNCI); Smoky Falls, Mattagami River, 8 Jun 1934, G.S. Walley (1, CNCI); Ottawa Co., Ottawa, 7 May 1960 - 11 June 1965, A.T. Howden (17, CMNC); Ottawa Co., Kanata, Corkstown Rd., 11 May 1975, J.D. Read (1, CMNC); Ottawa Co., North Gower, 28 Apr - 4 May 1987, L. LeSage, old beaver meadow, attracted with allylthiocyanate (2, CMNC); Ottawa Co., Ottawa, Mer Bleue Bog (45.3973°N, 75.5144°W), 13 May 2021, R.S. Anderson, beaten off Salix sp. (15, CMNC), BOLD ID#; Mer Bleue, 10 May 1933, W.J. Brown (75, CNCI); Wellington Co., Guelph, 3 May 1982, D. Morris (1, DEBU), DEBU01089097; Marmora, 22 Apr – 8 Aug 1952, J.C. Mitchell, on Salix (6, CNCI); **Quebec:** Aylmer (Gatineau), Pink Road (near CMN natural history campus), 21 Apr 2008, L. LeSage, on Salix discolor catkins (8, CNCI); Cantley, dune on hill near Ch. Holmes (45.591708°N, 75.784886°W), 22 May 2020, J.H. Lewis, on female Salix catkins (1, CMNC), LEW_68; Gatineau, Mont Cascades (45.590976°N, 75.850493°W), 14 May 2021, J.H. Lewis, beaten from Salix (1, CMNC); Gatineau, disturbed lot across road from 1740 Pink Road Hull (45.441961°N, 75.811547°W), 10 May 2020, J.H. Lewis, on female Salix catkins (1, CMNC), LEW_70; Gatineau, Boucher Forest (45.416327°N, 75.826699°W), 18 May 2021, J.H. Lewis, beaten from Salix (3, CMNC); 28 Jun 1965, Malaise trap (1, CNCI); Kazubazua, 25 May 1933, W.J. Brown (1, CNCI); Mont St. Hilaire, May 1909 (1, CNCI); **Saskatchewan:** Saskatoon, 21 May 1940, A.R. Brooks (1, CNCI); Canora, 28 May 1954, Brooks – Wallis (1, CNCI); Fish Creek, 20 May 1921, K.M. King (1, CNCI); **UNITED STATES OF AMERICA: Maine:** Penobscot Co., Orono, 24 May 1948 (2, ASUIC), UAIC1072936, UAIC1072937; **Minnesota:** Otter Tail Co., Maplewood St. Pk., 28

May 1983, I.S. Askevold, sweeps of *Salix* (2, CMNC; 1, ASUIC); **Wisconsin:** University of Wisconsin-Madison Arboretum, 30 April 1949, R.H. Jones (7, CNCI).

Dianosis: Length (1.9-2.2mm). Distinct swelling at tip of last sternite of males, not extending over more than half of last sternite (occasionally absent). Scales on pronotum usually not uniform in color, with discolored central and lateral bands. Metasternum and metepisternum with scales relatively sparse not obscuring underlying cuticle. Apex of aedeagus sinuate and coming to broad, rounded point. Apical edges of aedeagus flattened laterally, with hairs distributed along the surface. Aedeagus with protruding basal structures. Femora lacking minute tooth. Metasternum (black to dark red) usually not contrasting with femora, tibiae or latter half of rostrum. Elytral coloration distinct; largely black with contrasting orange band in apical half. Distributed across temperate North America (absent from the southwest and southeast).

Distribution in North America (Figure 2.37): This species is broadly distributed across Canada, from Quebec to Alberta, and is also known from several northern U.S. states including Maine, Minnesota, and Wisconsin. The species has been collected in large series from the Ottawa-Gatineau region where it is often the most abundant species of *Ellescus*; it is only known from short series or single specimens in most other provinces and states.

Natural history: This species occurs across temperate North America. It has been collected from *Salix* (*S. discolor* Muhlenberg, *S. petiolaris* Sm., *S. bebbiana* Sargent). Collections for this study revealed that this species emerges in early spring, and can be found inactive on female *Salix* catkins on colder mid to late April days.

Remarks: The combination of unique male genitalia, color, and ITS2 sequence data (see section 2.3.3) support this species as a new species. This species is closely related to *E.*

ephippiatus, as is evidenced by CO1 and ITS2 molecular data and morphology. This species appears to be active earlier in the spring than *E. ephippiatus*, and there is limited evidence of differing *Salix* host plant use in these two species. Furthermore, *Ellescus michaeli* n. sp. has yet to be collected from *Populus* species. Further sampling and recording of host plant use is required to make stronger claims of differing host plant preferences.

Etymology: This species is named after Dr. Michael Košťál (Slovakia), an independent researcher and weevil expert who graciously collected and mailed fresh specimens for use in this project.

***Ellescus ephippiatus* (Say, 1831)**
Figures 2.2, 2.3e, 2.26-2.34, 2.38

Erirhinus ephippiatus Say 1831: 25 (original description)

Erirhinus rufous Say 1831: 25 (description)

Erirhinus rufus; O'Brien and Wibmer 1982: 116 (catalogue, distribution)

Elleschus ephippiatus; Blatchley and Leng 1916: 284 (identification; distribution); Hatch 1971: 342 (identification; catalogue); O'Brien and Wibmer 1982: 116 (catalogue, distribution).

Ellescus ephippiatus; Hight 1988: 276 (apparent alternate host use; dubious); Ciegler 2010: 71 (identification, distribution); Prena 2018: 382 (taxonomic comments, syntype location); Bouquet et al. 2013: 326 (catalogue, distribution); Webster et al. 2016: 374 (distribution).

Type locality and data: This species was described from an unknown number of specimens collected in Indiana, U.S.A. Here, we adopt a definition of *E. ephippiatus* which is based on a syntype specimen that is deposited in the Swedish Museum of Natural History (NHRS) Schönherr collection (NHRS-JLKB000073656), the type locality, and the original description. The syntype specimen was not examined, but a high-quality photograph (Figures

2.32-2.34) of the syntype provided by NHRS confirms the specimen as *E. ephippiatus* as defined here. See Taxonomic Notes.

Diagnosis: Length (2.0-2.9 mm). Distinct swelling at tip of last sternite of males, not extending over more than half of last sternite (occasionally absent). Scales on pronotum usually not uniform in color, with discolored central and lateral bands. Metasternum and metepisternum with scales relatively sparse not obscuring underlying cuticle. Apex of aedeagus rounded and coming to a distinct point, and with two tufts of hairs (prominent in lateral view against a dark background). Aedeagus with protruding basal structures. Femora lacking minute tooth. Widespread across North America (apparently absent from California and southern Oregon).

Distribution in North America (Figure 2.38): This is the most widespread species of *Ellescus* in North America, with records across Canada and the United States (except for California and the southern tip of Oregon). Records from southern Texas and southern Arizona suggest that the species may also be present in Mexico.

Specimens examined: CANADA: Alberta: Calgary, 31 Jun 1957 – 19 May 1978, B.F. & J.L. Carr (3, CNCI); Elbow Falls, 13 Jun 1959, B.F. & J.L. Carr (5, CNCI); Exshaw, 27 Jun 1954, B.F. & J.L. Carr (1, CNCI); Fort Macleod, 20 Jun 1976 – 23 Jul 1977, B.F. & J.L. Carr (3, CNCI); Fitzgerald, 15 Jun 1988, B.F. & J.L. Carr (1, CNCI); Lethbridge, 24 Jun 1956, O. Peck (1, CNCI); Lethbridge, Oldman River, 22 Jun 1956, E.E. Sterns (3, CNCI); Lethbridge, 6 May – 30 Jun 1930, J.H. Pepper (9, CNCI); Writing-on-Stone Provincial Park (25 miles east), 15 Jul 1980, G. Gibson, sweeps (2, CMNC); Writing-on-Stone Provincial Park (0.5 miles east), 14 June 1982, R.S. Anderson & G.A.P. Gibson, sweeping (6, CMNC); Sandy Point campground, 32 kilometers south of Empress, 2 Aug 1981, R.S. Anderson (2, CMNC); Medicine Hat, Kin Coulee Park, 13 Jun 1982, R.S. Anderson & G.A.P. Gibson (13, CMNC); Medicine Hat, 3 Jun 1920 –

Jun 1930, F.S. Carr (3, CNCI); Medicine Hat, 17 Jun 1930, J.H. Pepper (1, CNCI); Medicine Hat, 21 Jul 1962 – 5 Aug 1979, B.F. & J.L. Carr (8, CNCI); Manyberries, 30 Jun 1930, J.H. Pepper (2, CNCI); McMurray, 4 Jun 1953, W.J. Brown, on *Salix* (6, CNCI); Edmonton, 27 May 1985, B.F. & J.L. Carr (1, CNCI), CNC COLEO DNA 00157038; **British Columbia:** Oliver (5-7 miles north), 21 May – 2 Jun 1958, H. & A. Howden (2, CNCI); Oliver, 15 May 1959, L.A. Kelton (11, CNCI); **Manitoba:** Churchill, 10 July 2008, S. McCubbin & J. Sones (1, CBG), CHU-MXC-090, JSCOC148-09; Churchill, 13-17 Jun 1952, J.G. Chillcott (2, CNCI); Aweme, 26 May 1903, N. Criddle (1, DEBU), DEBU01089099; Aweme, 28 Jun 1920, N. Criddle (1, CNCI); Glenboro, 9 Jun 1920, S. Criddle (1, CNCI); Eastman Region, Victoria Beach, 6 Jul 1985, I.S. Askevold (2, CMNC); Eastman Region, Brokenhead, road crossing at Highway #15, 20 kilometers east of Anola, I.S. Askevold & D.A. Pollock (4, CMNC); Wapusk National Park (58.7231°N, 93.4597°), 15 June – 22 June 2014, D. Iles (1, CBG), BIOUG17638-A05, CNWAA700-14; Gillam, 18 Jun – 1 Jul 1950, W.J. Brown (3, CNCI); Carberry, 20 May 1953, Brooks - Kelton (4, CNCI); Riding Mountain National Park, 5-11 June 1938, W.J. Brown (2, CNCI); Warkworth Creek (near Churchill), 21 Jun 1952, J.G. Chillcott (6, CNCI); **New Brunswick:** Sunbury Co., Maugerville (off Route 105 (45.8662°N, 66.4559°W), 4 June 2014, R.P. Webster, flood plain forest, sweeping roadside foliage (1, RWC); **Northwest Territories:** Yellowknife, 29 May 1953, J.G. Chillcott (2, CNCI); Yellowknife, 24 Jun 1949, R.R. Hall (1, CNCI); **Ontario:** Prince Edward Co., 19 Aug 1923 – 17 May 1950, J.F. Brimley (15, CNCI); Rouge National Urban Park, west of Glen Rouge campground (43.8040°N, 79.1455°W), 7 June 2013 (1, CBG), BIOUG13533-G06, SSROB2985-14; Kitchener, Huron Heights Secondary School (43.3924°N, 80.4663°W), 22 April - 3 May 2013, Chris Roth (1, CBG), BIOUG05652-F10, SMTPB14415-13; Bruce Co., Inverhuron Provincial Park (front dunes) (44°17'33"N, 81°35'28"W), 15 June 2003, M. Buck (1, DEBU), DEBU01127408; Huron Co., Goderich, 22 June 1977, K. Barber (1, DEBU), DEBU01089105; Wellington Co., Fergus, 5 Apr 1997, S.A. Marshall (1, DEBU), DEBU01068482; Middlesex Co., Ailsa Craig, 22-22 June 1983, E. Zaborski, flight intercept trap in turnip field (1, DEBU), DEBU01089107; Northumberland Co., Presqu'île Provincial Park, Stonehedge Cottage, 16 June 2003, P.D. Careless (1, DEBU), DEBU01089106; Wellington Co., Guelph, 10 July 1978, K.N. Barber, reared from *Populus tremuloides* cotton balls (9, DEBU), DEBU01089088-DEBU01089096; Kenora District, 14 miles south of Pickle Lake, 20 Jun 1973, Henry & Anne Howden (1, CMNC); Wellington Co.,

Erin, 17 Jun 1979, Jessica Ernst (1, CMNC); **Quebec:** Nord-du- Québec, James Bay, Long Point (53.9752°N, 79.0685°W), 7 Jul 2018, Pierre de Tonnacour, beaten from *Salix brachycarpa* var. *brachycarpa* (6, CMNC; 2, CNCI); Kazubazua, 25 May 1933, W.J. Brown (7, CNCI);

Saskatchewan: Elbow, 23 Jun 1954, Brooks – Kelton (7, CNCI); Saskatoon, 3 Jun 1949, J.R. Vockeroth (3, CNCI); Saskatchewan Landing Provincial Park (50°39'N, 107°56'W), 25 May 1955, J.R. Vockeroth (8, CNCI); Hatton, 11 Jun 1929, K.M. King & R. Glen (5, CNCI); Township 6, Region 28, 23 Jun 1990, B.F. & J.L. Carr (1, CNCI), CNC COLEO DNA 00157037; Township 8, Region 29, 23 Jun 1990, B.F. & J.L. Carr (1, CNCI), CNC COLEO DNA 00157036; **Yukon:** Tatchun Creek, 25 Jun 1979, B.F. & J.L. Carr (2, CNCI), CNC COLEO DNA 00157039; Mayo, 5, Jul 1955, B.F. & J.L. Carr (5, CNCI); Willow Creek, at kilometer 621 along Klondike Highway, 5 Jun 1980, R.J. Cannings (2, CMNC); McQuesten (10 kilometers east), 1 Jun 1980, R.J. Cannings (1, CMNC); Ross River, 9 kilometers south on Campbell Highway (61°54'N, 132°25'W), 12 Jun 1981, C. Guppy (2, CMNC); Ross River (132°30'N, 61°56'W), 20-22 Jun 1960, J.E.H. Martin & E.W. Rockburne (5, CNCI); Stewart Crossing (18 kilometers west), 2 Jun 1980, R.J. Cannings (2, CMNC); Highway 2, 22 miles north of Highway 1, 25 Jun 1979, B.F. & J.L. Carr (2, CNCI), CNC COLEO DNA 00157040; Kirkman Creek, 13 Jun 1928, A.C. Davis (36, CNCI); Swim Lakes (133°N, 62°13'W), 15 Jun 1960, J.E.H. Martin (1, CNCI); **UNITED STATES OF AMERICA: Alabama:** Monroe Co., Haines Island Park (31°43'23"N, 87°28'10"W), 26-31 May 1995, T.L. Schiefer, mercury vapour and blacklight trap (3, MEM); Macon Co., 4 miles east Shorter along I-85, 16 Apr 1984, D.A. Rider (1, LSAM); LSAM0144883, LSAM0144885; **Alaska (new state record):** Eagle, 18 Jun 1928 (10, CNCI); Washington Creek, 21 June 1977, J. Cowling (1, UAMIC), UAM100008612; Selawik National Wildlife Refuge (66.85873°N, 158.16618°W), 24 June 2010, D.S. Sikes, open sand dunes sweep (11, UAMIC), UAM100283227, UAM100283242, UAM100283250, UAM100283254, UAM100283256, UAM100283481 (LEW_78), UAM100283503, UAM100283520 (LEW_79), UAM100283521, UAM100283568, UAM100283596; Nogahabara Dunes (Koyukuk National Wildlife Refuge) (65.65846°N, 157.49608°W), 13 June 2010, J.A. Slowik, willow sweep (1, UAMIC) UAM100327118 (LEW_77); **Arizona (new state record):** Santa Cruz Co., 4 April 1937, R.A. Flock (2, ASUIC), UAIC1072923; Coconino Co., Oak Creek, Chavez King south of Sedona, 14 Jun 1978, M.W. Sanderson (1, ASUIC), UAIC1072941; Graham Co., Aravaipa Canyon, 24 Aug 1976, D.S. Chandler, sift oak litter (1,

ASUIC), UAIC1072925; Pima Co., Tucson, “Apr, 191” (2, ASUIC), UAIC1072924, UAIC1072939; Yavapai Co., Oak Creek, Baldwin’s King, 9 Jun 1978, M.W. Sanderson (1, ASUIC), UAIC1072940; Yavapai Co., Dead Horse Ranch State Park, Verde River near bridge (34.7502°N, 112.0217°W), 5 June 2011 (1, CBG), BIOUG02273-E11, BBCCA2871-12; Maricopa Co., 4 miles east of Buckeye along Gila River, 1 May 2018, Kyle E. Schnepf, beaten from willow (*Salix* sp.) (3, CMNC); Maricopa Co., Pheonix, 3 Apr 1942, P.C. Grassman (3, ASUIC), UAIC1072926; Maricopa Co., Pheonix, 9 Mar 1941, T. Parker (7, ASUIC), UAIC1072928-UAIC1072934; Javapaj Co., Cottonwood, 24-25 June 1978, C.V. Nidek (3, CMNC); “Ariz” (2, DEBU), DEBU01089100, DEBU01089102; **Arkansas (new state record)**: Washington Co., 2 miles southwest of West Fork, 12 May 1991, C. Carleton (1, LSAM), LSAM0210499; Washington Co., Devil’s Den State Park, 1-2 July 1984, E.G. & M.A. Riley (1, LSAM), LSAM0143750; Toad Suck Park, campground transition between park and forest (35.0724°N, 92.5449°W), 2 July 2011 (1, CBG), BIOUG02327-D08, BBCCA4766-12; **Colorado (new state record)**: La Veta Pass (13 miles west), 12 Jun 1982, B.F. & J.L. Carr (1, CNCI); Chaffee Co., O’Hayer Lake, 22 June 1983, I.S. Askevold (1, ASUIC); Las Animas, 15-16 Jun 1982, B.F. & J.L. Carr (5, CNCI); Pueblo Co., Colorado City, 9 Jun 1982, B.F. & J.L. Carr (1, CNCI); **Florida**: DeSoto Co., Fort Ogden, 8 Apr 1952, J.R. Vockeroth (4, CNCI); Seminole Co., Sanford, 19 Apr 1927, A.C. Davis (1, CNCI); Liberty Co., Apalachicola River, Highway 20 near Bristol (30°26.2’N, 85°00.0’W), 17 Apr 2010, S.M. Clark (2, ASUIC); Leon Co., Tall Timbers Res. Station, Sheep Island, 17 Apr 1978, G.J. Wibmer (1, ASUIC); Leon Co., Tallahassee, 3 April 1976, G.B. Marshall, on fruiting *Salix* (2, CMNC; 60, ASUIC); Alachua Co., Archer (2 miles west), 23 Mar 1953, Henry F. Howden (1, CMNC); Alachua Co., Gainesville NATL, 23 Mar 2016, Kyle E. Schnepf, beaten from *Salix* (5, CMNC), LEW_74; Glades Co., Palmdale, 31 March 2018, Kyle E. Schnepf, swept from vegetation (1, CMNC); Miami-Dade Co., Paradise Key, 6 Apr 1951, Henry & Anne Howden (1, CMNC); Volusia Co., South Daytona, 17 Apr 1959, “S.F.B.” (1, CMNC); Miami-Dade Co., Chekika Street (recreation area), 50 milometers southwest of Miami, 1 November 1984 - 3 Dec 1985, Stewart & Jarmila Peck, “Grossman HammockFor. Malaise-FIT” (1, CMNC); **Idaho (new state record)**: Cassia Co., Burley, 2 Jun 1986, B.F. & J.L. Carr (1, CNCI); Nez Perce Co., Lewiston, 22 May 1986, B.F. & J.L. Carr (2, CNCI); **Illinois (new state record)**: Union Co., Dongola (Hogam Bottoms), 19 June - 4 September 1968, D. Dillow, maple/cypress/beechnut buttress (6, LSAM)

LSAM0210492-LSAM0210497; Coles Co., Charleston, 12 May 1951, D.M. Tuttle Collection (39, ASUIC), UAIC1072875, UAIC1072878-UAIC1072898, UAIC1072905-UAIC1072914, UAIC1072916-UAIC1072921, UAIC1072935; **Indiana:** Jackson Co., Muscatatuck N.W.R., 26 Apr 2013, Gareth S. Powell (1, CMNC); Jackson Co., Muscatatuck N.W.R., 10 May - 7 Jun 2013, Kyle E. Schnepf, Lindgren funnel trap (1, CMNC); **Iowa:** Clinton Co., DeWitt, 21 May 1928, G.S. Walley (1, CNCI); **Kansas:** Riley Co., 20 April 1958 - 9 May 1960, J.R. McCoy Collection (7, MEM); Logan Co., State Lake, 7 June 1964, C.W. O'Brien (5, ASUIC); Douglas Co., F.H. Snow (4, CNCI); Douglas Co., Lawrence, Apr 1930 – 23 Apr 1962, L.W. Brown & W.J. Brown (13, CNCI); Reno Co., Medora, 2 May 1962, W.J. Brown (2, CNCI); **Louisiana (new state record):** East Baton Rouge Parish, Baton Rouge, Mississippi River Levee, 21 March 2003, M.G. Radtke, sweepnet (1, LSAM); East Baton Rouge Parish, Louisiana State University campus (30°24.5'N, 91°10.5'W), 22-23 May 2010, J.S. Park (1, LSAM), LSAM0201337, MDM2010; East Baton Rouge Parish, Baton Rouge, 12 May 1986, E.G. Riley (1, LSAM); East Baton Rouge Parish, Baton Rouge, 26 April 1986, E.G. Riley, flight trap (1, LSAM), LSAM0143498; East Baton Rouge Parish, Baton Rouge GSRI, 1 Apr 1991, V.L. Moseley, mating on willow fruit (9, LSAM), LSAM0145660-LSAM0145664, LSAM0145673, LSAM0145675, LSAM0145679, LSAM0145681; East Baton Rouge Parish, Baton Rouge, 8 May 1988 – 17 May 1989, D.A. Rider, mercury vapour and blacklight (6, LSAM), LSAM0143031, LSAM0143038, LSAM0146124, LSAM0146127, LSAM0146137, LSAM0146167; East Baton Rouge Parish, Baton Rouge, 28 May - 3 June 1986, C.B. Barr & E.G. Riley, yellow sticky board trap in cypress tree (3, LSAM), LSAM0142726-LSAM0142728; East Baton Rouge Parish, Louisiana State University Campus, 16 May 1984 - 15 July 1985, D.A. Rider (17, LSAM), LSAM0143151, LSAM0143173, LSAM0143175, LSAM0143177, LSAM0143197, LSAM0143200, LSAM0143203, LSAM0143315, LSAM0143319, LSAM0143357, LSAM0143358, LSAM0143816, LSAM0144843, LSAM014497, LSAM0145201, LSAM0145277, LSAM0145281; Jefferson Parish, Harahan, Apr 1944, F.G. Werner (1, ASUIC), UAIC1072871; Jefferson Parish, Jean Lafitte National Park, 8 May 1983, C.B. Barr (1, LSAM), LSAM0145397; East Baton Rouge Parish, "E of LA 3025, 1.2 mi. S. Central", 27 Apr – 12 May 1985, C.B. Barr, mercury vapour and black light (3, LSAM), LSAM0144680, LSAM0145456, LSAM0145457; St. Tammany Parish, I-10 at Pearl River, 17 Jun 1982, C.B. Barr & E.G. Riley, black light (1, LSAM), LSAM0145329; Tangipahoa Parish,

Tangipahoa River between Arcola and Tickfaw, 12 Apr 1986, C.B. Barr (1, LSAM), LSAM0144655; Orleans Parish, New Orleans, 3 July 1969, light trap (1, MEM); Orleans Parish, New Orleans, LA Nature Center, 25 April 1984, C.B. Barr, black light trap (2, LSAM), LSAM0144780, LSAM0144782; Vermillion Parish, Pecan Island, 11 March 1982, C.B. Barr (1, LSAM), LSAM0145323; Vermillion Parish, White Lake Research Station (29°55.141'N, 92°30.830'W), 20 March 2009, M. Gimmel, UV light (1, LSAM); West Feliciana Parish, Feliciana Preserve (30°47'N, 91°15'W), 14 Apr 2003, A. Tishechkin, beating low vegetation (1, LSAM); Corney Lake, 25-28 Jun 1983, B.F. & J.L. Carr (6, CNCI); **Mississippi (new state record)**: Attala Co., 1 mile east McCool (33°12'46"N, 89°23'21"W), 23 May 2005, J.A. MacGown & J.G. Hill, Berlese - soil & litter in mixed forest (1, MEM); Claiborne Co., Natchez Trace Parkway, Grindstone Ford (32°00'10", 90°53'41"W), 7 March 2005, J.G. Hill, Berlese litter (1, MEM); Harrison Co., Gulfport C.T. Nature Ar. (30°22'55"N, 89°03'23"W), 9 May 2000, T.L. Schiefer, blacklight trap (1, MEM); Oktibbeha Co., 3 miles west of Adaton (33°29'00"N, 88°58'13"W), 26 Mar 2000, T.L. Schiefer, on flowering *Prunus serotina* (1, MEM); Oktibbeha Co., near Noxubee National Wildlife Refuge (33°18'03"N, 88°46'08"W), 3 April 1998, T.L. Schiefer, on flowering *Salix nigra* (2, MEM); Oktibbeha Co., 8.5 miles south Starkville (33°19'58"N, 88°49'05"W), 20 April 1995, T.L. Schiefer, beating recently cut oaks (1, MEM); Oktibbeha Co., Starkville, MSU north of North Farm, 21 April 1994, D.M. Pollock, sweeping (1, MEM); Oktibbeha Co., Starkville, 13 March 1985, W.P. Chan (1, MEM); Oktibbeha Co., Noxubee Refugee, 5 Feb 1982, G.L. Snodgrass, Berlese - litter hardwood forest (1, MEM); Oktibbeha Co., Noxubee National Wildlife Refuge (33°24'11"N, 88°53'33"W), 6 Oct 2008, J. MacGown & J.G. Hill, litter mixed pine hardwood forest (1, CMNC); Oktibbeha Co., 4 miles east of Mississippi State University (33°23'04"N, 88°46'02"W), 23 Feb 2014, J.L. Gesell (1, MEM); Issaquena Co., Shipland W.M.A. (32°45'15"N, 91°07'44"W), 3 August 2005, J.A. MacGown & J.G. Hill, in leaf litter in floodplain hardwood forest (4, MEM); Jackson Co., Ocean Springs, 13 May 1984, Paul K. Lago (1, CMNC); Lafayette Co., Oxford, 4 June 1982 - 30 June 1983, Paul K. Lago (2, CMNC); Oktibbeha Co., Sessums (33°23'31"N, 88°42'40"W), 22 April - 28 April 1998, J.A. MacGown, Malaise trap (47, MEM); Sharkey Co., Sunflower WMA (32°50'55"N, 90°48'24"W), 21 July 2003, R.L. Brown, Berlese trap - bottomland hardwood litter (2, MEM); Noxubee Co., Noxubee National Wildlife Refuge (33°16'44"N, 88°46'39"W), 8 June 1998, T.L. Schiefer (8, MEM); Noxubee Co., Noxubee National Wildlife Refuge

(33°15'04"N, 88°44'51"W), 31 July 2008, J.A. MacGown, litter in pine-hardwood forest (2, MEM); Lee Co., Tombigbee State Park, 7 Jun 1995, T.L. Schiefer, beating trees and shrubs (1, MEM); Smith Co., 1 mile North of Raleigh, 14-15 June 1985, R. Brown & G. Burrows (1, MEM); Tate Co., Senatobia Wetlands (34°37'05"N, 89°56'50"W), 31 May 2018, R.J. Whitehouse, sweeping grasses and plants along water edge and field (1, MEM); Winston Co., Tombigbee National Forest (33°11'50"N, 89°03'20"W), 5 April 1999, T.L. Schiefer, blacklight trap in mixed mesic forest (1, MEM); **Missouri:** St. Louis, M. Schuster (1, DEBU), DEBU01089103; **Montana (new state record):** Deer Lodge Co., Anaconda, 4 July 1962, J.B. Karren, on *Salix* (1, ASUIC); Fergus Co., Little Box Elder Creek (by Highway 19), 13 Aug 1990, B.F. & J.L. Carr (1, CNCI); **Nebraska (new state record):** Thomas Co., Halsey, 29-31 July 1973, Henry & Anne Howden (1, CMNC); **Nevada (new state record):** Elko Co., North Fork, Humboldt River at Elburz, 22 June 1995, R.W. Baumann (1, ASUIC); **Oklahoma (new state record):** Pushmataha Co., Clayton, 21 Jun 1983, B.F. & J.L. Carr (1, CNCI); Cimarron Co., Lake Etling, 10 Jun 1983, B.F. & J.L. Carr (1, CNCI); Cleveland Co., Norman (Oliver Woods), 30 Jun 1969, W. Suter, nest in stump (1, LSAM), LSAM0142642; Marshall Co., 2 miles southeast of Willis, 5 Apr 1968 - 24 Jun 1969, willow swamp cedar, W. Suter (12, LSAM; 2, ASUIC), LSAM0141740, LSAM0142383, LSAM0142623, LSAM0142629, LSAM0210485-LSAM0210491; Marshall Co., 2 miles east Willis, Buncombe Creek Campground, 24 June 1969, W. Suter, oak forest floor (1, ASUIC); Marshall Co., University of Oklahoma Biological Station on Lake Texoma (Willis), 17 Apr 1968, W. Suter (2, LSAM), LSAM0141724, LSAM0141725; **Oregon:** Grant Co., John Day, U.S. Highway 30, 23 May 1959, Joe Schuh, on willow (1, CMNC); Columbia Co., 5 miles north - 2 miles east of Burlington app. 30, 7 October 1972, oak tree hollow debris and soil, E.M. Benedict (12, CMNC); Minam, 13 Jun 1984, B.F. & J.L. Carr (1, CNCI); **Pennsylvania:** Westmoreland Co., Jeannette, H.G. Klages (1, CNCI); **South Carolina:** Clemson College, 20 June 1936, O.L. Cartwright (1, CUAC); Georgetown Co., Georgetown, 4 Apr 1957, Chamberlain (1, CUAC); **Tennessee (new state record):** Hamilton Co., 10 miles south of Chattanooga, 8 April 1981, P. Ramey, sweeping (1, MEM); **Texas:** Bandera Co., Bear Creek, 7 miles northeast of Bandera, 4 May 1999, S.M. Clark (4, ASUIC); Berleson Co., 5 Feb 1958 (1, ASUIC); Briscoe Co., Quitaque Creek, 6 May 1970, on *Salix* (4, ASUIC); Burnett Co., Inks Lake State Park, 21 May 1989, E.G. Riley (1, ASUIC); Cameron Co., Brownsville, Apr 1978, W.L. Johnson, in Boll Weevil Mitchell trap on cottonfield margin (1,

MEM); Cameron Co., Brownsville, Palmetto Grove, 5-9 May 1978, A.E. & D.S. Lewis (1, ASUIC); Crosby Co., White River, 3 miles east of Crosbyton, 3 May 1970, C.W. O'Brien (1, ASUIC); Dickens Co., White River Lake, 16 Apr - 8 Jun 1989, R. Morris (4, CMNC); Culberson Co., Guadalupe National Park, Choza Springs, 22 July 1982, G.A.P. Gibson (1, CMNC); Grayson Co., Juniper Pt., L. Texoma (12 miles north of Whitesboro), 6 Apr 1969, W. Suter, litter around log (2, ASUIC); Gonzales Co., Palmetto St. Park, 30 May 1977, E.G. Riley, blacklight (2, ASUIC); Gray Co., Lake McClellan, 4 July 1970, G.B. Marshall (2, ASUIC); Eastland Co., 1 mile south of Eastland, 4 April 1985, G.B. Marshall (1, ASUIC); Harris Co., Highway 59 at San Jacinto, 4 June 1968 (1, ASUIC); Jim Wells Co., Lake Corpus Christi, 7 June 1954, Henry F. Howden (1, CMNC); Floyd Co., Lake Marvin, 14 miles east Canadian, 23 Apr 1970, L.B. & C.W. O'Brien (7, ASUIC); Brazos Co., Peach Creek at Highway 6, 8 miles south of College Station, 13 Apr 1987, R.S. Anderson (57, CMNC); Cameron Co., Southpoint Nursery (1 mile south of Southmost Ranch), 5-6 July 1982, G.A.P. Gibson (2, CMNC); Jeff Davis Co., 3.4 kilometers east of Fort Davis, 10-11 Sep 1988, R.S. Anderson, Berlese funnel trap with mixed hardwood litter (6, CMNC); Jeff Davis Co., 5 miles north of Fort Davis, 14 Jul 1956, Henry & Anne Howden (1, CMNC); Red River Co., 2 miles northwest of Talco, 8 June 1965, C.W. O'Brien, on *Salix nigra* var. *vallicola* (6, ASUIC); Val Verde Co., Langtry, 4 Nov 1982, J. Huber & A. Gonzalez, sweep along river edge (2, CMNC); Kerr Co., Kerrville, 4 Apr – 22 Apr 1959, Becker & Howden, beating *Salix* (92, CNCI); Brewster Co., Lajitas, 19 May 1959, Howden & Becker (1, CNCI); Bandera Co., Vanderpool, 18 Apr 1987, B.F. & J.L. Carr (5, CNCI); **Utah:** Provo, Utah Lake, 4 July 1964, L.B. & C.W. O'Brien (2, ASUIC); Sevier Co., Sevier River, 24 June 1966, W. Gagne (1, ASUIC); Wayne Co., Torrey (2 miles east), 2 Aug 1968, Anne T. Howden (2, CMNC); Washington Co., 7 miles west Sta. Clara, 8 Apr 1963, L.B. & C.W. O'Brien, on *Salix laevigata* Bebb (5, ASUIC); Washington Co., 7 miles east Gunlock, 8 Apr 1963, C.W. O'Brien, on *Salix laevigata* Bebb (1, ASUIC); Washington Co., Virgin - 3400', 7 Apr 1963, C.W. O'Brien, swept from *Salix* (3, ASUIC); Washington Co., St. George, 8 Apr 1963, C.W. O'Brien, on *Salix exigua* (1, ASUIC); Washington Co., Virgin, 10 Apr 1963, L.B. & C.W. O'Brien, at night on fruiting *Populus fremontii* (1, ASUIC); **Virginia (new state record):** Accomack Co., Deep Creek, Dismal Swamp, 5 May 1951, G.H. Nelson (1, CMNC); **Washington:** Snohomish Co., Sulton, 11 May 1933, R.A. Flock (2, ASUIC), UAIC1072872, UAIC1072873; **West Virginia (new state record):** Cabell Co., Green Bottom Wildlife

Management Area, 25 April 1996, L. Torres-Miller (1, CMNC); **Wyoming (new state record):** Park Co., junction between Highway 120 and Highway 296, 27 Jun 1982, B.F. & J.L. Carr (1, CNCI).

Natural history: This species occurs throughout North America, although it appears to be absent from California and southern Oregon (Figure 2.38). It has been collected on a wide variety of *Salix* and *Populus* species, including *Populus tremuloides*, *Salix brachycarpa* var. *brachycarpa* Nuttall, and *Salix laevigata* Bebb. Hight (1988) reported collecting the larvae of *E. ephippiatus* from the terminal bud of *Lythrum salicaria* L.; however, this identification is questionable given the lack of other such reports and the strong association of the family with members of Salicaceae. Additionally, Blatchley and Leng (1916) detailed the rearing of *E. ephippiatus* from the galls of *Rhabdophaga strobiloides* Osten Sacken, 1862 (Willow Pinecone Gall Midge). A large series from northern Oregon, U.S.A. were taken from Oak tree hollows, debris, and soil. This species is known to be attracted to lights (Ciegler 2010).

Taxonomic Notes: Despite being described from specimens collected in Indiana, U.S.A., label data for a syntype of *E. ephippiatus* deposited in the Carl Schönherr collection (NHRS) reads as follows: “ephippiatus [sic]/ Say./ Connecticut./Say” (Prena 2018). The locality (Connecticut) appears to have been erroneously introduced by Say during preparation of a list of specimens shipped to Schönherr, or by Schönherr who may have misinterpreted Say’s list. A handwritten copy of the list of specimens sent by Say is preserved in the Academy of Natural Sciences of Philadelphia (ANSP; Figure 2.39), and suggests that the Connecticut collection locality for *Phyxelis rigidus* (Say, 1831) may have been erroneously mistaken for the collection locality for *E. ephippiatus*, which follows subsequently in the same list.

2.3.4 Barcoding (CO1) + ITS2

Barcode (CO1) and ITS2 DNA sequence data were obtained for all *Ellescus* species present in North America (except for the west coast endemic species *E. californicus*). Sequence data for both genes were also obtained from European specimens of *E. bipunctatus*, as well as *E. scanicus* and *E. infirmus* (Old World endemic species). The neighbor-joining trees (Figures 2.40, 2.41) generated from these sequence data are in agreeance with the morphology-based species delineation results presented in the previous sections. The pairwise percent identity is also presented for both markers in Tables 2.5 and 2.6. The widespread hypervariable species *E. ehippiatus* does not separate from *Ellescus michaeli* n. sp. when using CO1, but does when using ITS2. Although the degree of ITS2 divergence from *E. ehippiatus* is relatively small (Figure 2.41; Table 2.6), specimens of *Ellescus michaeli* n. sp. consistently cluster together even when compared with *E. ehippiatus* collected across North America (Florida, Louisiana, Alaska). The North American and European populations of *E. bipunctatus* consistently separate using CO1 and ITS2, however dissections and examinations of external morphology did not uncover any significant differences in forms warranting separate status.

2.4 Discussion

Prior to the beginning of this research, four *Ellescus* species were recorded from North America, namely, *E. bipunctatus*, *E. scanicus*, *E. ehippiatus*, and *E. borealis* (O'Brien and Wibmer 1982; Bousquet et al. 2013). Here, this revisionary work used a combination of morphological and molecular techniques, and resulted in several new discoveries. Firstly, while *E. scanicus* is a valid species that is morphologically and molecularly distinct from other

congeners, no specimens of this taxon were present in any of the North American collections examined. Other members of the genus, especially aberrant forms of *E. bipunctatus*, were commonly mistaken for this species in collections. Thus, *E. scanicus* should be removed from the North American faunal checklists. This does not however, rule out the possibility of this species occurring on the continent, and collections not examined in this study should make use of the key provided here to further verify the lack of North American *E. scanicus* specimens. Secondly, *E. borealis* was determined on morphological grounds to simply be an all-red form of *E. bipunctatus*; a color form especially common in western Canada. Although there is some degree of molecular differentiation (CO1, ITS2) amongst the North American and European populations of *E. bipunctatus*, it is assumed that these do not represent different species because they lack any morphological or other character differences. Thirdly, *E. californicus* is resurrected from synonymy under *E. ephippiatus* as dissections revealed significant biogeographical differences as well as differences in the male genitalia. This species is restricted to California and southern Oregon, a faunistically unique region which harbors high numbers of endemic plant and animal species, including endemic members of Salicaceae (Little 1979; Harrison 2013). Lastly, this research revealed the presence of a new species, *Ellescus michaeli* n. sp., which occurs across temperate North America from Maine to northwestern Alberta. This species is not only genetically distinct, but also differs genitally and in color from all other congeners. Thus, based on this research it was found that four species of *Ellescus* are present in North America, namely, *E. bipunctatus*, *E. californicus*, *E. ephippiatus*, and *Ellescus michaeli* n. sp.

In addition to formally adding the above species to the Nearctic list and describing *Ellescus michaeli* n. sp., this research also uncovered potentially new synonyms during the

course of comparing North American material to the Old World fauna. In particular, dissections of the Old World species *E. brevirostris* (specimens procured from ZSM) revealed that this species is possibly *E. infirmus*, with which it appears to be virtually indistinguishable based on external morphology. The far-east species *E. languidus* (ZSM specimens) does however, appear to be distinct and is separable from other *Ellescus* by external morphology and genitalia. Specimens of the Japanese endemic species *E. bicoloripes* (Figure 2.42; HKC) were also examined and it is clear that these do not fit within *Ellescus*; specimens examined possess a prominent tooth on the femora (lacking in *Ellescus*), as well as a region of the elytra which is expanded laterally over the anterior quarter of the metepisternum (Figure 2.43). This laterally expanded region of the elytra is lacking in *Ellescus*, and is unique to *Adorytomus* (Kojima and Truong 2012). Thus, it is clear that a world revision of this group would be beneficial, and should not fail to use molecular data, as some species can be deceptively similar morphologically. Although specimens across the Nearctic region were examined for this study, further sampling is also encouraged particularly in California and Oregon, as well as in Arizona, New Mexico, Texas, and northern Mexico as these regions are generally not as well sampled. Although the validity of *E. californicus* as a unique species is well substantiated by morphology and biogeography, it is also suggested that future studies of the genus include molecular data for this species.

Table 2.1

Updated status of species of *Ellescus* (Coleoptera: Curculionidae) and their distributions based on O'Brien and Wibmer (1982), Bousquet et al. (2013), Alonso-Zarazaga et al. (2017) and the current study. The rightmost column reflects the updated distributional and taxonomic results of this study.

Species	Author / date of description	Distribution	Updated status / distribution
<i>E. bicoloripes</i>	(Voss and Chujo, 1960)	Japan	Misplaced species; likely belongs to <i>Adorytomus</i>
<i>E. bipunctatus</i>	(Linnaeus, 1758)	Europe (widespread), Japan, Russia, eastern Canada and northeastern United States	No changes
<i>E. borealis</i>	(Carr, 1920)	Canada and northern United States	Junior synonym of <i>E. bipunctatus</i>
<i>E. brevisrostris</i>	(Desbrochers des Loges, 1875)	Romania, Russia	No changes
<i>E. ephippiatus</i>	(Say, 1831)	Canada and United States (widespread)	No changes
<i>E. infirmus</i>	(Herbst, 1795)	Europe (widespread), Japan, Russia	No changes
<i>E. languidus</i>	(Faust, 1882)	Eastern China, Eastern Russia, Mongolia	No changes
<i>E. pauper</i>	(Chujo and Voss, 1960)	Japan	Misplaced species; likely belongs to <i>Adorytomus</i>
<i>E. scanicus</i>	(Paykull, 1792)	Europe (widespread), Armenia, east Russia, central Canada and northeastern United States	Only present in Europe; erroneously reported from North America
<i>E. michaeli</i> n. sp.	Lewis and Anderson, 2022	Canada and north United States	New species
<i>E. californicus</i>	(Casey, 1885)	California and southern Oregon	Resurrected from synonymy

Table 2.2

Notes on the type specimen repositories of the currently recognized species of *Ellescus* (Coleoptera: Curculionidae) reported from North America (O'Brien and Wibmer 1982; Bousquet et al. 2013). Note that this includes *E. scanicus* (Paykull, 1792), a species erroneously reported from North America, and *E. californicus* (Casey, 1885), a species which is currently treated as either a junior synonym or subspecies of *E. ephippiatus* (Say, 1831) (O'Brien and Wibmer 1982; Poole and Gentili 1996).

<i>E. bipunctatus</i> (Linnaeus, 1758)	No specimens in the collection of The Linnean Society of London (https://www.linnean.org/research-collections/linnaean-collections - accessed 23 November 2021) or Naturhistoriska riksmuseet (NHRS) (http://www2.nrm.se/en/col_preface.html - accessed 23 November 2021). Possibly not extant.
<i>E. borealis</i> (Carr, 1920)	holotype; The Canadian National Collection of Insects, Arachnids and Nematodes (CNC), Ottawa, Canada.
<i>E. californicus</i> (Casey, 1885)	holotype; Smithsonian National Museum of Natural History, Washington, D.C., U.S.A.
<i>E. ephippiatus</i> (Say, 1831)	syntype; Naturhistoriska riksmuseet (NHRS), Stockholm, Sweden (Prena 2018).
<i>E. scanicus</i> (Paykull, 1792)	holotype; Naturhistoriska riksmuseet (NHRS), Stockholm, Sweden (http://www2.nrm.se/en/col_preface.html - accessed 23 November 2021).

Table 2.3

List of COI and ITS2 primers used in the generic study of *Elleus* (Coleoptera: Curculionidae). Forward primers are listed first, followed by reverse primers.

Gene & primers	Primer sequence (5' - 3')	Reference
COI LCO1490 / HCO2198	GGTCAACAAATCATAAAGATATTGG / TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. (1994), Pentinsaari et al. (2014)
ITS2 ITS2F / ITS2R	GTGGATCCTGTGAACTGCAGGACACATG / GTGAATTCATGCTTAAATTTAGGGGGTA	Andreev et al. (1998)

Table 2.4

Collection data for specimens used in the neighbor-joining CO1 and ITS2 gene trees for *Ellescus* (Coleoptera: Curculionidae). The identities of specimens associated with sequences downloaded from GenBank or BOLD were verified morphologically through institutional loans.

Ellescus bipunctatus : **LEW_69** : CANADA, Quebec, Cantley, 45.591726°N, 75.784856°W, 17 May 2020, J.H. Lewis, CMNC.

Ellescus bipunctatus : **LEW_71** : CANADA, Quebec, near Ch. Holmes (Cantley), 45.598843°N, 75.798396°W, on female *Salix* catkins, J.H. Lewis, CMNC.

Ellescus bipunctatus : **LEW_90** : CANADA, Quebec, Mont Cascades ski hill (Gatineau), 45.588793°N, 75.845822°W, 13 May 2021, J.H. Lewis, beaten of *Salix* sp., CMNC.

Ellescus bipunctatus : **LEW_95 / LEW_97** : SLOVAKIA, Čačín, Bystrická vrchovina hills, 48.671667°N (original label 48°40.3'N), 19.256667°E (original label 19°15.4'E), 6 July 2020, Michael Košťál, CMNC.

Ellescus bipunctatus : **LEW_104** : U.S.A., Indiana, Morgan Co., 14 July – 7 August 2012, Kyle E. Schnepf, Malaise Trap, CMNC.

Ellescus bipunctatus : **LEW_118** : CANADA, Quebec, Boucher Forest (Gatineau), 45.427673°N, 75.823561°W, 2 May 2021, J.H. Lewis, beaten from *Populus tremuloides* (Michaux) female catkins, CMNC.

Ellescus bipunctatus : **LEW_135** : CANADA, Quebec, Cantley, 45.59468°N, 75.794389°W, 24 April 2021, J.H. Lewis, beaten of *Salix* sp., CMNC.

Ellescus bipunctatus : **BIOUG35446-H11 : ELPCH211-17 (BOLD)** : CANADA, Ontario, Hartington, Eel Lake – South Frontenac, 44.563°N, 76.5522°W, 6 September 2017, Jeremy deWaard, CBG.

Ellescus bipunctatus : **BIOUG35442-G10 : ELPCG9470-17 (BOLD)** : CANADA, Ontario, Hartington, Eel Lake – South Frontenac, 44.5645, 76.5516, 25 July – 8 August 2017, Gergin Blagoev, CBG.

Ellescus bipunctatus : **ZMUO.004794 : KJ962308 (GenBank)** : FINLAND, Espoo, Vanttila, 60.1829°N, 24.6157°E, 6 August 2012, Eero Helve, ZMUO.

Ellescus bipunctatus : **ZMUO.016797 : COLFH362-14 (BOLD)** : FINLAND, Kuusamo, Aronemi, 66.2741°N, 29.7033°E, 14 June 2014, Mikko Pentinsaari, ZMUO.

Ellescus bipunctatus : **ZMUO.016798 : COLFH363-14 (BOLD)** : FINLAND, Kuusamo, Aronemi, 66.2741°N, 29.7033°E, 14 June 2014, Mikko Pentinsaari, ZMUO.

Ellescus ehippiatus : **BIOUG02273-E11 : BBCCA2871-12 (BOLD)** : U.S.A. Arizona, Yavapai, Dead Horse Ranch State Park, Verde River near bridge, 34.7502°N, 112.0217°W, 5 June 2011, CBG.

Ellescus ehippiatus : **BIOUG05652-F10 : SMTPB14415-13 (BOLD)** : CANADA, Ontario, Kitchener, Huron Heights Secondary School, 43.3924°N, 80.4663°W, 22 April – 3 May 2013, Chris Roth, CBG.

Ellescus ehippiatus : **BIOUG17638-A05 : CNWAA700-14 (BOLD)** : CANADA, Manitoba, Wapusk National Park, 58.7231°N, 93.4597°W, 15-22 June 2014, D. Iles, CBG.

Ellescus ehippiatus : **BIOUG13533-G06 : SSROB2985-14 (BOLD)** : CANADA, Ontario, Rouge National Urban Park, west of Glen Rouge campground, 43.804°N, 79.1455°W, 7 June 2013, CBG.

Ellescus ehippiatus : **LEW_74** : U.S.A., Florida, Alachua Co., Gainesville NATI, 23 March 2016, Kyle E. Schnepf, beaten off *Salix* sp., CMNC.

Ellescus ehippiatus : **LEW_77** : U.S.A., Alaska, Nogahabara Dunes [Koyukuk NWR], 65.65846°N, 157.49608°W, 13 June 2010, J.A. Slowik, willow sweep, UAMIC, UAM100327118.

Ellescus ehippiatus : **LEW_78** : U.S.A., Alaska, Selawik NWR, 66.85873°N, 158.16618°W, 24 June 2010, D.S. Sikes, open sand dunes, sweep, UAMIC, UAM100283481.

Ellescus ehippiatus : **LEW_79** : U.S.A., Alaska, Selawik NWR, 66.85873°N, 158.16618°W, 24 June 2010, D.S. Sikes, open sand dunes, sweep, UAMIC, UAM100283520.

Ellescus ehippiatus : **LEW_109** : U.S.A., Louisiana, Vermillion Par. White Lake Research Station, 29.919017°N (original label 29°55'08.5"N), 92.513833°W (original label 92°30'49.8"W), 20 March 2009, M. Gimmel, UV light, CMNC.

Ellescus infirmus : **LEW_98 / LEW_99 / LEW_100** : SLOVAKIA, Cerová vrchovina, Jesnske – Barta, 48.296667°N (original label 48°17.8'), 20.070000°E (original label 20°04.2'), 9 October 2020, Michael Košťál, sift under *Salix alba*, CMNC.

Ellescus michaeli n. sp. : **LEW_68** : CANADA, Cantley, 45.591708°N, 75.784886°W, 22 May 2020, on *Salix* female catkins, J.H. Lewis, CMNC.

Ellescus michaeli n. sp. : **LEW_70** : CANADA, Quebec, across road from 1740 Pink Road (Gatineau), 45.441961°N, 75.811547°W, 10 May 2020, on female *Salix* catkins, J.H. Lewis, CMNC.

Ellescus michaeli n. sp. : **LEW_130** : CANADA, Quebec, Cantley, 45.591637°N, 75.785395°W, 23 April 2021, J.H. Lewis, beaten off *Salix* sp., CMNC.

Ellescus michaeli n. sp. : **LEW_145 / LEW_146 / LEW_148 / LEW_149** : CANADA, Ontario, Mer Bleue Bog, 45.3973°N, 75.5144°W, 13 May 2021, R. Anderson, beaten off *Salix* sp., CMNC.

Ellescus scanicus : **LEW_91** : SLOVAKIA, Podunajská nížina, Šoporňa, 48.240000°N (original label 48°14.4'), 17.811667°E (original label 17°48.7'), 5 January 2021, Michael Košťál, sift under *Populus alba*, CMNC.

Ellescus scanicus : **ZMUO.005338 : KJ964000.1 (GenBank)** : Finland, Kirkkonummi 6678:3369 Sundsberg, 21 May 2012, Mikko Tiusanen, ZMUO.

Table 2.5

Percent identity of CO1 sequences for representatives of species of *Ellescus* (Coleoptera: Curculionidae) (Aligned CO1 length: 702). Note that this measure specifies the percentage of sites (columns) in the DNA sequence alignment that are identical between sequences.

	LEW_99	LEW_98	LEW_100	LEW_78
<i>Ellescus infirmus</i> : LEW_99 : Cerova_vrchovina, Slovakia		98.1	98.8	88.9
<i>Ellescus infirmus</i> : LEW_98 : Cerova_vrchovina, Slovakia	98.1		99.4	88.2
<i>Ellescus infirmus</i> : LEW_100 : Cerova_vrchovina, Slovakia	98.8	99.4		88.6
<i>Ellescus ehippiatus</i> : LEW_78 : Selawik_NWR, Alaska	88.9	88.2	88.6	
<i>Ellescus ehippiatus</i> : LEW_79 : Selawik_NWR, Alaska	88.7	88.2	88.4	98
<i>Ellescus ehippiatus</i> : LEW_77 : Nogahabara Dunes, Alaska	86.4	86.1	86.1	93.4
<i>Ellescus ehippiatus</i> : CNWAA700-14 : Wapusk_National_Park, Manitoba	85.5	85.4	85.5	92.8
<i>Ellescus michaeli</i> n. sp. : LEW_145 : Mer_Bleue_Bog, Ontario	88.2	88.8	88.9	95
<i>Ellescus michaeli</i> n. sp. : LEW_146 : Mer_Bleue_Bog, Ontario	88.4	88.4	88.5	95.2
<i>Ellescus michaeli</i> n. sp. : LEW_68 : Cantley, Quebec	88.4	88	88.4	95.7
<i>Ellescus michaeli</i> n. sp. : LEW_67 : Cantley, Quebec	88.4	88	88.4	95.4
<i>Ellescus michaeli</i> n. sp. : LEW_70 : Gatineau, Quebec	87.9	87.8	87.9	95.3
<i>Ellescus michaeli</i> n. sp. : LEW_130 : Cantley, Quebec	89	89	89.4	95.8
<i>Ellescus michaeli</i> n. sp. : LEW_148 : Mer_Bleue_Bog, Ontario	87.9	87.7	87.9	94.9
<i>Ellescus michaeli</i> n. sp. : LEW_149 : Mer_Bleue_Bog, Ontario	87.2	87.1	87.2	94.6
<i>Ellescus ehippiatus</i> : SSROB2985-14 : Rouge_National_Urban_Park, Ontario	78.6	78.5	78.6	85.3
<i>Ellescus ehippiatus</i> : SMTPB14415-13 : Kitchener, Ontario	51.1	51	51	54.3
<i>Ellescus ehippiatus</i> : BBCCA2871-12 : Yavapai, Arizona	86	85.9	86	92.8
<i>Ellescus ehippiatus</i> : LEW_74 : Gainesville, Florida	88.3	88.2	88.3	95.4
<i>Ellescus ehippiatus</i> : LEW_109 : Vermillion_Park, Louisiana	89.4	89	89.4	96.4
<i>Ellescus scanicus</i> : KJ964000.1 : Kirkkonummi, Finland	81	80.6	80.7	82.6
<i>Ellescus scanicus</i> : LEW_91 : Soporna, Slovakia	84.1	83.9	84	85.2
<i>Ellescus bipunctatus</i> : LEW_95 : Cacin, Slovakia	85.6	86.3	86.5	86.6
<i>Ellescus bipunctatus</i> : LEW_97 : Cacin, Slovakia	85.9	85	85.6	86.6
<i>Ellescus bipunctatus</i> : COLFH363-14 : Kuusamo, Finland	82.2	81.8	81.9	83.5
<i>Ellescus bipunctatus</i> : COLFH362-14 : Kuusamo, Finland	77.4	77	77.1	78.5
<i>Ellescus bipunctatus</i> : KJ962308.1 : Espoo, Finland	82.3	81.9	82.1	83.7
<i>Ellescus bipunctatus</i> : ELPCH211-17 : Harington, Ontario	82.2	82.1	82.2	83
<i>Ellescus bipunctatus</i> : LEW_71 : Cantley, Quebec	84.9	84.8	84.9	85.6
<i>Ellescus bipunctatus</i> : ELPCG9470-17 : Harington, Ontario	82.1	81.9	82.1	82.8
<i>Ellescus bipunctatus</i> : LEW_118 : Boucher_Forest, Quebec	86.2	85.8	86.4	85.7
<i>Ellescus bipunctatus</i> : LEW_90 : Gatineau, Quebec	85.7	85.8	85.9	85.2
<i>Ellescus bipunctatus</i> : LEW_69 : Cantley, Quebec	85.8	85.5	85.8	85.9
<i>Ellescus bipunctatus</i> : LEW_135 : Cantley, Quebec	85.9	85.5	85.9	85.9

	LEW_79	LEW_77	CNWAA700-14	LEW_145
Ellescus_infirmus_: LEW_99_: Cerova_vrchovina, Slovakia	88.7	86.4	85.5	88.2
Ellescus_infirmus_: LEW_98_: Cerova_vrchovina, Slovakia	88.2	86.1	85.4	88.8
Ellescus_infirmus_: LEW_100_: Cerova_vrchovina, Slovakia	88.4	86.1	85.5	88.9
Ellescus_ephippiatus_: LEW_78_: Selawik_NWR, Alaska	98	93.4	92.8	95
Ellescus_ephippiatus_: LEW_79_: Selawik_NWR, Alaska		93.4	92.8	95
Ellescus_ephippiatus_: LEW_77_: Nogahabara_Dunes, Alaska	93.4		93.8	95.7
Ellescus_ephippiatus_: CNWAA700-14_: Wapusk_National_Park, Manitoba	92.8	93.8		94.3
Ellescus_michaeli_n.sp._: LEW_145_: Mer_Bleue_Bog, Ontario	95	95.7	94.3	
Ellescus_michaeli_n.sp._: LEW_146_: Mer_Bleue_Bog, Ontario	95.1	95.7	94.6	97.9
Ellescus_michaeli_n.sp._: LEW_68_: Cantley, Quebec	95.5	95.7	94.6	97.3
Ellescus_michaeli_n.sp._: LEW_67_: Cantley, Quebec	95.2	95.4	94.3	97
Ellescus_michaeli_n.sp._: LEW_70_: Gatineau, Quebec	95.2	95.5	94.6	97.1
Ellescus_michaeli_n.sp._: LEW_130_: Cantley, Quebec	95.7	95.8	94.5	98.5
Ellescus_michaeli_n.sp._: LEW_148_: Mer_Bleue_Bog, Ontario	94.8	95.5	94.5	97.1
Ellescus_michaeli_n.sp._: LEW_149_: Mer_Bleue_Bog, Ontario	94.5	95	94.8	96.5
Ellescus_ephippiatus_: SSROB2985-14_: Rouge_National_Urban_Park, Ontario	85.2	86.1	86.8	86.3
Ellescus_ephippiatus_: SMTPB14415-13_: Kitchener, Ontario	54.3	55	55	55
Ellescus_ephippiatus_: BBCCA2871-12_: Yavapai, Arizona	92.7	92.5	92.8	92.7
Ellescus_ephippiatus_: LEW_74_: Gainesville, Florida	95.3	94	93.1	95.3
Ellescus_ephippiatus_: LEW_109_: Vermillion_Park, Louisiana	96.2	94.4	93.5	95.9
Ellescus_scanicus_: KJ964000.1_: Kirkkonummi, Finland	82.5	81.7	82	82
Ellescus_scanicus_: LEW_91_: Soporna, Slovakia	85.1	83.5	82.4	85.6
Ellescus_bipunctatus_: LEW_95_: Cacin, Slovakia	86.5	83.8	83.7	86.8
Ellescus_bipunctatus_: LEW_97_: Cacin, Slovakia	86.4	84	83.2	85.7
Ellescus_bipunctatus_: COLFH363-14_: Kuusamo, Finland	83.5	82.7	83.4	83.2
Ellescus_bipunctatus_: COLFH362-14_: Kuusamo, Finland	78.4	77.9	78.3	78.1
Ellescus_bipunctatus_: KJ962308.1_: Espoo, Finland	83.6	82.5	83.2	83
Ellescus_bipunctatus_: ELPCH211-17_: Harington, Ontario	82.9	82.5	83.2	83
Ellescus_bipunctatus_: LEW_71_: Cantley, Quebec	85.6	83.7	83.2	85.3
Ellescus_bipunctatus_: ELPCG9470-17_: Harington, Ontario	82.8	82.4	83.1	82.9
Ellescus_bipunctatus_: LEW_118_: Boucher_Forest, Quebec	85.7	83.4	82.9	85.8
Ellescus_bipunctatus_: LEW_90_: Gatineau, Quebec	85.1	83.7	83.2	86
Ellescus_bipunctatus_: LEW_69_: Cantley, Quebec	85.7	83.7	83.2	85.3
Ellescus_bipunctatus_: LEW_135_: Cantley, Quebec	85.8	83.8	83.3	85.4

	LEW_146	LEW_68	LEW_67	LEW_70
Ellescus_infirmus_: LEW_99_: Cerova_vrchovina, Slovakia	88.4	88.4	88.4	87.9
Ellescus_infirmus_: LEW_98_: Cerova_vrchovina, Slovakia	88.4	88	88	87.8
Ellescus_infirmus_: LEW_100_: Cerova_vrchovina, Slovakia	88.5	88.4	88.4	87.9
Ellescus_ephippiatus_: LEW_78_: Selawik_NWR, Alaska	95.2	95.7	95.4	95.3
Ellescus_ephippiatus_: LEW_79_: Selawik_NWR, Alaska	95.1	95.5	95.2	95.2
Ellescus_ephippiatus_: LEW_77_: Nogahabara_Dunes, Alaska	95.7	95.7	95.4	95.5
Ellescus_ephippiatus_: CNWAA700-14_: Wapusk_National_Park, Manitoba	94.6	94.6	94.3	94.6
Ellescus_michaeli_n.sp._: LEW_145_: Mer_Bleue_Bog, Ontario	97.9	97.3	97	97.1
Ellescus_michaeli_n.sp._: LEW_146_: Mer_Bleue_Bog, Ontario		97.6	97.4	97.5
Ellescus_michaeli_n.sp._: LEW_68_: Cantley, Quebec	97.6		97.9	97.6
Ellescus_michaeli_n.sp._: LEW_67_: Cantley, Quebec	97.4	97.9		97.3
Ellescus_michaeli_n.sp._: LEW_70_: Gatineau, Quebec	97.5	97.6	97.3	
Ellescus_michaeli_n.sp._: LEW_130_: Cantley, Quebec	98.2	98	97.8	97.5
Ellescus_michaeli_n.sp._: LEW_148_: Mer_Bleue_Bog, Ontario	97.4	97.4	97.1	97.3
Ellescus_michaeli_n.sp._: LEW_149_: Mer_Bleue_Bog, Ontario	96.8	96.8	96.5	96.8
Ellescus_ephippiatus_: SSROB2985-14_: Rouge_National_Urban_Park, Ontario	86.6	86.6	86.3	86.6
Ellescus_ephippiatus_: SMTPB14415-13_: Kitchener, Ontario_	55.1	55.1	55.1	55.1
Ellescus_ephippiatus_: BBCCA2871-12_: Yavapai, Arizona	93	93	92.7	93
Ellescus_ephippiatus_: LEW_74_: Gainesville, Florida	95.6	95.6	95.3	95.6
Ellescus_ephippiatus_: LEW_109_: Vermillion_Park, Louisiana	96.3	96.6	96.3	96.2
Ellescus_scanicus_: KJ964000.1_: Kirkkonummi, Finland	82	82	81.8	81.9
Ellescus_scanicus_: LEW_91_: Soporna, Slovakia	85.6	84.9	84.7	84.8
Ellescus_bipunctatus_: LEW_95_: Cacin, Slovakia	86.5	86.3	86.1	86
Ellescus_bipunctatus_: LEW_97_: Cacin, Slovakia	85.8	85.9	85.8	85.6
Ellescus_bipunctatus_: COLFH363-14_: Kuusamo, Finland	83.2	83.2	83	83.3
Ellescus_bipunctatus_: COLFH362-14_: Kuusamo, Finland	78.1	78.1	78	78.2
Ellescus_bipunctatus_: KJ962308.1_: Espoo, Finland	83	83	82.9	83.1
Ellescus_bipunctatus_: ELPCH211-17_: Harington, Ontario	83.3	83.3	83.2	83.2
Ellescus_bipunctatus_: LEW_71_: Cantley, Quebec	85.7	86	85.9	85.6
Ellescus_bipunctatus_: ELPCG9470-17_: Harington, Ontario	83.2	83.2	83	83
Ellescus_bipunctatus_: LEW_118_: Boucher_Forest, Quebec	86.1	85.9	85.8	85.4
Ellescus_bipunctatus_: LEW_90_: Gatineau, Quebec	86.5	85.6	85.4	85.4
Ellescus_bipunctatus_: LEW_69_: Cantley, Quebec	85.7	86.2	86.1	85.6
Ellescus_bipunctatus_: LEW_135_: Cantley, Quebec	85.8	86.3	86.1	85.7

	LEW_130	LEW_148	LEW_149	SSROB2985-14
Ellescus_infirmus : LEW_99 : Cerova_vrchovina, Slovakia	89	87.9	87.2	78.6
Ellescus_infirmus : LEW_98 : Cerova_vrchovina, Slovakia	89	87.7	87.1	78.5
Ellescus_infirmus : LEW_100 : Cerova_vrchovina, Slovakia	89.4	87.9	87.2	78.6
Ellescus_ephippiatus : LEW_78 : Selawik_NWR, Alaska	95.8	94.9	94.6	85.3
Ellescus_ephippiatus : LEW_79 : Selawik_NWR, Alaska	95.7	94.8	94.5	85.2
Ellescus_ephippiatus : LEW_77 : Nogahabara_Dunes, Alaska	95.8	95.5	95	86.1
Ellescus_ephippiatus : CNWAA700-14 : Wapusk_National_Park, Manitoba	94.5	94.5	94.8	86.8
Ellescus_michaeli_n.sp. : LEW_145 : Mer_Bleue_Bog, Ontario	98.5	97.1	96.5	86.3
Ellescus_michaeli_n.sp. : LEW_146 : Mer_Bleue_Bog, Ontario	98.2	97.4	96.8	86.6
Ellescus_michaeli_n.sp. : LEW_68 : Cantley, Quebec	98	97.4	96.8	86.6
Ellescus_michaeli_n.sp. : LEW_67 : Cantley, Quebec	97.8	97.1	96.5	86.3
Ellescus_michaeli_n.sp. : LEW_70 : Gatineau, Quebec	97.5	97.3	96.8	86.6
Ellescus_michaeli_n.sp. : LEW_130 : Cantley, Quebec		97.3	96.6	86.5
Ellescus_michaeli_n.sp. : LEW_148 : Mer_Bleue_Bog, Ontario	97.3		96.6	86.5
Ellescus_michaeli_n.sp. : LEW_149 : Mer_Bleue_Bog, Ontario	96.6	96.6		86.8
Ellescus_ephippiatus : SSROB2985-14 : Rouge_National_Urban_Park, Ontario	86.5	86.5	86.8	
Ellescus_ephippiatus : SMTPB14415-13 : Kitchener, Ontario	55.1	55.1	55.1	51.9
Ellescus_ephippiatus : BBCCA2871-12 : Yavapai, Arizona	92.9	93.2	93.2	85.2
Ellescus_ephippiatus : LEW_74 : Gainesville, Florida	95.5	95.8	95.1	85.5
Ellescus_ephippiatus : LEW_109 : Vermillion_Park, Louisiana	96.7	96.1	95.5	85.8
Ellescus_scanicus : KJ964000.1 : Kirkkonummi, Finland	82.1	82.1	82	76
Ellescus_scanicus : LEW_91 : Soporna, Slovakia	85.8	85	84.2	75.9
Ellescus_bipunctatus : LEW_95 : Cacin, Slovakia	87.3	85.9	85.2	77.1
Ellescus_bipunctatus : LEW_97 : Cacin, Slovakia	86.5	85.4	84.8	76.8
Ellescus_bipunctatus : COLFH363-14 : Kuusamo, Finland	83.3	83.3	83.3	76.9
Ellescus_bipunctatus : COLFH362-14 : Kuusamo, Finland	78.3	78.3	78.3	76.7
Ellescus_bipunctatus : KJ962308.1 : Espoo, Finland	83.2	83.2	83.2	76.8
Ellescus_bipunctatus : ELPCH211-17 : Harington, Ontario	83.2	83.5	83.2	77.2
Ellescus_bipunctatus : LEW_71 : Cantley, Quebec	85.9	85.7	84.8	77.2
Ellescus_bipunctatus : ELPCG9470-17 : Harington, Ontario	83	83.3	83	77.1
Ellescus_bipunctatus : LEW_118 : Boucher_Forest, Quebec	86.5	85.4	84.5	76.9
Ellescus_bipunctatus : LEW_90 : Gatineau, Quebec	86.3	85.7	84.8	77.1
Ellescus_bipunctatus : LEW_69 : Cantley, Quebec	86.1	85.7	84.8	77.1
Ellescus_bipunctatus : LEW_135 : Cantley, Quebec	86.1	85.8	84.9	77.1

	SMTPB14415-13	BBCCA2871-12	LEW_74	LEW_109
Ellescus_infirmus : LEW_99 : Cerova_vrchovina, Slovakia	51.1	86	88.3	89.4
Ellescus_infirmus : LEW_98 : Cerova_vrchovina, Slovakia	51	85.9	88.2	89
Ellescus_infirmus : LEW_100 : Cerova_vrchovina, Slovakia	51	86	88.3	89.4
Ellescus_ephippiatus : LEW_78 : Selawik_NWR, Alaska	54.3	92.8	95.4	96.4
Ellescus_ephippiatus : LEW_79 : Selawik_NWR, Alaska	54.3	92.7	95.3	96.2
Ellescus_ephippiatus : LEW_77 : Nogahabara_Dunes, Alaska	55	92.5	94	94.4
Ellescus_ephippiatus : CNWAA700-14 : Wapusk_National_Park, Manitoba	55	92.8	93.1	93.5
Ellescus_michaeli_n.sp. : LEW_145 : Mer_Bleue_Bog, Ontario	55	92.7	95.3	95.9
Ellescus_michaeli_n.sp. : LEW_146 : Mer_Bleue_Bog, Ontario	55.1	93	95.6	96.3
Ellescus_michaeli_n.sp. : LEW_68 : Cantley, Quebec	55.1	93	95.6	96.6
Ellescus_michaeli_n.sp. : LEW_67 : Cantley, Quebec	55.1	92.7	95.3	96.3
Ellescus_michaeli_n.sp. : LEW_70 : Gatineau, Quebec	55.1	93	95.6	96.2
Ellescus_michaeli_n.sp. : LEW_130 : Cantley, Quebec	55.1	92.9	95.5	96.7
Ellescus_michaeli_n.sp. : LEW_148 : Mer_Bleue_Bog, Ontario	55.1	93.2	95.8	96.1
Ellescus_michaeli_n.sp. : LEW_149 : Mer_Bleue_Bog, Ontario	55.1	93.2	95.1	95.5
Ellescus_ephippiatus : SSROB2985-14 : Rouge_National_Urban_Park, Ontario	51.9	85.2	85.5	85.8
Ellescus_ephippiatus : SMTPB14415-13 : Kitchener, Ontario		54.4	54.4	54.4
Ellescus_ephippiatus : BBCCA2871-12 : Yavapai, Arizona	54.4		94.2	94.3
Ellescus_ephippiatus : LEW_74 : Gainesville, Florida	54.4	94.2		96.9
Ellescus_ephippiatus : LEW_109 : Vermillion_Park, Louisiana	54.4	94.3	96.9	
Ellescus_scanicus : KJ964000.1 : Kirkkonummi, Finland	49.7	82.4	82.4	82.5
Ellescus_scanicus : LEW_91 : Soporna, Slovakia	50	83	85.3	85.6
Ellescus_bipunctatus : LEW_95 : Cacin, Slovakia	50.6	83.5	86.1	87
Ellescus_bipunctatus : LEW_97 : Cacin, Slovakia	50.6	83	85.7	86.8
Ellescus_bipunctatus : COLFH363-14 : Kuusamo, Finland	50.6	82.9	83.3	83.8
Ellescus_bipunctatus : COLFH362-14 : Kuusamo, Finland	49.5	78.1	78.4	78.6
Ellescus_bipunctatus : KJ962308.1 : Espoo, Finland	50.6	83	83.5	83.6
Ellescus_bipunctatus : ELPCH211-17 : Harington, Ontario	50.3	83.5	83.5	83.5
Ellescus_bipunctatus : LEW_71 : Cantley, Quebec	50.3	83.5	85.8	86.1
Ellescus_bipunctatus : ELPCG9470-17 : Harington, Ontario	50.1	83.3	83.4	83.3
Ellescus_bipunctatus : LEW_118 : Boucher_Forest, Quebec	50.3	82.9	85.3	86.1
Ellescus_bipunctatus : LEW_90 : Gatineau, Quebec	50.3	82.9	85.6	85.6
Ellescus_bipunctatus : LEW_69 : Cantley, Quebec	50.3	82.9	85.6	86.1
Ellescus_bipunctatus : LEW_135 : Cantley, Quebec	50.4	83	85.6	86.1

	KJ964000.1	LEW_91	LEW_95	LEW_97
Ellescus_infirmus_: LEW_99_: Cerova_vrchovina, Slovakia	81	84.1	85.6	85.9
Ellescus_infirmus_: LEW_98_: Cerova_vrchovina, Slovakia	80.6	83.9	86.3	85
Ellescus_infirmus_: LEW_100_: Cerova_vrchovina, Slovakia	80.7	84	86.5	85.6
Ellescus_ephippiatus_: LEW_78_: Selawik_NWR, Alaska	82.6	85.2	86.6	86.6
Ellescus_ephippiatus_: LEW_79_: Selawik_NWR, Alaska	82.5	85.1	86.5	86.4
Ellescus_ephippiatus_: LEW_77_: Nogahabara_Dunes, Alaska	81.7	83.5	83.8	84
Ellescus_ephippiatus_: CNWAA700-14_: Wapusk_National_Park, Manitoba	82	82.4	83.7	83.2
Ellescus_michaeli_n.sp._: LEW_145_: Mer_Bleue_Bog, Ontario	82	85.6	86.8	85.7
Ellescus_michaeli_n.sp._: LEW_146_: Mer_Bleue_Bog, Ontario	82	85.6	86.5	85.8
Ellescus_michaeli_n.sp._: LEW_68_: Cantley, Quebec	82	84.9	86.3	85.9
Ellescus_michaeli_n.sp._: LEW_67_: Cantley, Quebec	81.8	84.7	86.1	85.8
Ellescus_michaeli_n.sp._: LEW_70_: Gatineau, Quebec	81.9	84.8	86	85.6
Ellescus_michaeli_n.sp._: LEW_130_: Cantley, Quebec	82.1	85.8	87.3	86.5
Ellescus_michaeli_n.sp._: LEW_148_: Mer_Bleue_Bog, Ontario	82.1	85	85.9	85.4
Ellescus_michaeli_n.sp._: LEW_149_: Mer_Bleue_Bog, Ontario	82	84.2	85.2	84.8
Ellescus_ephippiatus_: SSROB2985-14_: Rouge_National_Urban_Park, Ontario	76	75.9	77.1	76.8
Ellescus_ephippiatus_: SMTPB14415-13_: Kitchener, Ontario	49.7	50	50.6	50.6
Ellescus_ephippiatus_: BBCCA2871-12_: Yavapai, Arizona	82.4	83	83.5	83
Ellescus_ephippiatus_: LEW_74_: Gainesville, Florida	82.4	85.3	86.1	85.7
Ellescus_ephippiatus_: LEW_109_: Vermillion_Park, Louisiana	82.5	85.6	87	86.8
Ellescus_scanicus_: KJ964000.1_: Kirkkonummi, Finland		93.7	84.8	84.5
Ellescus_scanicus_: LEW_91_: Soporna, Slovakia	93.7		88.6	87.9
Ellescus_bipunctatus_: LEW_95_: Cacin, Slovakia	84.8	88.6		97.6
Ellescus_bipunctatus_: LEW_97_: Cacin, Slovakia	84.5	87.9	97.6	
Ellescus_bipunctatus_: COLFH363-14_: Kuusamo, Finland	84.4	85	94.3	94.9
Ellescus_bipunctatus_: COLFH362-14_: Kuusamo, Finland	79.3	79.7	88.4	88.8
Ellescus_bipunctatus_: KJ962308.1_: Espoo, Finland	84.5	85.2	94.4	95
Ellescus_bipunctatus_: ELPCH211-17_: Harington, Ontario	83.5	83.3	88.7	88.3
Ellescus_bipunctatus_: LEW_71_: Cantley, Quebec	83.5	85.6	91.4	91
Ellescus_bipunctatus_: ELPCG9470-17_: Harington, Ontario	83.5	83.3	88.6	88.3
Ellescus_bipunctatus_: LEW_118_: Boucher_Forest, Quebec	84.1	87.2	92.3	92
Ellescus_bipunctatus_: LEW_90_: Gatineau, Quebec	84.3	87.4	91.9	91.3
Ellescus_bipunctatus_: LEW_69_: Cantley, Quebec	84.3	86.6	91.8	91.6
Ellescus_bipunctatus_: LEW_135_: Cantley, Quebec	84.3	86.6	91.7	91.6

	COLFH363-14	COLFH362-14	KJ962308.1	ELPCH211-17
Ellescus_infirmus : LEW_99 : Cerova_vrchovina, Slovakia	82.2	77.4	82.3	82.2
Ellescus_infirmus : LEW_98 : Cerova_vrchovina, Slovakia	81.8	77	81.9	82.1
Ellescus_infirmus : LEW_100 : Cerova_vrchovina, Slovakia	81.9	77.1	82.1	82.2
Ellescus_ephippiatus : LEW_78 : Selawik_NWR, Alaska	83.5	78.5	83.7	83
Ellescus_ephippiatus : LEW_79 : Selawik_NWR, Alaska	83.5	78.4	83.6	82.9
Ellescus_ephippiatus : LEW_77 : Nogahabara_Dunes, Alaska	82.7	77.9	82.5	82.5
Ellescus_ephippiatus : CNWAA700-14 : Wapusk_National_Park, Manitoba	83.4	78.3	83.2	83.2
Ellescus_michaeli_n.sp. : LEW_145 : Mer_Bleue_Bog, Ontario	83.2	78.1	83	83
Ellescus_michaeli_n.sp. : LEW_146 : Mer_Bleue_Bog, Ontario	83.2	78.1	83	83.3
Ellescus_michaeli_n.sp. : LEW_68 : Cantley, Quebec	83.2	78.1	83	83.3
Ellescus_michaeli_n.sp. : LEW_67 : Cantley, Quebec	83	78	82.9	83.2
Ellescus_michaeli_n.sp. : LEW_70 : Gatineau, Quebec	83.3	78.2	83.1	83.2
Ellescus_michaeli_n.sp. : LEW_130 : Cantley, Quebec	83.3	78.3	83.2	83.2
Ellescus_michaeli_n.sp. : LEW_148 : Mer_Bleue_Bog, Ontario	83.3	78.3	83.2	83.5
Ellescus_michaeli_n.sp. : LEW_149 : Mer_Bleue_Bog, Ontario	83.3	78.3	83.2	83.2
Ellescus_ephippiatus : SSROB2985-14 : Rouge_National_Urban_Park, Ontario	76.9	76.7	76.8	77.2
Ellescus_ephippiatus : SMTPB14415-13 : Kitchener, Ontario	50.6	49.5	50.6	50.3
Ellescus_ephippiatus : BBCCA2871-12 : Yavapai, Arizona	82.9	78.1	83	83.5
Ellescus_ephippiatus : LEW_74 : Gainesville, Florida	83.3	78.4	83.5	83.5
Ellescus_ephippiatus : LEW_109 : Vermillion_Park, Louisiana	83.8	78.6	83.6	83.5
Ellescus_scanicus : KJ964000.1 : Kirkkonummi, Finland	84.4	79.3	84.5	83.5
Ellescus_scanicus : LEW_91 : Soporna, Slovakia	85	79.7	85.2	83.3
Ellescus_bipunctatus : LEW_95 : Cacin, Slovakia	94.3	88.4	94.4	88.7
Ellescus_bipunctatus : LEW_97 : Cacin, Slovakia	94.9	88.8	95	88.3
Ellescus_bipunctatus : COLFH363-14 : Kuusamo, Finland		89	95.2	88.2
Ellescus_bipunctatus : COLFH362-14 : Kuusamo, Finland	89		89.1	83.1
Ellescus_bipunctatus : KJ962308.1 : Espoo, Finland	95.2	89.1		88.3
Ellescus_bipunctatus : ELPCH211-17 : Harington, Ontario	88.2	83.1	88.3	
Ellescus_bipunctatus : LEW_71 : Cantley, Quebec	88.2	83.1	88.3	95.3
Ellescus_bipunctatus : ELPCG9470-17 : Harington, Ontario	88.2	83	88.3	95
Ellescus_bipunctatus : LEW_118 : Boucher_Forest, Quebec	88.3	83.1	88.5	93
Ellescus_bipunctatus : LEW_90 : Gatineau, Quebec	88.5	83.3	88.7	92.5
Ellescus_bipunctatus : LEW_69 : Cantley, Quebec	88.6	83.4	88.7	92.6
Ellescus_bipunctatus : LEW_135 : Cantley, Quebec	88.5	83.3	88.7	92.5

	LEW_71	ELPCG9470-17	LEW_118	LEW_90
Ellescus_infirmus_: LEW_99_: Cerova_vrchovina, Slovakia	84.9	82.1	86.2	85.7
Ellescus_infirmus_: LEW_98_: Cerova_vrchovina, Slovakia	84.8	81.9	85.8	85.8
Ellescus_infirmus_: LEW_100_: Cerova_vrchovina, Slovakia	84.9	82.1	86.4	85.9
Ellescus_ephippiatus_: LEW_78_: Selawik_NWR, Alaska	85.6	82.8	85.7	85.2
Ellescus_ephippiatus_: LEW_79_: Selawik_NWR, Alaska	85.6	82.8	85.7	85.1
Ellescus_ephippiatus_: LEW_77_: Nogahabara_Dunes, Alaska	83.7	82.4	83.4	83.7
Ellescus_ephippiatus_: CNWAA700-14_: Wapusk_National_Park, Manitoba	83.2	83.1	82.9	83.2
Ellescus_michaeli_n.sp._: LEW_145_: Mer_Bleue_Bog, Ontario	85.3	82.9	85.8	86
Ellescus_michaeli_n.sp._: LEW_146_: Mer_Bleue_Bog, Ontario	85.7	83.2	86.1	86.5
Ellescus_michaeli_n.sp._: LEW_68_: Cantley, Quebec	86	83.2	85.9	85.6
Ellescus_michaeli_n.sp._: LEW_67_: Cantley, Quebec	85.9	83	85.8	85.4
Ellescus_michaeli_n.sp._: LEW_70_: Gatineau, Quebec	85.6	83	85.4	85.4
Ellescus_michaeli_n.sp._: LEW_130_: Cantley, Quebec	85.9	83	86.5	86.3
Ellescus_michaeli_n.sp._: LEW_148_: Mer_Bleue_Bog, Ontario	85.7	83.3	85.4	85.7
Ellescus_michaeli_n.sp._: LEW_149_: Mer_Bleue_Bog, Ontario	84.8	83	84.5	84.8
Ellescus_ephippiatus_: SSROB2985-14_: Rouge_National_Urban_Park, Ontario	77.2	77.1	76.9	77.1
Ellescus_ephippiatus_: SMTPB14415-13_: Kitchener, Ontario_	50.3	50.1	50.3	50.3
Ellescus_ephippiatus_: BBCCA2871-12_: Yavapai, Arizona	83.5	83.3	82.9	82.9
Ellescus_ephippiatus_: LEW_74_: Gainesville, Florida	85.8	83.4	85.3	85.6
Ellescus_ephippiatus_: LEW_109_: Vermillion_Park, Louisiana	86.1	83.3	86.1	85.6
Ellescus_scanicus_: KJ964000.1_: Kirkkonummi, Finland	83.5	83.5	84.1	84.3
Ellescus_scanicus_: LEW_91_: Soporna, Slovakia	85.6	83.3	87.2	87.4
Ellescus_bipunctatus_: LEW_95_: Cacin, Slovakia	91.4	88.6	92.3	91.9
Ellescus_bipunctatus_: LEW_97_: Cacin, Slovakia	91	88.3	92	91.3
Ellescus_bipunctatus_: COLFH363-14_: Kuusamo, Finland	88.2	88.2	88.3	88.5
Ellescus_bipunctatus_: COLFH362-14_: Kuusamo, Finland	83.1	83	83.1	83.3
Ellescus_bipunctatus_: KJ962308.1_: Espoo, Finland	88.3	88.3	88.5	88.7
Ellescus_bipunctatus_: ELPCH211-17_: Harington, Ontario	95.3	95	93	92.5
Ellescus_bipunctatus_: LEW_71_: Cantley, Quebec		95	95.7	94.8
Ellescus_bipunctatus_: ELPCG9470-17_: Harington, Ontario	95		93	92.5
Ellescus_bipunctatus_: LEW_118_: Boucher_Forest, Quebec	95.7	93		96.9
Ellescus_bipunctatus_: LEW_90_: Gatineau, Quebec	94.8	92.5	96.9	
Ellescus_bipunctatus_: LEW_69_: Cantley, Quebec	95.3	92.6	96.9	97.5
Ellescus_bipunctatus_: LEW_135_: Cantley, Quebec	95.2	92.5	96.8	97.4

	LEW_69	LEW_135
Ellescus_infirmus_: LEW_99_: Cerova_vrchovina, Slovakia	85.8	85.9
Ellescus_infirmus_: LEW_98_: Cerova_vrchovina, Slovakia	85.5	85.5
Ellescus_infirmus_: LEW_100_: Cerova_vrchovina, Slovakia	85.8	85.9
Ellescus_ephippiatus_: LEW_78_: Selawik_NWR, Alaska	85.9	85.9
Ellescus_ephippiatus_: LEW_79_: Selawik_NWR, Alaska	85.7	85.8
Ellescus_ephippiatus_: LEW_77_: Nogahabara_Dunes, Alaska	83.7	83.8
Ellescus_ephippiatus_: CNWAA700-14_: Wapusk_National_Park, Manitoba	83.2	83.3
Ellescus_michaeli_n.sp._: LEW_145_: Mer_Bleue_Bog, Ontario	85.3	85.4
Ellescus_michaeli_n.sp._: LEW_146_: Mer_Bleue_Bog, Ontario	85.7	85.8
Ellescus_michaeli_n.sp._: LEW_68_: Cantley, Quebec	86.2	86.3
Ellescus_michaeli_n.sp._: LEW_67_: Cantley, Quebec	86.1	86.1
Ellescus_michaeli_n.sp._: LEW_70_: Gatineau, Quebec	85.6	85.7
Ellescus_michaeli_n.sp._: LEW_130_: Cantley, Quebec	86.1	86.1
Ellescus_michaeli_n.sp._: LEW_148_: Mer_Bleue_Bog, Ontario	85.7	85.8
Ellescus_michaeli_n.sp._: LEW_149_: Mer_Bleue_Bog, Ontario	84.8	84.9
Ellescus_ephippiatus_: SSROB2985-14_: Rouge_National_Urban_Park, Ontario	77.1	77.1
Ellescus_ephippiatus_: SMTPB14415-13_: Kitchener, Ontario_	50.3	50.4
Ellescus_ephippiatus_: BBCCA2871-12_: Yavapai, Arizona	82.9	83
Ellescus_ephippiatus_: LEW_74_: Gainesville, Florida	85.6	85.6
Ellescus_ephippiatus_: LEW_109_: Vermillion_Park, Louisiana	86.1	86.1
Ellescus_scanicus_: KJ964000.1_: Kirkkonummi, Finland	84.3	84.3
Ellescus_scanicus_: LEW_91_: Soporna, Slovakia	86.6	86.6
Ellescus_bipunctatus_: LEW_95_: Cacin, Slovakia	91.8	91.7
Ellescus_bipunctatus_: LEW_97_: Cacin, Slovakia	91.6	91.6
Ellescus_bipunctatus_: COLFH363-14_: Kuusamo, Finland	88.6	88.5
Ellescus_bipunctatus_: COLFH362-14_: Kuusamo, Finland	83.4	83.3
Ellescus_bipunctatus_: KJ962308.1_: Espoo, Finland	88.7	88.7
Ellescus_bipunctatus_: ELPCH211-17_: Harington, Ontario	92.6	92.5
Ellescus_bipunctatus_: LEW_71_: Cantley, Quebec	95.3	95.2
Ellescus_bipunctatus_: ELPCG9470-17_: Harington, Ontario	92.6	92.5
Ellescus_bipunctatus_: LEW_118_: Boucher_Forest, Quebec	96.9	96.8
Ellescus_bipunctatus_: LEW_90_: Gatineau, Quebec	97.5	97.4
Ellescus_bipunctatus_: LEW_69_: Cantley, Quebec		98.1
Ellescus_bipunctatus_: LEW_135_: Cantley, Quebec	98.1	

Table 2.6

Percent identity of ITS2 sequences for representatives of species of *Ellescus* (Coleoptera: Curculionidae) (Aligned ITS2 length: 795). Note that this measure specifies the percentage of sites (columns) in the DNA sequence alignment that are identical between sequences.

	LEW_91	LEW_95	LEW_97	LEW_90	LEW_69	LEW_71
<i>Ellescus_scanicus</i> _: LEW_91_: Soporna, Slovakia		52.9	52.9	53	53.2	53.2
<i>Ellescus_bipunctatus</i> _: LEW_95_: Cacin, Slovakia	52.9		100	76.8	77	77
<i>Ellescus_bipunctatus</i> _: LEW_97_: Cacin, Slovakia	52.9	100		76.8	77	77
<i>Ellescus_bipunctatus</i> _: LEW_90_: Gatineau, Quebec	53	76.8	76.8		99.8	99.8
<i>Ellescus_bipunctatus</i> _: LEW_69_: Cantley, Quebec	53.2	77	77	99.8		100
<i>Ellescus_bipunctatus</i> _: LEW_71_: Cantley, Quebec	53.2	77	77	99.8	100	
<i>Ellescus_bipunctatus</i> _: LEW_104_: Morgan County, Indiana	53.2	77	77	99.8	100	100
<i>Ellescus_bipunctatus</i> _: LEW_135_: Cantley, Quebec	53	76.7	76.7	99.4	99.6	99.6
<i>Ellescus_infirmus</i> _: LEW_100_: Cerova vrchovina, Slovakia	65	53.7	53.7	56.1	56.3	56.3
<i>Ellescus_michaeli</i> _n.sp._: LEW_68_: Cantley, Quebec	63.1	55.6	55.6	57	57.2	57.2
<i>Ellescus_michaeli</i> _n.sp._: LEW_147_: Mer Bleue Bog, Ontario	63.6	55.8	55.8	57.2	57.4	57.4
<i>Ellescus_michaeli</i> _n.sp._: LEW_148_: Mer Bleue Bog, Ontario	63.6	55.8	55.8	57.2	57.4	57.4
<i>Ellescus_michaeli</i> _n.sp._: LEW_145_: Mer Bleue Bog, Ontario	63.5	55.7	55.7	57	57.2	57.2
<i>Ellescus_ephippiatus</i> _: LEW_74_: Gainesville, Florida	62.9	55.2	55.2	57.5	57.7	57.7
<i>Ellescus_ephippiatus</i> _: LEW_78_: Selawik NWR, Alaska	63.5	56	56	57.9	58	58
<i>Ellescus_ephippiatus</i> _: LEW_79_: Selawik NWR, Alaska	63.5	56	56	57.9	58	58
<i>Ellescus_ephippiatus</i> _: LEW_109_: Vermillion Park, Louisiana	63.1	55.6	55.6	57.5	57.6	57.6

	LEW_104	LEW_135	LEW_100	LEW_68	LEW_147	LEW_148
Ellescus_scanicus_:LEW_91_:Soporna,Slovakia	53.2	53	65	63.1	63.6	63.6
Ellescus_bipunctatus_:LEW_95_:Cacin,Slovakia	77	76.7	53.7	55.6	55.8	55.8
Ellescus_bipunctatus_:LEW_97_:Cacin,Slovakia	77	76.7	53.7	55.6	55.8	55.8
Ellescus_bipunctatus_:LEW_90_:Gatineau,Quebec	99.8	99.4	56.1	57	57.2	57.2
Ellescus_bipunctatus_:LEW_69_:Cantley,Quebec	100	99.6	56.3	57.2	57.4	57.4
Ellescus_bipunctatus_:LEW_71_:Cantley,Quebec	100	99.6	56.3	57.2	57.4	57.4
Ellescus_bipunctatus_:LEW_104_:Morgan_County,Indiana		99.6	56.3	57.2	57.4	57.4
Ellescus_bipunctatus_:LEW_135_:Cantley,Quebec	99.6		56.3	57.2	57.4	57.4
Ellescus_infirmus_:LEW_100_:Cerova_vrchovina,Slovakia	56.3	56.3		85.7	86.4	86.4
Ellescus_michaeli_n.sp.:LEW_68_:Cantley,Quebec	57.2	57.2	85.7		99.3	99.3
Ellescus_michaeli_n.sp.:LEW_147_:Mer_Bleue_Bog,Ontario	57.4	57.4	86.4	99.3		100
Ellescus_michaeli_n.sp.:LEW_148_:Mer_Bleue_Bog,Ontario	57.4	57.4	86.4	99.3	100	
Ellescus_michaeli_n.sp.:LEW_145_:Mer_Bleue_Bog,Ontario	57.2	57.2	86.2	99	99.7	99.7
Ellescus_ephippiatus_:LEW_74_:Gainesville,Florida	57.7	57.7	87.1	90	90.6	90.6
Ellescus_ephippiatus_:LEW_78_:Selawik_NWR,Alaska	58	58	87.7	91.4	92	92
Ellescus_ephippiatus_:LEW_79_:Selawik_NWR,Alaska	58	58	87.7	91.4	92	92
Ellescus_ephippiatus_:LEW_109_:Vermillion_Park,Louisiana	57.6	57.6	87.4	90.9	91.6	91.6

	LEW_145	LEW_74	LEW_78	LEW_79	LEW_109
Ellescus_scanicus_: LEW_91_: Soporna, Slovakia	63.5	62.9	63.5	63.5	63.1
Ellescus_bipunctatus_: LEW_95_: Cacin, Slovakia	55.7	55.2	56	56	55.6
Ellescus_bipunctatus_: LEW_97_: Cacin, Slovakia	55.7	55.2	56	56	55.6
Ellescus_bipunctatus_: LEW_90_: Gatineau, Quebec	57	57.5	57.9	57.9	57.5
Ellescus_bipunctatus_: LEW_69_: Cantley, Quebec	57.2	57.7	58	58	57.6
Ellescus_bipunctatus_: LEW_71_: Cantley, Quebec	57.2	57.7	58	58	57.6
Ellescus_bipunctatus_: LEW_104_: Morgan County, Indiana	57.2	57.7	58	58	57.6
Ellescus_bipunctatus_: LEW_135_: Cantley, Quebec	57.2	57.7	58	58	57.6
Ellescus_infirmus_: LEW_100_: Cerova vrchovina, Slovakia	86.2	87.1	87.7	87.7	87.4
Ellescus_michaeli_n.sp._: LEW_68_: Cantley, Quebec	99	90	91.4	91.4	90.9
Ellescus_michaeli_n.sp._: LEW_147_: Mer Bleue Bog, Ontario	99.7	90.6	92	92	91.6
Ellescus_michaeli_n.sp._: LEW_148_: Mer Bleue Bog, Ontario	99.7	90.6	92	92	91.6
Ellescus_michaeli_n.sp._: LEW_145_: Mer Bleue Bog, Ontario		90.5	91.9	91.9	91.4
Ellescus_ephippiatus_: LEW_74_: Gainesville, Florida	90.5		98.2	98.2	97.9
Ellescus_ephippiatus_: LEW_78_: Selawik NWR, Alaska	91.9	98.2		100	99.4
Ellescus_ephippiatus_: LEW_79_: Selawik NWR, Alaska	91.9	98.2	100		99.4
Ellescus_ephippiatus_: LEW_109_: Vermillion Park, Louisiana	91.4	97.9	99.4	99.4	

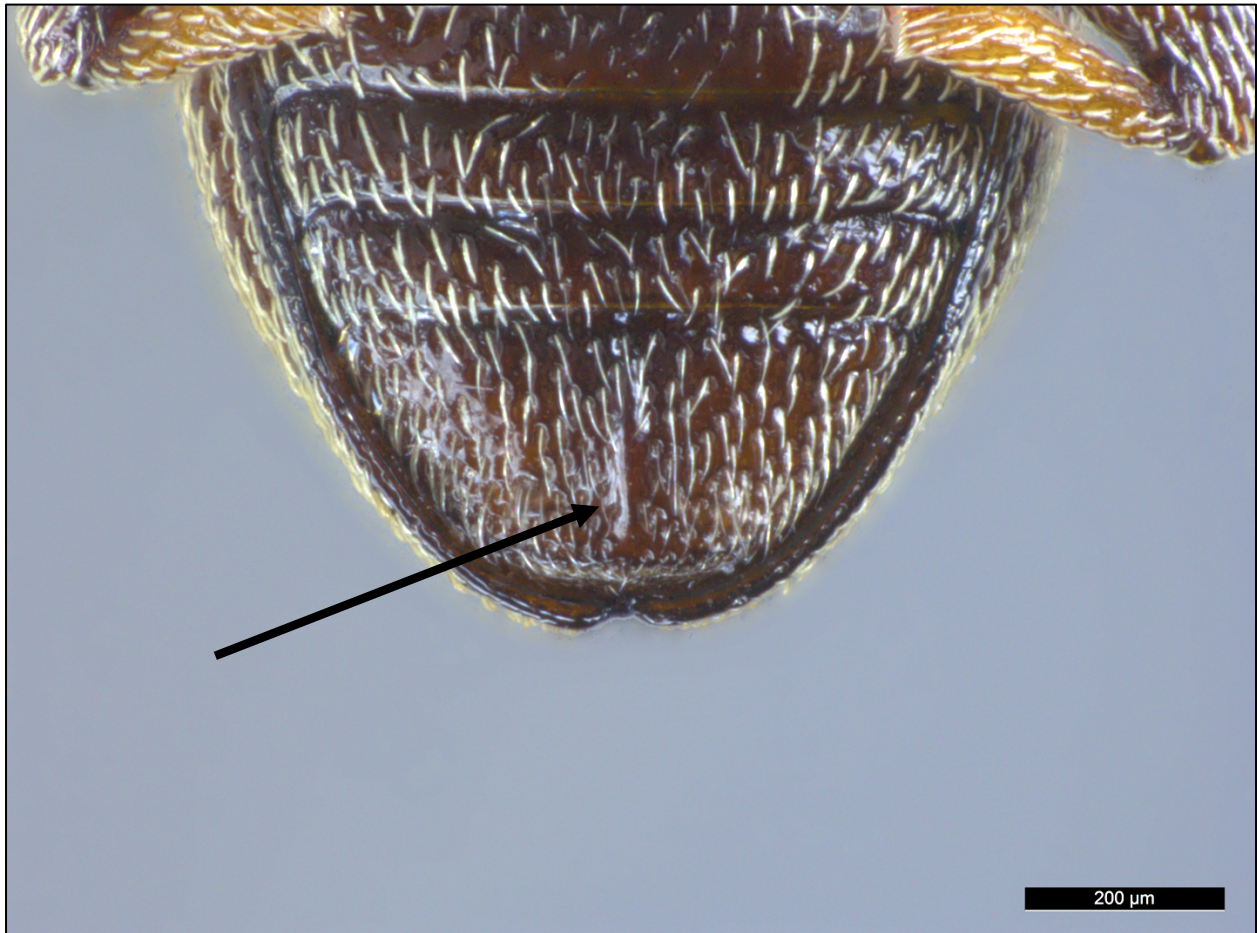


Figure 2.1 Male *Ellescus bipunctatus* (Linnaeus, 1758) with conspicuous glabrous carina running longitudinally over more than half of the last sternite.



Figure 2.2 Male *Ellescus ephippiatus* (Say, 1831) with distinct swelling at tip of last sternite, not extending over more than half of the last sternite. Note that this is reduced or absent in some specimens.

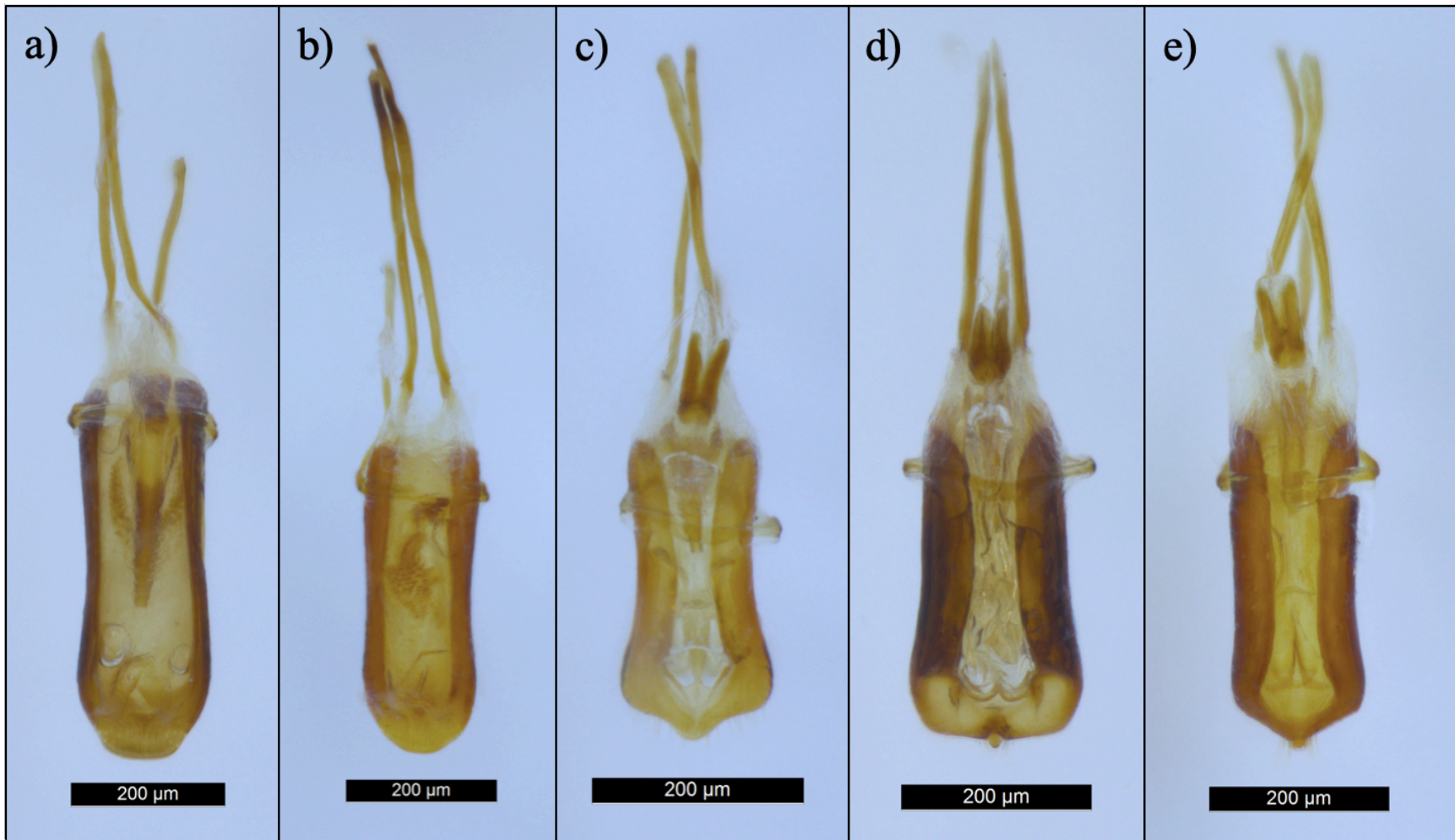


Figure 2.3 Dorsal photograph of the aedeagi of *Ellescus*: **a)** *E. scanicus* (Paykull, 1792), **b)** *E. bipunctatus* (Linnaeus, 1758), **c)** *Ellescus michaeli* n. sp., **d)** *E. californicus* (Casey, 1885) **e)** *E. ephippiatus* (Say, 1831).



Figure 2.4 Metepisternum and lateral half of metasternum densely punctate and covered with appressed white-yellow scales, cuticle in those regions mostly concealed - *Ellescus scanicus* (Paykull, 1792).



Figure 2.5 Metepisternum and lateral half of metasternum not densely punctate or covered with appressed white-yellow scales, with cuticle in those regions mostly concealed - *Ellescus bipunctatus* (Linnaeus, 1758).



Figure 2.6 Lateral photograph of *Ellescus scanicus* (Paykull, 1792).



Figure 2.7 Dorsal photograph of *Ellescus scanicus* (Paykull, 1792).



Figure 2.8 Lateral photograph of *Ellescus bipunctatus* (Linnaeus, 1758) (specimen #1).



Figure 2.9 Dorsal photograph of *Ellescus bipunctatus* (Linnaeus, 1758) (specimen #1).



Figure 2.10 Lateral photograph of *Ellescus bipunctatus* (Linnaeus, 1758) (specimen #2).



Figure 2.11 Dorsal photograph of *Ellescus bipunctatus* (Linnaeus, 1758) (specimen #2).



Figure 2.12 Lateral photograph of *Ellescus bipunctatus* (Linnaeus, 1758) (specimen #3).



Figure 2.13 Dorsal photograph of *Ellescus bipunctatus* (Linnaeus, 1758) (specimen #3).



Figure 2.14 Lateral photograph of the *Ellescus borealis* (Carr, 1920) holotype (CNC 379701). Note that *E. borealis* is a junior synonym of *E. bipunctatus* (Linnaeus, 1758), as evidence by the lack of morphological characters separating these taxa.



Figure 2.15 Dorsal photograph of the *Ellescus borealis* (Carr, 1920) holotype (CNC 379701). Note that *E. borealis* is a junior synonym of *E. bipunctatus* (Linnaeus, 1758), as evidence by the lack of morphological characters separating these taxa.



Figure 2.16 Photograph of the 5th sternite of the *Ellescus borealis* (Carr, 1920) holotype (CNC 379701). Note the carina running longitudinally along the center of the 5th sternite, a character only present in males of *E. bipunctatus* and *E. scanicus* (Paykull, 1792).

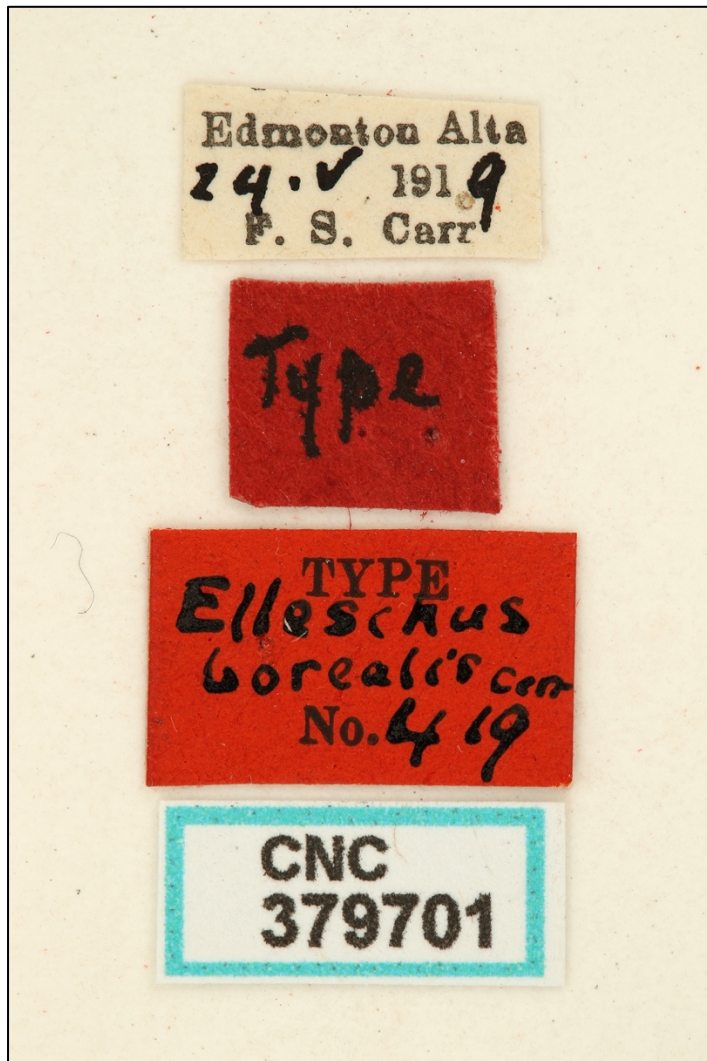


Figure 2.17 *Ellescus borealis* (Carr, 1920) holotype labels.



Figure 2.18 Lateral photograph of *Ellescus californicus* (Casey, 1885).



Figure 2.19 Dorsal photograph of *Ellescus californicus* (Casey, 1885).



Figure 2.20 Lateral photograph of the *Ellescus californicus* (Casey, 1885) holotype (USNMENT 01448984 / TYPE USNM 36721). Note the discolored lateral bands on the pronotum, and contrast in cuticular coloration between the metasternum (black), legs (rufous), and rostrum (rufous). The collection locality (California) further substantiates this as the same distinct form described in this work.

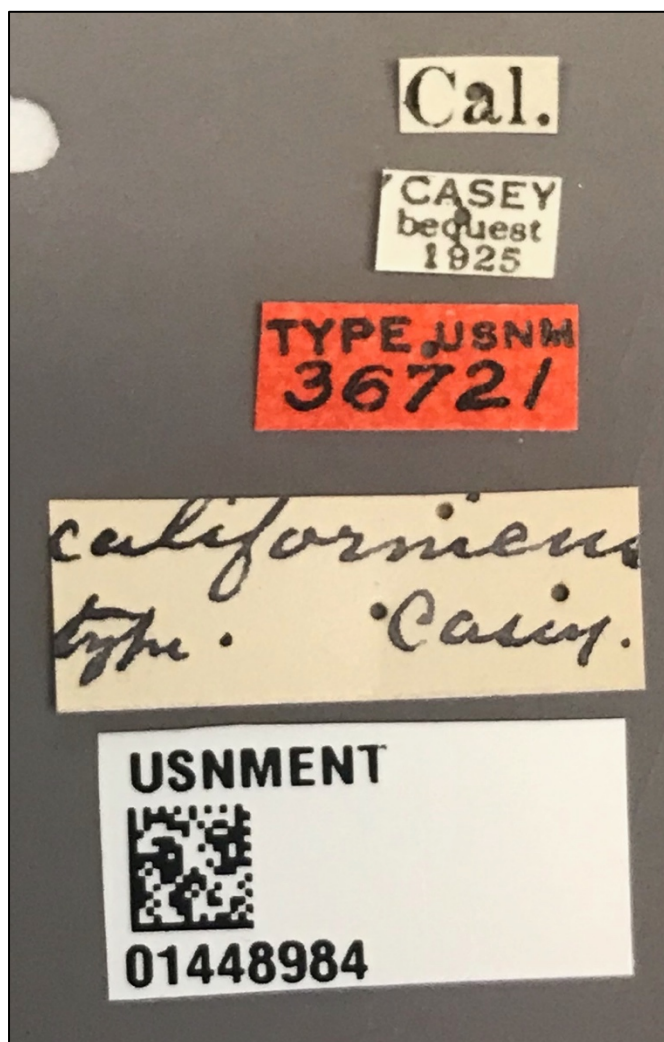


Figure 2.21 *Ellescus californicus* (Casey, 1885) holotype labels.



Figure 2.22 Lateral photograph of *Ellescus michaeli* n. sp. (specimen #1).



Figure 2.23 Dorsal photograph of *Ellescus michaeli* n. sp. (specimen #1).



Figure 2.24 Lateral photograph of *Ellescus michaeli* n. sp. (specimen #2).



Figure 2.25 Dorsal photograph of *Ellescus michaeli* n. sp. (specimen #2).



Figure 2.26 Lateral photograph of *Ellescus ehippiatus* (Say, 1831) (specimen #1).



Figure 2.27 Dorsal photograph of *Ellescus ehippiatus* (Say, 1831) (specimen #1).



Figure 2.28 Lateral photograph of *Ellescus ehippiatus* (Say, 1831) (specimen #2).



Figure 2.29 Dorsal photograph of *Ellescus ehippiatus* (Say, 1831) (specimen #2).



Figure 2.30 Lateral photograph of *Ellescus ehippiatus* (Say, 1831) (specimen #3).



Figure 2.31 Dorsal photograph of *Ellescus ehippiatus* (Say, 1831) (specimen #3).



Figure 2.32 Lateral photograph of the *Ellescus ephippiatus* (Say, 1831) syntype (NHRS-JLKB000073656). Note the largely red body contrasting with the black metasternum and metepisternum (not found in *E. michaeli*, the only other similar species in the region).



Figure 2.33 Dorsal photograph of the *Ellescus ehippiatus* (Say, 1831) syntype (NHRJLKB000073656). Note the weak lateral discolored pronotal bands, which are not found in *E. bipunctatus* (Linnaeus, 1758).

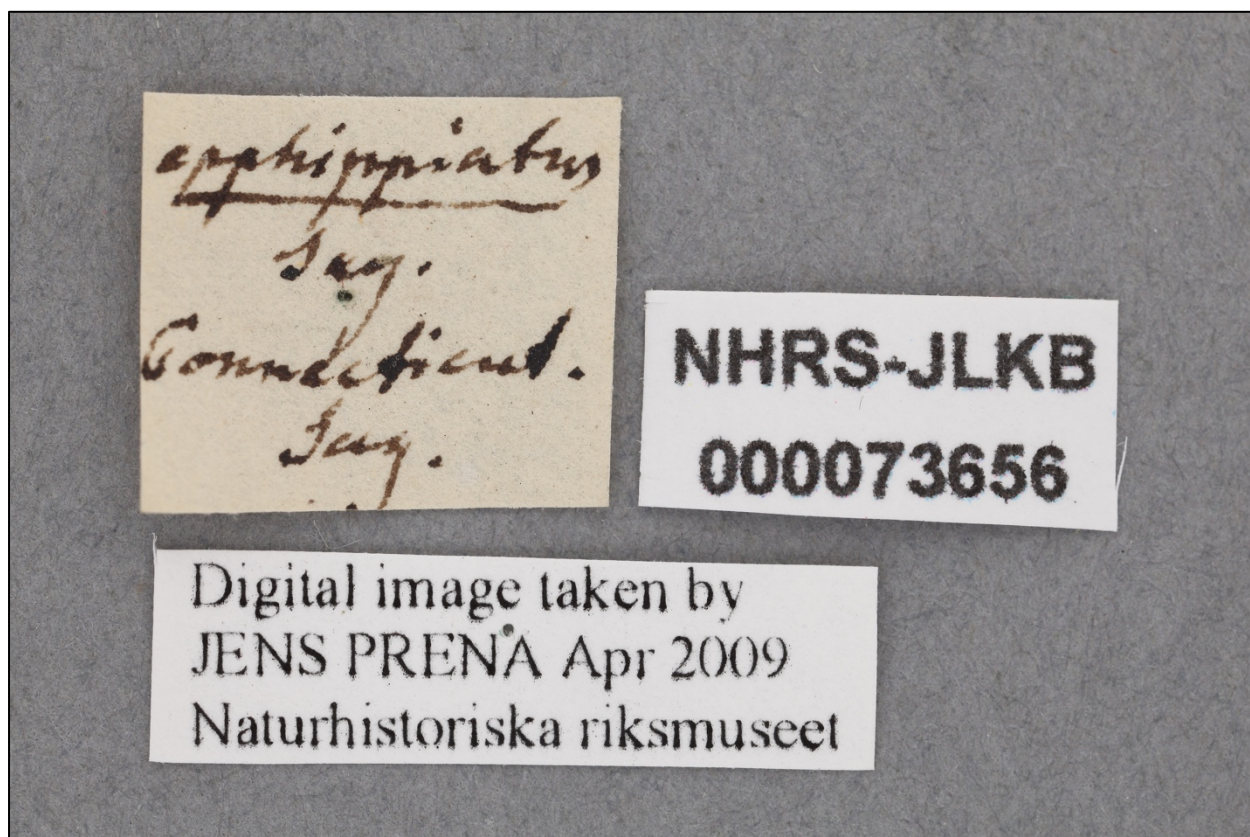


Figure 2.34 *Ellescus ehippiatus* (Say, 1831) syntype labels.



Figure 2.35 North American range map for *Ellescus bipunctatus* (Linnaeus, 1758). Provinces, territories, and states for which the species is recorded are shaded in gray. Maps were generated in SimpleMappr (Shorthouse 2010).

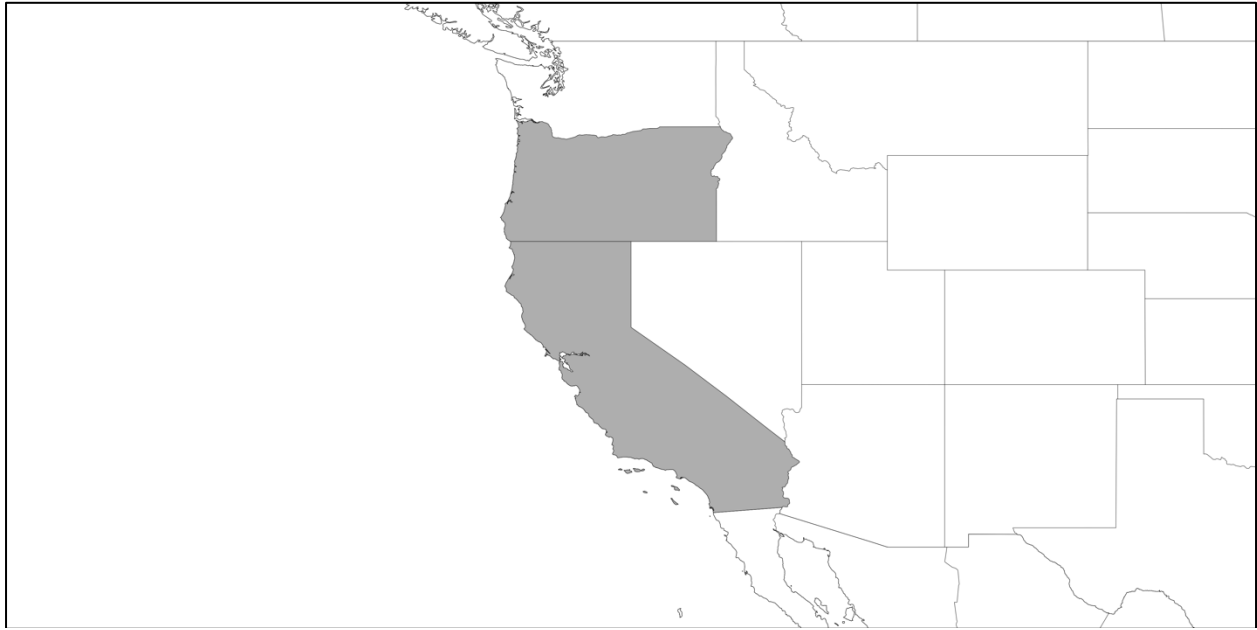


Figure 2.36 North American range map for *Ellescus californicus* (Casey, 1885). Provinces, territories, and states for which the species is recorded are shaded in gray. Maps were generated in SimpleMappr (Shorthouse 2010).

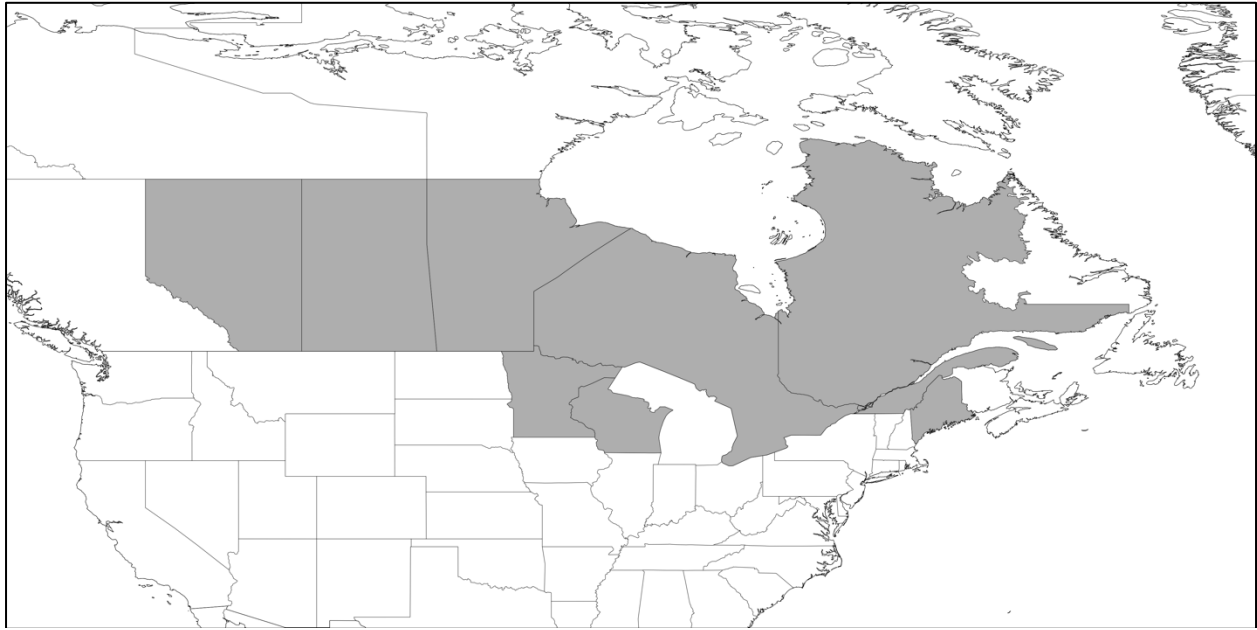


Figure 2.37 North American range map for *Ellescus michaeli* n. sp. Provinces, territories, and states for which the species is recorded are shaded in gray. Maps were generated in SimpleMapppr (Shorthouse 2010).



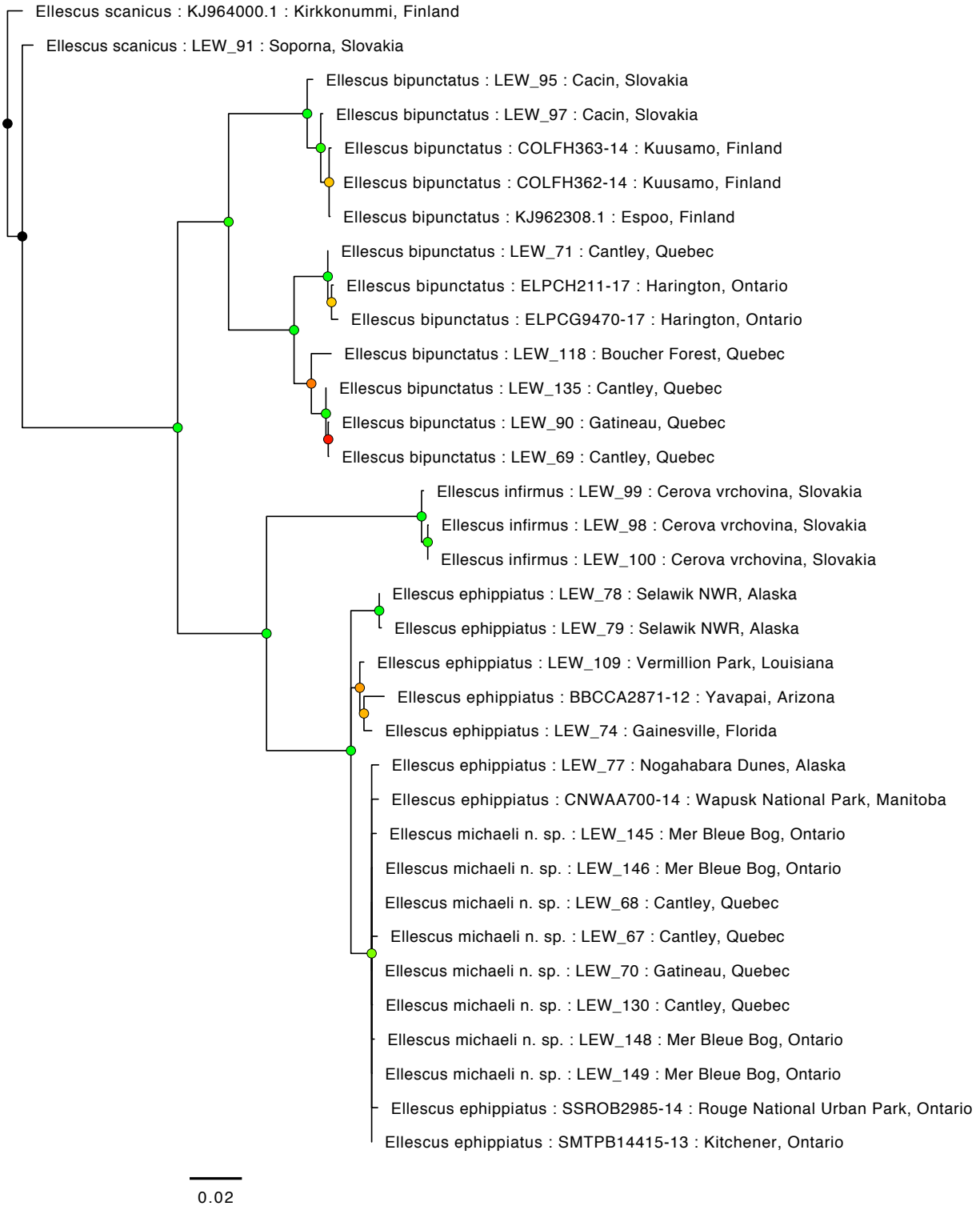


Figure 2.40 Neighbor-joining barcode tree (CO1) for *Ellescus* species under a Jukes-Cantor substitution model (10000 bootstrap replicates, support threshold of 70%). Branch support values are represented by color (green > 90%, red-orange 70-90%). Note that *Ellescus ehippiatus* and *Ellescus michaeli* n. sp. do not separate using CO1, but do when using the faster evolving marker ITS2.

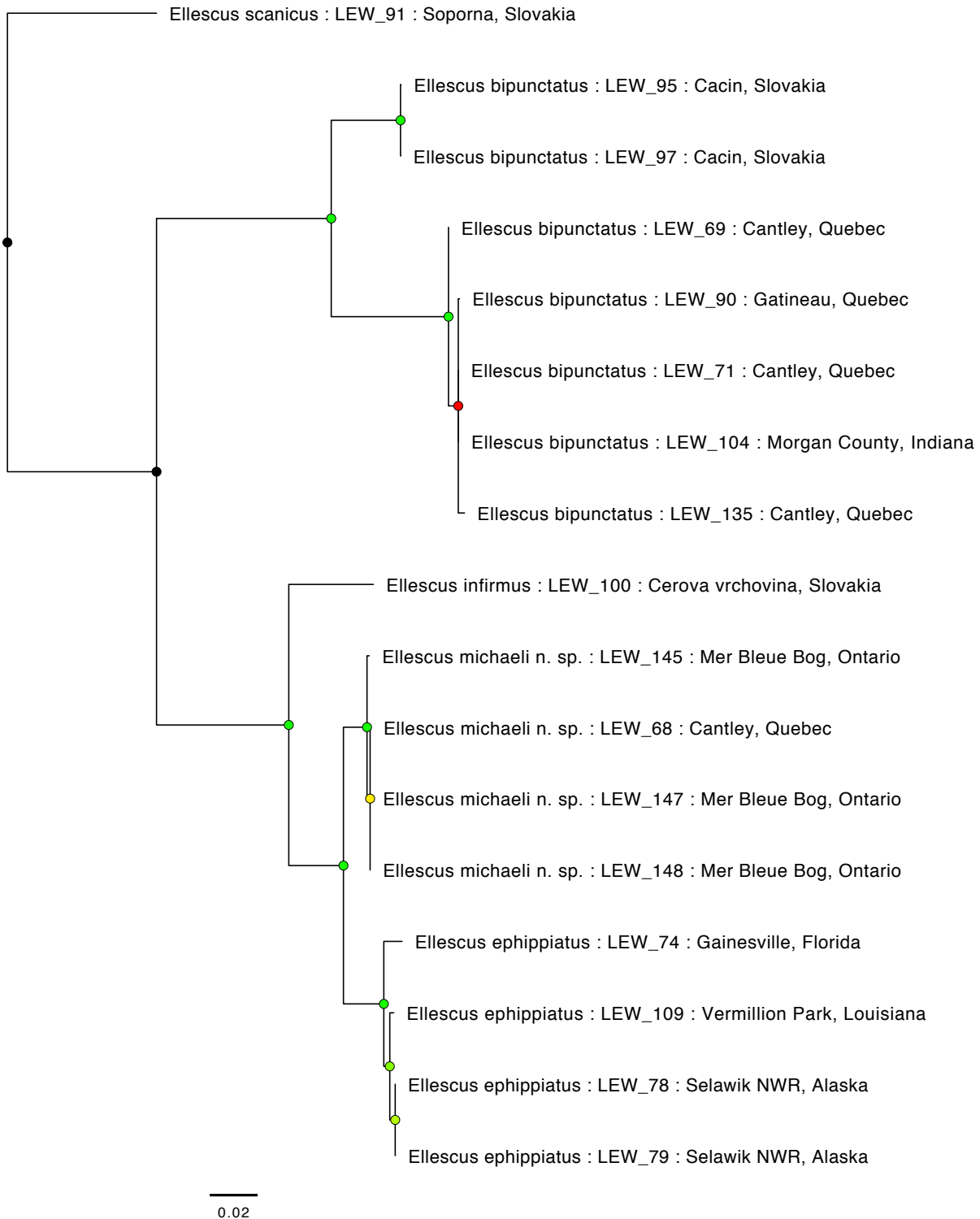


Figure 2.41 Neighbor-joining barcode tree (ITS2) for *Ellescus* species under a Jukes-Cantor substitution model (10000 bootstrap replicates, support threshold of 70%). Branch support values are represented by color (green > 90%, red-yellow 70-90%). Note that *Ellescus ehippiatus* and *Ellescus michaeli* n. sp. do not separate using CO1, but do when using the faster evolving marker ITS2.



Figure 2.42 Lateral photograph of *Adorytomus* sp. (Japan, Aichi Prefecture, Mt. Ouyama, 25 March 1992, H. Kojima; HKC).



Figure 2.43 *Adorytomus* sp., showing the laterally expanded region of the elytra over the anterior quarter of the metepisternum, which is covered in hairs ventrally (shown by white arrow).

Chapter 3: A Multi-locus Molecular Phylogeny of the Core Genera of Ellescini

3.1 Introduction

The tribe Ellescini (Curculionidae: Curculioninae) contains several genera (*Dorytomus*, *Elleus*, *Proctorus* and *Rodotymus*), all of which occur in the northern hemisphere and on members of Salicaceae (Alonso-Zarazaga and Lyal 1999; Anderson 2002; Clark 2010; Caldara 2014). Two genera which were previously included in Alonso-Zarazaga and Lyal (1999) under Ellescini, namely, *Minyrus* Schoenherr and *Ontoctetorus* Faust, were moved to Anthonomini (Clark 2010). Furthermore, the Australian genus *Sellechus* Lea was recently moved to Storeini (Pullen et al. 2014), and the placement of the Indomalaysian genera *Bathrorygma* Marshall and *Minyrophilus* Voss within Ellescini is dubious (Caldara et al. 2014). The east Asian genus *Adorytomus* (several spp.) occurs on members of Theaceae and Ericaceae (Kojima and Truong 2012; pers. comm. Dr. Hiroaki Kojima, Tokyo University of Agriculture), and the remaining four genera (*Dorytomus* Germar, *Elleus*, *Proctorus* LeConte, and *Rodotymus* Zumpt) occur exclusively on Salicaceae (Alonso-Zarazaga and Lyal, 1999; Anderson 2002; Alonso-Zarazaga et al. 2017). The genera *Dorytomus* (100+ species) and *Elleus* (6-7 species) are Holarctic in distribution, *Proctorus* (2 spp.) is endemic to North America, and *Rodotymus* (monotypic) occurs in Kazakhstan and its bordering countries (Alonso-Zarazaga and Lyal, 1999; Anderson 2002). As many of the genera are non-Salicaceae feeding (Marshall 1926; Kojima and Truong 2012), Asian and Australasian in distribution (Alonso-Zarazaga and Lyal, 1999), monotypic or small (Marshall 1926; Kojima and Truong 2012; Pullen et al. 2014) and are largely thought to not belong to Ellescini (Clark 2010; Caldara et al. 2014), this study focuses primarily on the phylogenetic relationships of the four willow-feeding genera (*Elleus*, *Dorytomus*, *Proctorus*, *Rodotymus*), that are hereafter referred to as the “core genera” of the Ellescini.

The tribe Ellescini is usually divided into two tribes, namely Ellescina Thompson, 1859 (generally includes *Ellescus*, *Proctorus*) and Dorytomina Bedel, 1886 (generally includes *Dorytomus*, *Rodotymus*) (Alonso-Zarazaga and Lyal 1999; Anderson 2002; Bousquet et al. 2013; Caldara et al. 2014; Alonso-Zarazaga et al. 2017). The subtribe Dorytomina Bedel, 1886 is treated as a synonym of Ellescini in Legalov (2015), however is treated as valid in Bukejs and Legalov (2019). Legalov (2020a, 2020b) resurrected the tribe Dorytomini Bedel, 1886 (includes *Dorytomus*) and placed it in the subfamily Erirhininae (Curculionidae) based on the presence of anal setae on the 5th ventrite and antennal scrobes oriented towards the eyes. Legalov (2021) also develops a key to the subtribes and genera of Ellescini and places *Ellescus*, *Tsherepanovia* Korotyaev, 1992 and *Pachytychius* Jekel, 1861 in Ellescina. This key does not include *Proctorus*, *Adorytomus*, or any of the other genera placed in Ellescini by Alonso-Zarazaga and Lyal (1999). A recent study which also takes larval morphology, bionomic data, and CO1 DNA sequence data into account does not support the placement of *Pachytychius* by Legalov (2021), but rather places that genus in Smicronychini (Curculionidae: Curculioninae) (Caldara et al. 2018). The placement of *Dorytomus* in another subfamily separate from *Ellescus* (as in Legalov 2020a, 2020b) is not a widely accepted hypothesis (Alonso-Zarazaga and Lyal 1999; Anderson 2002; Bousquet et al. 2013; Caldara et al. 2014; Alonso-Zarazaga et al. 2017). Indeed, the great morphological similarity of some *Dorytomus* to *Ellescus* and the presence of seemingly intermediate forms (e.g. *D. imbecillus* Faust, 1882, see below), shared host use (Salicaceae), the Holarctic distributions of both genera, and molecular data (this research) all suggest a close relationship between these genera. For this reason, the taxonomic proposals of Legalov (2020a, 2020b) are not further discussed. Although *Dorytomus* was previously divided into several subgenera in Alonso-Zarazaga and Lyal (1999), those subgenera were later synonymized by

Colonnelli (2003). Except for *Ellescus* (see Chapter 2), the species within the core genera of Ellescini are generally well known. The species of *Dorytomus* have been revised (regionally) in Japan (Morimoto and Enda 1962), North America (O'Brien 1970), and the Far East (Korotyaev 1976, 1996). The two species in the Nearctic genus *Proctorus* are well known and easily distinguished (Hubbard et al. 1878). The genus *Rodotymus* is monotypic and has been distinguished from *Dorytomus* on the basis of possessing dentate foretibiae.

Historically, the core genera of Ellescini have been separated on the basis of two characters: (1) the possession (or lack of) femoral teeth, or (2) the possession of basal teeth on the tarsal claws. Anderson (2002) separates *Ellescus* from the related genera *Dorytomus* and *Proctorus* by the possession of basal teeth on the tarsal claws and by the lack of femoral teeth (*Dorytomus*: simple tarsal claws, with femoral tooth; *Proctorus*: basal teeth on tarsal claws, with femoral tooth). However, it has been noted that some *Dorytomus* species lack teeth on middle and hind femurs (e.g., *D. imbecillus* Faust, 1882 [= *D. alaskanus* Casey, 1892]) (O'Brien 1970; Korotyaev 1976). Preliminary work for this study found that some specimens of *D. imbecillus* lack teeth on all femurs. Furthermore, some larger specimens of *E. scanicus* do possess minute femoral teeth or tubercles (see previous chapter). Lastly, one of the more prominent features of *Proctorus* males, namely, the possession of sternites with large ventral projections (Figures 3.1, 3.2), is also shared with the males of one Old World species of *Dorytomus* (*D. dorsalis* (Linnaeus, 1758); Figures 3.3, 3.4). Although the function of this unique character is unknown, it may be related to some aspect of sexual behavior as the projections are only possessed by males and the fact that weevils generally mate by males mounting females from behind. The curved nature of the projections suggest they might be suited to “hugging” the abdomen of females, or

in the case of *P. armatus* LeConte, 1876 which possesses an additional medio-basal projection on the last sternite, they might be suited to grasping or securing females. It is suspected that given the uniqueness of this character, *Proctorus* might be more closely related to *Dorytomus* than previously thought. Homology of the ventral projections in *D. dorsalis* and *Proctorus* also appears possible as the relative position, shape, and angle of insertion into the last sternite is similar in both genera (see Figures 3.2, 3.4). Thus, it is apparent that intermediate character states and special cases appear frequently enough within the tribe to cast doubt on the current generic taxonomy and warrant further investigation. In particular, do *Ellescus* and *Dorytomus* form monophyletic groups? Additionally, what is the phylogenetic placement of genera *Proctorus* and *Rodotymus*? Here, I use both morphology and molecular techniques to investigate these questions. Based on these findings, an updated key to the core genera of Ellescini is also produced.

3.2 Methods

3.2.1 Specimen acquisition and taxon sampling

Specimens were borrowed from museum and university insect collections in North America, Europe, and Japan. Institution names and associated acronyms used in this work are presented below:

AFCF : Atlantic Forestry Centre, Canadian Forest Service, Natural Resources Canada, Fredericton, New Brunswick, Canada

ASUIC : Arizona State University Insect Collection, Tempe, Arizona, U.S.A. (includes specimens from the Charles W. O'Brien collection)

CBG : Center for Biodiversity Genomics, Guelph, Ontario, Canada

CMNC : Canadian Museum of Nature, Ottawa, Ontario, Canada

CNCI : Canadian National Collection of Insects, Ottawa, Ontario, Canada

CUAC : Clemson University Arthropod Collection, Clemson, South Carolina, U.S.A.
DEBU : University of Guelph Insect Collection, Guelph, Ontario, Canada
HKC : Hiroaki Kojima Collection, Tokyo University of Agriculture, Tokyo, Japan
KSC : Kyle Schnepf Collection, Gainesville, Florida, U.S.A.
LSAM : Louisiana State Arthropod Museum, Baton Rouge, Louisiana, U.S.A.
MEM : Mississippi State Entomology Museum, Mississippi, U.S.A.
MIZ : Museum of the Institute of Zoology, Polish Academy of Sciences, Warsaw, Masovia, Poland
NBMIC : New Brunswick Museum Insect Collection, Saint John, New Brunswick, Canada
RBCM : Royal British Columbia Museum, Victoria, British Columbia, Canada
RWC : Reginald Webster Collection, Charters Settlement, New Brunswick, Canada
UAMIC : University of Alaska Museum Insect Collection, Fairbanks, Alaska, U.S.A.
ZMUO : University of Oulu Zoological Museum, Oulu, North Ostrobothnia, Finland
ZSM : Zoologische Staatssammlung München, München, Bavaria, Germany

Specimens were also procured through targeted collecting efforts in Quebec, Ontario, and New Brunswick, Canada, as well as by Dr. Michael Košťál in Slovakia. Collection efforts concentrated on *Salix* and *Populus* and consisted of beating branches and sifting leaf litter at the base of plants from mid-April to mid-July for 2020 and 2021 field seasons. For large trees, a 7-meter extendable net was used to beat branches that otherwise would have been out of reach. Specimens collected in the field were placed in individual vials (i.e., 1 vial per specimen) with >95% ethanol and stored in a freezer at -23°C until DNA extraction. All specimens collected in the field were deposited in the Canadian Museum of Nature Collection (CMNC). In total, DNA sequence data was obtained for five species of *Elleus* (5 / 6-7 valid species), eleven species of *Dorytomus* (11 / ~100 valid species), and both *Proctorus* species (2 / 2 valid species) (Table 3.3) (O'Brien and Wibmer 1982; Bousquet et al. 2013; Caldara et al. 2014; Alonso-Zarazaga et al. 2017). The species *Anthonomus signatus* Say, 1831 (Curculioninae: Anthonomini) and *Tychius*

meliloti Stephens, 1831 (Curculioninae: Tychiini) were chosen as outgroup taxa, as these species belong to tribes which are thought to be closely related to, but not belonging to Ellescini (Caldara 2001; Franz 2006). Associated collection data for all specimens used in the phylogenetic analyses are given in Table 3.4.

3.2.2 Dissection protocol, morphological analysis and imaging

Male specimens were dissected using standard protocols and genitalia were cleared in a water + KOH solution (see Smith 1979). Specimens were viewed under a 50X WILD M5A microscope (WILD Heerbrugg, Heerbrugg, Switzerland). All images were taken using a Leica Z16 APOA camera and LAS images stacking software (Leica Microsystems, Wetzlar, Germany).

3.2.3 DNA extraction protocols

Specimens recently collected from the field and stored in 95% ethanol in a freezer at -23°C produced satisfactory sequence data using methods outlined in Ivanova et al. (2006). This technique requires removing 2-3 legs and crushing them into a powder form using a grinding medium (1 mm diameter silicon carbide beads). Dried museum specimens (pointed), however, did not produce adequate sequence data using the methods of Ivanova et al. (2006). For this reason, the methods of Santos et al. (2018) were used. In this latter method, rather than removing and grinding legs of specimens, entire specimens are placed in tubes with a digestion buffer (194 µl WID buffer (Whole Insect Digestion Buffer) + 6 µl Proteinase K) and incubated for 16 hours at 56°C. This allows for an extraction of all of the DNA contained within a specimen, which is necessary with small, older weevil specimens as single legs do not contain enough high-quality,

amplifiable DNA. Furthermore, the whole insect extraction technique is non-destructive and was also not found to alter the shape of the male genitalia, allowing for morphological study afterwards. All DNA tubes were labeled with unique identifiers (LEW_###) and stored in a fridge at 4°C throughout the course of this study.

3.2.4 Gene and primer selection, polymerase chain reaction (PCR) and sequencing reactions

Several factors were taken into consideration when choosing genes for the Ellescini phylogenetic study. Firstly, markers appropriate for a phylogenetic study at the tribal level were chosen. This is important as markers which evolve too rapidly become saturated (i.e. not phylogenetically informative), and markers that are highly conserved do not contain enough any or enough phylogenetically useful information to reconstruct relationships (Tarasov and Dimitrov 2016). Secondly, despite early criticisms of the use of mtDNA in phylogenetics (e.g., Shaw 2002; Ballard and Whitlock 2003), the current trend is to use a combination of different types of markers (mtDNA, nDNA, ribosomal, etc.) (e.g., Chatzimanolis et al. 2010; Osswald et al. 2013; Grebennikov 2018; Letsch et al. 2019). Here, a combination of protein- coding mitochondrial (mtDNA), mitochondrial ribosomal (mtrRNA), and nuclear ribosomal (nrRNA) genes were chosen. Lastly, the ease of amplification of genes must also be taken into consideration. This is particularly important as 25-30% of the taxa used in this study were only available as dry-mounted museum specimens, many of which contained highly degraded DNA. When working with older specimens it is also often advisable to choose shorter markers that are present in large numbers in each cell as these are easily amplified even when the DNA is highly degraded (Rach et al. 2017). Finally, it should also be mentioned that the motivation for using

multiple markers lies in the fact that single-gene trees are not always congruent with species trees (see Maddison 1997).

The nuclear ribosomal gene 28S (large subunit 28S rRNA) (596-673 bp), mitochondrial ribosomal gene 16S (rrn1) (608-704 bp), and mitochondrial protein-coding genes CO1 (Cytochrome c oxidase subunit I) (713-824 bp) and CytB (Cytochrome B) (426-502 bp) were chosen for use in this study. In addition to being easily amplified and suitable for phylogenetic work at the tribal level, these four genes have also been extensively used in beetle phylogenetics studies (e.g., Chatzimanolis et al. 2010; Osswald et al. 2013; Grebennikov 2018; Letsch et al. 2019; Grebennikov and Anderson 2021). Huang et al. (2020) found that amongst 11 mitochondrial and nuclear markers commonly used in phylogenetics, CO1 was the most phylogenetically informative amongst all 11 genes, and 28S was the most phylogenetically informative amongst the four rRNA genes tested (followed by 16S, 12S, and 18S). Primers and thermal profiles used to amplify these genes are outlined in Tables 3.1 and 3.2. PCR recipes were as follows: Per sample - DNA grade water (9.05µl), Q5 buffer (5X) (3µl) (<https://www.neb.ca/q5>), dNTPs (10mM concentration) (0.3µl), primers (10µM concentration) (0.75µl each) (Table 2.3), Q5 polymerase (2 U/µl) (0.15µl), and template DNA (1µl). In instances where PCR products remained weak despite altering thermocycler programs, the amount of template DNA was doubled, which occasionally produced a satisfactory amount of amplicon suitable for sequencing. For older, mounted specimens the following PCR recipe was used: Per sample - DNA grade water (32.5µl), Q5 buffer (5X) (10µl), dNTPs (10mM concentration) (1µl), primers (10µM concentration) (2.5µl each), Q5 polymerase (2 U/µl) (0.5µl), and template DNA (1µl). PCR products were visualized using agarose gel electrophoresis with

TAE buffer (Tris-acetate-EDTA buffer) and ethidium bromide stain. Strong products were selected for sequencing. Satisfactory PCR products were diluted and sequencing reactions were carried out using the following recipe: Per sample - DNA grade water (6.2µl), ABI buffer (5X) (1.8µl), primer (10µM concentration) (0.5µl), BigDye (0.5µl) (<https://www.thermofisher.com/ca/en/home.html>), and PCR product (1µl). The thermocycler program for sequencing reactions was as follows: Initial (95°C / 3 min.) - [Denaturing (96°C / 30 sec.) - Annealing (50°C / 20 sec.) - Extension (60°C / 4 min.)] (30 cycles). To ensure that unused dye-labelled ddNTPs are removed from the sequencing reactions purification was carried out through an ethanol/EDTA/sodium acetate precipitation process. Double stranded sequencing was performed on an ABI 3500XL sequencer (Applied Biosystems™, ThermoFisher Scientific, Waltham, Massachusetts).

3.2.5 Sequence editing, alignment and phylogenetic analysis

All sequence editing was performed in Geneious Prime ver. 2021.1.1 (<https://www.geneious.com>). Raw sequences were first trimmed using the built in “Trim Ends” function (error probability limit = 0.05; default settings), and visually scanned afterwards. When ambiguous regions still occurred near the ends despite the automatic trimming, sequences were manually trimmed to remove these ambiguities. Low quality sequences (HQ < 50%) were removed from the analysis. As a precautionary step, all sequences were run through BLAST (Altschul et al. 1990) to ensure that non-target DNA had not been amplified. Alignment of CO1 and CytB sequences was straightforward and was performed in MUSCLE ver. 3.8.425 (Edgar 2004) using default settings. As amino-acid sequences are more conserved, the CO1 and CytB alignments were further verified by translating nucleotide sequences into amino-acid sequences

using the Geneious Prime “Translate” function (translation table set to [Invertebrate Mitochondrial]). Taking secondary structure into account when aligning rRNA has been shown to improve alignment accuracy (Kjer 1995; Letsch et al. 2010; Zhang et al. 2015). For this reason, alignment of the rRNA genes 16S and 28S was performed in MAFFT 7 ver. 7.490 (Kato et al. 2019; <http://mafft.cbrc.jp/alignment/server/>) using the Q-INS-i secondary structure algorithm (Kato and Toh 2008), which has been widely used to structurally align beetle and weevil rRNA sequences (Bocak et al. 2016; Cai et al. 2019; Grebennikov and Anderson 2021). Statistics relating to the alignments of each gene are presented in Table 3.5. The Maximum Likelihood (ML) and Bayesian Inference (BI) analyses were performed on the same all-nucleotide 2,372 bp concatenated alignment.

Partition model selection was performed in IQ-TREE ver. 1.6.12 (Nguyen et al. 2015). Furthermore, as CO1 and CytB are protein-coding genes (Zardoya and Meyer 1996) partitions were also considered for each codon position in the alignment. A partition scheme varying by codon position is worth considering when working with protein-coding genes as natural selection acts differently on bases in different codon positions (Koonin and Wolf 2010). Similarly, evolutionary processes differ between nuclear and mitochondrial genes (Nylander et al. 2004). As the rRNA genes 28S and 16S are non-protein coding, no codon partitioning was considered for these genes. An optimal partition model search was run (-m TESTMERGE) with the aim of reducing over-parametrization and improving model fit. The “TESTMERGE” program searches for a partition scheme that minimizes the number of partitions through a process of merging (Chernomor et al. 2016). The optimal partition scheme determined by IQ-TREE was as follows: 16S (GTR+F+I+G4); 28S, CO1 (1st codon position), CytB (1st codon position) (SYM+I+G4);

CO1 (2nd codon position), CytB (2nd codon position) (F81+F+I); CO1 (3rd codon position), CytB (3rd codon position) (HKY+F+G4) (see Minh et al. (2021) for acronym explanations). This optimal partition scheme was used in all subsequent ML and BI tree searches. All trees were stylized in FigTree ver. 1.4.4 (Rambaut 2009).

Maximum Likelihood analysis was performed in IQ-TREE ver.1.6.12 (Nguyen et al. 2015), with a heuristic search of 100000 initial trees (-ninit 100000) (see Appendix B for Bayes Block code). Missing data and gaps were treated as unknown characters (see Minh et al. 2021). Both ultrafast bootstrap (UFboot) values (-bb 10000) and Shimodaira-Hasegawa approximate Likelihood Ratio Test (SH-aLRT) values (-alrt 10000) were included as measures of branch support. Ultrafast bootstrap approximation is less biased than regular bootstrapping and has a different but more natural interpretation than standard bootstrap values (Minh et al. 2013). Generally, clades with UFboot values greater than 95% are considered strongly supported, as opposed to 70-80% which are standard cut-off values for regular bootstraps (Lemoine et al. 2018; Minh et al. 2021). When using UFboot values, it is also recommended to use an SH-aLRT test (Guindon et al. 2010; Minh et al. 2021), which represents an alternative measure of clade support. In an SH-aLRT test, clades with percent values greater than 80% are considered well supported (Minh et al. 2021).

Bayesian analysis was performed in MrBayes ver. 3.2.7a (Huelsenbeck and Ronquist 2001) using the optimal partitioning scheme produced in IQ-TREE. Two tree searches (nchains = 4, starttree = random) were run for 3.5 million generations with sampling every 1000 generations. The convergence of parameter values was verified in Tracer ver.1.7.1 (Rambaut et

al. 2018), by ensuring that all parameters had approximately normal distributions, PSRF (potential scale reduction factor) greater than or equal to one, and ESS (effective sample size) greater than 1000 (Nascimento et al. 2017; Rambaut et al. 2018). Convergence of runs was also verified by ensuring that average standard deviation of split frequencies (ASDSF) had stabilized below 0.01 (see Ronquist et al. 2020). A 50% majority rule consensus tree was then generated with the first 25% of trees discarded as burn-in.

3.3 Results

The ML and BI trees (Figures 3.5, 3.6) were topologically similar, and showed moderate to high support (SH-aLRT: 94 / UFBoot: 83 / posterior probability : 1) for *Ellescus* as a clade sister to all other ingroup taxa. Within the genus *Ellescus*, moderate to high support (SH-aLRT: 93 / UFBoot: 86 / posterior probability : 1) was found for the existence of two main clades, one containing *E. scanicus* and *E. bipunctatus*, and the other containing *E. infirmus*, *Ellescus michaeli* n. sp., and *E. ephippiatus*. Although support for the precise placement of *Proctorus* was low, moderate to high support (SH-aLRT: 94 / UFBoot: 83 / posterior probability : 1) was found in both ML and BI trees for the genus *Proctorus* as a nested lineage within the genus *Dorytomus*. The genus *Proctorus* **new synonym** is thus synonymized under *Dorytomus*.

Rodotymus dentimanus was differentiated from species of *Dorytomus* on the basis of possessing dentate foretibiae (Zumpt 1932). Based on a comparison of specimens of *R. dentimanus* and similar long-legged species of *Dorytomus* from North America and Europe (*D. longimanus* (Forster, 1771), *D. mucidus* (Say, 1831), *D. inaequalis* Casey, 1892, and *D. brevisetosus* Casey, 1892), it was found that specimens of *D. inaequalis* also has dentate foretibiae. This finding

coupled with similar geographic distribution and general similarity to other members of the long-legged *Dorytomus* clade suggest that *Rodotymus* actually belongs to *Dorytomus*. As such, *Rodotymus* **new synonym** is synonymized under *Dorytomus*.

Updated key to the core genera of Ellescini

1a) At least fore-femora with well-developed tooth [sometimes lacking (e.g. *Dorytomus imbecillus* Faust, 1882)]. Tarsal claws simple or with basal tooth; if with basal tooth, then also possessing distinct, elongate tooth on all femora.....*Dorytomus* Germar, 1817

2a) All femora lacking prominent teeth [some specimens of *Ellescus scanicus* (Paykull, 1792) may have extremely minute teeth]. Tarsal claws with basal tooth.....*Ellescus* Dejean, 1821

3.4 Discussion

Although predictions of the monophyly of *Ellescus* were hindered by the lack of informative morphology, it was previously suspected that *Proctorus* could belong to *Dorytomus* as male *Proctorus* (Figures 3.1, 3.2) and one species of *Dorytomus* (*D. dorsalis*; Figures 3.3, 3.4) possess distinct ventral projections on the 5th sternite. This study provides the first strong molecular evidence of the monophyly of the genus *Ellescus*, and the first to find high support for the hypothesis that *Proctorus* is a lineage nested within *Dorytomus*. Given the strong molecular support for the placement of *Proctorus*, and the possible homology of projections on the 5th sternite in *Proctorus* and *Dorytomus dorsalis*, the genus *Proctorus* is determined here to be a junior synonym of *Dorytomus*. Based on the possession of basal teeth on the tarsal claws, *Proctorus* was previously placed in the subtribe Ellescina with *Ellescus* (Alonso-Zarazaga and Lyal 1999; Anderson 2002; Bousquet et al. 2013; Caldara et al. 2014; Alonso-Zarazaga et al. 2017). Although support for the exact phylogenetic placement of *Proctorus* within *Dorytomus* is not high, the more derived position of *Proctorus* within *Dorytomus* suggests that toothed tarsal

claws evolved in the former lineage, as all other members of *Dorytomus* lack toothed tarsal claws. The phylogenetic relationships of members of *Ellescus* were also highly supported and align with morphological evidence presented in the previous chapter. In particular, the clade containing *E. scanicus* and *E. bipunctatus*) is defined by the possession of a carina on the 5th sternite (only males; reduced in *E. scanicus*) (Figure 2.2), a lack of prominent, protruding basal v-shaped structures in the aedeagus (Figure 2.4a, b), and by the apex of the aedeagus, which is not pointed (Figure 2.4a, b). The second clade, which contains *E. infirmus*, *Ellescus michaeli* n. sp., and *E. ephippiatus* is defined by the possession of a tubercle on the apex of the 5th ventrite (only males) (Figure 2.3), an aedeagus with prominent, protruding basal v-shaped structures (Figure 2.4c-e), and by the pointed apex of the aedeagi (Figure 2.4c-e). Based on these defining morphological characters, the west-coast endemic *E. californicus* also clearly falls into this latter clade.

This research can be improved in several ways. Firstly, it is suggested that taxon sampling be improved to include more species of *Dorytomus*, as only ~11% (11/~100) of species were included here. In particular, it would be worth including sequence data for *Dorytomus dorsalis*, which would help establish the homology of the projections of the 5th sternite also found in *Proctorus*. It would also be worth including members of the “long-legged” *Dorytomus* (e.g., *D. longimanus* Forster, 1771), as these appear to represent a distinct clade within the genus, and whose phylogenetic relationships to other members of *Dorytomus* are unknown. Additionally, the East Asian genus *Adorytomus* is currently placed within Ellescini, but this may not be correct as members of this genus do not use members of Salicaceae as hosts and are morphologically quite divergent from the core genera. Additionally, it is suggested that gene sampling be

increased to improve the robustness of phylogenetic analyses. Preliminary work for this study explored the possibility of using the protein-coding nuclear gene ArgK (Arginine Kinase); however, this gene was found to be too conserved for use in this research. If fresher material is available, the protein-coding nuclear gene CAD might be worth investigating, as this gene resolves deeper as well as more recent phylogenetic relationships (Wild and Maddison 2008). Of course, the advent of next-generation sequencing (NGS) and phylogenomic methods (e.g. McKenna et al. 2009; Shin et al. 2017) has revolutionized the field of systematics in recent years. Such a phylogenomic study of the tribe with extensive taxon sampling would be especially useful for resolving phylogenetic relationships among species within the large genus *Dorytomus*, and would also allow for the development of an accurate system of subgeneric classification.

Table 3.1

List of primers used in the phylogenetic study of Ellescini (Coleoptera: Curculionidae). Forward primers are listed first, followed by reverse primers.

Gene & primers	Primer sequence (5' - 3')	Reference
CO1 LCO1490 / HCO2198	GGTCAACAAATCATAAAGATATTGG / TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. (1994), Pentinsaari et al. (2014)
CytB SytB_F / SytB_R	TGAGGNCAAATATCHTTYTGAGG / GCAAATARRAARTATCATTCDDGG	Gillet (2014), Timmermans et al. (2010)
16S (rrnI) 16S-ar-JJ / 16S1472-JJ-al	CRCCTGTTTATTAAAAACAT / GGTCCTTTCGTACTAA	Astrin et al. (2012)
28S D2B / D3Ar	GTCGGGTTGCTTGAGAGTGC / TCCGTGTTTCAAGACGGGTC	Grebennikov (2018)

Table 3.2

List of PCR thermal cycling conditions and DNA polymerase used for each gene used in the phylogenetic study of Ellescini (Coleoptera: Curculionidae).

Gene	Thermal profile	DNA Polymerase	Reference (if applicable)
COI	Initial (98°C / 30 sec.) - [Denaturing (94°C / 35 sec.) - Annealing (70°C / 35 sec.) (-2°C per cycle) - Extension (72°C / 60 sec.)] (15 cycles) – [Denaturing (94°C / 35 sec.) - Annealing (55°C / 35 sec.) - Extension (72°C / 60 sec.)] (25 cycles) - Final (72°C / 5 minutes)	Q5 (https://www.neb.ca/q5)	Astrin (2012)
CytB	Initial (98°C / 30 sec.) - [Denaturing (94°C / 35 sec.) - Annealing (70°C / 35 sec.) (-1°C per cycle) - Extension (72°C / 60 sec.)] (15 cycles) – [Denaturing (94°C / 35 sec.) - Annealing (55°C / 35 sec.) - Extension (72°C / 60 sec.)] (25 cycles) - Final (72°C / 5 min.)	Q5	Astrin (2012)
16S (rrnI)	Initial (98°C / 30 sec.) - [Denaturing (94°C / 35 sec.) - Annealing (55°C / 35 sec.) (-1°C per cycle) - Extension (72°C / 60 sec.)] (15 cycles) – [Denaturing (94°C / 35 sec.) - Annealing (40°C / 35 sec.) - Extension (72°C / 60 sec.)] (25 cycles) - Final (72°C / 5 min.)	DreamTaq (https://www.thermofisher.com/ca/en/home.html)	Astrin (2012)
28S	Initial (98°C / 30 sec.) - [Denaturing (98°C / 10 sec.) - Annealing (56°C / 30 sec.) - Extension (72°C / 30 sec.)] (34 cycles) - Final (72°C / 5 min.)	DreamTaq	

Table 3.3

A list of taxa and genes used in the phylogenetic analyses of Ellescini (Coleoptera: Curculionidae). The DNA extraction number is given for each gene (format: LEW_AAA). In some cases it was not possible to obtain all sequence data from one specimen; in those cases sequence data from another specimen of the same species was used. When a taxon lacks sequence data this is indicated by an “X” under the associated gene.

Species	CO1	CytB	28s	16s
<i>Anthonomus signatus</i> Say, 1831	LEW_21	LEW_21	LEW_21	X
<i>Tychius meliloti</i> Stephens, 1831	LEW_22	LEW_22	LEW_20	LEW_20
<i>Ellescus scanicus</i> (Paykull, 1792)	LEW_91	LEW_91	LEW_91	LEW_91
<i>Ellescus bipunctatus</i> (Linnaeus, 1758) (Europe)	LEW_97	LEW_95	LEW_97	LEW_95
<i>Ellescus bipunctatus</i> (Linnaeus, 1758) (North America)	LEW_135	LEW_135	LEW_135	LEW_135
<i>Ellescus ephippiatus</i> (Say, 1831)	LEW_78	LEW_78	LEW_78	LEW_78
<i>Ellescus michaeli</i> n. sp.	LEW_145	LEW_147	LEW_72	LEW_147
<i>Ellescus infirmus</i> (Herbst, 1795)	LEW_99	LEW_124	LEW_124	LEW_124
<i>Proctorus armatus</i> LeConte, 1876	LEW_155	LEW_152	LEW_155	X
<i>Proctorus decipiens</i> (LeConte, 1876)	LEW_142	LEW_142	LEW_123	LEW_123
<i>Dorytomus dejeani</i> Faust, 1882	LEW_101	LEW_102	LEW_102	LEW_101
<i>Dorytomus puberulus</i> (Boheman, 1843)	LEW_93	LEW_93	LEW_93	LEW_94
<i>Dorytomus leucophyllus</i> (Motschulsky, 1845)	LEW_75	LEW_75	LEW_75	LEW_75
<i>Dorytomus tremulae</i> (Fabricius, 1787)	LEW_120	LEW_143	LEW_120	LEW_143
<i>Dorytomus rubrirostris</i> (Gravenhorst, 1807)	LEW_144	LEW_144	LEW_144	LEW_144
<i>Dorytomus marmoreus</i> Casey, 1892	LEW_108	LEW_108	LEW_108	X
<i>Dorytomus parvicollis</i> Casey, 1892	LEW_139	LEW_139	LEW_139	LEW_139
<i>Dorytomus hirtus</i> LeConte, 1876	LEW_153	LEW_153	LEW_153	LEW_153
<i>Dorytomus vagenotatus</i> Casey, 1892	LEW_154	LEW_154	LEW_154	LEW_154
<i>Dorytomus laticollis</i> LeConte, 1876	LEW_158	LEW_158	LEW_158	LEW_158
<i>Dorytomus imbecillus</i> Faust, 1882	LEW_161	LEW_161	LEW_161	LEW_161

Table 3.4

Associated collection data for specimens used in the phylogenetic analyses of Ellescini (Coleoptera: Curculionidae).

Anthonomus signatus : **LEW_21** : CANADA, Quebec, Cantley, 45.599159°N, 75.798685°W, 4 September 2020, J.H. Lewis, sweeping *Rubus* sp., CMNC.

Tychius meliloti : **LEW_20** / **LEW_22** : CANADA, Quebec, Gatineau, 45.426794°N, 75.824132°W, 3 September 2020, J.H. Lewis, sweeping clover, CMNC.

Ellescus scanicus : **LEW_91** : SLOVAKIA, Podunajská nížina, Šoporňa, 48.240000°N (original label 48°14.4'), 17.811667°E (original label 17°48.7'), 5 January 2021, Michael Košťál, sift under *Populus alba*, CMNC.

Ellescus bipunctatus : **LEW_95** / **LEW_97** : SLOVAKIA, Čačín, Bystrická vrchovina hills, 48.671667°N (original label 48°40.3'N), 19.256667°E (original label 19°15.4'E), 6 July 2020, Michael Košťál, CMNC.

Ellescus bipunctatus : **LEW_135** : CANADA, Quebec, Cantley, 45.59468°N, 75.794389°W, 24 April 2021, J.H. Lewis, beaten off *Salix* sp., CMNC.

Ellescus ephippiatus : **LEW_78** : U.S.A., Alaska, Selawik NWR, 66.85873°N, 158.16618°W, 24 June 2010, D.S. Sikes, open sand dunes, sweep, UAMIC, UAM100283481.

Ellescus michaeli n. sp.: **LEW_72** : CANADA, Quebec, Cantley, 45.591828°N, 75.784871°W, 20 May 2020, J.H. Lewis, beaten off *Salix* sp., CMNC.

Ellescus michaeli n. sp.: **LEW_145** / **LEW_147** : CANADA, Ontario, Mer Bleue Bog, 45.3973°N, 75.5144°W, 13 May 2021, R. Anderson, beaten off *Salix* sp., CMNC.

Ellescus infirmus : **LEW_99** / **LEW_124** : SLOVAKIA, Cerová vrchovina, Jesnske – Barta, 48.296667°N (original label 48°17.8'), 20.070000°E (original label 20°04.2'), 9 October 2020, Michael Košťál, sift under *Salix alba*, CMNC.

Proctorus armatus : **LEW_152** : CANADA, Ontario, Constance Bay, 17 May 2003, Henry & Anne Howden, CMNC.

Proctorus armatus : **LEW_155** : CANADA, New Brunswick, York Co., Douglas - Currie Mountain, 45.9844°N, 66.7592°W, 3-15 May 2013, C. Alderson and V. Webster, mixed forest with *Quercus rubra*, Lindgren funnel trap in canopy of *Quercus rubra*, NBM, NBM-070121.

Proctorus decipiens : **LEW_123** : CANADA, Quebec, (Mont Cascades) Gatineau, 45.590941°N, 75.850435°W, 13 May 2021, J.H. Lewis, beaten off *Salix* sp., CMNC.

Proctorus decipiens : **LEW_142** : CANADA, Quebec, (Mont Cascades) Gatineau, 45.590941°N, 75.850435°W, 12 May 2021, J.H. Lewis, beaten off *Salix* sp., CMNC.

Dorytomus dejeani : **LEW_101 / LEW_102** : SLOVAKIA, Podunajská nížina, 48°14.4'N, 17°48.7'E, 5.i.2021, Michael Košťál, CMNC.

Dorytomus puberulus : **LEW_93 / LEW_94** : SLOVAKIA, Podunajská nížina, 48°14.4'N, 17°48.7'E, 5.i.2021, Michael Košťál, CMNC.

Dorytomus leucophyllus : **LEW_75** : U.S.A., Alaska, Serpentine H.S., 65.85183°N, 164.69884°W, 10 July 2016, D.S. Sikes, *Salix*, moss leaf litter, Berlese, UAMIC, UAM100416529.

Dorytomus tremulae : **LEW_120 / LEW_143** : SLOVAKIA, Podunajská nížina, 48°14.4'N, 17°48.7'E, 5.i.2021, Michael Košťál, CMNC.

Dorytomus rubrirostris : **LEW_144** : SLOVAKIA, Podunajská nížina, 48°14.4'N, 17°48.7'E, 5.i.2021, Michael Košťál, CMNC.

Dorytomus marmoreus : **LEW_108** : CANADA, New Brunswick, Queens Co., C.F.B. Gagetown, 45.7516°N, 66.1866°W, 15-31 July 2013, C. Alderson and V. Webster, Old mixed forest with *Quercus rubra*, Lindgren funnel trap in canopy of *Populus grandifolia*, CMNC.

Dorytomus parvicollis : **LEW_139** : CANADA, New Brunswick, Madawaska Co., Edmundston (behind Tim Hortons and Jessome's La Source; exit 16), 47.3891°N, 68.343053°W, 5 July 2021, J.H. Lewis, beaten from *Populus* sp., CMNC.

Dorytomus hirtus : **LEW_153** : CANADA, New Brunswick, Restigouche Co., Jacquet River Gorge PNA, 47.8257°N, 66.0764°W, 15-29 May 2014, C. Alderson and V. Webster, Old *Populus balsamifera* stand near river; Lindgren funnel trap in canopy of *P. balsamifera*, CMNC.

Dorytomus vagenotatus : **LEW_154** : CANADA, New Brunswick, York Co., Currie Mountain, 45.9832°N, 66.7564°W, 24 June - 9 July 2013, C. Alderson and V. Webster, Old *Pinus strobus* stand; Lindgren funnel trap in canopy of *P. strobus*, CMNC.

Dorytomus laticollis : **LEW_158** : U.S.A., Alaska, White Mtns Rec. Area, 65.33469°N, 146.83997°W, 17 June - 1 July 2016, J. Hagelin, 10m b. and w. spruce, willow, hanging malaise, UAMIC, UAM100412661.

Dorytomus imbecillus : **LEW_161** : U.S.A., Alaska, Anaktuvuk Pass, 68.14957°N, 151.7746°W, 21 May 2016, D. Sikes & K. Daly, 697m el., 100m *Salix*, *Betula*, *Alnus*, grasses, sweeping, UAMIC, UAM100387574.

Table 3.5

Statistics for genes used in the phylogenetic analysis of Ellescini (Coleoptera: Curculionidae). Note that pairwise identity (%) represents the average percent identity across the entire alignment.

	CO1	CytB	16S	28S
Individuals (n)	21	21	18	21
Aligned length	705	401	604	662
Pairwise identity (%)	78.6	78.8	92.6	86.9
Identical sites	288	144	399	345



Figure 3.1 Lateral photograph of *Proctorus armatus* (LeConte, 1876). Note the ventral projections on the 5th sternite.



Figure 3.2 Oblique-angle photograph of the last sternite of *Proctorus armatus* (LeConte, 1876), illustrating the ventral projections.



Figure 3.3 Lateral photograph of *Dorytomus dorsalis* (Linnaeus, 1758). Note the ventral projections on the 5th sternite.

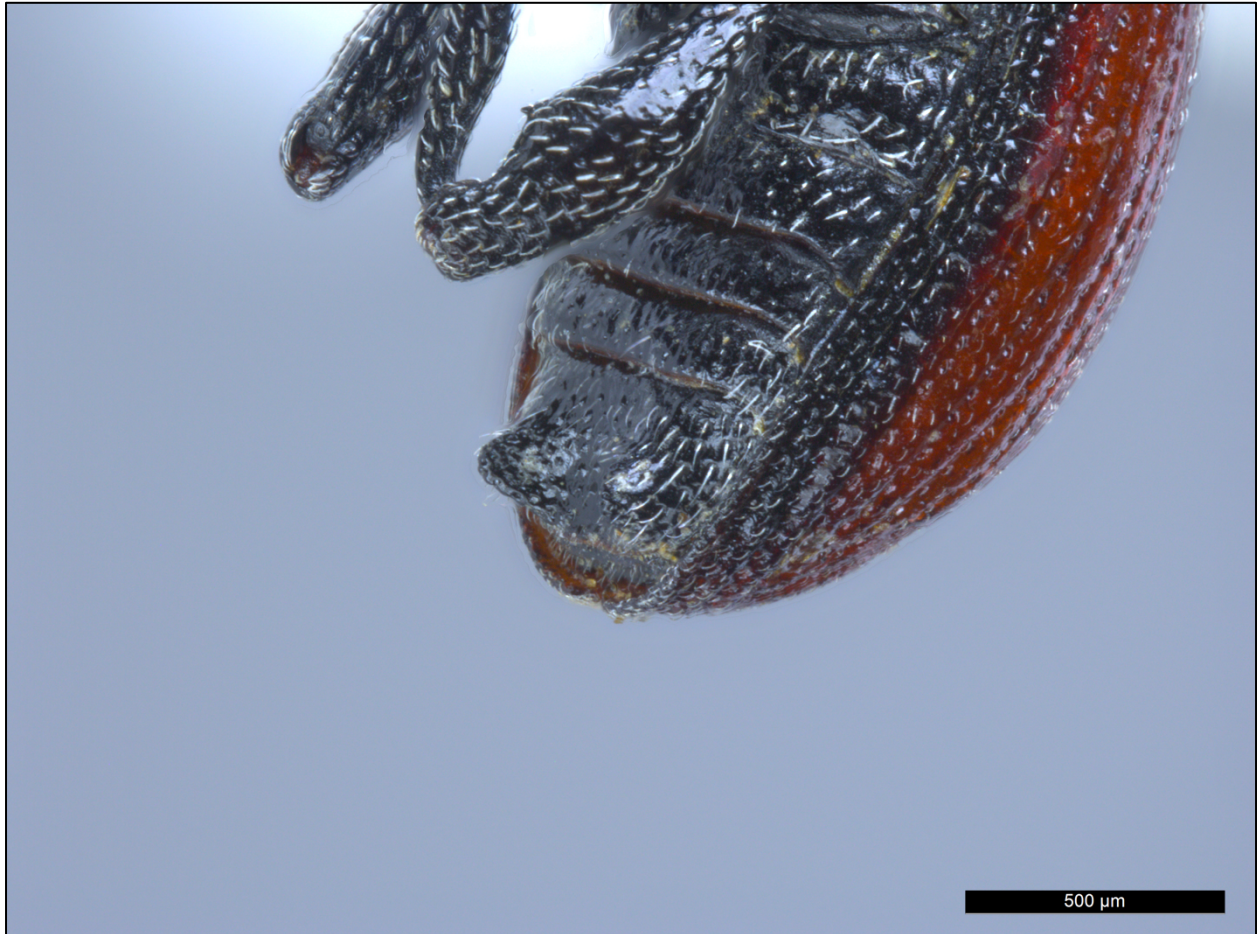


Figure 3.4 Oblique-angle photograph of the last sternite of *Dorytomus dorsalis* (Linnaeus, 1758), illustrating the ventral projections.

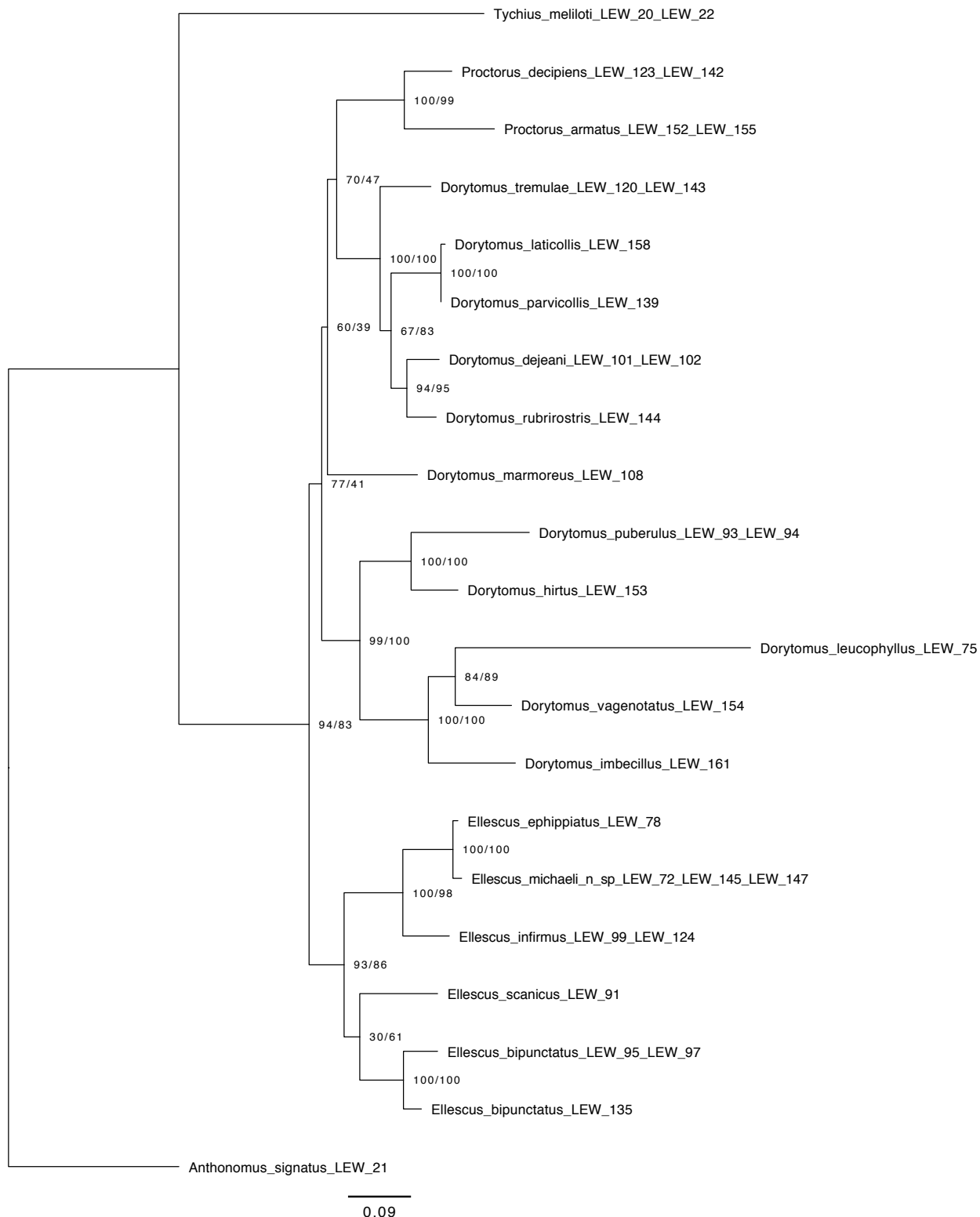


Figure 3.5 Maximum Likelihood tree of the core genera of Ellescini (Coleoptera: Curculionidae: Curculioninae) using CO1, CytB, 16S, and 28S sequence data with gene and codon partitioning (codon partitioning only in CO1/CytB). Branch support values at the nodes represent SH-aLRT values and ultrafast bootstrap values, respectively.

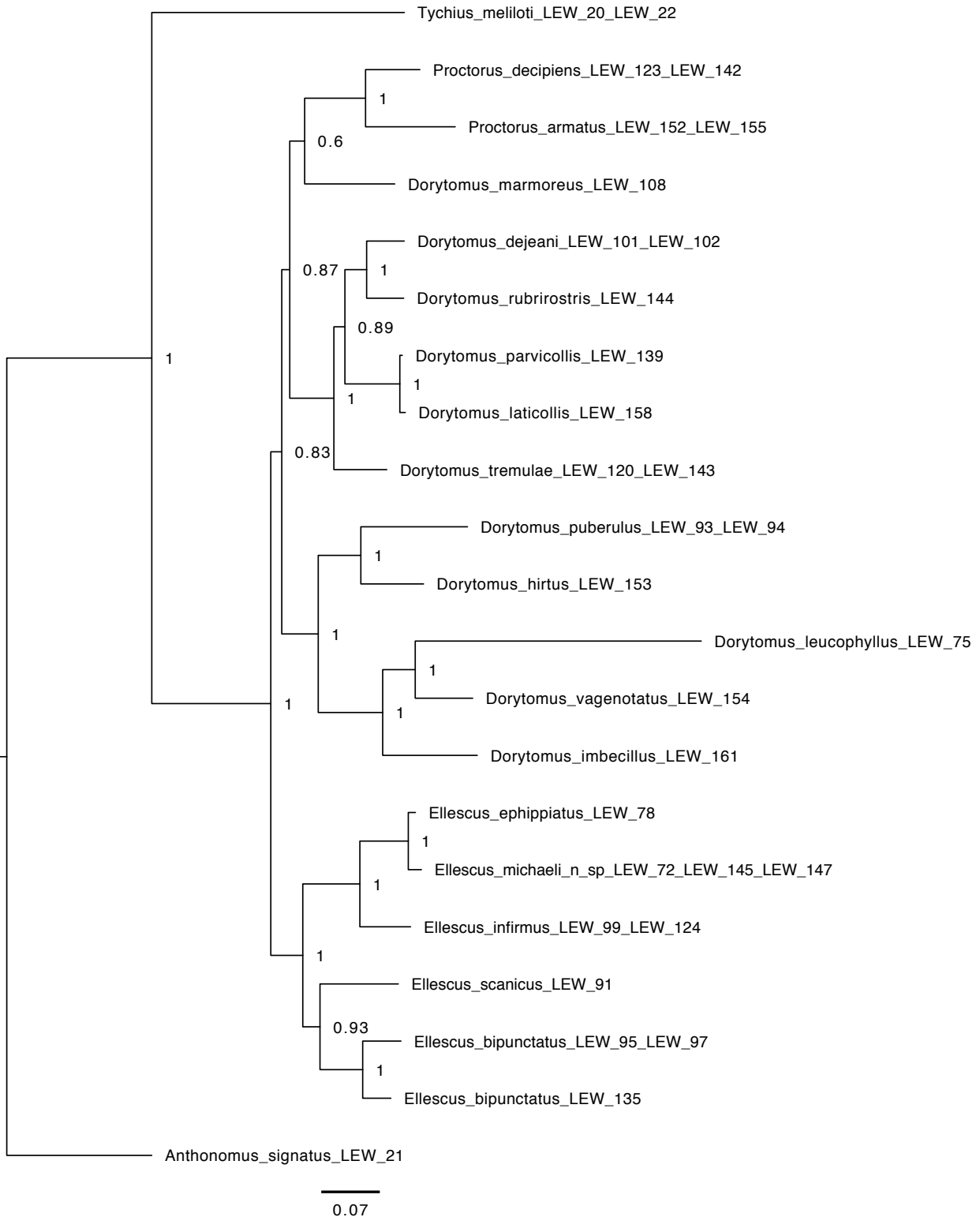


Figure 3.6 Majority rule consensus tree (Bayesian) of the core genera of Ellescini (Coleoptera: Curculionidae: Curculioninae) using CO1, CytB, 16S, and 28S sequence data with gene and codon partitioning (codon partitioning only in CO1/CytB). Branch support values at the nodes represent posterior probabilities.

References

- Abeille, de Perrin E. 1910. Diagnose provisoire d'un *Mecinus* nouveau. Bulletin de la Société Entomologique de France 1910: 83.
- Alonso-Zarazaga, M.A., and Lyal, C.H.C. 1999. A world catalogue of families and genera of Curculionoidea (Insecta: Coleoptera) (excepting Scolytidae and Platypodidae). Entomopraxis, S.C.P., Barcelona, Spain. pp. 315.
- Alonso-Zarazaga, M.A., Barrios, H., Borovec, R., Bouchard, P., Caldara, R., Colonnelli, E., Gültekin, L., Hlavá, P., Korotyaev, B., Lyal, C.H.C., Machado, A., Meregalli, M., Pierotti, H., Ren, L., Sánchez-Ruiz, M., Sforzi, A., Silfverberg, H., Skuhrovec, J., Trýzna, M., Velázquez de Castro, A.J., and Yunakov, N.N. 2017. Cooperative catalogue of Palearctic Coleoptera Curculionoidea. Monografías electrónicas SEA 8. Sociedad Entomológica Aragonesa S.E.A, Zaragoza, Spain. pgs. 729.
- Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. 1990. Basic local alignment search tool. Journal of Molecular Biology 215: 403-410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- Anderson, R.S. 1995. An evolutionary perspective on diversity in Curculionoidea. Memoirs of the Entomological Society of Washington 14: 103-114.
- Anderson, R.S. 2002. *Family 131 - Curculionidae* in Arnett, R.H., Thomas, M.C., Skelley, P.E., and Frank, J.H. (eds.) - American Beetles - Volume 2 - Polyphaga: Scarabaeoidea through Curculionoidea. CRC Press, Florida, U.S.A. pgs. 722-815.

- Anderson, R.S., Keller, O., and Prena, J. 2013. *Curculio rubidus* Gyllenhal, 1836 (Coleoptera: Curculionidae: Curculioninae), a European Weevil New to North America. The Coleopterists Bulletin 67: 368-369. <https://doi.org/10.1649/0010-065X-67.3.368>
- Anderson, R.S. 2016. A taxonomic revision of the genus *Lymantes* Schönherr, 1838 (Coleoptera: Curculionidae: Molytinae: Lymantini) in the United States of America. The Coleopterists Bulletin 70: 111-124. <https://doi.org/10.1649/072.070.0115>
- Anderson, R.S. 2018. *Purealus beckelorum*, a new genus and species of cleonine weevil from western Texas and eastern New Mexico (Coleoptera, Curculionidae, Lixinae, Cleonini). Zookeys 785: 1-10. <https://doi.org/10.3897/zookeys.785.26674>
- Andreev, D., Breilid, H., Kirkendall, L., Brun, L.O., and French-Constant, R.H. 1998. Lack of nucleotide variability in a beetle pest with extreme inbreeding. Insect Molecular Biology 7: 197-200. <https://doi.org/10.1046/j.1365-2583.1998.72064.x>
- Astrin, J.J., Stuben, P.E., Misof, B., Wagele, J.W., Gimnich, F., Raupach, M.J., and Ahrens, D. 2012. Exploring diversity in cryptorhynchine weevils (Coleoptera) using distance-, character- and tree-based species delineation. Molecular Phylogenetics and Evolution 63: 1-14. <https://doi.org/10.1016/j.ympev.2011.11.018>
- Austin, E.P. 1880. Supplement to the checklist of the Coleoptera of America, north of Mexico. S.E. Cassino, Boston. pp. 67.
- Ballard, J.W.O., and Whitlock, M.C. 2003. The incomplete natural history of mitochondria. Molecular Ecology 13: 729-744. <https://doi.org/10.1046/j.1365-294X.2003.02063.x>
- Bentz, B. 2008. *Mountain pine beetle, Dendroctonus ponderosae* (Coleoptera: Curculionidae, Scolytinae) in Capinera, John L. (ed.) - Encyclopedia of Entomology - 2nd Edition. Springer Science Business Media B.V., Heidelberg, Germany. pgs. 2494-2497.

- Blatchley, W.S., and Leng, C.W. 1916. Rhynchophora or weevils of Northeastern America. The Nature Publishing Company, Indiana, U.S.A. pp. 682.
- Bocak, L., Kundrata, R., Fernandez, C.A., and Vogler, A.P. 2016. The discovery of Iberobaeniidae (Coleoptera: Elateroidea): a new family of beetles from Spain, with immatures detected by environmental DNA sequencing. *Proceedings of the Royal Society B* 283: 20152350. <https://doi.org/10.1098/rspb.2015.2350>
- Bousquet, Y., Bouchard, P., Davies, A.E., and Sikes, D.S. 2013. Checklist of beetles (Coleoptera) of Canada and Alaska - 2nd edition. Pensoft *Series Faunistica* No. 109, Sofia, Bulgaria. pp. 402.
- Bright, D.E. 1976. The Insect and Arachnids of Canada Part 2 - The bark beetles of Canada and Alaska - Coleoptera: Scolytidae. Research Branch Agriculture Canada, Ottawa, Ontario. pp. 241.
- Bright, D.E. 1993. The Insect and Arachnids of Canada Part 21 - The weevils of Canada and Alaska - Volume 1 - Coleoptera: Curculionoidea, excluding Scolytidae and Curculionidae. Research Branch Agriculture Canada, Ottawa, Ontario. pp. 217.
- Bright, D.E., and Bouchard, P. 2008. The Insect and Arachnids of Canada Part 25 - The weevils of Canada and Alaska - Coleoptera: Curculionidae: Entiminae. Research Branch Agriculture Canada, Ottawa, Ontario. pp. 327.
- Bukejs, A., and Legalov, A.A. 2019. A new species of the genus *Dorytomus* Germar, 1817 (Coleoptera: Curculionidae) from Baltic amber. *Entomologica Fennica* 30: 173-178. <https://doi.org/10.33338/ef.87174>
- Cai, C.Y., Wang, Y.L., Liang, L., Yin, Z.W., Thayer, M.K., Newton, A.F., and Zhou, Y.L. 2019. Congruence of morphological and molecular phylogenies of the rove beetle subfamily

- Staphylininae (Coleoptera: Staphylinidae). Scientific Reports 9: 15137.
<https://doi.org/10.1038/s41598-019-51408-1>
- Caldara, R. 2001. Phylogenetic analysis and higher classification of the tribe Mecinini (Coleoptera: Curculionidae, Curculioninae). – Koleopterologische Rundschau 71: 171–203.
- Caldara, R., Skuhrovec, J., Gosik, R., and Marvaldi, A.E. 2018. On the affinities and systematic position of *Pachytychius* Jekel, a genus currently incertae sedis in Coleoptera, Curculionidae: Evidence from immature stages and the COI gene support its placement in Smicronychini. Zoologischer Anzeiger 277: 218-230.
<https://doi.org/10.1016/j.jcz.2018.08.007>
- Chatzimanolis, S., Cohen, I. M., Schomann, A., and Solodovnikov, A. 2010. Molecular phylogeny of the mega-diverse rove beetle tribe Staphylinini (Insecta, Coleoptera, Staphylinidae). Zoologica Scripta 39: 436-449. <https://doi.org/10.1111/j.1463-6409.2010.00438.x>
- Chernomor, O., von Haeseler, A., and Minh, B.Q. 2016. Terrace aware data structure for phylogenomic inference from supermatrices. Systematic Biology 65: 997-1008.
<https://doi.org/10.1093/sysbio/syw037>
- Chujo, M., and Voss, E. 1960. Neue Curculioniden-subfamilie, -gattungen und -arten von Japan (Coleoptera, Curculionidae). Memoirs of the Faculty of Liberal Arts and Education - Kanagawa University (Part II) 94: 1-17.
- Ciegler, J.C. 2010. Weevils of South Carolina (Coleoptera: Nemomychidae, Attelabidae, Brentidae, Ithyceridae, and Curculionidae). Biota of South Carolina - Volume 6. Clemson University, Clemson, South Carolina. pp. 276.

- Clark, W.E. 2010. List of species of Curculionidae (Coleoptera) assigned to the tribe Anthonomini. Accessed 26 October 2021 at <http://webhome.auburn.edu/~clarkwe/anthsp.htm>
- Collins, R.A., Boykin, L.M., Cruickshank, R.H., and Armstrong, K.F. 2012. Barcoding's next top model: an evaluation of nucleotide substitution models for specimen identification. *Methods in Ecology and Evolution* 3: 457–465. <https://doi.org/10.1111/j.2041-210X.2011.00176.x>
- Colonnelli, E. 2003. A revised checklist of Italian Curculionoidea (Coleoptera). *Zootaxa* 337: 1–142. <https://doi.org/10.11646/zootaxa.337.1.1>
- Cywinska, A., Hunter, F.F., and Hebert, P.D.N. 2006. Identifying Canadian mosquito species through DNA barcodes. *Medical and Veterinary Entomology* 20: 413-424. <https://doi.org/10.1111/j.1365-2915.2006.00653.x>.
- Davis, S.R. 2017. The weevil rostrum (Coleoptera: Curculionoidea): Internal structures and evolutionary trends. *Bulletin of the American Museum of Natural History* No. 416. pp. 76.
- Dejean, P.F.M.A. 1821. *Catalogue de la collection de Coleopteres de M. le Baron*. Paris, France. pp. 136.
- Desbrochers des Loges, J. 1875. *Opuscules Entomologiques (Coléoptères) - 1er cahier*. pp. 1-36.
- Edgar, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792-1797. <https://doi.org/10.1093/nar/gkh340>
- Didham, R.K., Basset, C., Collins, C.M., Leather, S.R., Littlewood, N.A., Menz, M.H.M., Muller, J., Packer, L., Saunders, M.E., Schonrogge, K., Stewart, A.J.A., Yanoviak, S.P.,

- and Hassall, C. 2020. Interpreting insect declines: seven challenges and a way forward. *Insect Conservation and Diversity* 13: 103-114. <https://doi.org/10.1111/icad.12408>
- Dieckmann, L. 1970. Zur nomenklatur einiger europaischer Russelkafer-Gattungen. *Beitrage zur Entomologie* 20: 111-128. <https://doi.org/10.21248/contrib.entomol.20.1-2.111-128>
- Elias, M., Hill, R.I., Willmott, K.R., Dasmahapatra, K.K., Brower, A.V.Z., Mallet, J., and Jiggins, C.D. 2006. Limited performance of DNA barcoding in a diverse community of tropical butterflies. *Proceedings of the Royal Society B* 274: 2881-2889. <https://doi.org/10.1098/rspb.2007.1035>.
- Evangelista, D., Buss, L., and Ware, J.L. 2013. Using DNA barcodes to confirm the presence of a new invasive cockroach pest in New York City. *Journal of Economic Entomology* 106: 2275-2279. <https://doi.org/10.1603/EC13402>.
- Faust, J. 1882. Rüsselkäfer aus dem Amurgebiet. *Deutsche Entomologische Zeitschrift* 26: 257-295.
- Fisher, J., and Bruck, D.J. 2004. A technique for continuous mass rearing of the black vine weevil, *Otiorhynchus sulcatus*. *Entomologia Experimentalis et Applicata* 113: 71-75.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294-299.
- Forister, M.L., Pelton, E.M., and Black, S.H. 2019. Declines in insect abundance and diversity: We know enough to act now. *Conservation Science and Practice* 1: e80. <https://doi.org/10.1111/csp2.80>

- Fossen, E.I.F., Ekrem, T., Nilsson A., and Bergsten, J. 2016. Species delimitation in northern European water scavenger beetles of the genus *Hydrobius* (Coleoptera, Hydrophilidae). *Zookeys* 564: 71-120. <https://doi.org/10.3897/zookeys.564.6558>
- Franklin, M.T., Hueppelsheuser, T.K., Abram, P.K., Bouchard, P., Anderson, R.S., and Gibson, G.A.P. 2021. The Eurasian strawberry blossom weevil, *Anthonomus rubi* (Herbst, 1795), is established in North America. *The Canadian Entomologist* 153: 579-585. <https://doi.org/10.4039/tce.2021.28>
- Franz, N.M., and O'Brien, C.W. 2001. Revision and phylogeny of *Perellesschus* (Coleoptera: Curculionidae) with notes on its association with *Carludovica* (Cyclanthaceae). *Transactions of the American Entomological Society* 127: 255-287.
- Franz, N.M. 2006. Towards a phylogenetic system of derelomine flower weevils (Coleoptera: Curculionidae). *Systematic Entomology* 31: 220–287. <https://doi.org/10.1111/j.1365-3113.2005.00308.x>
- Gallego, D., and Galian, J. 2001. The internal transcribed spacers (ITS1 and ITS2) of the rDNA differentiates the bark beetle forest pests *Tomicus destruens* and *T. piniperda*. *Insect Molecular Biology* 10: 415-420. <https://doi.org/10.1046/j.0962-1075.2001.00279.x>
- Gillet, C.P.D.T. 2014. Molecular phylogenetics of the superfamily Curculionoidea (Insecta: Coleoptera). PhD Thesis, University of East Anglia. pp. 242.
- Grebennikov, V.V. 2016. Neglected *Trichalophus* (Coleoptera: Curculionidae): DNA barcode and phylogeography of high-altitude flightless weevils rediscovered in Southwest China. *Bonn Zoological Bulletin* 64: 59-76.
- Grebennikov, V.V. 2018. Dryophthorinae weevils (Coleoptera: Curculionidae) of the forest floor in Southeast Asia: Three-marker analysis reveals monophyly of Asian Stromboscerini

- and new identity of rediscovered *Tasactes*. European Journal of Entomology 115: 437–444. <https://doi.org/10.14411/EJE.2018.044>
- Grebennikov, V.V., and Anderson, R.S. 2021. Late miocene eastwards transatlantic dispersal of flightless anchonine weevils (Coleoptera: Curculionidae: Molytinae). Zootaxa 4952: 55–70. <https://doi.org/10.11646/zootaxa.4952.1.3>
- Guindon, S., Dufayard, J-F., Lefort, V., Anisimova, M., Hordijk, W., and Gascuel, O. 2010. New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. Systematic Biology 59: 307-321. <https://doi.org/10.1093/sysbio/syq010>
- Gunter, N.L., Oberprieler R.G., and Cameron S.L. 2016. Molecular phylogenetics of Australian weevils (Coleoptera: Curculionoidea): exploring relationships in a hyperdiverse lineage through comparison of independent analyses. Australian Entomologist 552: 217-233. <http://dx.doi.org/10.1111/aen.12173>
- Hamada, N., Pepinelli, M., Mattos-Gloria, A., and Luz, S.L.B. 2010. A new black fly species from Brazil, closely related to *Simulium guianense* Wise (Diptera, Simuliidae), revealed by morphology and DNA barcoding. Zootaxa 2428: 22-36. <https://doi.org/10.11646/zootaxa.2428.1.2>
- Hamilton, J. 1889. Catalogue of the Coleoptera common to North America, northern Asia and Europe, with distribution and bibliography. Paul C. Stockhausen Entomological Printer, Philadelphia. pp. 162.
- Han, K., and Park, S. 2015. Additional contribution to weevil fauna of Korea (Coleoptera: Curculionidae). Entomological Research Bulletin 31: 180-185.

- Harrison, S. 2013. Plant and animal endemism in California. University of California Press, Berkeley, California. pp. 200. <https://doi.org/10.1525/9780520954731>
- Hatch, M.H. 1971. The beetles of the Pacific Northwest - Part 5: Rhipiceroidea, Sternoxi, Phytophaga, Rhynchophora, and Lamellicornia. University of Washington Publications in Biology Volume 16. pp. 662.
- Hebert, P.D.N., Cywinska, A., Ball, S.L., and deWaard, J.R. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society B 270: 313-321. <https://doi.org/10.1098/rspb.2002.2218>.
- Herbst, J.F.W. 1795. Natursystem aller bekannten in- und ausländischen Insekten, als eine Fortsetzung der von Büffonschen Naturgeschichte. Der Käfer sechster Theil. Berlin: Joachim Pauli. pp. 520.
- Hight, S.D. 1988. Available feeding niches in populations of *Lythrum salicaria* (Purple Loosestrife) in Northeastern United States. Proceedings of the VII International Symposium on Biological Control of Weeds, Rome, Italy. pgs. 269-278.
- Hoffmann, A. 1954. Faune De France No. 59 - Coléoptères Curculionides (Deuxième Partie). Fédération Française des Sociétés de Sciences Naturelles, Paris, France. pp. 1208.
- Howarth, F.G., and Stone, F.D. 1990. Elevated Carbon Dioxide Levels in Bayliss Cave, Australia: Implications for the Evolution of Obligate Cave Species. Pacific Science 44: 207-218.
- Huang, W., Xie, X., Peng, F., Liang, X., Wang, X., and Chen, X. 2020. Optimizing the widely used nuclear protein-coding gene primers in beetle phylogenies and their application in the genus *Sasajiscymnus* Vandenberg (Coleoptera: Coccinellidae). Ecology and Evolution 10: 7731-7738. <https://doi.org/10.1002/ece3.6497>

- Huelsenbeck, J.P., and Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17: 754-755.
- Hubbard, H.G., Schwarz, E.A., and LeConte, J.L. 1878. The Coleoptera of Michigan. *Proceedings of the American Philosophical Society* 17, No. 101: 593-669.
- Ivanova, N.V., deWaard, J., and Hebert, P.D.N. 2006. An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes* 6: 998-1002. <https://doi.org/10.1111/j.1471-8286.2006.01428.x>
- Katoh, K., and Toh, H. 2008. Improved accuracy of multiple ncRNA alignment by incorporating structural information into a MAFFT-based framework. *BMC Bioinformatics* 9: 212. <https://doi.org/10.1186/1471-2105-9-212>
- Katoh, K., Rozewicki, J., and Yamada, K.D. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20: 1160-1166. <https://doi.org/10.1093/bib/bbx108>
- Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide-sequences. *Journal of Molecular Evolution* 16:111-120. <https://doi.org/10.1007/BF01731581>
- King, J.N., Yanchuk, A.D., Kiss, G.K., and Alfaro, R.I. 1997. Genetic and phenotypic relationships between weevil (*Pissodes strobi*) resistance and height growth in spruce populations of British Columbia. *Canadian Journal of Forest Research* 27: 732-739. <https://doi.org/10.1139/x97-009>
- Kjer, K.M. 1995. Use of rRNA Secondary structure in phylogenetic studies to identify homologous positions: An example of alignment and data presentation from the frogs.

- Molecular Phylogenetics and Evolution 4: 314-330.
<https://doi.org/10.1006/mpev.1995.1028>
- Korotyaev, B.A. 1976. Review of weevils of the genus *Dorytomus* Germ. (Coleoptera, Curculionidae) of North-East Asia. Entomologicheskoe Obozrenie 55: 124–136.
- Korotyaev, B.A. 1996. 131 - *Dorytomus* Germ. in Egorov, A.B., Zherichin, V.V. and Korotyaev, B.A. (eds.) - Sem. Curculionidae - Dolgonosiki - Key to the Insects of the Russian Far East - Volume 3 - Coleoptera - Part 3 - Supplement. Vladivostok, Dal'nauka. pgs. 468-478
- Kojima, H., and Truong, X.L. 2012. Weevils of the genus *Adorytomus* Voss (Coleoptera, Curculionidae) from Vietnam. Esakia 52: 35-39.
- Koonin, E.V., and Wolf, Y.I. 2010. Constraints and plasticity in genome and molecular-phenome evolution. Nature Reviews Genetics 11: 487-498. <https://doi.org/10.1038/nrg2810>
- Legalov, A.A. 2015. Fossil mesozoic and cenozoic weevils (Coleoptera, Obrienioidea, Curculionoidea). Paleontological Journal 49: 1442-1513.
<https://doi.org/10.1134/S0031030115130067>
- Legalov, A.A. 2020a. Revised checklist of weevils (Coleoptera: Curculionoidea excluding Scolytidae and Platypodidae) from Siberia and the Russian Far East. Acta Biologica Sibirica 6: 437-549. <https://doi.org/10.3897/abs.6.e59314>
- Legalov, A.A. 2020b Annotated key to weevils of the world. Part 4. Subfamilies Eirrhiniinae, Dryophthorinae and Cossoninae (Curculionidae). Ukrainian Journal of Ecology 10: 319-331. https://doi.org/10.15421/2020_104

- Legalov, A.A. 2021. First description of the male of *Tsherepanovia globosa* Korotyaev, 1992 (Coleoptera, Curculionidae) with new taxonomic data. *Ecologica Montenegrina* 41: 10-12. <http://dx.doi.org/10.37828/em.2021.41.3>
- Lawrence, J.F., and Newton, A.F. 1982. Evolution and classification of beetles. *Annual Review of Ecology and Systematics* 13: 261-290.
- Lemoine, F., Domelevo Entfellner, J.B., Wilkinson, E., Correia, D., Felipe, M.D., Oliveira, T., and Gascuel, O. 2018. Renewing Felsenstein's phylogenetic bootstrap in the era of big data. *Nature* 556: 452-456. <https://doi.org/10.1038/s41586-018-0043-0>
- Letsch, H., Kuck, P., Stocsits, R.R., and Misof, B. 2010. The impact of rRNA secondary structure consideration in alignment and tree reconstruction: simulated data and a case study on the phylogeny of hexapods. *Molecular Biology and Evolution* 27: 2507-2521. <https://doi.org/10.1093/molbev/msq140>
- Letsch, H., Balke, M., Toussaint, E.F.A., and Riedel, A. 2019. Historical biogeography of the hyperdiverse hidden snout weevils (Coleoptera, Curculionidae, Cryptorhynchinae). *Systematic Entomology* 45: 312-326. <https://doi.org/10.1111/syen.12396>
- Lewis, J.H., McAlpine, D.F., and Smith, A.B.T. 2019. *Gryllus veletis* (Orthoptera: Gryllidae) in New Brunswick: first detection in Maritime Canada. *Northeastern Naturalist* 26: N18-N20. <https://doi.org/10.1656/045.026.0214>
- Li, Y., Zhou, X., Feng, G., Hu, H., Niu, L., Hebert, P.D.N., and Huang, D. 2010. COI and ITS2 sequences delimit species, reveal cryptic taxa and host specificity of fig-associated *Sycophila* (Hymenoptera, Eurytomidae). *Molecular Ecology Resources* 10: 31-40. <https://doi.org/10.1111/j.1755-0998.2009.02671.x>

- Linnaeus, C. 1758. *Systema Naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis*. Tomus I. Edition decima, reformata. Holmiae: Laurentii Salvii. pp. 824.
- Little, E.L. 1979. Checklist of United States trees (native and naturalized). Forest Service - United States Department of Agriculture - Agriculture Handbook No. 541. pp. 375.
- Maddison, W.P. 1997. Gene trees in species trees. *Systematic Biology* 46: 523-536.
<https://doi.org/10.1093/sysbio/46.3.523>
- Marshall, G.A.K. 1926. On new species of Curculionidae (Coleopt.) from Java and Sumatra. *Treubia* 8: 342-351.
- Marshall, S.A. 2006. *Insects: Their natural history and diversity*. Firefly Books Ltd., Richmond Hill, Ontario. pp. 732.
- Marshall, S.A. 2018. *Beetles: The natural history and diversity of Coleoptera*. Firefly Books Ltd., Richmond Hill, Ontario. pp. 784.
- Masaki, M., Ohmura, K., and Ichinohe, F. 1984. Host range studies of the Black Vine Weevil, *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae). *Applied Entomology and Zoology* 19: 95-106. <https://doi.org/10.1303/aez.19.95>
- Masly, J.P. 2012. 170 years of “Lock-and-Key”: genital morphology and reproductive isolation. *International Journal of Evolutionary Biology* 2012: 247352.
<https://doi.org/10.1155/2012/247352>
- Mattson, W.J., Niemela, P., Millers, I., and Inguanzo, Y. 1994. Immigrant phytophagous insects on woody plants in the United States and Canada: an annotated list. United States Department of Agriculture – Forest Service – General Technical Report NC-169. pp. 27.

- McKenna, D.D., Sequeira, A.S., Marvaldi, A.E., and Farrell, B.D. 2009. Temporal lags and overlap in the diversification of weevils and flowering plants. *Proceedings of the National Academy of Sciences of the United States of America* 106: 7083-7088. <https://doi.org/10.1073/pnas.0810618106>
- McKenna, D.D., Shin, S., Ahrens, D., Balke, M., Beza-Beza, C., Clarke, D.J., Donath, A., Escalona, H.E., Friedrich, F., Letsch, H., Liu, S., Maddison, D., Mayer, C., Misof, B., Murin, P.J., Niehuis, O., Peters, R.S., Podsiadlowski, L., Pohl, H., Scully, E.D., Yan, E.V., Zhou, X., Ślipiński, A., and Beutel R.G. 2019. The evolution and genomic basis of beetle diversity. *Proceedings of the National Academy of Sciences of the United States of America* 116: 24729-24737. <https://doi.org/10.1073/pnas.1909655116>
- Morimoto, K. and Enda, N. 1962. Taxonomic and biological studies on the genus *Eteophilus* from Japan. *Bulletin of the Government Forest Experiment Station* 146: 13–31.
- Nasimento, F.B., dos Reis, M., and Yang, Z. 2017. A biologist's guide to Bayesian phylogenetic Analysis. *Nature Ecology and Evolution* 1: 1446-1454. <https://doi.org/10.1038/s41559-017-0280-x>
- Slosson, A.T. 1897. Additional list of insects taken in alpine region of Mt. Washington. *Entomological News* 8: 237-240.
- Su, Z., Zhang, R., and Qiu, J. 2011. Decline in the diversity of willow trunk-dwelling weevils (Coleoptera: Curculionoidea) as a result of urban expansion in Beijing, China. *Journal of Insect Conservation* 15: 367-377. <https://doi.org/10.1007/s10841-010-9310-6>
- Meier, R., Shiyang, K., Vaidya, G., and Ng, P.K.L. 2006. DNA barcoding and taxonomy in Diptera: A tale of high intraspecific variability and low identification success. *Systematic Biology* 55: 715-728. <https://doi.org/10.1080/10635150600969864>.

- Minh, B.Q., Nguyen, M.A.T., and von Haeseler, A. 2013. Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution* 30: 1188-1195.
<https://doi.org/10.1093/molbev/mst024>
- Minh, B.Q., Lanfear, R., Trifinopoulos, J., Schrempf, D. and Schmidt, H.A. 2021. IQ-TREE version 2.1.2: Tutorials and manual phylogenomic software by maximum likelihood. Accessed 3 April 2021 at <http://www.iqtree.org/doc/>
- Morris, M.G. 1995. Surface swimming in some Curculionidae. *Memoirs of the Entomological Society of Washington* 14: 129-136.
- Nguyen, L.-T., Schmidt, H.A., von Haeseler, A., and Minh, B.Q. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Molecular Biology and Evolution* 32: 268-274. <https://doi.org/10.1093/molbev/msu300>
- Nylander, J.A., Ronquist, F., Huelsenbeck, J.P., and Nieves-Aldrey, J. 2004. Bayesian phylogenetic analysis of combined data. *Systematic Biology* 53: 47-67.
<https://doi.org/10.1080/10635150490264699>
- Oberprieler, R.G., Marvaldi, A.E., and Anderson, R.S. 2007. Weevils, weevils, weevils everywhere. *Zootaxa* 1668: 491-520. <https://doi.org/10.11646/zootaxa.1668.1.24>
- Caldara, R., Franz, N.M., and Oberprieler, R.G. 2014. 3.7.10 *Curculioninae Latreille, 1802* in Leschen R.A.B. and Beutel, R.G. (eds.) - *Handbook of Zoology. Coleoptera, Beetles (Phytophaga: Chrysomeloidea, Curculionoidea): Morphology and systematics - Volume 3*. De Gruyter, Berlin, Germany. pgs. 589-628.
- Oberprieler, R.G., Anderson, R.G., and Marvaldi, A.E. 2014. 3. *Curculionoidea Latreille, 1802: Introduction, Phylogeny* in Leschen R.A.B. and Beutel, R.G. (eds.) - *Handbook of*

- Zoology. Coleoptera, Beetles (Phytophaga: Chrysomeloidea, Curculionoidea):
Morphology and systematics - Volume 3. De Gruyter, Berlin, Germany. pgs. 285-300.
- O'Brien, C.W. 1970. A Taxonomic Revision of the weevil genus *Dorytomus* in North America.
(Coleoptera: Curculionidae). University of California Press, Berkeley, California. pp. 82.
- O'Brien, C.W. 1981. The larger (4.5+ mm) *Listronotus* of America north of Mexico
(Cylindrorhininae, Curculionidae, Coleoptera). Transactions of the American
Entomological Society 107: 69-123. <https://www.jstor.org/stable/25078279>
- O'Brien, C.W., and Wibmer, G.J. 1982. Annotated checklist of the weevils (Curculionidae sensu
lato) of North America, Central America, and the West Indies (Coleoptera:
Curculionoidea). Memoirs of the American Entomological Institute - Number 34. pp.
382.
- Olivier, A.G. 1791. Encyclopédie méthodique, ou par ordre des matières; par une société de gens
de lettres, de savants et d'artistes. Précédée d'un vocabulaire universel, servant de table
pour l'Ouvrage, ornée des Portraits de MM Diderot et d'Alembert, premiers Editeurs de
l'Encyclopédie. Histoire naturelle. Insectes. Tome cinquième. Part 2. pgs. 369-793.
- Osswald, J., Bachmann, L., and Gusarov, V. 2013. Molecular phylogeny of the beetle tribe
Oxypodini (Coleoptera: Staphylinidae: Aleocharinae). Systematic Entomology 38: 507-
522. <https://doi.org/10.1111/syen.12011>
- Painting, C.J., and Holwell, G.I. 2013. Exaggerated trait allometry, compensation and trade-offs
in the New Zealand Giraffe Weevil (*Lasiornychus barbicornis*). PLoS One 8: e82467.
<https://doi.org/10.1371/journal.pone.0082467>
- Paquin, P., and Anderson, R.S. 2009. *The first troglobitic weevil (Coleoptera: Curculionidae) in*
North America? Description of a new eyeless species of Lymantes Schönherr from

- Central Texas caves* in Reddell, J.R. and Cokendolpher, J.C. (eds.) - Studies on the Cave and Endogean Fauna of North America V. Texas Memorial Museum, Speleological Monographs 7. Pgs. 115-123.
- Pauly, A., Devalez, J., Sonet, G., Nagy, Z.T., and Boeve, J. 2015. DNA barcoding and male genital morphology reveal five new cryptic species in the West Palearctic bee *Seladonia smaragdula* (Vachal, 1895) (Hymenoptera: Apoidea: Halictidae). *Zootaxa* 4034: 257-290. <https://doi.org/10.11646/zootaxa.4034.2.2>
- Paykull, G. 1792. *Monographia Curculionum Sveciae*. Upsaliae: Litt. Viduae Direct. Joh. Edman. pp. 152.
- Pentinsaari, M., and Hebert, P.D.N., and Mutanen, M. 2014. Barcoding beetles: A regional survey of 1872 species reveals high identification success and unusually deep interspecific divergences. *PLoS One*: e108651. <https://doi.org/10.1371/journal.pone.0108651>
- Pentinsaari, M., Anderson, R.S., Borowiec, L., Bouchard, P., Brunke, A., Douglas, A., Smith, A.B.T., and Hebert, P.D. 2019. DNA barcodes reveal 63 overlooked species of Canadian beetles (Insecta, Coleoptera). *ZooKeys* 894: 53-150. doi:10.3897/zookeys.894.37862.
- Poole, R.W., and Gentili, P. 1996. *Nomina insecta nearctica: a check list of the insects of North America* Vol. 1, Coleoptera, Strepsiptera. Entomological Information Services, Rockville, Maryland. pp. 827.
- Prena, J. 2018. An annotated inventory of the weevils (Coleoptera: Curculionoidea) described by Thomas Say. *Bulletin of the Museum of Comparative Zoology* 161: 1-401.

- Pullen, K.R., Jennings, D., and Oberprieler, R.G. 2014. Annotated catalogue of Australian weevils (Coleoptera: Curculionidae). Zootaxa 3896: 1-481.
<http://dx.doi.org/10.11646/zootaxa.3896.1.1>
- Rach, J., DeSalle, R., Sarkar, I.N., Schierwater, B., and Hadrys, H. 2007. Character-based DNA barcoding allows discrimination of genera, species and populations in Odonata. Proceedings of the Royal Society B 275: 1632. <https://doi.org/10.1098/rspb.2007.1290>.
- Rach, J., Bergmann, T., Paknia, O., DeSalle, R., Schierwater, B., and Hadrys, H. 2017. The marker choice: Unexpected resolving power of an unexplored CO1 region for layered DNA barcoding approaches. PLoS One 12: e0174842.
<https://doi.org/10.1371/journal.pone.0174842>
- Radcliffe, E.B., and Flanders, K.L. 1998. Biological control of alfalfa weevil in North America. Integrated Pest Management Reviews 3: 225-242.
- Rambaut, A. 2009. FigTree ver. 1.4.4. Available at <http://tree.bio.ed.ac.uk/software/figtree/>
- Rambaut, A., Drummond, A.J., Xie, D., Baele, G., and Suchard, M.A. 2018. Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67: 901-904. <https://doi.org/10.1093/sysbio/syy032>
- Ratnasingham, S., and Hebert, P.D. 2007. BOLD: Barcode of Life Data Systems (<http://www.barcodinglife.org>). Molecular Ecology Notes 7: 1-10.
- Riedel, A., Daawia, D., and Balke, M. 2009. Deep cox1 divergence and hyperdiversity of *Trigonopterus* weevils in a New Guinea mountain range (Coleoptera, Curculionidae). Zoologica Scripta 39: 63-74. <https://doi.org/10.1111/j.1463-6409.2009.00404.x>.

- Riedel, A., Sagata, K., Surbakti, S., Tanzler, R., and Balke, M. 2013. One hundred and one new species of *Trigonopterus* weevils from New Guinea. *ZooKeys* 280: 1-150.
<https://doi.org/10.3897/zookeys.280.3906>.
- Rivera, J., and Currie, D.C. 2009. Identification of Nearctic black flies using DNA barcodes (Diptera: Simuliidae). *Molecular Ecology Resources* 2009: 224-236. doi:10.1111/j.1755-0998.2009.02648.x
- Ronquist, F., Huelsenbeck, J.P., Teslenko, M., Zhang, C., and Nylander, J.A.A. 2020. MrBayes version 3.2 Manual: Tutorials and Model Summaries. Accessed 3 April 2021 at <https://nbsweden.github.io/MrBayes/manual.html>
- Ruchin, A.B., Egorov, L.V., and Khapugin, A.A. 2021. Usage of fermental traps for the study of the species diversity of Coleoptera. *Insects* 12: 407.
<https://doi.org/10.3390/insects12050407>
- Santos, D., Ribeiro, G.C., Cabral, A.D., Speranca, M.A. 2018. A non-destructive enzymatic method to extract DNA from arthropod specimens: Implications for morphological and molecular studies. *PloS ONE* 13: e0192200.
<https://doi.org/10.1371/journal.pone.0192200>
- Saitou, N., and Nei, M. 1987. The neighbor-joining method: a new method for reconstruction of phylogenetic trees. *Molecular Biology and Evolution* 4: 406-425.
<https://doi.org/10.1093/oxfordjournals.molbev.a040454>
- Scherf, H. 1964. Die Entwicklungsstadien der mitteleuropaischen Curculioniden (Morphologie, Bionomie, Okologie). *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft* 506: 1-335.

- Schoenherr, C.J. 1838. Genera et species Curculionidum, cum synonymia hujus familiae.
- Species novae aut hactenas minus cognitae, descriptionibus a Dom. Leonardo Gyllenhal, C.H. Boheman, et entomologis aliis illustratae. Volume 4 - Part 2. Paris, France. pgs. 601-1121.
- Shaw, K.L. 2002. Conflict between nuclear and mitochondrial DNA phylogenies of a recent species radiation: What mtDNA reveals and conceals about modes of speciation in Hawaiian crickets. Proceedings of the National Academy of Sciences of the United States of America 99: 16122-16127. <https://doi.org/10.1073/pnas.242585899>
- Shelef, O., Helman, Y., Friedman, A., Behar, A., and Rachmilevitch, S. 2013. Tri-party underground symbiosis between a weevil, bacteria and a desert plant. PLoS One 8: e76588. <https://doi.org/10.1371/journal.pone.0076588>
- Shin, S., Clarke, D.J., Lemmon, A.R., Lemmon, E.M., Aitken, A.L., Haddad, S., Farrell, B.D., Marvaldi, A.E., Oberprieler, R.G., and McKenna, D.D. 2017. Phylogenomic data yield new and robust insights into the phylogeny and evolution of weevils. Molecular Biology and Evolution 35: 823-836. <https://doi.org/10.1093/molbev/msx324>
- Shorthouse, D.P. 2010. SimpleMappr, an online tool to produce publication-quality point maps. Available at from <http://www.simplemappr.net>
- Skevington, J.H. 2005. Revision of Nearctic *Nephrocerus* Zetterstedt (Diptera: Pipunculidae). Zootaxa 977: 1-36. <https://doi.org/10.11646/zootaxa.977.1.1>
- Smith, E.H. 1979. Techniques for the dissection and mounting of the male (aedeagus) and female (spermatheca) genitalia of the Chrysomelidae (Coleoptera). The Coleopterists Bulletin 33: 93-103. <https://www.jstor.org/stable/4000169>

- Sprick, P., and Floren, A. 2018. Diversity of Curculionoidea in humid rain forest canopies of Borneo: A Taxonomic Blank Spot. *Diversity* 10: 116. <https://doi.org/10.3390/d10040116>
- Srivathsan, A., and Meier, R. 2012. On the inappropriate use of Kimura-2-parameter (K2P) divergences in the DNA-barcoding literature. *Cladistics* 28: 190-194. <https://doi.org/10.1111/j.1096-0031.2011.00370.x>
- Stepanovic, S., Kosovac, A., Krstic, O., Jovic, J., and Tosevski, I. 2015. Morphology versus DNA barcoding: two sides of the same coin. A case study of *Ceutorhynchus erysimi* and *C. contractus* identification. *Insect Science* 23: 638-648. <https://doi.org/10.1111/1744-7917.12212>.
- Su, Z., Huang, D., Omar, Y., Ren, L., Alonso-Zarazaga, M., Majer, J., and Zhang, R. 2012. What's under a plastic strip? Hidden urban biodiversity in the Beijing metropolitan area, China. *The Coleopterists Bulletin* 66(3): 289-293.
- Sweeney, J.D., Anderson, R.S., Webster, R.P., and Neville, R. 2012. First records of *Orchestes Fagi* (L.) (Coleoptera: Curculionidae: Curculioninae) in North America, with a checklist of the North American Rhamphini. *The Coleopterists Bulletin* 66(4): 297-304. <https://doi.org/10.1649/072.066.0401>
- Takakuwa, M., and Fujita, H. 2010. Spring research on insect fauna near Hananoego of the montane zone of Yaku-shima Island, Southwest Japan - 屋久島高地帯花之江河周辺における昆虫調査. *Bulletin of the Kanagawa Prefecture Museum of Natural History* 39: 35-38.

- Tarasov, S., and Dimitrov, D. 2016. Multigene phylogenetic analysis redefines dung beetles relationships and classification (Coleoptera: Scarabaeidae: Scarabaeinae). *BMC Evolutionary Biology* 16: 257. <https://doi.org/10.1186/s12862-016-0822-x>
- Timmermans, M.J.T.N., Dodsworth, S., Culverwell, C.L., Bocak, L., Ahrens, D., Littlewood, D.T.J., Pons, J., and Vogler, A.P. 2010. Why barcode? High throughput multiplex sequencing of mitochondrial genomes for molecular systematics. *Nucleic Acids Research* 38: e197. <https://doi.org/10.1093/nar/gkq807>
- Vanin, S.A., and Guerra, T.J. 2012. A remarkable new species of flesh-fly mimicking weevil (Coleoptera: Curculionidae: Conoderinae) from Southeastern Brazil. *Zootaxa* 3413: 55-63. <https://doi.org/10.11646/zootaxa.3413.1.5>
- Voss, E., and Chujo, M. 1960. Curculionid-beetles of Niigata Prefecture, Honshu, Japan, collected by Dr. K. Baba (IV). *Niponius* 1: 1-10.
- Wassell, J.L.H. 1966. Coprophagous weevils (Coleoptera: Curculionidae). *Journal of the Entomological Society of Queensland* 5: 73-74.
- Webster, R.P., Anderson, R.S., Webster, V.L., Alderson, C.A., Hughes, C.C., and Sweeney, J.D. 2016. New Curculionoidea records from New Brunswick, Canada with an addition to the fauna of Nova Scotia. *Zookeys* 573: 367-386. <https://doi.org/10.3897/zookeys.573.7444>
- Wheeler, Q.D., and Platnick, N.I. 2000. *The phylogenetic species concept (sensu Wheeler and Platnick)* in Wheeler, Q.D. and Meier, R. (eds.) - *Species Concepts and Phylogenetic Theory: A Debate*. Columbia University Press, New York, U.S.A. pgs. 56-69.
- Whitehead, D.R., Chamorro, M.L., and Anderson R.S. 2018. An illustrated key to the species of *Curculio* Linnaeus (Coleoptera: Curculionidae) of North America east of the Mississippi

- River. Proceedings of the Entomological Society of Washington 120: 616-641.
<https://doi.org/10.4289/0013-8797.120.3.616>
- Whitworth, T.L., Dawson, R.D., Magalon, H., and Baudry, E. 2007. DNA barcoding cannot reliably identify species of the blowfly genus *Protophormia* (Diptera: Calliphoridae). Proceedings of the Royal Society B 274: 1619. <https://doi.org/10.1098/rspb.2007.0062>.
- Wild, A.L., and Maddison, D.R. 2008. Evaluating nuclear protein-coding genes for phylogenetic utility in beetles. Molecular Phylogenetics and Evolution 48: 877-891.
<https://doi.org/10.1016/j.ympev.2008.05.023>
- Wilts, B.D., Michielsen, K. Kuipers, J., De Raedt, H., and Stavenga, D.G. 2012. Brilliant camouflage: photonic crystals in the diamond weevil, *Entimus imperialis*. Proceedings of the Royal Society – Biological Sciences 279: 2524-2530.
<https://doi.org/10.1098/rspb.2011.2651>
- Yao, H., Song, J., Liu, C., Luo, K., Han, J., Li, Y., Pang, X., Xu, H., Zhu, Y., Xiao, P., and Chen, S. 2010. Use of ITS2 Region as the universal DNA barcode for plants and animals. PLoS One 5: e13102. <https://doi.org/10.1371/journal.pone.0013102>
- Yusseff-Vanegas, S.Z., and Agnarsson I. 2017. DNA-barcoding of forensically important blow flies (Diptera: Calliphoridae) in the Caribbean Region. PeerJ 5: e3516.
<https://doi.org/10.7717/peerj.3516>
- Zardoya, R., and Meyer, A. 1996. Phylogenetic performance of mitochondrial protein-coding genes in resolving relationships among vertebrates. Molecular Biology and Evolution 13: 933-942. <https://doi.org/10.1093/oxfordjournals.molbev.a025661>

- Zetterstedt, J.W. 1837. Coleoptera. Columns 7-192. In: Insecta Lapponica descripta. Sectio prima. Coleoptera, Orthoptera et Hemiptera. Lipsiae: Leopoldi Voss, vi pp. + 1140 columns.
- Zhang, SQ., Che, L-H., Li, Y., Liang, D., Pang, H., Slipinski, A., and Zhang, P. 2018. Evolutionary history of Coleoptera revealed by extensive sampling of genes and species. Nature Communications 9: 205. <https://doi.org/10.1038/s41467-017-02644-4>
- Zhang, W., Yuan, Y., Yang, S., Huang, J., and Huang, L. 2015. ITS2 secondary structure improves discrimination between medicinal “Mu Tong” species when using DNA barcoding. PLoS ONE 10: e0131185. <https://doi.org/10.1371/journal.pone.0131185>
- Zumpt, F. 1932. Curculioniden-Studien II. Systematisch-synonymische Bemerkungen über palaearktische Russeikafer. Coleopterologisches Centralblatt 6: 38-44.
- Zwolfer, H., and Bennet, F.D. 1969. *Ludovix fasciatus* Gyll. (Col., Curculionidae), an entomophagous weevil. The Entomologists Monthly Magazine 105: 122-123.

Appendix A: Supplementary Material for Chapter 3

Bayes Block code used in the Bayesian analysis:

```
BEGIN MRBAYES;

log start filename=Bayes_log.txt;

charset 16s = 1-604;
charset 28s_col_3_cytb_1 = 605-1266 1269-1971\3 1972-2372\3;
charset col_1_cytb_2 = 1267-1971\3 1973-2372\3;
charset col_2_cytb_3 = 1268-1971\3 1974-2372\3;

partition bestScheme = 4: 16s, 28s_col_3_cytb_1, col_1_cytb_2, col_2_cytb_3;
set partition = bestScheme;

lset applyto=(1) nst=6 rates=invgamma;
lset applyto=(2) nst=6 rates=invgamma;
lset applyto=(3) nst=1 rates=propinv;
lset applyto=(4) nst=2 rates=gamma;

prset apply=(1,3,4) statefreqpr=fixed(empirical);
prset apply=(2) statefreqpr=fixed(equal);

unlink statefreq=(all) revmat=(all) shape=(all) pinvar=(all) tratio=(all);

mcmcp ngen=3500000 printfreq=2000 samplefreq=1000 nruns=2 nchains=4 starttree=random
relburnin=yes burninfrac=0.25 savebrlens=yes;
mcmc;

sump;
sumt contype=halfcompat;

log stop;

END;
```