Placement of Micropezinae (Micropezidae) on the Diptera Tree of Life: a Molecular Phylogenetic Approach

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ABSTRACT

PLACEMENT OF MICROPEZINAE (MICROPEZIDAE) ON THE DIPTERA TREE OF LIFE: A MOLECULAR PHYLOGENETIC APPROACH

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This thesis is divided into two parts centred around the higher relationships of Micropezinae (Diptera: Micropezidae). The first chapter is a multi-gene molecular phylogenetic analysis of Nerioidea (Diptera: Schizophora). The relationships of the four families of Nerioidea (Micropezidae, Neriidae, Pseudopomyzidae, and Cypselosomatidae), as well as the internal relationships and subfamilial classification of Micropezidae, have been the source of considerable debate. These relationships are tested using 13 genetic loci sampled for 77 Nerioidea species and 39 outgroup taxa from across Schizophora and analyzed using Bayesian Inference and Maximum Likelihood. Nerioidea was recovered as monophyletic with strong support, as were each of the four families within, with Micropezidae being returned as the sister group to the remaining Nerioidea. Internal relationships of Micropezidae included strong support for each of the subfamilies with the exception of Eurybatinae, which was recovered as polyphyletic, with the Metopochetini forming a well-supported sister group relationship with Micropezinae. The remaining Eurybatinae were well-supported as the sister group to Taeniapterinae. The implications for the taxonomy and classification of Micropezidae subfamilies are addressed. The tribe Metopochetini is elevated to subfamily rank (Metopochetinae).

The second chapter of this thesis aims to clarify the higher relationships of Nerioidea within Schizophora. The relationships between superfamilies of Schizophora have proven difficult to establish on the basis of morphology and molecular data to date, resulting in unstable classifications and evolutionary hypotheses. Using a supermatrix composed of previously published DNA sequences from 23 genetic loci sampled across 2300 schizophoran taxa, the relationships of Schizophora are estimated using Maximum Likelihood with multiple data subsampling and substitution model strategies. Two preferred trees were recovered which are strongly congruent with both morphological and genomic approaches, signifying supermatrix analyses represent a viable alternative for elucidating schizophoran relationships. Taxon availability and sampling, particularly among Carnoidea and Sphaeroceroidea, remain a significant barrier to a fully resolved phylogeny of Schizophora. Higher relationships of Schizophora were found to be highly sensitive to taxon sampling and substitution model parameters, while there is significant evidence of differential phylogenetic signal between nucleotide sites and taxonomic lineages, indicative of heterotachy. Implications for the future of Schizophora systematics are discussed.

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INTRODUCTION AND LITERATURE REVIEW

Diptera are one of the most diverse groups of animals on the planet and are vital to global ecosystem health and function. Found natively on every continent including Antarctica, flies inhabit nearly every terrestrial ecosystem, from the driest deserts to the wettest rainforests, from crystal clear mountain streams to rain-filled backyard containers, and from pristine plains to putrid pits of petroleum. This ability to adapt to most biological situations has resulted in a diversity of forms and features that have been recognized as more than 160,000 species (Pape et al. 2011), with likely at least as many remaining to be described (Borkent et al. 2018), with some extreme estimates placing the total species-level diversity of flies at more than ten times what is presently known (Hebert et al. 2016). While many fly species remain unknown and awaiting description, the systematics and higher classification of those we do know about remains a work in progress, particularly among Schizophora.

At the root of the dipteran family tree are a series of clades that have traditionally been known collectively as the "Nematocera", most of which are delicate flies that possess long, highly segmented antennal flagella. This group, which includes commonly encountered flies such as mosquitoes, midges, and black flies among its roughly 55,000 described species (Pape et al. 2011), are largely aquatic as larvae, although the highly diverse Bibionomorpha have transitioned to terrestrial habitats (Marshall 2012). The remaining flies, characterized by a stockier body and with shorter, abbreviated antennae among other features, together form a clade known as the Brachycera. Among the lower Brachycera, which includes the familiar horse, soldier, bee, and robber flies, the relationships between major groupings remain in flux, with several competing hypotheses currently within the literature based on differing datasets and methodologies (Yeates 2002, Wiegmann et al. 2011, Lambkin et al. 2013, Shin et al. 2018). Whatever the relationships between the lower Brachycera, the Empidoidea, the dance flies and relatives, are more closely related to the higher Brachycera, and together with the relictual family Apistomyidae form the Eremoneura along with the higher Diptera of the Cyclorrhapha (Sinclair and Cumming 2006, Wiegmann et al. 2011). The Cyclorrhapha, characterized by a specialized puparium (itself the ultimate larval exuvia that is retained and inflated as a protective barrier during pupation) with an anterior circular weakening that allows the adult fly to break out upon completion of pupation, includes the families historically labelled "Aschiza" (also recently shown to be another paraphyletic grade of superfamilies and families) with the Pipunculidae (not the Syrphoidea) repeatedly being recovered as sister to Schizophora, one of the most important groups of flies on the planet (Wiegmann et al. 2011, Tachi 2014, Young et al. 2016, Pauli et al. 2018).

Schizophora is one of the most diverse groups of Diptera, and includes those flies possessing a ptilinum (an inflatable membranous sac on the head that is used to break out of the puparium following pupation) and associated suture and musculature as well as circumversion of the post-abdomen (360º dextral rotation) completed entirely (and permanently) within the puparium (but see Lambkin et al. 2013 for additional synapomorphies). The Schizophora are thought to have evolved approximately 70 million years ago (Wiegmann et al. 2011, Cerretti et al. 2017), and are believed to have radiated explosively at or shortly following the Cretaceous-Paleogene (K-Pg) boundary, a radiation event regarded by Grimaldi and Engel (2005) as the "largest Tertiary (=Paleogene) radiation of insects alongside ditrysian Lepidoptera", and by extension, of all other animals. Thus, while the Chicxulub asteroid impact abruptly ended the age of non-avian dinosaurs (Schulte et al. 2010), it ultimately ushered in the age of schizophoran Diptera. The reasons for this explosive radiation at or following the K-Pg boundary are not entirely clear, however some authors have proposed that the additional resistance to desiccation provided by the puparium allowed these flies to survive the scorched Earth conditions immediately following the asteroid impact (Lambkin et al. 2013), while diversifying alongside the various plants, mammals, and other insects rebounding from the K-Pg extinction event would have provided the wide variety of biological and functional niches that modern Diptera fill (Grimaldi et al. 2005). This radiation is believed to have occurred remarkably quickly, evidenced by numerous specimens trapped in tree sap ~45 million years ago, when Europe was covered with subtropical forest reminiscent of that found in southeast China today (Alekseev and Alekseev 2016), which have been identified as belonging to at least 39 of the 82 modern families of schizophoran Diptera (von Tschirnhaus and Hoffeins 2009). Today, the Schizophora represent more than one-third of the species-level diversity of flies (56,000 species), organized into more than half of the recognized dipteran families (82; Pape et al. 2011), and unravelling the evolutionary history and relationships of the rapidly radiating Schizophora has proven a consistent challenge for systematists, remaining "one of the most difficult questions in systematic entomology" (Wiegmann and Yeates 2017).

Beginning with Linnaeus, who grouped what would eventually be recognized as the Schizophora into four genera (*Conops* L. 1758, *Musca* L. 1758, *Oestrus* L. 1758, and *Hippobosca* L. 1758), Diptera systematists have been slowly dividing up species into a dizzying number of higher taxa, often with conflict between concepts (Frey 1921, Hendel 1928, Crampton 1944). While many early workers left their mark upon the classification systems, it wasn't until Hennig's phylogenetic approach of creating a higher classification of Schizophora based on synapomorphies (Hennig 1958)

did the classification schema begin to approach stabilization (Table 1). By studying a remarkable diversity of taxa, Hennig grouped the Schizophora into 14 superfamilies and similar higher taxa, while also admitting his inability to place a large section of acalyptrate Diptera (a collection he labelled as having "Confused or Vague Affinities"). This work would prove to be one of the most influential dipterological works of the 20th century, and is still referred to in modern works for its role in shaping the classification of higher Diptera (Meier 2005, Woodley et al. 2009). Importantly, and in what would go on to become a trend among dipteran systematics, Hennig's publication of his classification alongside the characters supporting it allowed other dipterists to not just argue over classifications, but to actually test them, using character sets and systems of their own design set against the theory of apomorphy and shared evolutionary novelty. First in this new wave of scientific dipterology came Rohdendorf (1964, 1974), who, while noting the excellence and value of Hennig's attention to morphological detail, placed greater importance in the functional and ecological roles of phenotypes, and developed a classification of his own based primarily on these roles and on hypothetical ancestral reconstructions, concluding with 17 superfamily-level taxa (Table 1). The resulting classification differed quite radically from Hennig (1958), and from all subsequent attempts, including Griffiths (1972) and the near-concurrent publication by Hennig of a greatly expanded update to his assessment of schizophoran relationships (1973). Griffiths (1972), whose classification was based predominantly on characters of the male genitalia, suggested the Schizophora were properly classified into 13 higher groupings (Table 1), while Hennig's (1973) revised classification settled on 15 (Table 1). This textbook treatment of Schizophoran diversity and morphology by Hennig was the most comprehensive overview thus far developed, not least because Hennig included in his classification all of the fossil Schizophora thus far known, many of which he had previously examined and described himself (Hennig 1965, 1971), in addition to larval characters he similarly developed previously (Hennig 1948a, 1950, 1952). Yet, despite meticulously considered and supported synapomorphies for grouping families together, these works provided few hypotheses about how family groups were related to one another. Griffiths proposed a few relationships, namely the nine prefamilies constituting the "Muscoidea", as well as a few "family-group" assignments therein (Table 1), but Hennig, Rohdendorf and Griffiths explicitly discussed in their works the difficulty of reconstructing relationships among higher groups due to the extreme species diversity and amount of homoplasy seen across the Schizophora. It wasn't until J.F. McAlpine published his Phylogeny and Classification of the Muscomorpha (1989) as a part of the Manual of Nearctic Diptera Volume 3 (McAlpine and Wood 1989) did anyone provide a fully resolved Schizophora phylogeny using a cladistic methodology. This phylogeny and associated classification invoking 13 superfamilies (Table 1) had as significant an impact on the dipterological

community as Hennig did 30 years prior, and remains one of the most cited resources regarding the relationships of higher flies (Yeates and Wiegmann 2012), particularly as the dipterological community moved away from the qualitative systematics of Hennig, Griffiths, and McAlpine, and towards quantitative and molecular phylogenetics.

As algorithmic solutions for reconstructing phylogenies from character matrices became more accessible via computer software packages, dipterists increasingly experimented with new methods of translating flies into phylogenies, particularly among the lower Diptera (Wiegmann et al. 1993, Cumming et al. 1995, Oosterbroek and Courtney 1995, Michelsen 1996, Yeates 2002). However, the relationships of the Schizophora remained poorly tested using quantitative methods and morphology, save for a supermatrix approach (Yeates et al. 2007) that collated morphological character data presented in Griffiths (1972), Hennig (1973), and McAlpine (1989), and which returned a phylogeny very similar to that of McAlpine (1989). While this was the only broad phylogenetic study attempting to quantitatively test the relationships of the Schizophora in full, several other studies looked at relationships within families (e.g. Grimaldi 1990, 1997, McAlpine 1996) or superfamilies (e.g. Pape 1992, 2001). A similar trend was occurring with recently accessible and affordable DNA data, with most studies focusing on relationships within families (e.g. Desalle 1992, Smith and Bush 1997, Baker et al. 2001, Meier and Wiegmann 2002, Remsen and O'Grady 2002, Su et al. 2008, Marshall et al. 2009). It wasn't until the culmination of the Fly Tree of Life project that higher level relationships across the Schizophora were tested as comprehensively as Griffiths (1972), Hennig (1973), or McAlpine (1989), first using molecular data (Wiegmann et al. 2011), and later with quantitative analysis of morphology, albeit with a greatly reduced taxon set compared to Hennig, McAlpine, or Wiegmann et al. (Lambkin et al. 2013). Comparing the relationships proposed by these five major Schizophoran systematic studies (Griffiths 1972, Hennig 1973, McAlpine 1989, Wiegmann et al. 2011, Lambkin et al. 2013), it is immediately apparent how little congruency there is between proposed hypotheses; while Hennig (hesitantly) and McAlpine (definitively) proposed that Acalyptratae and Calyptratae were monophyletic sister taxa, Griffiths, Wiegmann et al., and Lambkin et al., suggested an acalyptrate grade with a monophyletic Calyptratae. Additionally, there is very little consensus regarding the relationships of the subordinate families and superfamilies within the Schizophora, including the sister taxon to the Calyptratae; Griffiths left the relationships of his broadly defined "Muscoidea" (which included the majority of schizophoran groups, including the Calyptratae) unresolved, while Wiegmann et al., returned Ephydroidea as sister to the Calyptratae, and Lambkin et al. suggested Tephritidae (representing the larger Tephritoidea) as sister to the calyptrates. The instability

of relationships among Schizophora continued as several more studies focusing on familial or superfamilial relationships included broad outgroups for comparison, with each resulting in a new series of hypotheses on the relationships of schizophoran Diptera (e.g. Han et al. 2002, Han et al. 2005, 2009, Han and Ro 2016, Kutty et al. 2008, Gibson et al. 2010, Kutty et al. 2010, Winkler et al. 2010, Tóthová et al. 2013). Pape et al. (2011) provided a synopsis of the relationships of Schizophora based on the broad evidence at their disposal via a classification schema that largely reflects the works of Hennig and McAlpine (Table 1), although they adopted a few relationships on the basis of Wiegmann et al. (2011) (see placement of Megamerinidae and Pallopteridae within Opomyzoidea). However, while there remains considerable debate regarding schizophoran relationships, four groups have largely been agreed upon as being monophyletic by modern systematists: Calyptratae, Sciomyzoidea, Lauxanioidea, and importantly, Nerioidea.

Nerioidea (=Micropezoidea prior to McAlpine (1989); see Appendix 1: Annotated Catalog of Family-group Names in the Nerioidea) are predominantly long-legged, charismatic flies that are largely saprophagous as larvae (Marshall 2012), although some lineages are much more enigmatic (e.g. Pseudopomyzidae). Historically, the membership of the Nerioidea has been a matter of considerable debate, and many taxa now placed elsewhere in the Schizophora have been allied with the Nerioidea: Tanypezidae (P. J. M. Macquart 1835, Blanchard 1840, J. Macquart 1843, Collin 1945, Martin L Aczél 1949b, 1951, 1954, 1955, 1959, 1961), Gobryidae (Enderlein 1922), Nothybidae (Osten-Sacken et al. 1882, Cresson 1912, Enderlein 1922), Scathophagidae (Loew 1862, Osten-Sacken 1878), Sciomyzidae (Westwood 1840), Sepsidae (Robineau-Desvoidy 1830), Richardiidae (Macquart 1835, 1843), Ulidiidae (Loew 1862), and Megamerinidae (McAlpine 1966, Colless and McAlpine 1970, 1991, McAlpine 1996, 1997b, 1997a, 1998). Similarly, nerioid taxa have been referred to several other families, including Dolichopodidae (Neriidae (*Longina* Wiedemann 1830): Bigot 1852, 1853), Opomyzidae (Micropezidae (*Calobata* Meigen 1803): Fallén 1820), Sepsidae (Micropezidae (*Calobata*): Walker 1860), Psilidae (Micropezidae (*Micropeza*), Neriidae (*Nerius*): Walker 1860), Milichiidae (Pseudopomyzidae (*Pseudopomyza* Strobl 1893): Hennig 1941, Frey 1952), Heleomyzidae (Pseudopomyzidae (*Heloclusia* Malloch 1933): Malloch 1933, Hennig 1958), Clusiidae (Cypselosomatidae (*Cypselosoma* Hendel 1913): Hennig 1948), Anthomyzidae (Pseudopomyzidae (*Pseudopomyza*, *Latheticomyia* Wheeler 1953): Hennig 1958) and Tanypezidae (Micropezidae (*Calobata*, *Micropeza* Meigen 1803, *Tanipoda* Rondani 1856 (=*Rainieria* Rondani 1843)): Rondani 1856; Micropezidae (*Calobata)*, Neriidae (*Nerius* Fabricius 1805): Tillyard 1926). However, following Hennig (1958) and the application of apomorphic characters to establish relationships and define taxa, most recent authors consider the

superfamily as containing four families, Neriidae (120 spp., pantropical), Micropezidae (<700 spp., worldwide), Cypselosomatidae (6 spp., Oriental), and Pseudopomyzidae (30 spp., worldwide but not in Africa). Proposed synapomorphies for the Nerioidea are reviewed by Buck and McAlpine (2010), and include several characters of the male and female genitalia. While the monophyly of the Nerioidea is largely accepted, the relationships of the Nerioidea, both internally and with respect to the remaining Schizophora, remain a matter of considerable debate.

Looking outwardly, Nerioidea are frequently considered to be one of the more "primitive" groups of acalyptrate Schizophora (Hendel 1916, Crampton 1944b, 1944a, McAlpine 1966, Shatalkin 1994, McAlpine and Shatalkin 1998) although there is little quantitative support for this hypothesis. Hendel (1916) regarded the Tyloidea (=Micropezidae + Neriidae) as related to his Sciomyzoidea, Tephritoidea and Sepsoidea based primarily on characters of the wing costa and head chaetotaxy. Séguy (1934), treating acalyptrate families "from the simple to the compound", placed the Tylidae (=Micropezidae) between the Lauxaniidae and Tanypezidae. Hendel (1936) placed Tylidae (=Micropezidae) and Neriidae within a larger group he called "Trypetides" and which consisted of nine families that are generally referred to the Tephritoidea today, plus Agromyzidae and Tanypezidae. Crampton (1944b, 1944a), who's concept of ancestry is not based on modern evolutionary theory, noted that the Calobatoidea (=Micropezidae + Calobatidae + Neriidae) are an "isolated, primitive group" on analysis of male genitalia, and suggests that the Calobatoidea are closely related to both the Platypezidae and Cordyluridae (=Scathophagidae), even going so far as to suggest that the lower Calyptratae, including the Cordyluridae, may be derived from Calobatoidea (and more specifically Micropezidae) "forebears among the Acalypteratae". Hennig (1952), in his Die Larvenformen der Dipteren III considers the Tanypezidae, Megamerinidae, and Clusiidae as being related to Tylidae (=Micropezidae including *Cypselosoma*, *Formicosepsis*, and Neriidae), together aligned with the Trypetoidea (=Tephritoidea), on the shared form and structure of the anterior spiracles. He would again draw connections between Nerioidea and Clusiidae by noting similarities of the aedeagus, as well as facial morphology between Clusiidae and *Pseudopomyza* and *Heloclusia* (Pseudopomyzidae), although he did not go so far as to suggest any formal relationships between these taxa (Hennig, 1958). Rohdendorf (1964, 1974) placed the Micropezidae (including Neriidae) and Cypselosomatidae in his Psilidea alongside Psilidae, Diopsidae, Megamerinidae, and Nothybidae. Hackman and Väisänen (1985) consider the Micropezoidea as being closely related to Conopidae+Otitoidea based on "primitive antenna characters". J.F. McAlpine (1989) treated the Diopsoidea as sister to the Nerioidea on the basis of wing vein morphology and general body form, while D.K. McAlpine (1996) provided characters of the head, wing, and male

aedeagus that he tentatively suggested may indicate relatedness between Cypselosomatidae and Clusiidae, but espoused more confident support in a sister group relationship between Nerioidea and Heleomyzoidea, specifically relying on characters shared between Pseudopomyzidae and Heleomyzidae with respect to head morphology and chaetotaxy, wing venation and chaetotaxy, and foreleg morphology. Entering the molecular era, nerioid exemplars were recovered as sister to a wide variety of taxa depending on gene region and analysis method, including Tephritoidea (Han et al. 2002, Gibson et al. 2010), Psilidae (Gibson et al. 2010), Aulacigastridae (Wiegmann et al. 2011), Tanypezidae (Han et al. 2016), Schizophora minus Conopidae+Diopsoidea (Han et al. 2005), and even polyphyletic, with nerioid taxa being allied with Psilidae, Sphaeroceridae, Heleomyzidae, Anthomyzidae, and Sepsidae when using short segments of cytochrome-c oxidase I ("DNA Barcodes" sensu Hebert et al. 2003) (Galinskaya et al. 2016).

While the placement of Nerioidea within the Schizophora has proven to be a challenge, the relationships within Nerioidea have been equally difficult to reconstruct, with nearly every possible arrangement of families and subfamilies having been suggested at one time or another. To complicate matters further, since 1900, Neriidae have been treated as a subordinate group of Micropezidae (Hendel 1903, Cresson 1912, Lamb 1914, Enderlein 1922, Cole 1923, Enderlein 1923, Cresson 1926, Curran 1934a, Hennig 1936, 1938, 1941c, Aczél 1949a, Hennig 1952, James 1954), Cypselosomatidae as a subordinate group of Micropezidae (Hennig 1941b, 1941c), and Pseudopomyzidae as a subordinate group of Cypselosomatidae (Griffiths 1972, Prado 1984, Hackman et al. 1985, McAlpine 1987, 1989, Wiegmann et al. 2011, Pape et al. 2011), while Micropezidae has been repeatedly split into either two (Curran 1934b, Crampton 1944b, 1944a, Martin L Aczél 1954, 1955, Frey 1958) or three family-level taxa (Hennig 1958, 1965, 1973). Several times, authors have flip-flopped on their family-level concepts of Neriidae and Micropezidae, with the most notable example, Hennig, repeatedly alternating (often without explanation or justification for his variations) between treating the Neriidae as a distinct family (Hennig 1936a, 1937, 1958) or as a subfamily of Micropezidae (Hennig 1936b, 1938, 1941c, 1952), before eventually settling on the most liberal family-level concept of Nerioidea thus far proposed, and consisting of Cypselosomatidae, Pseudopomyzidae, Neriidae, Micropezidae, Calobatidae, and Taeniapteridae (Hennig 1971, 1973). However, most subsequent authors have rejected Hennig's six-family treatment, choosing instead to recognize three or four families depending on their treatment of Pseudopomyzidae, and constraining the most speciose lineage of Nerioidea to a single family, Micropezidae.

Micropezidae is composed of nearly 700 species distributed around the world, from the subantarctic Kerguelen archipelago and Heard Island (*Calycopteryx moselyi* Eaton 1875) to north of the Arctic Circle (*Cnodacophora* Czerny, Merritt & Peterson 1976; *Compsobata* Czerny, *Paracalobata* Hendel, and *Calobata* Meigen, Greve & Nielsen 1991) (Fig. 1), although the greatest diversity can be found in the neotropics. Of the larvae that are known, most are saprophagous, living in dead, decaying plant material (Marshall 2010), with the exception of *Micropeza corrigiolata* (Linnaeus 1767), which inhabits healthy root nodules of legumes (Müller 1957). The family is divided into five subfamilies: Calycopteryginae, comprising a single species, *Calycopteryx moselyi*, restricted to the Kerguelen archipelago and Heard Island; Calobatinae, consisting of a few dozen Holarctic species; Taeniapterinae, the most diverse and widely spread of the Micropezidae, with more than 500 species found around the globe; Eurybatinae, representing roughly 50 species almost entirely restricted to the Oriental and Australasian regions save for a single outlier recently described from Costa Rica (Marshall 2002); and Micropezinae, composed of 84 species spread across the Palearctic, western Nearctic, and Neotropical region. The relationships between the subfamilies have been a matter of considerable debate, with Hennig (1936b, 1952, 1958), Aczél (1949b, 1949c, 1951), and later McAlpine (1975, 1998) each proposing their own arrangements of relationships between subfamilies, arrangements which often underwent considerable evolution as they studied additional specimens and species. Micropezidae are well-represented within Eocene Baltic amber deposits (Hennig 1965, Evenhuis 1994, von Tschirnhaus et al. 2009), and are also known from European Oligocene compression fossils (Statz 1940), Dominican amber from the Miocene (Grimaldi et al. 2005), and recent Afrotropical copal (Meunier 1906), although the systematic positions of most of these fossils remain debated and unresolved (McAlpine 1998). The relationships of Micropezidae, particularly Taeniapterinae, Calobatinae, and Eurybatinae, are currently the subject of considerable revision (see for example Ozerov 1991, McAlpine 1998, Marshall 2011, 2013, 2016, 2017, Ferro and Marshall 2018), leaving the relationships and diversity of Micropezinae yet to be addressed.

Micropezinae, defined by a circular antennal flagellomere, the complete absence of frontal bristles, and the absence of wing vein bm-m leaving a confluent wing cell bm+dm, has a complicated nomenclatural history. The genus *Tylos* was first described without the designation of a type species by Meigen (1800). Coquillet (1910) would eventually designate *Micropeza corrigiolata* as the type species, fulfilling the requirements of the Code, however by this time *Tylos* Andouin had already been established for a genus of Isopoda, making *Tylos* Meigen a junior homonym, which would eventually be suppressed by the International Commission on Zoological Nomenclature (1955), almost a decade before the ICZN suppressed Meigen's 1800 pamphlet in its entirety (ICZN 1963).

However, thanks to Hendel (1908), *Tylos* remained in use by many European authors, including Hennig, until the ICZN's 1955 decision. Three years after his original preview pamphlet, Meigen properly described the genus *Micropeza*, designating *Micropeza corrigiolata* as the type species by monotypy (Meigen 1803), and which would ultimately come to be the available, valid name for the genus. Over the course of the 20th century, multiple authors would erect new names to subdivide *Micropeza* into smaller genera or subgenera (Enderlein 1922, Cresson 1926, 1938, Hendel 1932, Aczél 1949c, Ozerov 1997), although today only two genera are typically recognized, *Cryogonus* Cresson 1926, and *Micropeza* Meigen 1803 (Marshall 2010).

Biogeographically, Micropezinae are most diverse in the New World, particularly the Neotropics, with 48 of the 77 described species occurring south of the United States, while 16 species are native to North America north of Mexico, and 16 species restricted to the Palaearctic (Fig. 2). This strong diversity within the neotropics, along with the presumably plesiomorphic *Cryogonus* naturally occurring in southern South America, lead Hennig (1936b) and Aczél (1949) to conclude that the subfamily originated in South America. Micropezinae was recorded from the Afrotropical region by Meunier (1906), in which he described a specimen preserved in Eocene Copal as *Micropeza prompta* Meunier, 1906. Upon examination of the illustrations in Meunier (1906) (the original fossil, supposedly deposited in the private collection of a "J. Evers", cannot be located), it is apparent that this specimen does not belong to *Micropeza* given the presence of vein bm-m which is clearly illustrated in his Figure 4 (reproduced in Fig. 3). Given the structure of the male terminalia as illustrated in his Figure 5 (see Fig. 3), it seems likely that this species should be transferred to Taeniapterinae, and potentially the genus *Aristobatina* Verbeke given Meunier's description of a very long fore first tarsomere, which is treated by Marshall (2014) as characteristic of this African-endemic genus. However, the western European *Micropeza corrigiolata* has been secondarily introduced to coastal South Africa (Barraclough 1996) in addition to eastern North America (Hoebeke and Wheeler 1993). *Micropeza lateralis* Meigen, 1826, a species native to western Europe, has been introduced to the Pacific Northwest region of North America (Waloff 1966, Hoebeke and Wheeler 2016). Important works in Micropezinae systematics and identification include Hennig (1936c) and Aczél (1949c) for their treatments of Neotropical species, Merritt and James (1973) and Merritt and Peterson (1976) for the Nearctic fauna, and Hennig (1936c), Freidberg (1984), and Ozerov (2008) for the Palaearctic fauna.

Little is known about the biology of micropezine flies, although Séguy (1951) reports that adults of *Micropeza corrigiolata* are "zoophagous", presumably in a similar fashion to the leaf surface

scavenging behaviour recorded in Calobatinae (Marshall 2006). Unlike Taeniapterinae, in which courtship and mating behaviours are well-reported (Wheeler 1924, Marshall 2006), there are no published observations for Micropezinae; Müller (1957) reports seeing no specific behaviours during his time rearing *Micropeza corrigiolata*. New data suggest Micropezinae exhibit a novel form of male grappling during copulation, with the male grasping the base of one of the female's femora using his fore tibiofemoral joint (Fig. 4). There are no recognized morphological ornamentations of the male fore legs, and it is unknown at this time whether the male is physically restraining the female, gaining leverage to avoid being dislodged, or whether this behaviour is a benign form of courtship (Baena and Eberhard 2007).

Micropezinae larvae appear to be closely associated with legumes (Fabaceae), with all known larval biologies and associations involving species of Fabaceae that possess indeterminate root nodules (Table 2). Müller (1957) recorded the larval biology and morphology of *Micropeza corrigiolata* in great detail, noting that larvae bore into the root nodules of peas and several common leguminous forage crops, feeding on the interior contents of the root nodule without consuming the nodule's cortex and parenchyma, suggesting that the larvae are preferentially feeding on the nitrogen-fixing rhizobia bacteria contained within the infection zone of the nodules (Foucher and Kondorosi 2000), in what could be considered a highly specialized form of microbial grazing. It is not known whether *Micropeza corrigiolata* larvae consume the contents of multiple nodules, or whether they remain within a single nodule feeding on the continually replaced plant tissue and associated bacteria. Upon completing their larval development, 3rd instar larvae leave the nodule and migrate into the soil to overwinter, completing pupation in the spring (Hoebeke and Wheeler 1994). Müller (1957) reports that *Micropeza corrigiolata* is univoltine in temperate areas but may be bivoltine in the southern reaches of its European range. The agricultural impact of *Micropeza corrigiolata* on peas or forage crops, either in regard to yield loss or nitrogen fixation in agroecosystems, has never been assessed. The larvae of all other species remain unknown and undescribed.

This thesis will assess the systematic position and status of Micropezinae using a molecular phylogenetic approach. First, a molecular phylogeny of Nerioidea will be produced to test the monophyly of Micropezidae and the relationships among its subfamilies. Next, a supermatrix of molecular data for the Schizophora will be analyzed in an attempt to unravel relationships among the higher flies, and particularly to place Nerioidea within Schizophora, hopefully revealing the ancestral relationships of the superfamily.

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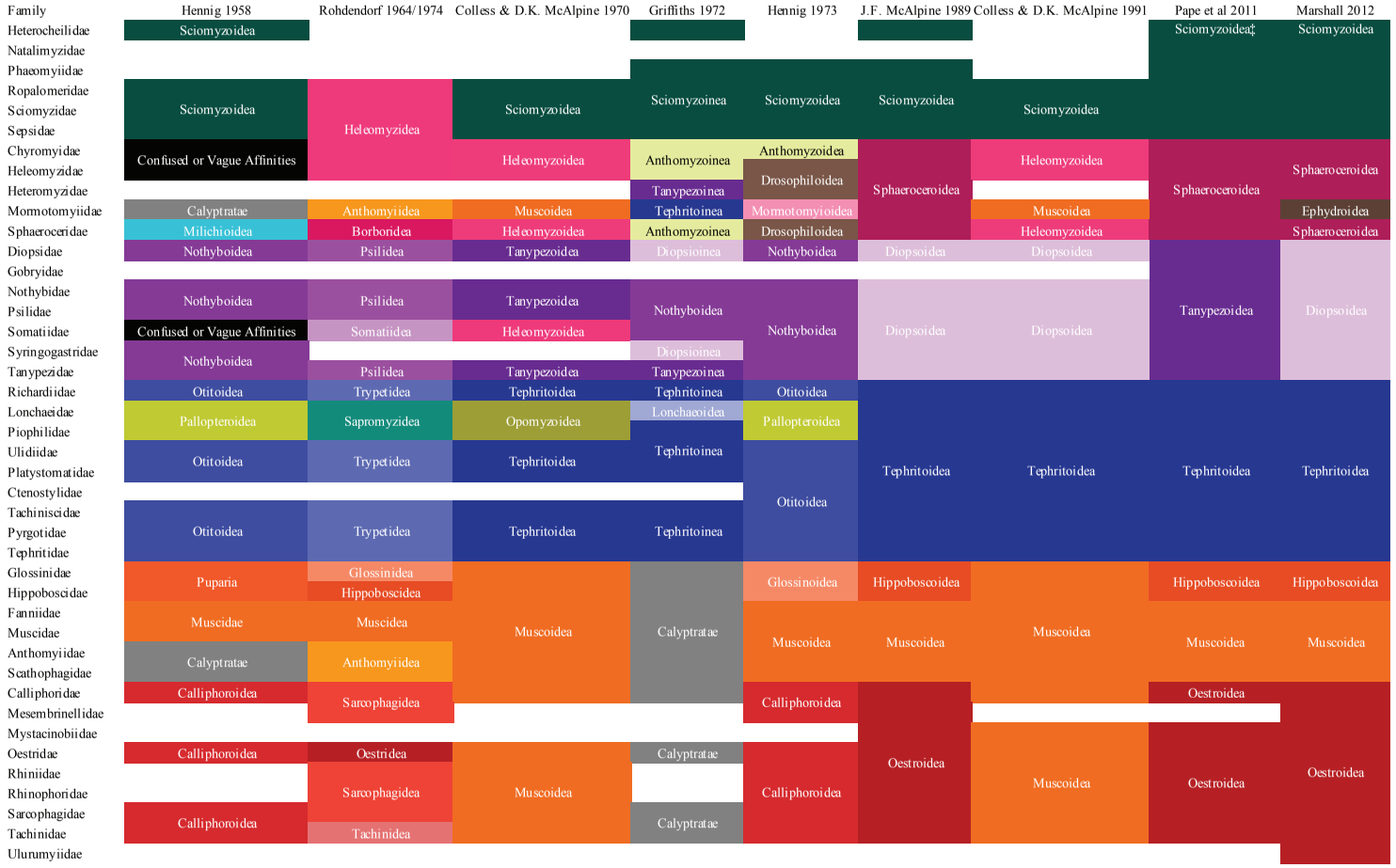
Table 1: Schizophora classification schemes through time. Names are coloured by superfamilies as arranged by Pape et al. 2011, with different shades indicating unique names but related names.

Table 1 (continued): Schizophora classification schemes through time. Names are coloured by superfamilies as arranged by Pape et al. 2011, with different shades indicating unique names but related names.



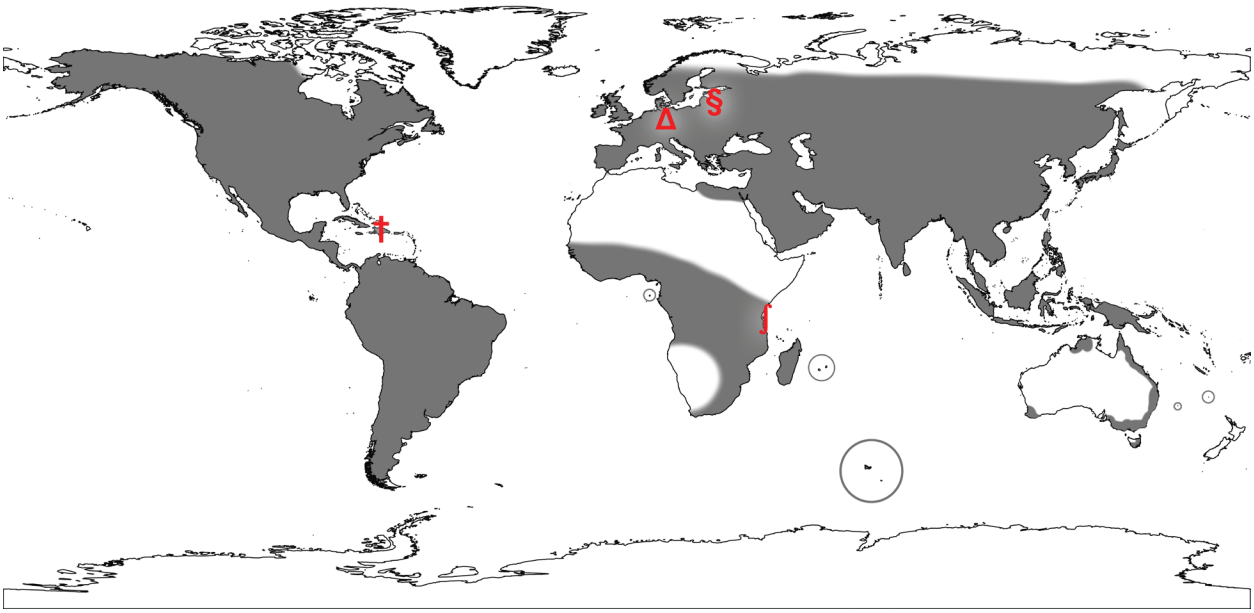


Figure 1: Global distribution of extant Micropezidae. Known Micropezidae fossils are represented as follows: ∫ – copal fossil, Pleistocene/Holocene, Taeniapterinae (putatively) (Meunier 1906); † – amber fossils, Oligocene/Miocene, Micropezinae (unpublished), Taeniapterinae (Grimaldi and Engel 2005); Δ – compression fossil, Oligocene, Calobatinae (Statz 1940); § – amber fossils, Eocene/Oligocene, Eurybatinae (Hennig 1965, von Tschirnhaus and Hoffeins 2009).

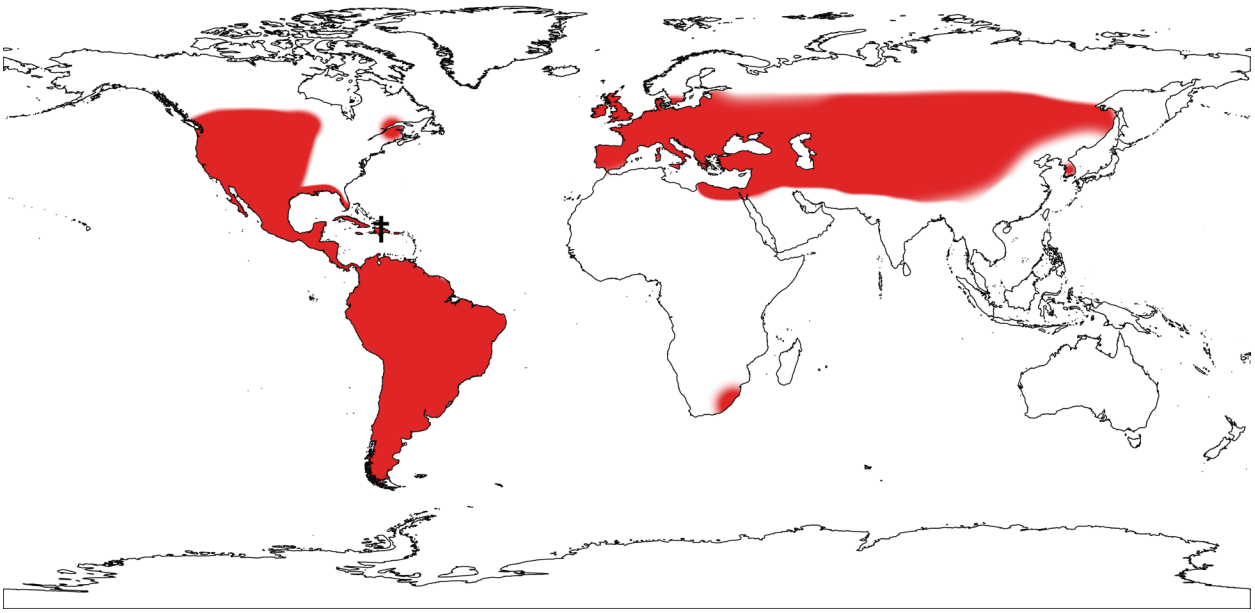


Figure 2: Global distribution of extant Micropezinae. Known Micropezinae fossils are represented as follows: † – amber fossil, Oligocene/Miocene, Micropezinae (unpublished).

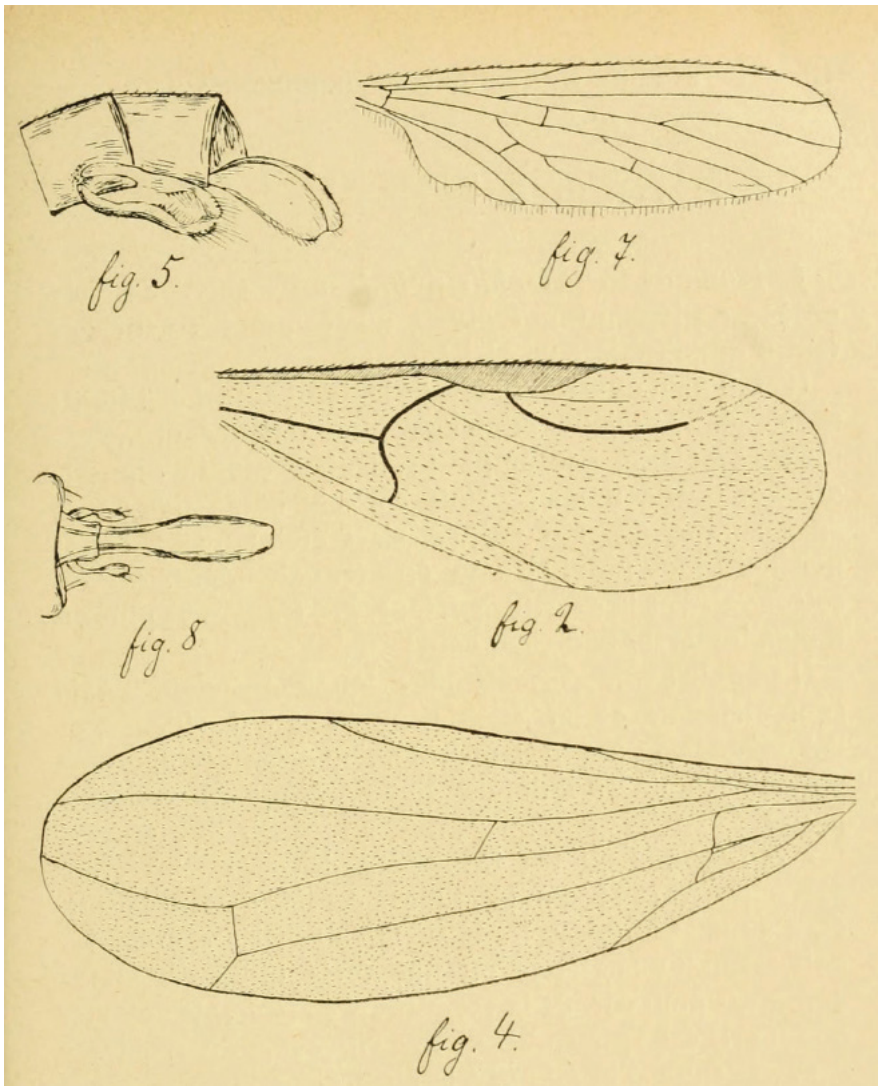


Figure 3: Illustrations of the copal fossil species *Micropeza prompta*, reproduced from Meunier (1906); “fig. 4” and “fig. 5” represent *Micropeza prompta*. Original figure captions from Meunier (1906): “Fig. 4. – Aile de *Micropeza prompta*, nov sp. No 15. (Copal fossile de Zanzibar.) 66 d.”, “Fig. 5. – Extrémité de l’abdomen de ce *Tanypezinæ*. 66 et 124.”.

Figure 4: *Micropeza stigmatica* in copula, Pima County, Arizona, USA, 24.v.2018. Photo by Chris Mallory, reproduced via CC-BY-NC license. iNaturalist observation no. 12799188 (https://www.inaturalist.org/observations/12799188).

Table 2: Summary of known larval hosts and associations for Micropezinae. All known host plants belong to Fabaceae.

	Natural Range	Host plant species	Known Biology	Reference
Micropeza corrigiolata (Linneaus)	Western Europe	*Pisum sativa* L. (Faboideae: Fabeae)	Host; larvae burrow into root nodules	Müller (1957)
		Trifolium pratense L. (Faboideae: Trifolieae)	Host; larvae burrow into root nodules	Müller (1957)
		Medicago sativa L. (Faboideae: Trifolieae)	Host; larvae burrow into root nodules	Müller (1957)
Micropeza lateralis (Fabricius)	Western Europe	*Cytisus scoparius* (L.) Link (Faboideae: Genisteae)	Association	Waloff (1966); Allen (1982); Hoebeke and Wheeler (2016); S. McCann (pers. comm.)
Micropeza littoralis (Albuquerque)	Brazil	'Leguminosae-Papillionae" sp. (Faboideae)	Association	Albuquerque (1967)
Cryogonus descolei Aczél	Argentina	*Prosopis rubriflora* Hassl. (Caesalpinioidea)	"Host"	new record (3 specimens from USNM)

CHAPTER 1 – A MOLECULAR PHYLOGENY OF NERIOIDEA (DIPTERA: SCHIZOPHORA)

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This chapter represents a collaborative research project prepared for peer-reviewed publication and includes input and contributions of varying degrees from multiple authors. The contributions of all authors are as follows:

M.D. Jackson: Project design, DNA extraction, sequencing, sequence alignment, phylogenetic analyses, production of all writing and all figures except live photographs.

J.H. Skevington: Specimen collection, project conception and design, mentorship and training of M.D. Jackson during project design and analysis, manuscript editing and revision, provided funding.

S.A. Marshall: Majority of specimen collection, project conception and design, mentorship and training of M.D. Jackson during project design and interpretation of results, manuscript editing and revision, provided major funding, provided live photographs.

S. Kelso: DNA extraction, sequencing, protocol vetting, mentorship of M.D. Jackson during DNA sequencing lab work.

N.A.M. Yusof: Specimen identification, input and discussions regarding relationships of Eurybatinae.

G.B. Ferro: Specimen identification, input and discussions regarding relationships of Taeniapterinae.

Introduction

Nerioidea are morphologically diverse flies composed of roughly 800 species, and are found globally, from the subantarctic Kerguelen archipelago and Heard Island (*Calycopteryx moselyi* Eaton 1875;) to north of the Arctic Circle (*Cnodacophora*, Merritt & Peterson 1976; *Compsobata*, *Paracalobata*, and *Calobata*, Greve & Nielsen 1991), although the greatest diversity occurs pantropically. While the biology of nerioid flies is generally poorly known, particularly with respect to larval biology, some species have been the focus of physiological and evolutionary biology research (Barrio *et al.* 2014; Bonduriansky 2006; Bonduriansky & Head 2007; Kawasaki *et al.* 2008; Renault & Lalouette 2011), and their elaborate courtship rituals have drawn the attention of naturalists for generations (Dumbardon-Martial & Marshall 2015; Eberhard 1998; Wheeler 1924). Given the diversity of these flies, and their potential for ecological, evolutionary, and physiological research, it is of interest to understand how they are related to one another, and it is important that those relationships are reflected in their classification. Historically there has been a wide diversity of taxonomic concepts and classifications proposed, with little consensus among them.

Today, Nerioidea is widely considered to consist of Neriidae, Micropezidae, Pseudopomyzidae, and Cypselosomatidae (Buck and McAlpine 2010; Marshall 2010, 2012) (Fig. 1); however the composition of the superfamily has long been a matter of debate. The family classification of Nerioidea was unsettled in the early 20th century with several fluid concepts emerging, not only on alternative taxonomic compositions (Table 1), but also classificatory ranks, with Neriidae often being treated as a subordinate group of Micropezidae (Hendel 1903, Cresson 1912, Lamb 1914, Enderlein 1922, Cole 1923, Enderlein 1923, Cresson 1926, Hennig 1936, 1938, 1941c, Hennig 1952, James 1954), and Cypselosomatidae as a subordinate group of Micropezidae (Hennig 1941b, 1941c), while Micropezidae was repeatedly split into two family-level taxa (Aczél 1954, 1955, Crampton 1944b; a; Curran 1934b; Frey 1958). Several authors presented Neriidae as either a subordinate group of Micropezidae or as an independent family in contemporaneous publications (e.g. Curran 1934a, 1934b and Aczél 1949b, 1949c, 1949a), while Hennig, between 1936 and 1958, repeatedly alternated between treating Neriidae as a distinct family (Hennig 1936a, 1937, 1958) or as a subfamily of Micropezidae (Hennig 1936b, 1938, 1941c, 1952) before eventually proposing the most liberal family-level concept of Cypselosomatidae, Neriidae, Micropezidae, Calobatidae, and Taeniapteridae (Hennig 1958). Following McAlpine's (1966) erection of Pseudopomyzidae within Nerioidea, and subsequent reinforcement by Griffiths (1972) and Hennig (1971, 1973), Nerioidea (as Micropezoidea/oinea prior to McAlpine et al. 1981 and subsequent volumes) has been recognized as comprising four families, although several authors have treated Pseudopomyzidae

as a subordinate group of Cypselosomatidae (Griffiths 1972, Prado 1984, Hackman et al. 1985, McAlpine 1987, 1989, Wiegmann et al. 2011, Pape et al. 2011), and Hennig maintained his three family-level Micropezidae concept (Hennig 1971, 1973). So, although the taxonomic community has settled on the taxonomic composition of Nerioidea, the relationships of the superfamily, both externally and internally, are still very much up in the air.

Nerioidea have frequently been considered one of the more "primitive" groups of acalyptrate Schizophora (Crampton 1944b; 1944a; Hendel 1916; McAlpine 1966; McAlpine & Shatalkin 1998; Shatalkin 1994) although there is little quantitative support for this hypothesis. In the pre-cladistics era, Hendel (1916) regarded the Tyloidea (=Micropezidae + Neriidae) as related to his Sciomyzoidea, Tephritoidea and Sepsoidea based primarily on characters of the wing costa and head chaetotaxy. Séguy (1934), treating acalyptrate families "from the simple to the compound", placed the Tylidae (=Micropezidae) between the Lauxaniidae and Tanypezidae. Hendel (1936) referred Tylidae (=Micropezidae) and Neriidae to a larger group he called "Trypetides" and which consisted of 9 families that are generally referred to as Tephritoidea today, plus Agromyzidae and Tanypezidae. Crampton (1944b, 1944a) noted that the Calobatoidea (=Micropezidae + Calobatidae + Neriidae) are an "isolated, primitive group" on analysis of male genitalia, suggesting that Calobatoidea are closely related to both the Platypezidae and Cordyluridae (=Scathophagidae), even going so far as to suggest that the lower Calyptratae, including the Cordyluridae, may be derived from Calobatoidea or specifically Micropezidae. In the cladistic era, Hennig (1952), considers the Tanypezidae, Megamerinidae, and Clusiidae as being related to Tylidae (=Micropezidae, including *Cypselosoma* Hendel, *Formicosepsis* Meijere, and Neriidae), together aligned with the Trypetoidea (=Tephritoidea) on the shared form and structure of the anterior spiracles in third-instar larvae. Rohdendorf (1964, 1974) placed the Micropezidae (including Neriidae) and Cypselosomatidae in his Psilidea alongside Psilidae, Diopsidae, Megamerinidae, and Nothybidae. Hackman and Väisänen (1985) considered the Micropezoidea as being closely related to Conopidae+Otitoidea based on "primitive antenna characters". J.F. McAlpine (1989) treated the Diopsoidea as sister to the Nerioidea on the basis of wing vein morphology and general body form, while D.K. McAlpine (1996) provided characters supporting a sister group relationship between Nerioidea and Heleomyzoidea, specifically characters shared between Pseudopomyzidae and Heleomyzidae with respect to head morphology and chaetotaxy, wing venation and chaetotaxy, and foreleg morphology. In their supertree meta-analysis of morphological character data from Griffiths (1972), Hennig (1973), and McAlpine (1989), Yeates *et al.* (2007) recovered Diopsoidea as sister to Nerioidea. Finally, in the molecular era, nerioid exemplars were recovered as sister to a wide

variety of taxa depending on gene region sampled and analysis method, including Tephritoidea (Han et al. 2002, Gibson et al. 2010), Psilidae (Gibson *et al.* 2010b), Aulacigastridae (Wiegmann et al. 2011), Tanypezidae (Han et al. 2016), Schizophora minus Conopidae+Diopsoidea (Han et al. 2005), and even polyphyletic, with nerioid taxa being allied with Psilidae, Sphaeroceridae, Heleomyzidae, Anthomyzidae, and Sepsidae when using short segments of cytochrome-c oxidase I ("DNA Barcodes" sensu Hebert et al. 2003) (Galinskaya *et al.* 2016).

Within Nerioidea, following the erection of Pseudopomyzidae (McAlpine 1966), three hypotheses regarding the relationships of the four nerioid families emerged (excluding Megamerinidae *sensu* McAlpine 1966, 1997b, 1998). McAlpine (1966), despite an extensive morphological comparison between Cypselosomatidae, Neriidae and Micropezidae proposed the four families "may be arranged in a linear series according to degree of morphological specialization" with Pseudopomyzidae basally and a derived sister relationship between Neriidae and Micropezidae (Fig. 2a). This relationship would later be supported by Shatalkin's (1994) interpretation of male genitalia, particularly the structure and evolution of his aedeagal dorsal sclerite (= phallic plate of Marshall (2014)). While McAlpine (1996) explicitly stated his opinion that characters uniting Pseudopomyzidae and Cypselosomatidae were the result of sympleisomorphy or convergence, most other authors recognize Cypselosomatidae and Pseudopomyzidae as a monophyletic group defined by characters of the male abdomen and genitalia, thus leaving the greatest debate surrounding Neriidae and whether it is more closely related to Cypselosomatidae+Pseudopomyzidae as presented by McAlpine (1989), Wiegmann *et al.* (2011), and Koch *et al.* (2015) (Fig. 2b), or Micropezidae, as supported by Griffiths (1972), Hennig (1971, 1973), and Yeates *et al.* (2007) (Fig. 2c). As previously mentioned, Hennig's final conclusions (1971, 1973) on the relationships between Neriidae and Micropezidae *sensu lato* are complex.

The mid-20th century saw numerous family-level taxonomic concepts emerge as several authors worked to understand the relationships of nerioid specimens collected from around the world. Three authors in particular, Martín L. Aczél, Willi Hennig, and David K. McAlpine, were instrumental to the development of Nerioidea classification and relationships. While Hennig's application of Neriidae at the family-level between his 1936a work on copulatory organs in the higher Diptera and his 1952 work on larval Diptera was inconsistent, he quite clearly accepted throughout that Neriidae should be considered a subordinate group of Micropezidae, and was more closely related to his Tylinae (= Micropezinae, comprised of Tylini and Trepidariini (=Calobatini)) than to Taeniapterinae (Hennig 1936b, 1952) (Fig. 3a). Hennig's classification was

first challenged by Aczél, who first argued that Neriidae was a separate family sister to Tylidae while changing his mind on the subfamilial relationships of Micropezidae (Aczél 1949b; a) (Fig. 3b). Aczél (1951; pg. 513) subsequently agreed with Hennig's assessment, going so far as to present a classification system with Taeniapterinae elevated to the family rank (Fig. 3c), although he confusingly followed Micropezidae *s. lat.* (separate from Neriidae) for the remainder of the work. Aczél later (1954) revised the Neriidae, redefining the tribal classification of that family based solely on the structure of the antennal bases. Meanwhile, Hennig applied his newly minted theory of phylogenetic systematics to a broadened study of Schizophora without establishing synapomorphies for Micropezidae *s. lat*., and following Aczél's family-level treatment of Micropezidae while maintaining that Taeniapteridae was sister to Neriidae+Tylidae+Trepidariidae (=Neriidae+Micropezinae+(Calobatinae+Eurybatinae) (Hennig 1958) (Fig. 3d). Aczél would not live to see Hennig's treatment, having passed away in 1958 (Mello 2010), although in two works published posthumously (Aczél 1959, 1961), it seems he had come to accept Neriidae as an independent family sister to Micropezidae *s. str.*, while still considering Micropezinae and Trepidariinae as sister taxa (Fig. 3e). D.K. McAlpine entered the discussion in 1966, recognizing Neriidae as an independent family that was not the sister group of Micropezidae, and arguing that Micropezidae and Megamerinidae were sister taxa (Fig. 3f). This idea was rejected by Hennig (1971, 1973) in his final works involving Micropezidae. While he believed Neriidae arose from within Micropezidae, he was unable to find synapomorphies to support his theory, instead settling on an unresolved, four-family classification, explicitly noting he believed that his Trepidariidae was not monophyletic. Griffiths (1972) echoed Hennig's rejection of Megamerinidae belonging to Nerioidea, but chose to recognize Neriidae and Micropezidae *s. lat.* as individual families pending further research. Finally, in his first study devoted to Micropezidae systematics, McAlpine (1975) erected two new subfamilies (Calycopteryginae for the enigmatic and monotypic subantarctic genus *Calycopteryx* Eaton, and Eurybatinae for a collection of Australasian genera previously considered part of Calobatinae/Trepidariinae by previous authors, including Hennig), and proposed a sequential relationship between subfamilies, anchored by Calycopteryginae and with Taeniapterinae sister to Eurybatinae (Fig. 3g), relationships he maintained following his comprehensive review of the Australian Micropezidae (McAlpine 1998).

There has been little consensus on the relationships of Nerioidea based on morphological data, providing an opportunity to test these relationships with molecular characters. Here, we employ a large molecular dataset and diverse taxon sampling from across Schizophora to produce the first molecular phylogeny of Nerioidea.

Materials & Methods

Taxon and Gene Region Sampling

In order to thoroughly test the higher relationships of Nerioidea, 77 species representing all families, subfamilies, and tribes of Nerioidea were sampled or retrieved from GenBank, except for Calycopteryginae (Table 2, 3). Thirty-nine species of higher Diptera representing 18 families are used as outgroups, with Syrphidae rooting all analyses.

13 gene regions were sampled for analysis: Cytochrome c Oxidase I (COI), Cytochrome c Oxidase II (COII), Cytochrome b (cytB), 12S Ribosomal DNA (12S), 16S Ribosomal DNA (16S), 28S Ribosomal DNA (28S), Alanyl-tRNA synthetase (AATS), the carbamoyl phosphate synthetase region of CAD (CAD), elongation factor 1α (EF-1α), phosphogluconate dehydrogenase (PGD), triose phosphate isomerase (TPI), *white* (wht), and *wingless* (wng).

DNA Extraction, Amplification, and Sequencing

DNA extraction and polymerase chain reaction (PCR) amplification followed Gibson *et al.* (2010b), except ExTaq polymerase and associated mixture recipes were used for all reactions. PCR primers used and annealing temperatures for each gene region sampled are listed in Table 4. PCR amplification products were visualized on 1% agarose electrophoresis gel, and purified using the E-Gel® system (Invitrogen TM, Carlsbad, California, USA) following Gibson *et al.* (2010a). Sequencing of purified products followed Gibson *et al.* (2010b). GenBank accession numbers for previously published sequences are listed in Table 2.

Sequence Editing and Alignment

Sequence chromatograms were visualized and contigs built using Geneious® v10.2.2 (Biomatters Ltd., Auckland, New Zealand), and then exported for management and alignment in Mesquite v3.3 (Maddison & Maddison 2017). Protein coding genes were aligned by hand in Mesquite via amino acid translation, while ribosomal genes were aligned using the online server for MAFFT v7 (Katoh & Standley 2013), available at https://mafft.cbrc.jp/alignment/server/. A progressive method of alignment (FFT-NS-2) was used with default parameters. Ribosomal sequences were then checked by hand and minor modifications were made via Mesquite to fix alignments where

small regions of sequence were misaligned. A 76 bp intron was identified and excluded in the PGD dataset, corresponding to characters 528-604 of the alignment. 842 characters of 28S rDNA data (aligned positions: 426-521, 770-880, 1072-1110, 1125-1188, 1919-1929, 2194-2425, 2943-2999, 3065-3146, 3802-3922, 4605-4633) were excluded from final analysis because the characters could not be confidently aligned across all taxa with either MAFFTT or by hand. Sequences were concatenated using Mesquite. Sequence alignments are available at https://doi.org/10.5063/F1PK0DDV.

Data Analysis

PartitionFinder 2 (Lanfear *et al.* 2016) implemented on the CIPRES Science Gateway v3.3 (Miller *et al.* 2010) was used to assign partitions and evolutionary models for both nucleic acid and amino acid data under the Akaike Information Criterion c (AICc) and using the "greedy" scheme. A general time reversible model with gamma distribution and proportion of invariable sites (GTR+I+G) was returned as the best model for all genes and codon positions for the nucleic acid dataset (24 partitions analyzed), while the amino acid model selection is presented in Table 5.

Nucleic acid data were analyzed using Bayesian inference and maximum likelihood, while amino acid data were analysed using maximum likelihood. The Bayesian inference nucleic acid data were analysed using RAxML v8.2.10 (Stamatakis 2014) on the CIPRES Science Gateway v3.3 (Miller *et al.* 2010); a partition block produced by PartitionFinder2 was included in the analysis file, and GTRGAMMA+I was used for the rate model, with 100 bootstrap replications. The resulting tree was visualized in Mesquite. Maximum likelihood analysis of amino acids was performed in RAxML Next Generation (RAxML-ng ver. 0.6.0 BETA) (Kozlov *et al.* 2018) in macOS High Sierra (ver. 10.13.1) using the command "./raxml-ng --all --msa DATA.phy --model DATApartitions.txt --prefix DATA --threads 1 --brlen scaled --tree pars{10} --seed 12345". Bayesian inference of nucleic acids was performed using Mr.Bayes v3.2.6 (Ronquist & Huelsenbeck 2003) and Mr.Bayes 3.2.2 on XSEDE Restart Interface on the CIPRES Science Gateway; a partition block produced by PartitionFinder2 was included in the analysis file to set partitions and associated evolutionary model and rates. A Markov Chain Monte Carlo (MCMC) method was selected, with four chains (3 hot, 1 cold) run simultaneously for 50,000,000 generations, sampling trees every 1,000 generations. Tracer v1.7.1 (Rambaut *et al.* 2018) was used to assess stationarity by monitoring effective sample sizes (ESS) for all parameters of the combined analysis and to calculate the log-likelihood values for the best tree. A burn-in of 50% was specified, and the resulting majority-rule consensus tree

with posterior probability estimation for each node and relative branch lengths was visualized in Mesquite.

Results

Once introns and unalignable regions were excluded, a final concatenated dataset of 14,286 bp was analyzed (lengths, in bp, by loci: 12S, 643; 16S, 613; 28S, 4,033; AATS, 563; CAD, 2151; COI, 1509; COII, 537; cyt-B, 748; EF-1a, 1059; PGD, 790; TPI, 498; *white*, 461; *wingless*, 681). The Bayesian analysis was deemed to have reached stationarity after 5 million generations, with average standard deviation of split frequencies = 0.0476, log-likelihood values for the best tree of the Bayesian analysis = -186,732.376, and all ESSs above 350. Both nucleic acid analyses (ML and BI) returned trees with highly congruent and strongly supported nodes, particularly within the ingroup (Fig. 4A, but see Sup. Fig. 1. for differential topology of outgroups from BI), while ML analysis of amino acid data returned a tree with weak branch supports and differences in topology (Fig. 4B). The nucleic acid dataset and resulting tree are considered the preferred analysis for purposes of further discussion.

Discussion

Relationships of Schizophora

Relationships and branch supports of outgroup taxa differed between analysis methods (See Supplementary Figure 1 for outgroup relationships using Bayesian inference analysis of nucleic acids), however we found no support for the hypothesis that Nerioidea are a basal lineage of Schizophora, instead recovering Conopidae+Lauxaniidae as the strongly supported sister group to the remaining Schizophora. This relationship between Conopidae, Lauxaniidae, and the remaining Schizophora was also recovered by Gibson *et al.* (2010b) with DNA data analyzed using Bayesian inference. Wiegmann *et al.* (2011), when analyzing a combined dataset of morphological and molecular characters under Bayesian inference (Fig. S2 in Wiegmann *et al.* 2011), also recovered Conopidae+Lauxanioidea as sister to the remaining Schizophora, although their primary analysis with increased taxon coverage placed Conopidae as paraphyletic within Sciomyzoidea, itself deep within Schizophora. Lambkin *et al.* (2013) recovered Conopidae as sister to the remaining Schizophora using morphological evidence, but Lauxaniidae was considered only distantly related.

We found no evidence of a sister group relationship between Nerioidea and Diopsoidea *sensu* McAlpine (1989), and further, with comprehensive family-level sampling, did not recover

a monophyletic Diopsoidea. Tanypezidae was recovered as sister to Platystomatidae, while Nothybidae was recovered as sister to Sphaeroceridae. However, while we generally recovered strong branch supports for individual families, backbone support between families was weak, as was taxon sampling across Schizophora, and relationships between outgroup taxa should be considered preliminary at best. We also found no evidence of a relationship between Megamerinidae and Nerioidea, with the former being recovered as polyphyletic due to a lack of overlap in gene regions analyzed for the two species included. Wiegmann *et al.* (2011) recovered Megamerinidae as sister to Schizophora minus Ephydroidea+Calyptratae when only molecular data was analyzed (Fig. 1 in that study), and while further research will be necessary to fully address the relationships of this family, molecular data do not support McAlpine's placement of Megamerinidae within Nerioidea (McAlpine 1997c).

Regarding the sister taxa to Nerioidea, we recovered a poorly supported clade composed of families representing Sphaeroceroidea, Diopsoidea, and Opomyzoidea as sister to Nerioidea. While this assortment of taxa most closely associated with Nerioidea would seem to provide little insight into the evolution of Nerioidea, it is worth re-examining historical treatments of Nerioidea and the four families recovered here as potential sister taxa to Nerioidea. Nothybidae have been the least associated with Nerioidea historically, despite similarities in general appearance and behaviour, including courtship rituals involving nuptial gifts and trophallaxis (Paiero & Marshall 2014). *Nothybus* was treated as a genus of Micropezidae by several early authors (Cresson 1912; Enderlein 1922; Osten-Sacken 1882) before being removed to its own family by Frey (1927). In his early systematic treatment of Micropezidae, Hennig did not know how to deal with *Nothybus*, only stating that its relationships were unclear (Hennig 1936c), while Aczél (1955) postulated that the family may be sister to the remaining Acalyptratae. Subsequent workers rejected Aczél's theory (arguments summarized by Lonsdale & Marshall 2016), instead treating the family as part of Diopsoidea (sometimes deemed Nothyboidea), or more closely related to Teratomyzidae (Griffiths 1972) or Ephydroidea (McAlpine 1997b). The only quantitative treatment to include exemplars of Nothybidae and Nerioidea was Galinskaya *et al.* (2016), who recovered Nothybidae as sister to Neriidae+Sepsidae or Conopidae+Lauxaniidae depending on analysis method.

Opomyzoid taxa, particularly Clusiidae, have been repeatedly linked to Nerioidea. Hennig (1948b) transferred *Cypselosoma* and *Formicosepsis* into Clusiidae, and later drew comparisons between the larvae of Micropezidae, Neriidae, and Clusiidae (Hennig 1952), as well as the distinct basal cone of the aedeagus shared by Clusiidae and Micropezidae, while noting the sclerotization of the

face in Clusiidae was reminiscent of that found in *Pseudopomyza* and *Heloclusia* (Hennig 1958). Later, McAlpine would recognize similarities in head chaetotaxy, wing morphology, and aedeagal structure between Clusiidae and Cypselosomatidae, although he considered these to be the result of convergence and unlikely to support relationships between these taxa. The only quantitative study to include both Clusiidae and Nerioidea exemplars was Wiegmann *et al.* (2011), who recovered Clusiidae as sister to Pallopteridae+Neurochaetidae, far removed from Nerioidea. The inclusion of Clusiidae in Opomyzoidea has been called into question using both molecular data (Winkler *et al.* 2010), and morphological characters (Lonsdale *et al.* 2010), and the relationships of the family remain tentative. Comparison to Nerioidea may prove fruitful in future work on the relationships of Clusiidae.

Nerioidea species have at times also been treated with Anthomyzidae, another putative opomyzoid family. Few quantitative studies have included exemplars from both Anthomyzidae and Nerioidea, however Galinskaya *et al.* (2016) recovered *Rainieria* within a clade containing Anthomyzidae, Sphaeroceridae, and Heleomyzidae, while Wiegmann *et al.* (2011) found no relationship between Nerioidea and Anthomyzidae, with the latter being recovered as sister to Heleomyzidae. Hennig (1958) suggested *Pseudopomyza* and *Latheticomyia* (Pseudopomyzidae) could be aligned with either Anthomyzidae or Heleomyzidae before agreeing with D.K. McAlpine (1966) that both genera belong to Nerioidea (Hennig 1971). McAlpine (1966) considered Heleomyzoidea (=Sphaeroceroidea) as the probable sister group to Nerioidea by McAlpine (1996), particularly noting similarities between Heleomyzidae and Pseudopomyzidae, a pairing he considered to be the sister group to the remaining Nerioidea.

While each of the most closely related outgroups has at one point or another been linked to Nerioidea using morphology, better taxon sampling will be required to properly test and assess these relationships.

Monophyly of Nerioidea and Relationships of its Families

The four families traditionally treated as Nerioidea were recovered as monophyletic with strong branch support in both nucleic acid and amino acid analyses, as were each of the independent families. Monophyly of Nerioidea (excluding Megamerinidae) has historically been supported on the basis of abdominal and post-abdominal characters (Griffiths 1972; McAlpine 1989; Sinclair *et al.* 2013). In our analyses, Micropezidae was recovered as sister to the remaining Nerioidea with high support, corroborating the work of J.F. McAlpine (1989), Wiegmann *et al.* (2011) and

Koch *et al.* (2015). However, the relationships between Cypselosomatidae, Pseudopomyzidae, and Neriidae (termed CPN henceforth) remain less stable, with the preferred nucleic acid data returning Pseudopomyzidae sister to Cypselosomatidae (Fig. 4A) *sensu* McAlpine (1989), while amino acid data returned Pseudopomyzidae sister to Neriidae (Fig. 4B), a relationship considered plausible by McAlpine (1996). While J.F. McAlpine (1989), in treating Diopsoidea as sister to Nerioidea, provided several putative synapomorphies supporting CPN, D.K. McAlpine (1996, 1998) considered most of these characters to be plesiomorphic or weak for defining CPN when Sphaeroceroidea was treated as sister to Nerioidea.

A potential synapomorphy for CPN proposed by McAlpine (1998) is the number of slits in the posterior spiracle of third instar larvae. Ferrar (1987) discusses and illustrates these "unusual and distinctive" characters across Nerioidea (Table 7), and McAlpine proposed that three slits was the groundplan for Micropezidae, while the known larvae of Neriidae and Cypselosomatidae both have four. Looking more broadly at proposed and recovered sister taxa to Nerioidea, Sphaeroceroidea, Diopsoidea, Clusiidae, and Anthomyzidae, are all reported to have three slits where larvae are known (Ferrar 1987), lending support for the four slits shared by the few known CPN larvae as being apomorphic. Larvae of Pseudopomyzidae remain poorly known and undescribed (Krivosheina (1979) reared larvae of *Polypathomyia stackelbergi* to adulthood but did not describe the larvae, while Roháček (2012) reported a mass occurrence of adults and probable larval habitat but did not find larvae), and finding and describing third instar Pseudopomyzidae larvae will be vital to test and better understand the relationships of this clade.

The placement and family-level treatment of Pseudopomyzidae remains difficult to ascertain. While others have tested the relationships of Pseudopomyzidae (Wiegmann *et al.* 2011; as Cypselosomatidae) and Cypselosomatidae (Koch *et al.* 2015) with other Nerioidea, this is the first quantitative study to test the relationships of both with other Nerioidea, and we find the relationships between these two families to be dependent on the data analyzed. Better taxon sampling within both families will be necessary to fully resolve the relationships within the CPN clade, however it seems prudent that Cypselosomatidae and Pseudopomyzidae be maintained as independent families until further evidence can be acquired.

Within Neriidae we recovered a paraphyletic Neriinae and monophyletic Telostylinae, a result at odds with Buck (2010) who argued Telostylinae, with their simple antennal bases, was possibly paraphyletic and Neriinae, with their prominent antennal bases, was likely monophyletic. Koch *et*

al. (2015), the only other study to quantitatively test the internal relationships of Neriidae, failed to recover either subfamily as monophyletic, instead recovering *Telostylus* as sister to the remaining Neriidae and *Chaetotylus* sister to the New World Neriinae. With Neriinae at the base of the tree, it would appear the defining characters of the subfamily relationships are inadequate or incorrectly polarized. However, our taxon sampling is minimal, particularly among New World genera, and there remains much work left to fully test and resolve the relationships within this charismatic family.

Monophyly of Micropezidae and Relationships of its Subfamilies

We here recover a monophyletic Micropezidae, refuting Hennig's concept of relationships between Neriidae and Micropezidae *sensu lato* (Hennig 1971, 1973). The relationships of subfamilies in Micropezidae recovered by both nucleic acid and amino acid datasets represent novel hypotheses about the evolution and classification of the family. Most importantly, we recovered Eurybatinae as paraphyletic, with Metopochetini being strongly supported as sister to Micropezinae, while Eurybatini was recovered as sister to Taeniapterinae. Calobatinae was returned as sister to Metopochetini+Micropezinae in our preferred tree (Fig. 4A), but sister to all remaining Micropezidae using amino acids, however relationships between subfamilies and tribes using amino acids were statistically poor or unsupported (Fig. 4B). Calobatinae has often been associated with Micropezinae (Aczél 1949b, 1951, 1959; Cresson 1938; Griffiths 1972; Hennig 1936b, 1952; D.K. McAlpine 1966), albeit while concepts of the former still included what would become Eurybatinae. In the modern 5 subfamily classification, D.K. McAlpine (1975, 1998) treated Calobatinae as sister to all Micropezidae other than *Calycopteryx*, as here recovered by our amino acid analysis.

A sister group relationship between Metopochetini and Micropezinae was previously considered by McAlpine (1998), and is recovered with high support in our preferred analysis. McAlpine (1998) noted both groups lack the axillary fascicle on the wing base, and that both possessed a complete mesoscutal grove across the thorax, although he eventually placed greater confidence in the structure of the male sternite 6 that linked Metopochetini with Eurybatinae. It seems that McAlpine based his interpretation of sternite 6 in *Micropeza* on examination of two European species (*Micropeza corrigiolata* and *Micropeza nigra* Loew, 1873; McAlpine 1998; pg 127) in which the secondary sexual characters of sternite 5 in the male are greatly reduced. Sternite 6 of most Micropezinae in which the copulatory fork remains present conform to McAlpine's description of "triradiate", and thus a modified sternite 6, whether triradiate or otherwise, may be

better considered as in the groundplan of Micropezidae. McAlpine also notes that Metopochetini have cylindrical tibia (as in Calobatinae and Micropezinae), unlike the sulcate state he proposes as a synapomorphy for Taeniapterinae+Eurybatinae (although this character is also variable within Taeniapterinae), and that Metopochetini lack the unique setal pattern at the apex of the hind tibia that he recognizes as an apomorphy of his Eurybatinae (1998).

Taking these morphological characters and the relationships recovered here based on both DNA datasets analyzed, a sister relationship between Metopochetini and Micropezinae likely exists. In light of the relationships recovered here, it seems appropriate to consider the classification of Metopochetini. We see two available options to accurately reflect relationships through nomenclature: 1) expand the concept of Micropezinae to include Metopochetini, relegating Micropezinae *sensu stricto* to Micropezini, or 2) elevate Metopochetini to subfamily rank, distinctly maintaining it as separate from Micropezinae. Given micropezines and metopochetines are easily recognizable as independent entities based on morphology, in addition to their geographical separation and isolation, we are inclined to propose the latter option, recognizing Metopochetinae McAlpine, 1975 as a sixth subfamily of Micropezidae, as defined by McAlpine (1975). While McAlpine (1998) provided several autapomorphies for Metopochetini with respect to Eurybatini, these characters will need to be readdressed in light of our recovered affinity between Metopochetinae and Micropezinae. Within Metopochetinae, we confirm McAlpine's hypothesis that *Badisis ambulans*, a strange apterous species whose larvae inhabit the pitchers of a rare species of pitcher plant in southwestern Australia, is allied with the more diverse *Metopochetus* (1990, 1998).

Micropezinae, defined by the absence of fronto-orbital bristles and absence of wing vein bm-m (making cells bm and dm confluent), has received little phylogenetic attention. The recovery of a paraphyletic *Micropeza* with respect to *Cryogonus* conflicts with the work of Hennig (1936d) and Aczél (1949c, 1951), who both considered *Cryogonus* to represent the basal, primitive lineage of Micropezinae due to the presence of proepisternal setae and dorsocentral bristles, the latter of which are unrecorded in other Micropezinae. *Micropeza* has been repeatedly split into multiple genera or subgenera on the basis of a number of questionably homoplastic characters (Aczél 1949c; Cresson 1930; Enderlein 1922; Cresson 1938; Hendel 1932; Ozerov 1997), few of which are congruent with the phylogeny recovered here. Given the relationship between *Cryogonus* and *Micropeza* recovered here, and the lack of supportable alternative classifications available for *Micropeza* at this time, we propose that species currently treated as *Cryogonus* (*Cryogonus formicarius*, *Cryogonus descolei* Aczel, 1949, and *Cryogonus gibbivertex* Enderlein, 1922) be transferred to

Micropeza, and *Cryogonus* and its subgenera (*Henniginus* Aczél, 1949, and *Cressoninus* Aczél, 1949) be treated as synonyms of *Micropeza*.

A close relationship between Eurybatinae and Taeniapterinae has been widely accepted since the former was erected (Marshall 2012; McAlpine 1975, 1998), we here find strong support for a sister-group relationship between Eurybatinae *s.s.* and Taeniapterinae. The Eurybatinae *s.s.* (Eurybatini *sensu* McAlpine (1975)) was recovered with strong support in our analyses. Monophyly of Taeniapterinae is strongly suggested by the unique loss of surstyli in all known species, and we here recover high support for this clade. The Taeniapterinae are divided into the Taeniapterini, diagnosed and defined on the basis of a strikingly elongate anal cell, and a paraphyletic remainder (sometimes treated as the Rainieriini or Grallipezini) diagnosed on the absence of the derived anal cell. Data from Jackson *et al.* (2015) comprises the majority of Taeniapterinae taxa included in this study, and we recover the same topology with a basal, paraphyletic Rainieriini, and a derived, monophyletic Taeniapterini as in that study, although support for relationships in this subfamily remain weak. The Afrotropical genus *Aristobatina* and Oriental *Mimegralla* were added to the prior dataset, and *Aristobatina* was recovered as sister to the remaining Taeniapterinae, suggesting that Taeniapterinae may have its origins in the Old World. Our sampling of Old World taeniapterines is weak however, and conclusions regarding the origins and early relationships of the subfamily should be reserved until more taxa from the Oriental and Australian-Oceanic region are sampled.

Conclusions

Here, using nucleic and amino acid data from 13 genes, we present the first quantitative molecular phylogenetic assessment of the Nerioidea, along with outgroup taxa sampled from across Schizophora. While we could not recover with confidence a sister group to Nerioidea, we find evidence that several prior familial and superfamilial hypotheses regarding higher relationships of Nerioidea warrant further exploration. Within Nerioidea, our results indicate Micropezidae is sister to a clade comprised of Cypselosomatidae, Pseudopomyzidae, and Neriidae, but the internal relationships of this clade remain in conflict depending on the data used. Within Micropezidae, we find a novel association between Micropezinae and Metopochetini, the latter of which we propose elevating to subfamily rank on the basis of our data and a suite of previously proposed morphological synapomorphies. Future work on Nerioidea systematics should continue to expand our taxon sampling and include DNA sequence data from across the full geographic and phylogenetic range of Nerioidea. Also, a full-scale morphological analysis across Nerioidea will

be an important test of the phylogenetic signal provided by DNA, while also allowing the inclusion and placement of fossil Nerioidea (von Tschirnhaus & Hoffeins 2009).

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Tables and Figures

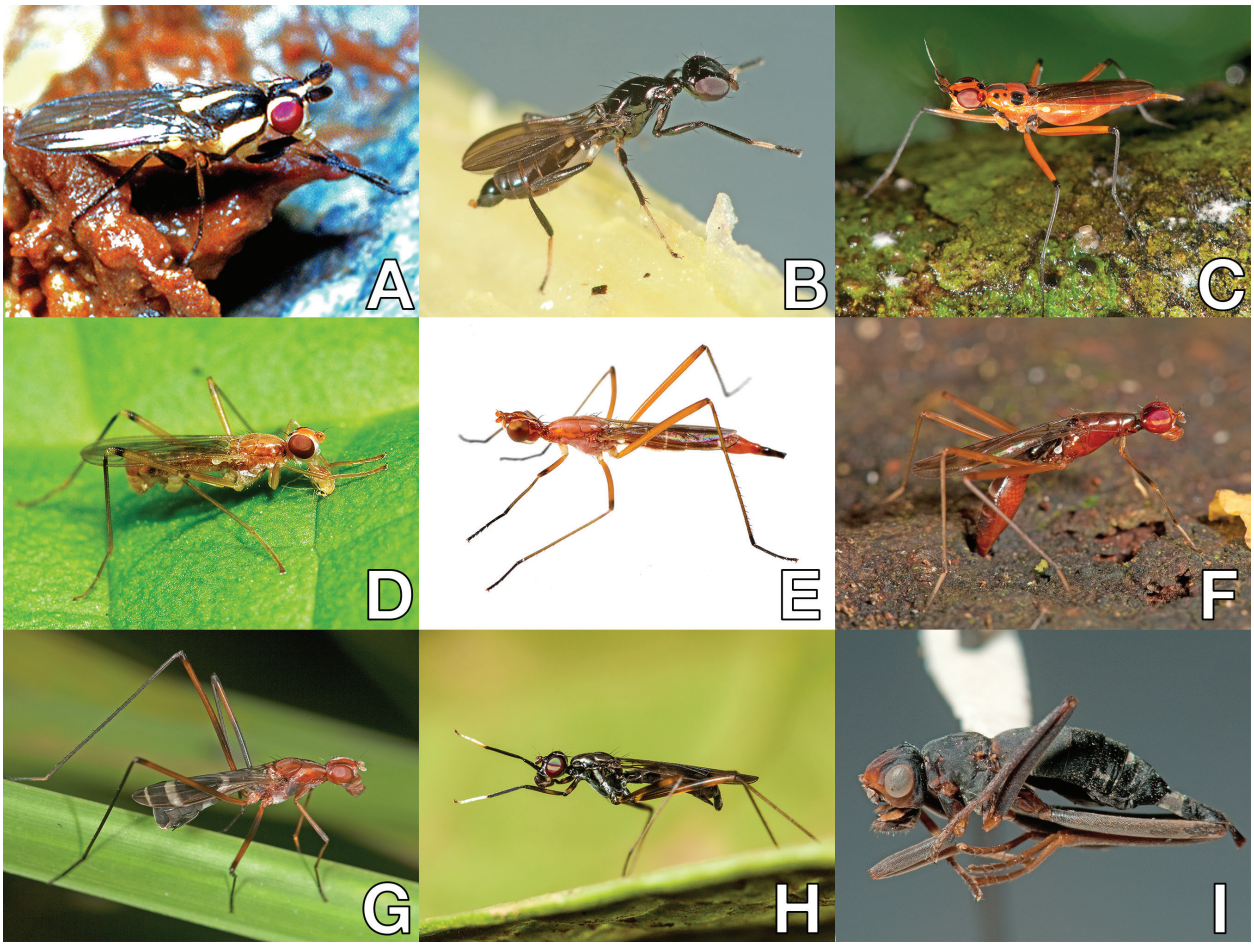
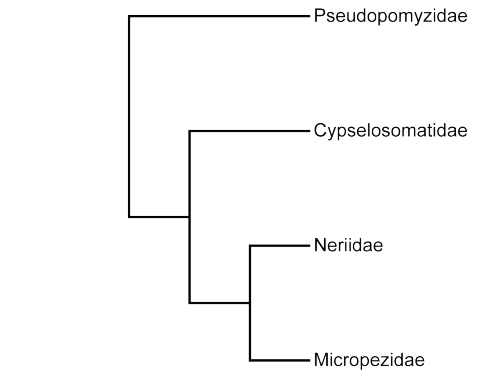


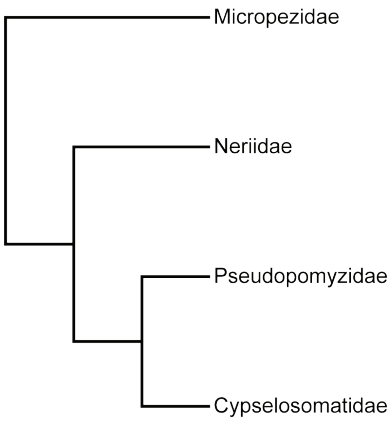
Figure 1. Diversity of Nerioidea (photos by SAM): A) *Latheticomyia* sp. (Pseudopomyzidae); B) *Formicosepsis* sp. (Cypselosomatidae); C) *Telostylus* sp. (Neriidae: Telostylinae); D) *Compsobata univitta* (Micropezidae: Calobatinae); E) *Micropeza ruficeps* (Micropezidae: Micropezinae); F) *Crosa semilauta* (Micropezidae: Eurybatinae: Eurybatini); G) *Metopochetus freyi* (Micropezidae: Eurybatinae: Metopochetini); H) *Rainieria* sp. (Micropezidae: Taeniapterinae) (photo by MDJ); I) *Calycopteryx moseleyi* (Micropezidae: Calycopteryginae), specimen from USNM.

Table 1: Alternative classifications of Nerioidea and subordinate taxa since 1900.

Families sometimes treated as belonging in Nerioidea		
Tanypezidae	Aczél 1949c, 1951, 1954, 1955, 1959, 1961; Collin 1945; Verbeke 1951	
Gobryidae	Enderlein 1922	
Nothybidae	Cresson 1912; Enderlein 1922	
Megamerinidae	Colless & McAlpine 1970, 1991; McAlpine 1966, 1996a, 1997a, c, 1998	
Nerioidea taxa treated as belonging elsewhere in Schizophora		
Pseudopomyzidae (*Pseudopomyza*)	Milichiidae (Hennig 1941a, Frey 1952)	Heleomyzidae or Anthomyzidae (Hennig 1958)
Pseudopomyzidae (*Latheticomyia*)	Heleomyzidae or Anthomyzidae (Hennig 1958)	incertae sedis (Steyskal 1965)
Pseudopomyzidae (*Heloclusia*)	Heleomyzidae (Malloch 1933, Hennig 1958)	
Cypselosomatidae (*Formicosepsis*)	Sepsidae (Enderlein 1920; Frey 1925, 1928, Meijere 1916, 1924)	
Cypselosomatidae (*Cypselosoma*)	Sphaeroceridae (Hendel 1931)	Clusiidae (Hennig 1948)
Micropezidae (*Calobata*)	Tanypezidae (Tillyard 1926)	
Neriidae (*Nerius*)	Tanypezidae (Tillyard 1926)	



2a



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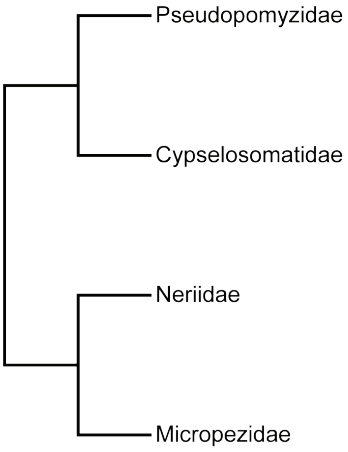
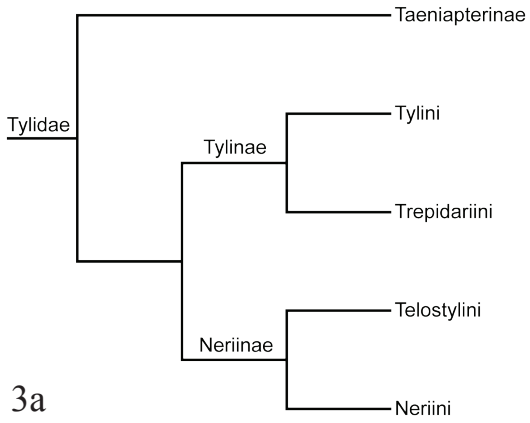


2c

Figure 2: Proposed familial relationships of Nerioidea. a) Relationships of Nerioidea as proposed by McAlpine 1966. b) Relationships of Nerioidea as proposed by McAlpine (1989), Wiegmann *et al.* (2011), and Koch *et al.* (2015). c) Relationships of Nerioidea as proposed by Hennig 1971, 1973, Griffiths 1972, and Yeates *et al.* 2007.



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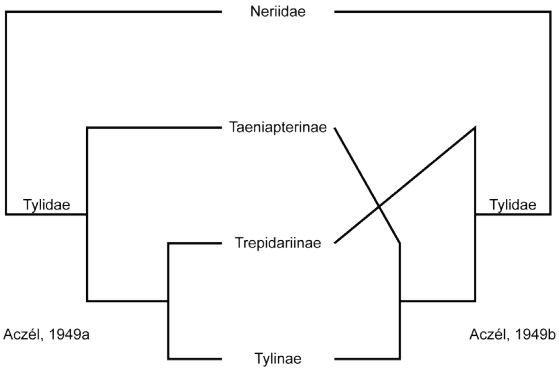

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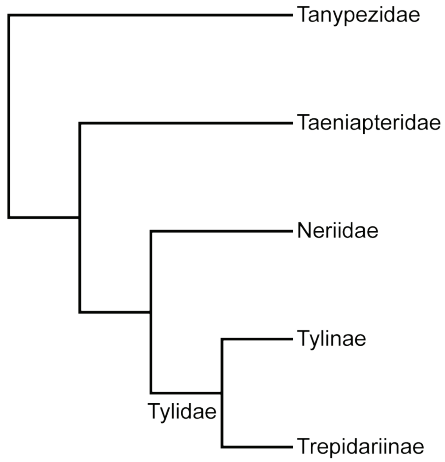
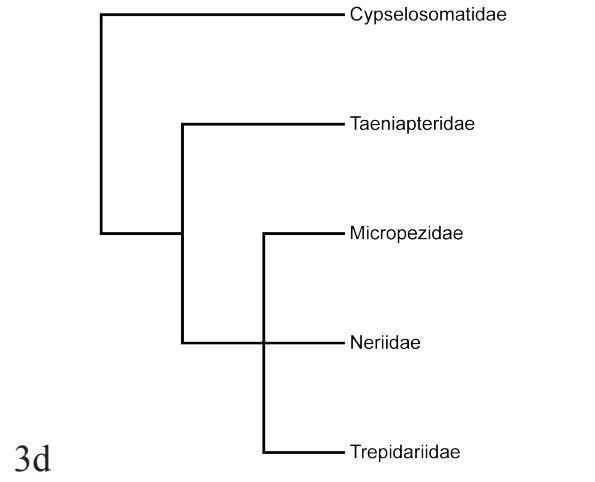


3c

Figure 3: Proposed subfamilial relationships of Micropezidae. a) Relationships of Micropezidae proposed by Hennig (1936b, 1952). b) Relationships of Micropezidae proposed by Aczél (1949b, c). c) Relationships of Micropezidae proposed by Aczél (1951).



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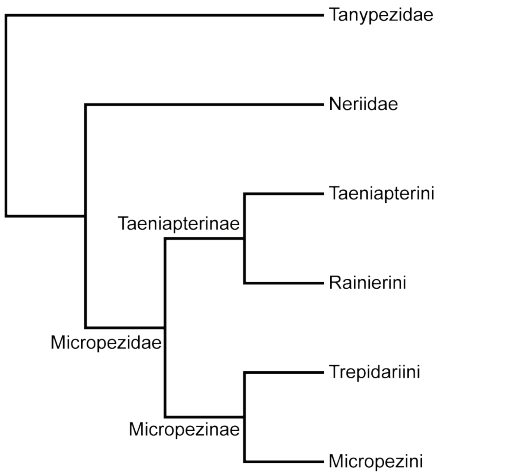

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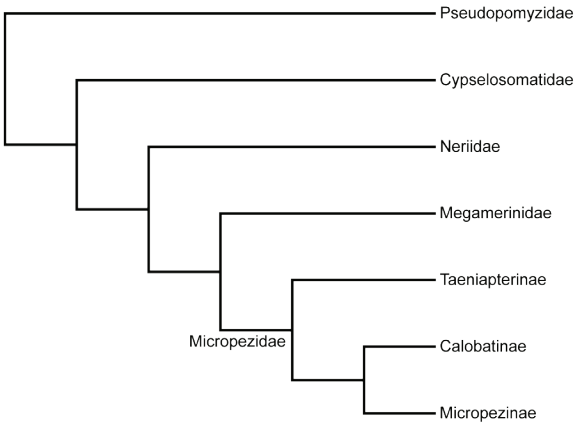

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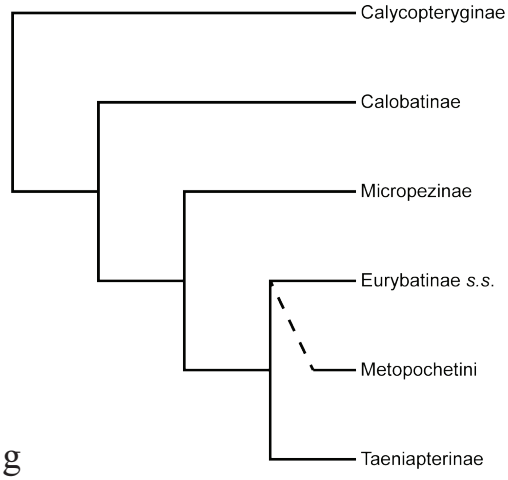


3g

Figure 3, continued: Proposed subfamilial relationships of Micropezidae. d) Relationships of Micropezidae proposed by Hennig (1958). e) Relationships of Micropezidae proposed by Aczél (1959). f) Relationships of Micropezidae proposed by McAlpine (1966). g) Relationships of Micropezidae proposed by McAlpine (1975, 1998).

Table 2: Taxa included in analysis, and sources of sequence data. Superfamily assignment follows Pape et al. (2011).

Superfamily	Family	Species	Source of Data
Syrphoidea	Pipunculidae	*Cephalops longistylis* De Meyer, 1990	Wiegmann et al. (2011)
		Pipunculus "sp.nov."	Gibson et al. (2010)
	Syrphidae	*Episyrphus balteatus* (De Geer, 1776)	Wiegmann et al. (2011)/Han et al. (2005)
		Rhingia nasica Say, 1823	Wiegmann et al. (2011)
		Toxomerus marginata (Say, 1823)	Gibson et al. (2010)
Archischiza	Conopidae	*Physocephala marginata* (Say, 1823)	Gibson et al. (2010)
		Zodion cinereum (Fabricius, 1794)	Gibson et al. (2010)
Carnoidea	Inbiomyiidae	*Inbiomyia mcalpineorum*	Wiegmann et al. (2011)
Lauxanioidea	Lauxaniidae	*Pseudocalliope* sp.	This study (Unique Specimen ID: JSM2132)
Nerioidea	Cypselsomatidae	*Formicosepsis barbipes* Andersson, 1976	This study (Unique Specimen ID: JSM485)
	Micropezidae		
	Calobatinae	*Cnodacophora sellata* (Meigen, 1826)	Wiegmann et al. (2011)
		Cnodocophora sellata (Meigen, 1826)	This study (Unique Specimen ID: MCNOS)
		Compsobata cibaria (Linnaeus, 1758)	This study (Unique Specimen ID: JSS17366)
		Compsobata cibaria (Linnaeus, 1758)	Wiegmann et al. (2011)
		Compsobata univitta (Walker, 1849)	This study (Unique Specimen ID: JSS17367)
	Eurybatinae (Eurybatini)	*Paraeurybata taeniata*	This study (Unique Specimen ID: JSM9778)
		Crepidochaetus argenteofascia Frey, 1958	This study (Unique Specimen ID: JSM9776)
		Crepidochaetus sp.	This study (Unique Specimen ID: JSM4764)
		Crosa semilauta (Osten Sacken, 1882)	This study (Unique Specimen ID: JSM9773)
		Crosa sp.	This study (Unique Specimen ID: JSM4699)
		Eurybata hexapla Osten Sacken, 1882	This study (Unique Specimen ID: JSM9771)
		Nestima pleuralis Steyskal, 1952	This study (Unique Specimen ID: JSM9785)
		Papeza "n.sp.1"	This study (Unique Specimen ID: JSM9777)

Table 2, contined: Taxa included in analysis, and sources of sequence data. Superfamily assignment follows Pape et al. (2011).

Superfamily	Family	Species	Source of Data
Nerioidea	Eurybatinae (Eurybatini)	*Trepidarioides territus* (Osten Sacken, 1882)	This study (Unique Specimen ID: JSM9800)
	Eurybatinae (Metopochetini)	*Badisis ambulans* McAlpine, 1990	This study (Unique Specimen ID: JSM9782)
		Metopochetus freyi McAlpine, 1998	This study (Unique Specimen ID: JSM4811)
		Metopochetus freyi McAlpine, 1998	This study (Unique Specimen ID: JSM4812)
		Metopochetus perclusus (Walker, 1864)	This study (Unique Specimen ID: JSM4706)
	Micropezinae	*Cryogonus formicarius* (Rondani, 1863)	Jackson et al. (2015)
		Cryogonus formicarius (Rondani, 1863)	This study (Unique Specimen ID: JSM9786)
		Cryogonus formicarius (Rondani, 1863)	This study (specimen lost)
		Micropeza albiseta (Czerny, 1932)	This study (Unique Specimen ID: JSM9762)
		Micropeza argentiniensis (Aczél, 1949)	Jackson et al. (2015)
		Micropeza argentiniensis (Aczél, 1949)	Jackson et al. (2015)
		Micropeza brasiliensis Schiner, 1868	Jackson et al. (2015)
		Micropeza brasiliensis Schiner, 1868	Jackson et al. (2015)
		Micropeza compar Cresson, 1938	This study (Unique Specimen ID: JSM9764)
		Micropeza corrigiolata (Linnaeus, 1767)	This study (Unique Specimen ID: JSM9755)
		Micropeza lateralis Meigen, 1826	This study (Unique Specimen ID: JSM9749)
		Micropeza nitidor Cresson, 1938	This study (Unique Specimen ID: JSM9766)
		Micropeza sp.	This study (Unique Specimen ID: JSM2107)
		Micropeza verticalis Cresson, 1930	This study (Unique Specimen ID: JSM9759)
	Taeniapterinae (Rainieriini)	*Aristobatina morogoro* Marshall, 2014	This study (Unique Specimen ID: JSM9799)
		Aristobatina rufithorax (Enderlein, 1922)	This study (Unique Specimen ID: JSM9781)

Table 2, contined: Taxa included in analysis, and sources of sequence data. Superfamily assignment follows Pape et al. (2011).

Superfamily	Family	Species	Source of Data
Nerioidea	Taeniapterinae (Rainieriini)	*Calosphen auristrigosus* group	Jackson et al. (2015)
		Globopeza venezuelensis Marshall, 2005	Jackson et al. (2015)
		Globopeza venezuelensis Marshall, 2005	Jackson et al. (2015)
		Grallipeza "glypha"	Jackson et al. (2015)
		Grallipeza "octa"	Jackson et al. (2015)
		Grallipeza "tapanti" group	Jackson et al. (2015)
		Grallipeza vicina Hennig, 1934	Jackson et al. (2015)
		Hoplocheiloma totliana Gmelin, 1970	Jackson et al. (2015)
		Mimegralla splendens (Wiedemann, 1830)	This study (Unique Specimen ID: JSM9780)
		Rainieria antennaepes (Say, 1823)	Han et al. (2002), Han and Ro (2005)
		Rainieria antennaepes (Say, 1823)	This study (specimen lost)
		Scipopus (*Phaeopterina*) sp.	Jackson et al. (2015)
		Scipopus sp.	Jackson et al. (2015)
		Scipopus sp.	Jackson et al. (2015)
	Taeniapterinae (Taeniapterini)	*Cardiacephala harenosus* (Cresson, 1930)	Jackson et al. (2015)
		Cardiacephala harenosus group	Jackson et al. (2015)
		Cardiacephala harenosus group	Jackson et al. (2015)
		Cardiacephala sp.	This study (specimen lost)
		Grallomyia tarsata (Wiedemann, 1830)	Jackson et al. (2015)
		Grallomyia tarsata (Wiedemann, 1830)	Jackson et al. (2015)
		Grallomyia tarsata group	Jackson et al. (2015)
		Hemichaeta scutellata (Cresson, 1930)	Jackson et al. (2015)
		Mitromyia gratula (Steyskal, 1967)	Jackson et al. (2015)

Table 2, contined: Taxa included in analysis, and sources of sequence data. Superfamily assignment follows Pape et al. (2011).

Superfamily	Family	Species	Source of Data
Nerioidea	Taeniapterinae (Taeniapterini)	*Mitromyia ichneumoneus* (Brauer, 1885)	Jackson et al. (2015)
		Paragrallomyia albibasis (Enderlein, 1922)	Jackson et al. (2015)
		Paragrallomyia aliacea group	Jackson et al. (2015)
		Paragrallomyia angulata (Loew, 1866)	Jackson et al. (2015)
		Paragrallomyia annulata (Fabricius, 1787)	Jackson et al. (2015)
		Paragrallomyia gorgonae (Hennig, 1934)	Jackson et al. (2015)
		Paragrallomyia thiemei (Enderlein, 1922)	Jackson et al. (2015)
		Paragrallomyia vulgata (Hennig, 1934)	Jackson et al. (2015)
		Poecilotylus "2FOnohd"	Jackson et al. (2015)
		Poecilotylus albitarsis (Enderlein, 1922)	Jackson et al. (2015)
		Poecilotylus balzapambana group	Jackson et al. (2015)
		Poecilotylus balzapambana group	Jackson et al. (2015)
		Poecilotylus balzapambana group	Jackson et al. (2015)
		Poecilotylus balzapambana group	Jackson et al. (2015)
		Poecilotylus paraguayensis (Enderlein, 1922)	Jackson et al. (2015)
		Poecilotylus trifasciatus (Wiedemann, 1830)	Jackson et al. (2015)
		Ptilosphen cyaneiventris (Macquart, 1846)	Jackson et al. (2015)
		Ptilosphen nr *enderleini*	This study (Unique Specimen ID: USNMENT01199767)
		Taeniaptera lasciva (Fabricius, 1798)	Jackson et al. (2015)
		Taeniaptera lasciva (Fabricius, 1798)	Jackson et al. (2015)

Table 2, contined: Taxa included in analysis, and sources of sequence data. Superfamily assignment follows Pape et al. (2011).

Superfamily	Family	Species	Source of Data
		Taeniaptera lasciva (Fabricius, 1798)	Jackson et al. (2015)
Nerioidea	Taeniapterinae (Taeniapterini)	*Taeniaptera trivittata* Macquart, 1835	Gibson et al. (2010)
		Taeniaptera trivittata Macquart, 1835	Jackson et al. (2015)
		Taeniaptera trivittata Macquart, 1835	This study (specimen lost)
	Neriidae		
	Neriinae	*Glyphidops* (*Glyphidops*) sp.	This study (Unique Specimen ID: JSM4807)
		Glyphidops sp.	This study (Unique Specimen ID: JSM4803)
		Gymnonerius sp.	This study (Unique Specimen ID: JSM4638)
		Gymnonerius sp.	This study (Unique Specimen ID: JSM4755)
		Nerius plurivittatus Bigot, 1886	This study (Unique Specimen ID: JSM9775)
		Nerius sp.	This study (Unique Specimen ID: JSM4735)
		Telostylinus sp.	This study (Unique Specimen ID: JSS17374)
		Telostylinus sp.	Wiegmann et al. (2011)
	Telostylinae	*Chaetonerius* sp.	This study (Unique Specimen ID: JSM4835)
		Chaetonerius sp.	This study (Unique Specimen ID: JSM4860)
		Chaetonerius sp.	This study (Unique Specimen ID: JSM4861)
		Chaetonerius sp.	This study (Unique Specimen ID: JSM4862)
		Telostylus sp.	This study (Unique Specimen ID: JSM4873)
	Pseudopomyzidae	*Heloclusia* sp.	This study (Unique Specimen ID: JSM6858)
		Rhinopomyzella sp.	Wiegmann et al. (2011)
Opomyzoidea	Anthomyzidae	*Anthomyza gracilis* complex	Wiegmann et al. (2011)
		Anthomyza gracilis Fallén, 1823	Rohacek et al. (2014)
		Stiphrosoma setipleurum Rohacek & Barber, 2005	Rohacek et al. (2014)
	Clusiidae	*Sobarocephala setipes* Melander & Argo, 1924	Lonsdale et al. (2010)
	Fergusoninidae	*Fergusoninia turneri*	This study (Unique Specimen ID: JSM2167)
	Megamerinidae	*Megamerina* sp.	Wiegmann et al. (2011)
		Texara sp.	This study (Unique Specimen ID: JSS17361)
Sphaeroceroidea	Sphaeroceridae	*Pterogramma* sp.	This study (Unique Specimen ID: JSM4553)
Diopsoidea	Diopsidae	*Eurydiopsis* sp.	This study (Unique Specimen ID: JSM4836)

Table 2, contined: Taxa included in analysis, and sources of sequence data. Superfamily assignment follows Pape et al. (2011).

Superfamily	Family	Species	Source of Data
		Sphyracephala brevicornis (Say, 1817)	This study (Unique Specimen ID: JSS17377)
Diopsoidea	Diopsidae	*Sphyracephala europaea* Papp & Foldvari, 1997	This study (Unique Specimen ID: JSS17376)
		Teleopsis dalmanni (Wiedemann, 1830)	Wiegmann et al. (2011), This study (Unique Specimen ID: JSM2175)
		Teloglabrus sp.	This study (Unique Specimen ID: MTELO)
	Gobryidae	*Gobrya* sp.	This study (Unique Specimen ID: JSM4474)
	Nothybidae	*Nothybus kuznetsovorum* Galinskaya & Shatalkin, 2015	This study (Unique Specimen ID: JSM4469)
		Nothybus longicollis (Walker, 1856)	This study (Unique Specimen ID: JSM4593)
	Psilidae	*Chamaepsila rosae* (Fabricius, 1794)	This study (Unique Specimen ID: JSM1987)
		Chyliza apicalis Loew, 1860	This study (Unique Specimen ID: JSM4480)
	Somatiidae	*Somatia aestiva* (Fabricius, 1805)	This study (Unique Specimen ID: JSS17363)
	Syringogastridae	*Syringogaster* "dactylogaster"	This study (Unique Specimen ID: JSM1819)
		Syringogaster "ecuadorensis"	This study (Unique Specimen ID: JSM1773)
		Syringogaster atricalyx Marshall & Buck, 2009	This study (Unique Specimen ID: JSM1821)
		Syringogaster brunnea Cresson, 1912	This study (Unique Specimen ID: JSM1771)
		Syringogaster brunnea Cresson, 1912	This study (Unique Specimen ID: JSM1822)
		Syringogaster brunneina Marshall & Buck, 2009	This study (Unique Specimen ID: JSM1820)
		Syringogaster sp.	This study (Unique Specimen ID: JSM1910)
	Tanypezidae	*Neotanypeza* sp.	This study (Unique Specimen ID: JSM2102)
		Strongylophthalmyia angustipennis Melander, 1920	This study (Unique Specimen ID: JSM2193)
		Strongylophthalmyia pengellyi Barber, 2006	This study (Unique Specimen ID: JSM2111)
		Tanypeza longimana Fallén, 1820	This study (Unique Specimen ID: JSM4491)
Tephritoidea	Platystomatidae	*Elassogaster* sp.	This study (Unique Specimen ID: JSM496)

Table 3: Specimen data for newly sequenced specimens included for this study.

Species	Unique Specimen Identifier	Locality	Date	Collector	Identified By
Pseudocalliope sp.	JSM2132	Canada, Ont, Elora, Pilkington overlook	2006.i	S.A. Marshall	S.M. Paiero
Formicosepsis barbipes Andersson, 1976	JSM4815	Philippines, University of the Philippines Los Bãnos	2010.iv.1	S. & S. Marshall	M.D. Jackson
Cnodocophora sellata (Meigen, 1826)	MCNOS				
Compsobata cibaria (Linnaeus, 1758)	JSS17366			B.M. Wiegmann	M. Buck
Compsobata univitta (Walker, 1849)	JSS17367	U.S.A., North Carolina, Sawin Co.[unty], GSMNP [Great Smoky Mountains National Park], Big Cove Road, Site #2	2001.v.19-26	B.M. Wiegmann and B.K. Cassel	S.A. Marshall
Paraeurybata taeniata	JSM9778	Mauritius, Black River Gorges N.P. Mare Longue	2016. xii.7-9	S.A. Marshall	S.A. Marshall
Crepidochaetus argenteofascia Frey, 1958	JSM9776	Philippines, Los Banos Mt Makiling Trail	2010.iii.20-30	S.A. Marshall	S.A. Marshall
Crepidochaetus sp.	JSM4764	Malaysia, Sabah, Crocker Range, Kipandi Butterfly Park	2012.x.26-xi.29	S. Bosuang	N.A.M. Yusof
Crosa semilauta (Osten Sacken, 1882)	JSM9773	Philippines, Los Banos Mt Makiling Trail	2010.iii.20-30	S.A. Marshall	S.A. Marshall
Crosa sp.	JSM4699	Indonesia, West Papua, Arfak Mountains	2012.xi.1	J.H. Skevington	N.A.M. Yusof
Eurybata hexapla Osten Sacken, 1882	JSM9771	Philippines, Los Banos Mt Makiling Trail	2010.iii.20-30	S.A. Marshall	S.A. Marshall
Nestima pleuralis Steyskal, 1952	JSM9785	Papua New Guinea, Wanang 3	2012		N.A.M. Yusof
Papeza "n.sp.1"	JSM9777	Papua New Guinea, Mt. Wilhelm	2012		N.A.M. Yusof
Trepidarioides territus (Osten Sacken, 1882)	JSM9800	Philippines, Los Banos Mt Makiling Trail	2010.iii.20-30	S.A. Marshall	S.A. Marshall
Badisis ambulans McAlpine, 1990	JSM9782	Australia, Western Australia, Denmark	2004.xii.1	S.A. Marshall	S.A. Marshall
Metopochetus freyi McAlpine, 1998	JSM4811	Australia, New South Wales, Oxley Highway, Ginger Creek	2010.i.5	S.A. Marshall	S.A. Marshall
Metopochetus freyi McAlpine, 1998	JSM4812	Australia, New South Wales, Oxley Highway, Ginger Creek	2010.i.5	S.A. Marshall	S.A. Marshall

Table 3, continued: Specimen data for newly sequenced specimens included for this study.

Species	Unique Specimen Identifier	Locality	Date	Collector	Identified By
Metopochetus perclusus (Walker, 1864)	JSM4706	Indonesia, West Papua, Waigeo Island	2012.x.25-26	J.H. Skevington	S.A. Marshall
Cryogonus formicarius (Rondani, 1863)	JSM9786	Chile, Cardenal Caro Province, PaÑul ~30k S Richelemu Molimo de Agua	2006.xi.25	S.A. Marshall	M.D. Jackson
Micropeza albiseta (Czerny, 1932)	JSM9762	Costa Rica, San Jose, Zurqui de Moravia	2013. viii.5-6	S.A. Marshall	M.D. Jackson
Micropeza compar Cresson, 1938	JSM9764	U.S.A., Arizona, Graham Co; PiÑaleno Mts; Twilighlight Campsite, Mt. Graham; 25 km SW Jct 191 on hwy 366	2015. vii.30-viii.7	M.E. Irwin	M.D. Jackson
Micropeza corrigiolata (Linnaeus, 1767)	JSM9755	Canada, New Brunswick, Sackville	2010. vii.2-3	J. Klymko	M.D. Jackson
Micropeza lateralis Meigen, 1826	JSM9749	Canada, British Columbia, Iona Beach	2014.vii.23	S. McCann	M.D. Jackson
Micropeza nitidor Cresson, 1938	JSM9766	U.S.A., Arizona, Graham Co; PiÑaleno Mts; Twilighlight Campsite, Mt. Graham; 25 km SW Jct 191 on hwy 366	2015. vii.30-viii.7	M.E. Irwin	M.D. Jackson
Micropeza sp.	JSM2107			B.M. Wiegmann	S.A. Marshall
Micropeza verticalis Cresson, 1930	JSM9759	Cuba, Parque Nacional Alexander Humboldt	2012.vi.6	S.A. Marshall	M.D. Jackson
Aristobatina morogoro Marshall, 2014	JSM9799	Tanzania, Morogoro Region, Udzungwa Mts Natl. Park	2009.xi.11-13	T. Pape & S.A. Marshall	S.A. Marshall
Aristobatina rufithorax (Enderlein, 1922)	JSM9781	Tanzania, Morogoro Region, Udzungwa Mts Natl. Park	2009.xi.11-13	T. Pape & S.A. Marshall	S.A. Marshall
Mimegralla splendens (Wiedemann, 1830)	JSM9780	Mauritius, Domaine Ylang Ylang	2016.xii.14	G. Kvifte	S.A. Marshall
Ptilosphen nr *enderleini*	USNMENT01199767	Peru: Cusco: Estacion Biologica Villa Carmen, trail 0, on narrow ridge, 12.88721ºS 71.41405ºW 780m, trap VC0ML-67	2014.i.11	M. Choque	S. A. Marshall
Glyphidops (*Glyphidops*) sp.	JSM4807	Peru, Madre de Dios, Los Amigos Biological Station	2006.vi.2-14	S. Paiero & J. Klymko	M.D. Jackson
Glyphidops sp.	JSM4803	Costa Rica, San Jose, Tarrazu, San Carlos, Reserva Riosparaiso, Albergue Pecari	2006.ii.26	S.M. Paiero	M.D. Jackson

Table 3, continued: Specimen data for newly sequenced specimens included for this study.

Species	Unique Specimen Identifier	Locality	Date	Collector	Identified By
Gymnonerius sp.	JSM4638	Malaysia, Sabah, Crocker Range, Kipandi Butterfly Park	2012.iii	S. Bosuang	M.D. Jackson
Gymnonerius sp.	JSM4755	Malaysia, Sabah, Crocker Range, Kipandi Butterfly Park	2011.x.28-2012.xi.31	S. Bosuang	M.D. Jackson
Nerius plurivittatus Bigot, 1886	JSM9775	Costa Rica, Guanacaste, Rin. de la Vieja Nat. Prk.	2010.viii.17	M.D. Jackson	M.D. Jackson
Nerius sp.	JSM4735	Vietnam, Cuc Phuong National Park	2011.viii.11	S.A. Marshall	M.D. Jackson
Telostylinus sp.	JSS17374	Fiji, Cakaudrove, Vanue Levu Island, Near Nauavanadi, mangroves and second growth along roadside	2006.i.27	J.H. Skevington	J. H. Skevington
Chaetonerius sp.	JSM4835	Malaysia, Sabah, Mount Kinabalu National Park, Poring Hot Springs	2012.viii.16	J.H., A.M. & A.W. Skevington	M.D. Jackson
Chaetonerius sp.	JSM4860	Malaysia, Sabah, Danum Valley Field Centre	2012.viii.5-10	J.H., A.M. & A.W. Skevington	M.D. Jackson
Chaetonerius sp.	JSM4861	Malaysia, Sabah, Danum Valley Field Centre	2012.viii.5-10	J.H., A.M. & A.W. Skevington	M.D. Jackson
Chaetonerius sp.	JSM4862	Malaysia, Sabah, Danum Valley Field Centre	2012.viii.5-10	J.H., A.M. & A.W. Skevington	M.D. Jackson
Telostylus sp.	JSM4873	Malaysia, Sabah, Danum Valley Field Centre	2012.viii.5-10	J.H., A.M. & A.W. Skevington	M.D. Jackson
Heloclusia sp.	JSM6858	Chile, hwy 201 betw. Liquine and Conaripe	2013.xii.1-4	S.A. Marshall	S.A. Marshall
Fergusoninia turneri	JSM2167	Australia	1999		O. Lonsdale
Texara sp.	JSS17361	Republic of Korea, South Chungcheong Province, keumsan Nami-myen Pohyeonsa Pohyeonsa P. Tripotn leg.	2005.vi.1-8		M. Buck
Pterogramma sp.	JSM4553				

Table 3, continued: Specimen data for newly sequenced specimens included for this study.

Species	Unique Specimen Identifier	Locality	Date	Collector	Identified By
Eurydiopsis sp.	JSM4836	Malaysia, Sabah, Mount Kinabalu National Park, Poring Hot Springs	2012.viii.16	J.H., A.M. & A.W. Skevington	O. Lonsdale
Sphyracephala brevicornis (Say, 1817)	JSS17377	Canada, Ontario, Cave, Hogsback	2006.vi.9	S.A. Marshall	S.A. Marshall
Sphyracephala europaea Papp & Foldvari, 1997	JSS17376	Hungary, Mako	2004.x.20	leg. Paulovics and Földvári	M. Foldvari
Teleopsis dalmanni (Wiedemann, 1830)	JSM2175	Lab colony?			B.M. Wiegmann
Teloglabrus sp.	MTELO				
Gobrya sp.	JSM4474	Vietnam, Catcat, Sapa District	2011.viii.18	S.A. Marshall	S.A. Marshall
Nothybus kuznetsovorum Galinskaya & Shatalkin, 2015	JSM4469	Vietnam, Cuc Phuong National Park	2011.viii.11	S.A. Marshall	S.A. Marshall
Nothybus longicollis (Walker, 1856)	JSM4593	Malaysia, Sabah, Penampang Distr., Crocker Range, Kipandi Butterfly park	2011.x.15	S. Gaimari, M. Hauser	S.A. Marshall
Chamaepsila rosae (Fabricius, 1794)	JSM1987	Lab colony?			B.M. Wiegmann
Chyliza apicalis Loew, 1860	JSM4480	Canada, New Brunswick, St. John Co., Fundy Bay Trail	2011.viii.3	O. Lonsdale	O. Lonsdale
Somatia aestiva (Fabricius, 1805)	JSS17363	Costa Rica, San Jose, Tarrazu, San Carlos, Reserva Riosparaiso, Albergue Pecari	2006.ii.22-26	S.A. Marshall	S.A. Marshall
Syringogaster "dactylogaster"	JSM1819	Peru	2006		S.A. Marshall
Syringogaster "ecuadorensis"	JSM1773	Peru	2006		S.A. Marshall
Syringogaster atricalyx Marshall & Buck, 2009	JSM1821	Peru	2006		S.A. Marshall
Syringogaster brunnea Cresson, 1912	JSM1771	Costa Rica	2006		S.A. Marshall
Syringogaster brunnea Cresson, 1912	JSM1822	Costa Rica	2006		S.A. Marshall
Syringogaster brunneina Marshall & Buck, 2009	JSM1820	Costa Rica	2006		S.A. Marshall
Syringogaster sp.	JSM1910				

Table 3, continued: Specimen data for newly sequenced specimens included for this study.

Species	Unique Specimen Identifier	Locality	Date	Collector	Identified By
Neotanypeza sp.	JSM2102	Costa Rica, San Jose, San Jose, Tarrazu, San Carlos, La Virgen	2006.ii.25	S.A. Marshall	M. Buck
Strongylophthalmyia angustipennis Melander, 1920	JSM2193	Canada, Ontario, Sault Saint Marie, Sault Coll. Outdoor Lab	2007.vii.17	K.N. Barber	K.N. Barber
Strongylophthalmyia pengellyi Barber, 2006	JSM2111	Canada, Ontario, Sault Ste. Marie, Finn Hill	2007.v.30	K.N. Barber	K.N. Barber
Tanypeza longimana Fallén, 1820	JSM4491	Canada, Ontario, Ottawa-Carleton, Mer Bleue Bog, 15 km E of Ottawa	2011.ix.11	S.H. Cumming	J.M. Cumming
Elassogaster sp.	JSM496	Australia: QLD: Cooloola National Park 26º03’S 153º01’E	2002.x.6-8	J. Skevington	M.D. Jackson

Table 4: DNA sequences used in this study. GenBank accession numbers from studies listed in Table 2. 'x' represent new sequences contributed by this study.

Species	12S	16S	28S	AATS	CAD	COI	COII	cyt-B	EF1a	PGD	TPI	white	wingless
Cephalops longistylis			KC177724	KC178226						KC177374	KC177951		
Pipunculus "sp.nov."	HM062589		HM062617	HM062643		HM062539			HM062665	HM062758	HM062686	HM062699	
Episyrphus balteatus	AY573076	AY573115	KC177727	KC178228		KC192981	KC192991	KC177603			KC177954		
Rhingia nasica			KC177725	KC178227	KC177156					KC177375	KC177952		
Toxomerus marginata	HM062596		HM062624	HM062649	HM062742	HM062546		HM062568	HM062672	HM062762	HM062690	HM062701	
Physocephala marginata	HM062570		HM062599	HM062626	HM062719				HM062651	HM062744	HM062674	HM062716	
Zodion cinereum	HM062575		HM062602	HM062629	HM062724	HM062525		JN664867	HM062655	HM062748	HM062680	HM062703	
Inbiomyia mcalpineorum			KC177817										
Pseudocalliope sp.	x		x	x		x	x		x			x	
Formicosepsis barbipes	x		x			x	x		x				x
Cnodacophora sellata			KC177732	KC178232	KC177160					KC177380	KC177959		
Cnodocophora sellata	x	x	x	x		x	x		x		x		x
Compsobata cibaria	x	x				x	x		x				x
Compsobata cibaria			KC177733	KC178233	KC177161					KC177381	KC177960		
Compsobata univitta	x	x	x	x		x			x	x	x		
Paraeurybata taeniata	x	x	x			x	x		x				x
Crepidochaetus argenteofascia	x	x	x		x	x	x	x	x				
Crepidochaetus sp.	x		x			x	x						
Crosa semilauta		x	x			x		x	x			x	x
Crosa sp.	x		x	x	x	x			x	x			
Eurybata hexapla	x	x	x	x		x	x	x	x			x	x
Nestima pleuralis	x	x	x			x							
Papeza "n.sp.1"	x	x	x			x						x	x
Trepidarioides territus		x				x		x					
Badisis ambulans	x	x	x			x	x		x				x

Table 4, continued: DNA sequences used in this study. GenBank accession numbers from studies listed in Table 2. 'x' represent new sequences contributed by this study.

Species	12S	16S	28S	AATS	CAD	COI	COII	cyt-B	EF1a	PGD	TPI	white	wingless
Metopochetus freyi	x		x			x			x		x		
Metopochetus freyi	x		x			x			x			x	
Metopochetus perclusus	x		x		x	x	x				x		
Cryogonus fomicarius	KM287333					KM287293							
Cryogonus fomicarius	x	x	x		x	x	x		x				
Cryogonus fomicarius					x	x				x	x		
Micropeza albiseta	x	x	x	x	x	x	x	x	x				
Micropeza argentiniensis	KM287343				KM287265	KM287301							
Micropeza argentiniensis	KM287346				KM287266	KM287304							
Micropeza brasiliensis	KM287345					KM287303							
Micropeza brasiliensis	KM287344					KM287302							
Micropeza compar	x	x	x	x	x	x	x	x	x				
Micropeza corrigiolata	x	x	x			x	x	x	x				
Micropeza lateralis	x	x	x	x		x	x	x	x				
Micropeza nitidor	x	x	x			x	x	x	x		x	x	
Micropeza sp.	x	x	x	x		x	x		x	x	x		x
Micropeza verticalis	x	x	x			x	x	x	x				
Aristobatina morogoro		x											
Aristobatina rufithorax	x	x	x		x	x	x		x	x		x	x
Calosphen auristrigosus group	KM287332					KM287292							KM287196
Globopeza venezuelensis	KM287335				KM287257								KM287198
Globopeza venezuelensis	KM287334				KM287256								KM287197
Grallipeza "glypha"	KM287337				KM287259	KM287295							KM287200
Grallipeza "octa"	KM287336				KM287258	KM287294							KM287199
Grallipeza "tapanti" group	KM287338				KM287260	KM287296							KM287201

Table 4, continued: DNA sequences used in this study. GenBank accession numbers from studies listed in Table 2. 'x' represent new sequences contributed by this study.

Species	12S	16S	28S	AATS	CAD	COI	COII	cyt-B	EF1a	PGD	TPI	white	wingless
Grallipeza vicina	KM287339				KM287261	KM287297							
Hoplocheiloma totliana	KM287342												
Mimegralla splendens	x	x	x	x	x	x	x			x		x	x
Rainieria antennaepes	AY573098	AY123347	AY123359				AY573173						
Rainieria antennaepes				x	x	x	x		x				
Scipopus (*Phaeopterina*) sp.	KM287354				KM287273	KM287312							
Scipopus sp.	KM287355				KM287274	KM287313							KM287209
Scipopus sp.	KM287356				KM287275	KM287314							KM287210
Cardiacephala harenosus	KM287347				KM287267	KM287305							KM287203
Cardiacephala harenosus group	KM287349				KM287269	KM287307							KM287205
Cardiacephala harenosus group	KM287348				KM287268	KM287306							KM287204
Cardiacephala sp.		x		x	x	x	x		x	x		x	
Grallomyia tarsata	KM287341				KM287263	KM287299							KM287202
Grallomyia tarsata	KM287340					KM287298							
Grallomyia tarsata group					KM287262								
Hemichaeta scutellata					KM287264	KM287300							
Mitromyia gratula	KM287362				KM287281	KM287320							
Mitromyia ichneumoneus	KM287363				KM287282	KM287321							KM287216
Paragrallomyia albibasis	KM287357		x		KM287276	KM287315			x				KM287211
Paragrallomyia aliacea group	KM287366												
Paragrallomyia angulata	KM287359		x		KM287278	KM287317			x				KM287213
Paragrallomyia annulata	KM287360		x		KM287279	KM287318			x				KM287214
Paragrallomyia gorgonae	KM287361		x		KM287280	KM287319			x				KM287215
Paragrallomyia thiemei	KM287370		x		KM287290	KM287329			x				KM287221
Paragrallomyia vulgata	KM287372		x			KM287331			x				KM287223

Table 4, continued: DNA sequences used in this study. GenBank accession numbers from studies listed in Table 2. 'x' represent new sequences contributed by this study.

Species	12S	16S	28S	AATS	CAD	COI	COII	cyt-B	EF1a	PGD	TPI	white	wingless
Poecilotylus "2FOnohd"	KM287351				KM287271	KM287309							
Poecilotylus albitarsis	KM287358		x		KM287277	KM287316							KM287212
Poecilotylus balzapambana group	KM287367				KM287287	KM287326							KM287218
Poecilotylus balzapambana group					KM287286	KM287325							KM287217
Poecilotylus balzapambana group	KM287368				KM287288	KM287327							KM287219
Poecilotylus balzapambana group	KM287369				KM287289	KM287328							KM287220
Poecilotylus paraguayensis	KM287350				KM287270	KM287308							KM287206
Poecilotylus trifasciatus	KM287352					KM287310							KM287207
Ptilosphen cyaneiventris	KM287353				KM287272	KM287311							KM287208
Ptilosphen nr *enderleini*	x	x				x	x	x					
Taeniaptera lasciva					KM287285	KM287324							
Taeniaptera lasciva	KM287364				KM287283	KM287322							
Taeniaptera lasciva	KM287365				KM287284	KM287323							
Taeniaptera trivittata	HM062585		HM062613	HM062639	HM062732	HM062535		HM062559	HM062662	HM062755	HM062693	HM062709	
Taeniaptera trivittata	KM287371		x		KM287291	KM287330			x				KM287222
Taeniaptera trivittata	x	x		x	x	x	x		x		x	x	
Glyphidops (*Glyphidops*) sp.	x	x	x	x		x		x	x	x	x		
Glyphidops sp.	x		x	x		x			x				
Gymnonerius sp.	x		x	x	x	x	x		x	x			x
Gymnonerius sp.	x		x			x			x				
Nerius plurivittatus	x	x	x			x							x
Nerius sp.	x	x	x			x	x	x		x			
Telostylinus sp.	x	x	x	x		x	x		x	x	x		
Telostylinus sp.			KC177734	KC178234	KC177162						KC177961		
Chaetonerius sp.	x		x	x	x	x	x		x				

Table 4, continued: DNA sequences used in this study. GenBank accession numbers from studies listed in Table 2. 'x' represent new sequences contributed by this study.

Species	12S	16S	28S	AATS	CAD	COI	COII	cyt-B	EF1a	PGD	TPI	white	wingless
Chaetonerius sp.	x	x	x	x		x	x						x
Chaetonerius sp.			x	x	x	x	x	x					x
Chaetonerius sp.	x	x	x	x	x	x	x	x					x
Telostylus sp.	x	x	x	x	x	x	x	x	x			x	
Heloclusia sp.		x	x	x		x	x	x	x			x	x
Rhinopomyzella sp.			KC177738	KC178238	KC177165						KC177965		
Anthomyza gracilis complex			KC177785	KC178282	KC177204					KC177417	KC178003		
Anthomyza gracilis	EU268515	EU268541	KJ418498			KJ418548/ KJ418592							
Stiphrosoma setipleurum	KJ418444	KJ418466	KJ418510			KJ418558/ KJ418604	KJ418633	KJ418653					
Sobarocephala setipes			FJ890879		FJ416888	FJ435896	FJ435929						
Fergusoninia turneri	x		x	x		x	x		x	x			
Megamerina sp.			KC177731	KC178231	KC177159					KC177379	KC177958		
Texara sp.	x						x		x				
Pterogramma sp.	x		x		x	x	x						
Eurydiopsis sp.	x		x		x	x	x					x	
Sphyracephala brevicornis	x	x	x	x		x	x		x		x	x	x
Sphyracephala europaea	x	x	x	x			x		x		x	x	
Teleopsis dalmanni	x	KC177441				KC192968	KC192987		x			x	x
Teloglabrus sp.	x		x	x					x		x		x
Gobrya sp.	x		x	x	x	x	x		x				
Nothybus kuznetsovorum	x		x	x	x	x	x		x	x			
Nothybus longicollis	x		x		x	x	x		x	x			x
Chamaepsila rosae	x	x				x	x		x			x	x
Chyliza apicalis	x		x			x	x						

Table 4, continued: DNA sequences used in this study. GenBank accession numbers from studies listed in Table 2. 'x' represent new sequences contributed by this study.

Species	12S	16S	28S	AATS	CAD	COI	COII	cyt-B	EF1a	PGD	TPI	white	wingless
Somatia aestiva	x	x	x	x		x	x		x	x	x		x
Syringogaster "dactylogaster"	x												
Syringogaster "ecuadorensis"	x												
Syringogaster atricalyx	x												
Syringogaster brunnea	x						x						
Syringogaster brunnea	x	x	x	x			x		x	x			x
Syringogaster brunneina	x												x
Syringogaster sp.			x	x	x	x	x		x		x		
Neotanypeza sp.	x	x	x	x		x	x		x	x	x		x
Strongylophthalmyia angustipennis	x		x	x	x	x	x		x		x		
Strongylophthalmyia pengellyi	x	x	x	x		x	x		x	x	x	x	x
Tanypeza longimana	x		x	x		x	x		x	x	x	x	
Elassogaster sp.	x		x	x		x			x	x		x	

Table 5: Primer sequences and PCR annealing temperatures for amplifying gene regions used in this study.

Gene Region	Primer Name	Primer Sequence 5'-> 3'	Reference	Annealing Temperature (ºC)
12S	12SAi	AAACTAGGATTAGATACCCTATTAT	(Simon *et al*. 1994)	54
	12SBi	AAGAGCGACGGGCGATGTGT	(Simon *et al*. 1994)	
16S	12727	AATTTATTGCACTAATCTGCC	(Baker *et al*. 2001)	45
	13270	GCTGGAATGAATGGTTGGACG	(Baker *et al*. 2001)	
28S	F-2	GGATTTTYTKAGTAGCGGCG	(Miranda *et al*. 2016)	48
	28S-Dipt-4632R	GGTTCATCCCACAGCGCC	(Gibson *et al*. 2011)	
	28S-Dipt-4534F	CCTATTCTCAAACTTTAAATGGG	(Gibson *et al*. 2011)	
	A28F	GGAACCGTATTCCCTTTCG	(Han *et al*. 2002)	
	28S-Dipt-4997F	GGAGGACTGAAGTGGAGAAGG	(Gibson *et al*. 2011)	
	28S-Dipt-5532R	CTCAATCTTCAGAGCCAATCC	(Gibson *et al*. 2011)	
	28S-Dipt-5497F	GGAAGTCGGCAAATTAGATCCG	(Gibson *et al*. 2011)	
	28S-Dipt-6647R	CGTCGCTATGAACGCTTGGCC	(Gibson *et al*. 2011)	
	28S-Dipt-6565F	CTCGGCCTATCGATCCTTTTGG	(Gibson *et al*. 2011)	
	28S-Dipt-7176R	CCACTTACAACACCTTGCC	(Gibson *et al*. 2011)	
AATS	IF40	GNATGAAYCARTTYAARCCNAT	(Feng-Yi Su *et al*. 2008)	45
	IR244	CATNCCRCARTCNATRTGYTT	(Feng-Yi Su *et al*. 2008)	

Table 5, continued: Primer sequences and PCR annealing temperatures for amplifying gene regions used in this study.

Gene Region	Primer Name	Primer Sequence 5'-> 3'	Reference	Annealing Temperature (ºC)
CAD	581F2-F	GGWGGWCAAACWGCWYTMAAYTGYGG	(Moulton & Wiegmann 2004)	45
	843-R	GCYTTYTGRAANGCYTCYTCRAA	(Moulton & Wiegmann 2004)	
	CAD-Dipt-2341F	TGGHAGYTCNATGAARAGYGT	(Gibson *et al.* 2011)	
	CAD-Dipt-3682F	CCNTTYAAYATGCARYTNATYGC	(Gibson *et al.* 2011)	
COI	LCO1490	GGTCAACAAATCATAAAGATATTGG	(Folmer *et al.* 1994)	45
	COI-Dipt-2183F	CARCAYYTATTYTGATTTTTTGG	(Gibson *et al.* 2011)	
	COI-Dipt-2183R	CCAAAAAATCARAATARRTGYTG	(Gibson *et al.* 2011)	
	UEA10	TCCAATGCACTAATCTGCCATATTA	(Lunt *et al.* 1996)	
COII	2.Strom	TAATTTGAACTATYTTACCIGC	(van Dorp 2004)	45
	mt3660	CCACAAATTTCTGAACATTGACCA	(Stern 1994)	
cyt-b	CB-J-10933-F	TATGTTTTACCTTGAGGACAAATATC	(Simon *et al.* 1994)	45
	TSI-N-11683-R	AAATTCTATCTTATGTTTTCAAAAC	(Simon *et al.* 1994)	
EF-1alpha	M46-F	GAGGAAATYAARAAGGAAG	(Cho *et al.* 1995)	50
	rcM4-R	ACAGCVACKGTYTGYCTCATRTC	(Cho *et al.* 1995)	
PGD	2F-F	ATHGARTAYGGNGAYATGCA	(Regier 2008)	45
	PGDR-R	GTRTGNGCNCCRAARTARTC	(Scott *et al.* 1993)	

Table 5, continued: Primer sequences and PCR annealing temperatures for amplifying gene regions used in this study.

Gene Region	Primer Name	Primer Sequence 5'-> 3'	Reference	Annealing Temperature (ºC)
TPI	111Fb-F	GGNAAYTGGAARATGAAYGG	(Bertone *et al*. 2008)	50
	275-R	GCCCANACNGGYTCRTANGC	(Bertone *et al*. 2008)	
white	11404S-F	TGYGCNTATGTNCARCARGAYGA	(Baker *et al*. 2001)	50
	11975-R	ACYTGNACRTAAAARTCNGCNGG	(Baker *et al*. 2001)	
wingless	Lep-Wg1-F	GARTGYAARTGYCAYGGYATGTCTGG	(Brower & Desalle 1998)	50
	Pomp-Wg2-R	ACTGCGCAGCACCAGTGGAATGTGCA	(Pilgrim *et al*. 2008)	

Table 6. PartitionFinder 2 results, models selected for amino acid data analyzed in RAxML-ng.

Amino Acid Partition	Best Model
AATS	LG4X
CAD	JTT+I+G
COI	MTZOA+G
COII	MTART+G
cytB	MTART+G
EF-1alpha	LG4X
PGD	LG4X
TPI	LG4X
white	LG+I+G
wingless	JTT+G

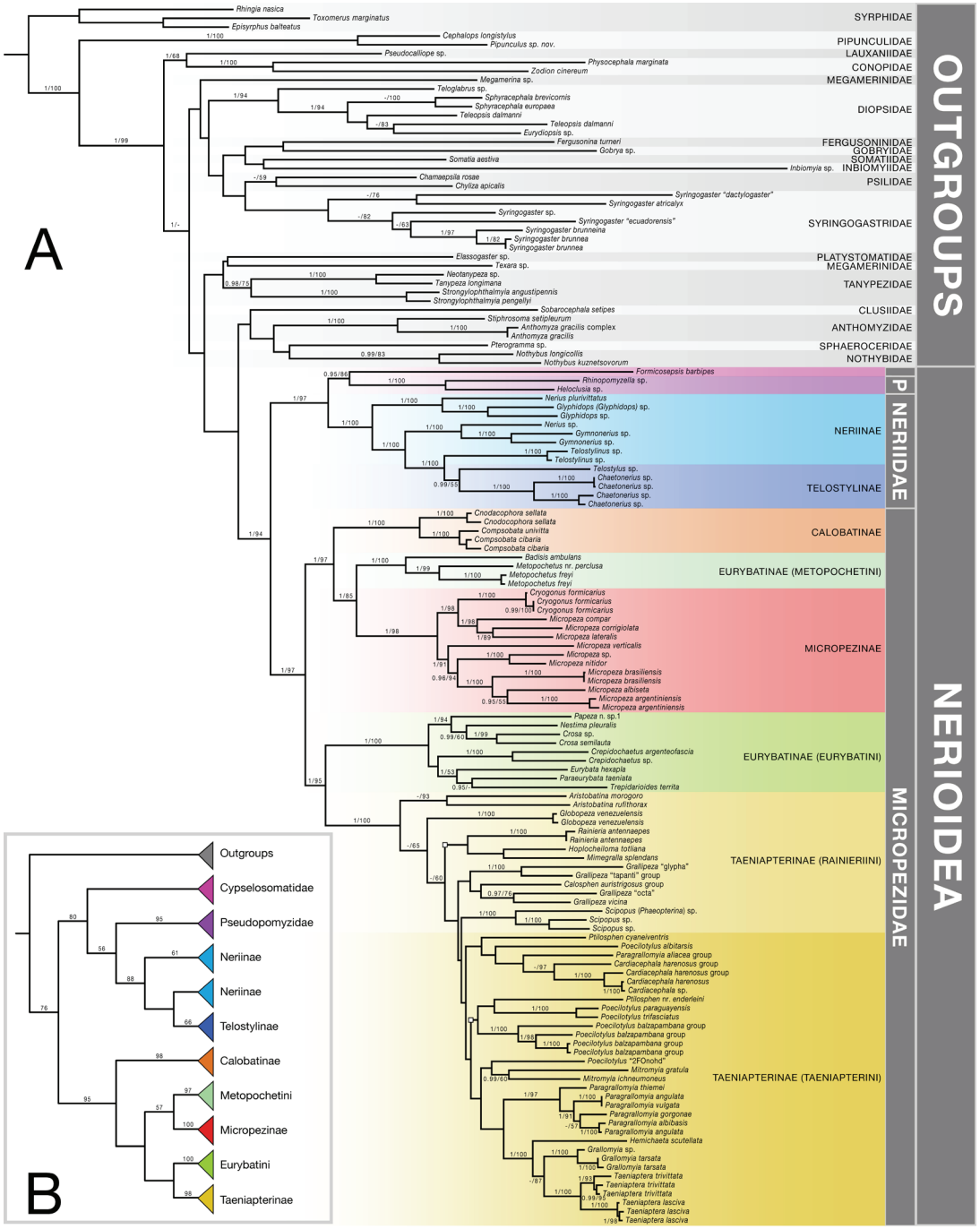
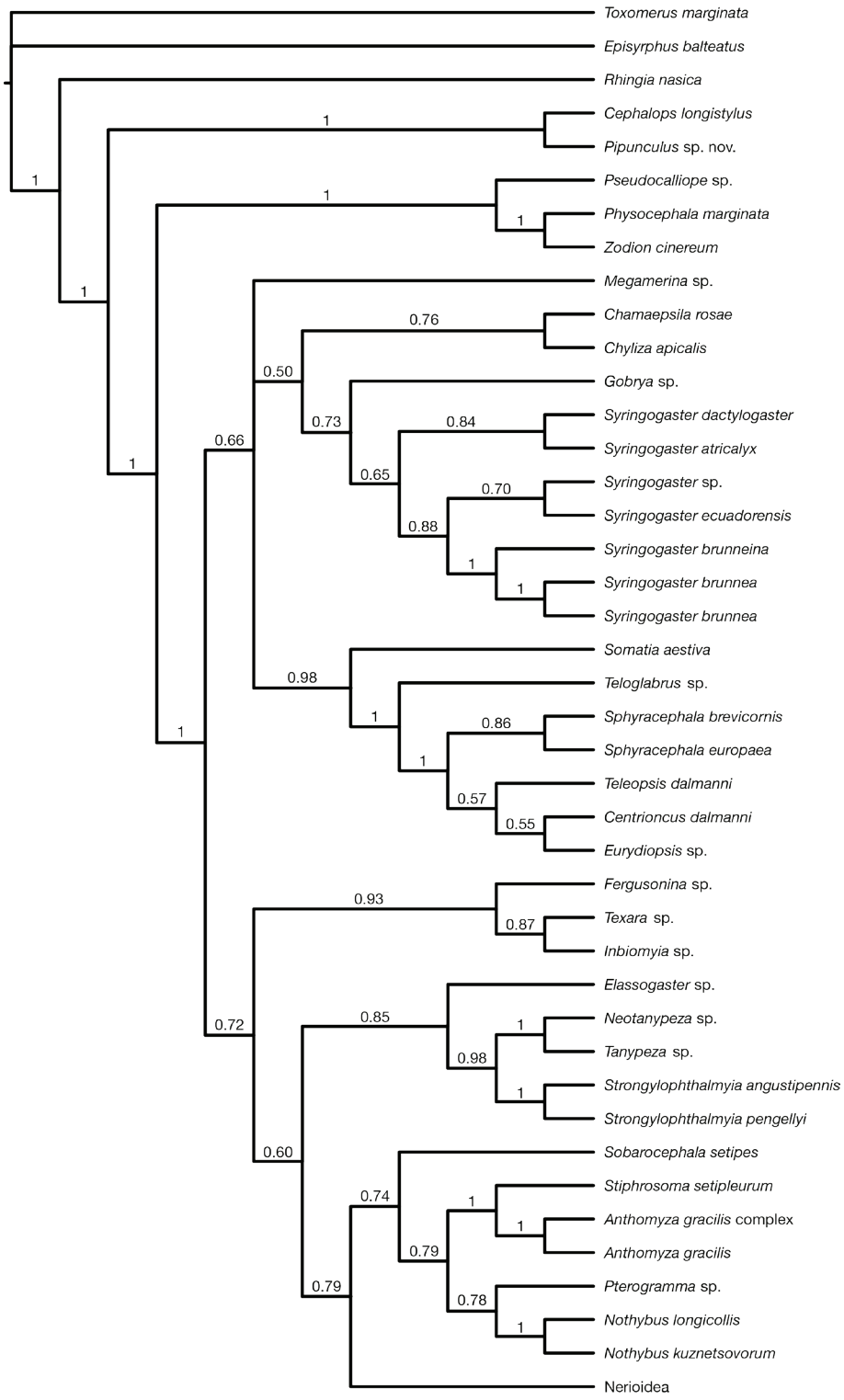


Figure 4: Phylogenetic results based on DNA data for 13 genes obtained from 117 species of higher Diptera. A) Nucleic acid data analysed using Maximum Likelihood and Bayesian Inference. Branch support numbers refer to posterior probabilities >0.95 and bootstrap values >50 (pp/bs). The branch lengths presented are from Maximum Likelihood analysis and the presented topology was largely consistent between Maximum Likelihood and Bayesian Inference analyses, with the exception of the outgroups (see Supplementary Figure 1 for Bayesian Inference cladogram and posterior probabilities for outgroup taxa) and the two nodes marked with white squares. At these points, Bayesian Inference returned collapsed polytomies of unsupported nodes within each of the two clades. B) Amino acid data analysed using Maximum Likelihood. Branch support numbers refer to bootstrap values >50.

Table 7: Number of posterior spiracle slits in third star larvae reported across Nerioidea.

Family	Subfamily	Species	Number of posterior spiracle slits	Reference
Micropezidae	Calobatinae	*Calobata petronella*	3	Ferrar (1987)
		Compsobata univitta	4	Ferrar (1987)
	Micropezinae	*Micropeza corrigiolata*	3	Ferrar (1987)
	Taeniapterinae	*Taeniaptera lasciva*	3	Ferrar (1987)
		Paragrallomyia annulata	3	Fischer (1932)
		Mimegralla spp.	3	Ferrar (1987)
		Rainieria antennaepes	50+	Ferrar (1987)
		Calobatina geometroides	40+	Ferrar (1987)
	Calycopteryginae	*Calycopteryx moseleyi*	3	Ferrar (1987)
	Eurybatinae (Metopochetini)	*Badisis ambulans*	0 (aquatic)	Yeates (1992)
Neriidae	Neriinae	*Odontoloxozus longicornis*	4	Ferrar (1987)
		Telostylinus lineolatus	4	Ferrar (1987)
Cypselosomatidae		*Cypselosoma australe*	4	Ferrar (1987)



Supplementary Figure 1: Outgroup relationships recovered from Bayesian Inference analysis of nucleotide data. Numbers above branches represent posterior probabilities

CHAPTER 2 – MOLECULAR SUPERMATRIX ANALYSIS OF ACALYPTRATE DIPTERA

Introduction

Schizophoran Diptera represent one of the largest recent radiations of life on Earth, with more than 56,000 species constituting 82 families, representing about one third the dipteran species diversity yet one half the family-level diversity (Pape et al. 2011). These flies, defined by a unique head structure called the ptilinum, used by newly emerged imagoes to escape their puparium, include many species of considerable importance to biomedical research, agriculture, and human health. Schizophoran Diptera influence our lives every day, from the model organism *Drosophila melanogaster,* whose study has saved untold numbers of lives, to countless vectors of disease that plague people, crops, and livestock and which cumulatively cost billions of dollars in damage and lost economic potential every year (Soroka et al. 2004, Colautti et al. 2006, Taylor et al. 2012, Oliveira et al. 2013, Malheiro et al. 2015, Ekesi et al. 2016, FAO 2017). Yet despite the global importance of these flies, and the continued application of cutting-edge technologies, techniques, and philosophies by the dipterological community, the evolutionary history and relationships of Schizophora remain poorly resolved.

The Calyptratae are widely recognized as monophyletic and consist of 15 families and more than 40% of the described schizophoran species, with the remaining schizophoran diversity spread across the families of acalyptrate Diptera. The classification of this remaining group, including their relationships with Calyptratae, has proven challenging for Diptera systematists, with many hypotheses having been put forward (Table 1). Of these proposed classifications, the work of 3 authors are routinely considered and referred to: Hennig, Griffiths, and J.F. McAlpine.

With the first cladistic consideration of higher dipteran relationships, Hennig (1958) subdivided Schizophora into 10 superfamilies alongside a suite of families he considered to have “confused or vague affinities”, but he was unable to provide any further insight into the higher relationships of Schizophora. He would gradually refine his theories (Hennig 1971), tentatively proposing that Acalyptratae was monophyletic, and subdividing it up into 11 superfamilies, but with little indication of how they were related to one another (Hennig 1973). Contemporaneously, Griffiths (1972) put forward a slightly more resolved classification of 13 higher schizophoran taxa, however he considered Calyptratae as arising from within a paraphyletic acalyptrate grade. The first fully resolved phylogeny of schizophoran relationships would eventually be published by J.F. McAlpine

(1989) in which he divided a monophyletic Acalyptratae into 10 superfamilies, providing detailed discussions of apomorphies he considered in support of his classification. Yeates et al. (2007) provided a supertree analysis of the Schizophora phylogenies put forward by Hennig, Griffiths, and J.F. McAlpine, a method whereby the nodes from a set of phylogenies are converted into a matrix and analyzed as more traditional morphological or molecular characters would be (Ragan 1992), and which, unsurprisingly given the unresolved nature of Hennig and Griffiths work, returned a topology of schizophoran relationships almost identical to that proposed by J.F. McAlpine (1989). Sinclair et al. (2013) were able to provide additional male genitalia characters in support of most of McAlpine's higher taxa (Tephritoidea, Nerioidea, Carnoidea, Lauxanioidea, Sciomyzoidea, and Ephydroidea), but were unable to provide synapomorphies for relationships between superfamilies. Classification schemes for Schizophora were also presented by Rohdendorf (1964, 1974) and Colless and McAlpine (1970, 1975, 1991), but across all of these proposed classifications (Table 1), only a handful of superfamilies–Lauxanioidea, Sciomyzoidea, and Nerioidea–were routinely supported by additional datasets, while concepts of Tephritoidea and Ephydroidea were mostly conserved between authors. While the composition of these few groups appeared largely settled, the relationships between them and the remaining Schizophora, however they were to be grouped or divided, remained largely unaddressed save for J.F. McAlpine's work. It would be these proposed relationships by McAlpine that would become the primary point of comparison for future attempts at discriminating schizophoran relationships as the dipterological community entered the age of molecular systematics.

Thanks to the long-established importance of Drosophilidae to the fields of genetics, medicine, and evolution, *Drosophila* (*Sophophora*) *melanogaster* was one of the first eukaryotic organisms to have nucleic acid sequences published (Pavlakis et al. 1979, Silverman et al. 1979), and it wasn't long until systematists began experimenting with this new source of data. The first systematic test of species relationships using molecular data in Schizophora used distance matrices developed from Alcohol Dehydrogenase gene sequences for three *Drosophila* species (Cohn et al. 1984), while the first cladistic treatment of molecular data involved mitochondrial data collected from Hawaiian *Drosophila* (DeSalle et al. 1987). Interestingly, the first test of schizophoran family-level relationships using DNA (28S ribosomal DNA; Vossbrinck and Friedman 1989) was published within months of McAlpine's (1989) morphological qualitative approach, and returned a paraphyletic acalyptrate grade approaching Calyptratae, opposing J.F. McAlpine's hypothesis of a monophyletic Acalyptratae. The 1990's would see an expansion of molecular data being used to test relationships among acalyptrate species and genera, particularly within Tephritidae and

Drosophilidae (reviewed by Caterino et al. 2000), but only one other study tested relationships of Schizophora, again returning a paraphyletic acalyptrate grade (Soto-Adames et al. 1994). The turn of the 21st Century saw the interfamilial relationships of several acalyptrate superfamilies being tested with molecular data (Han et al. 2002, Meier and Wiegmann 2002, O'Grady and Kidwell 2002, Shaw 2002), and whose results were encouraging. The prospect of resolving the higher relationships of acalyptrate Diptera using DNA seemed possible, with promises of a revolutionary new age of Diptera systematics arriving on the wings of molecular data (Yeates et al. 2007).

This new age of Diptera systematics began with a diversity of studies using nuclear genes in conjunction with the mitochondrial and ribosomal loci previously established. Internal relationships of Agromyzidae (Scheffer et al. 2007, Winkler et al. 2009), Clusiidae (Lonsdale et al. 2010), and Sepsidae (Feng-Yi Su et al. 2008) were all tested using new data sets, while others began to look more broadly, testing the relationships of families within and between established superfamilies using molecular datasets (Kutty et al. 2008, Gibson et al. 2010, Winkler et al. 2010). These works contributed to, and ultimately culminated in, the publication of the Fly Tree of Life project, which sampled exemplar taxa from nearly every family of Diptera, sequencing at least one of more than 20 target loci (Wiegmann et al. 2011). While Schizophora and Calyptratae were well-supported as expected, relationships among acalyptrate taxa were largely at odds with previously proposed classifications and were poorly supported. Of the superfamilial relationships returned, only Nerioidea and Lauxanioidea matched Hennig's (1973), Griffiths' (1972), and McAlpine's (1989) proposed concepts, while Tephritoidea, Ephydroidea, and Sciomyzoidea were each provided new concepts by Wiegmann et al. that varied, sometimes considerably, from those proposed by Hennig, Griffiths, and/or McAlpine. In addition, when the molecular dataset was restricted to only the most completely sampled taxa, and the accompanying morphological matrix was analyzed with (Wiegmann et al. 2011) or without DNA data (Lambkin et al. 2013), relationships among Schizophora changed significantly. So, while there were great expectations of "new and exciting insights" into acalyptrate phylogeny resulting from the FlyTree of Life project (Yeates et al. 2007; pg 582), these expectations were quickly tempered by ongoing instability in classification. Ultimately, unravelling and understanding the evolutionary history of Schizophora, particularly among acalyptrate lineages, came to be considered "one of the most difficult questions in systematic entomology" (Wiegmann and Yeates 2017; pg 258).

Following Wiegmann et al. (2011), DNA data were used extensively to test relationships within schizophoran families, leading to an ever-increasing pool of published data available for

systematists to use and recombine as they explored the relationships of Schizophora at multiple levels. However, while more and more systematists have begun sequencing the DNA of flies to create datasets from dozens of genes for scores of species, new techniques have emerged to generate larger and ever more expansive DNA datasets, and it is now possible to quickly and "affordably" retrieve molecular data from hundreds to tens of thousands of genetic loci using next generation sequencing technologies (Yeates et al. 2016). These new techniques allow for the accumulation of massive datasets for individual species, frequently utilizing transcriptomes (the sections of DNA that are actively being used at the time of the organism's collection) or even entire genomes. This new "postgenomic era" has been anointed the future of phylogenetic systematics (Trautwein et al. 2012; pg 461), a future which, echoing words published a decade earlier, comes "with the promise of exciting new hypotheses" for understanding major radiations of Diptera, including Schizophora (Wiegmann et al. 2017; pg. 260).

Along with the continued collection of DNA data from acalyptrate flies for varying purposes, phylogenetics software and resources have improved, and our general knowledge regarding factors influencing phylogenetic analyses, substitution models, and molecular evolution have all progressed, providing ample opportunity to revisit the problem of schizophoran relationships with fresh analyses and larger, more inclusive taxon sampling. Supermatrix analyses, which combine data from unrelated studies and sources into one single mega-analysis (de Queiroz and Gatesy 2007), have proven fruitful in wide-scale phylogenetic analyses, particularly among insects (Peters et al. 2011, Hedtke et al. 2013, Bocak et al. 2014, Chesters 2016), including Diptera (van der Linde et al. 2010, Piwczyński et al. 2014, Shin et al. 2018). This type of analysis is inherently high in its proportion of missing data as it pieces together sequences from sources of varying dimensions and depths. This degree of missing data has been proposed to have detrimental effects during analysis, particularly if the missing data is not randomly distributed across the matrix (Simmons 2012, Xi et al. 2016); however empirical examples have shown the significance of missing data to be minimal (Roure et al. 2013), and it is believed the advantages of additional taxa, even if they are missing a large proportion of data, outweigh the impacts of missing data when resolving deep phylogenies (Hillis 1996, Zwickl and Hillis 2002, Hedtke et al. 2006).

Methods

Taxonomy

Superfamily- and family-level classification and nomenclature used in this study are based on Pape et al. (2011) and Marshall (2012), with updated Calyptratae nomenclature drawn from Cerretti et al. (2017). While Pape et al. (2011) and Marshall (2012) are largely in agreeance, in the current work we follow Marshall rather than Pape et al. for the following points: Pallopteridae is treated as belonging to Tephritoidea, Acartophthalmidae as belonging to Carnoidea, and Heteromyzidae is treated as a subfamily of Heleomyzidae, while Pseudopomyzidae is recognized as distinct from Cypselosomatidae, and Mesembrinellidae as distinct from Calliphoridae.

Data Collection

A review of the Diptera systematics literature was undertaken searching for studies using DNA from acalyptrate Schizophora, and GenBank accession numbers for sequences and taxa of interest were compiled. Following this literature search, GenBank was searched using the GenBank Taxonomy database to locate additional sequences for the included gene loci for acalyptrate families that were published in non-systematics articles, excluding *cytochrome c oxidase* I data, as these data have become so ubiquitously collected and published that sorting through the nearly 200,000 sequences is beyond the scope of this project. Chesters (2016) performed a supermatrix analysis including nearly 50,000 species of insects, but the inclusion of thousands of COI DNA Barcodes resulted in massive taxonomic discordance and polyphyly of Schizophoran families. Species for which only COI data were available were not included here, unless they were included in one of the studies discovered via the literature review (i.e., miscellaneous DNA Barcodes from the Barcode of Life Database (Ratnasingham and Hebert 2007) were not included). While the internal relationships of Calyptratae were not the focus of this study, select data from a few large molecular phylogenetics projects (Petersen et al. 2007, Kutty et al. 2008, 2010) encompassing the spectrum of diversity of this clade were included to address the relationships between Calyptratae and other Schizophora. Non-schizophoran Eremoneura species were included as outgroups. In total, 2301 uniquely named taxa in GenBank were included in this study, across 92 families of higher Diptera, including representatives of all Schizophora families (Supplementary Table 1; 87 ingroup families, 5 outgroup families: available at https://doi.org/10.5063/F1PK0DDV).

DNA Sequences & Alignment

Twenty-three genes that have been used for estimating phylogenies in Schizophora were selected, including data from mitochondrial coding genes (ATP6, ATP8, COI, COII, COIII, CYTB, ND3, ND4L, ND6), nuclear coding genes (AATS, CAD, EF-1α, G6PD, PEPCK, PER, PGD, PUG, SINA, SYX1A, TPI), and ribosomal RNA genes from both mitochondrial (12S, 16S) and nuclear

(28S) sources. GenBank accession numbers for included data can be found in Supplementary Table 1. All sequences were imported into Mesquite 3.5 (Maddison and Maddison 2017), and aligned using the FFT-NS-i, FFT-NS-1, or FFT-NS-2 algorithm in MAFFT version 7 (Katoh and Standley 2013) using the MAFFT online service (Katoh et al. 2017). All alignments were then checked by eye and modified by hand where necessary (readframe shifts, misaligned gaps). 28S sequences from Su et al. (2008) and Zhao et al. (2013) were found to have regions 2 and 3 transposed relative to the remaining dataset; these regions were manually corrected, and were subsequently aligned with the remaining dataset. Data was concatenated in Mesquite. When a species had more than one sequence available for a given gene, the longest sequence was selected, except for 28S, where mosaics were built from data obtained from different specimens that had non-overlapping sites in order to maximize coverage and sequence completeness. Sequence alignment files are available at https://doi.org/10.5063/F1PK0DDV.

Excluded Data

A 74 bp unalignable intron in the PGD dataset (alignment positions 536-610) was excluded from analysis, as was a 104 bp unalignable region of CAD (alignment positions 943-1047). Due to computational limits imposed by a matrix of this size, a structural alignment could not be done with the 28S dataset, and ultimately several regions, totalling 1,306 bp (alignment positions 1355-1560; 1637-1674; 1992-2039; 2231-2252; 2597-2642; 4021-4510; 5065-5235; 6143-6155; 6289-6300; 6307-6576), were excluded from analysis because homology could not be confidently assured through visual inspection of the data.

Data Partitioning & Subsampling

To assess the influence on phylogeny reconstruction of missing data, taxon sampling, and gene sampling, several partitions were subsampled for analysis. To minimize the impact of gene trees on species tree estimation, all subsampling schemes included sequential restrictions in taxon sampling favoring taxa with greater coverage of the 23 sampled gene loci (Table 2).

ALL+ALL: all genes and all taxa were included for analysis.

ALL+2: all genes were included, but only taxa that had data for at least 2 genes were included for analysis. Datasets including and excluding 3rd codon positions of protein coding genes were analyzed.

ALL+5: all genes were included, but only taxa that had data for at least 5 genes were included for analysis. Datasets including and excluding 3rd codon positions of protein coding genes were analyzed.

Phylogenetic Analysis

Because of the size of the matrix and datasets, computational efficiency was a high priority to allow for accurate, timely analyses, and thus all datasets were analyzed under Maximum Likelihood using IQ-TREE ver. 1.6.6 (Nguyen et al. 2015). IQ-TREE is specifically optimized for large phylogenomic datasets, particularly those datasets with large degrees of missing data, by using Phylogenetic Terrace Aware data structure and partitioning assessments (Chernomor et al. 2016), and also allows for greater flexibility and specificity in applying substitution models to data partitions. All datasets were partitioned by gene, and protein coding genes were also partitioned by codon position, with the GTR+G model applied to all partitions using the -spp option for edge-linked branch lengths. Ultrafast Bootstrapping by site within partitions (Hoang et al. 2018) was used to provide branch supports, with 1000 replicates performed.

To test the influence of model optimization on the data, the ALL+2 dataset was run through ModelFinder (Kalyaanamoorthy et al. 2017) within IQ-TREE prior to analysis to find the best-fitting model for each partition. These partitions and model optimizations were then applied to the ALL+2 and ALL+5 datasets as well as each respective "no 3rds" variation. Partitioning and model schemes are provided in Table 3. All IQ-TREE analyses were run on the CIPRES Science Gateway (Miller et al. 2010).

Results & Discussion

The present study represents a taxonomically-rich quantitative phylogenetic study of schizophoran Diptera and reveals that most families and superfamilies of Schizophora as classified using morphological characters can be successfully recovered using DNA data by adding taxa (even at the expense of increasing total missing data), optimizing substitution models and partitioning schemes, and by controlling for differential phylogenetic signal in 3rd codon positions (Fig. 1-11, Supp. Fig. 1-2). Topologies between datasets and treatments are relatively stable, although some taxa exhibited significant shifts in higher relationships when 3rd codon positions were excluded from analyses. The ALL+2 analyses with optimized models (with and without 3rd codon positions; Fig. 3 and 6, respectively) successfully recovered most of J.F. McAlpine's (1989) superfamilies between them, and for this reason are selected as the preferred trees for purposes of this discussion. Relationships within Calyptratae largely reflect those found in previous studies (Petersen et al. 2007, Kutty et al. 2008, 2010, Cerretti et al. 2017, Marinho et al. 2017), although the relationship between Calyptratae and other Schizophora remains unresolved. Nerioidea, Tephritoidea, Lauxanioidea and Ephydroidea were recovered in most analyses, while Diopsoidea and Opomyzoidea appear

to be particularly vulnerable to perturbations in taxon selection and model optimization. The recovered higher relationships of Sciomyzoidea reveal several new hypotheses that will require critical re-examination of morphological concepts, and which highlight the importance of outgroup selection. Taxon availability within Carnoidea and Sphaeroceroidea remains a critical weakness, as does backbone support and stability, although it is proposed that the latter two weaknesses are a symptom of the former. Finally, the implications for the future of phylogenetic and phylogenomic studies into the relationships and systematics of Schizophora in light of the reported findings are considered.

Gene Trees, Taxon Sampling, Missing Data, and Substitution Models

While there is no expectation that J.F. McAlpine's morphological phylogeny is "correct" (i.e. perfectly approximating the true evolutionary history of Schizophora), one of the central assumptions of this study is if multiple, independent sources of data converge on similar phylogenetic solutions, then those congruent solutions should be considered to have greater merit than less congruent solutions. Comparing the topology of trees across dataset subsampling treatments against the topology of J.F. McAlpine's (1989) morphology-based phylogeny, the treatments displaying the best congruency were the ALL+2 treatments with optimized partitioning and substitution model schemes (Figs. 3 and 6) which are selected as the preferred trees. Comparing these preferred trees to the remaining treatments, the influence gene trees, taxon sampling, missing data, and substitution model choices have on the data can be elucidated.

The ALL+ALL analysis (Fig. 1), which included all species regardless of the number of genes sequenced for each, returned a general topology almost identical to that of the ALL+2 under similar parameters (Fig. 2), but with hundreds of species of Drosophilidae and Tephritidae being returned in discordant locations. Of these discordant species, almost all are represented in the matrix by a single mitochondrial sequence from either COI, COII, or COIII, or the nuclear gene SINA. Other species from these families which only have DNA sequences from other genes, such as 16S, 28S, or CAD are returned within the proper family, often alongside other species of their genus. This suggests that species for which limited DNA data are available, even as little as data from a single gene, can positively contribute to a large phylogeny like this if there are sufficient related species which have been sequenced for that same gene. However, caution is warranted when including species for which the only data available are from the cytochrome c oxidase mitochondrial complex, as these sequences appear to be more prone to substitution saturation and homoplasy. This is disappointing given that the largest potential source of Schizophora DNA are

COI sequences collected as DNA Barcodes (Hebert et al. 2003). It remains possible that including a large number of species spanning the diversity of Schizophora that are solely represented by DNA Barcodes may overcome this observed homoplasy problem as there are examples in Fig. 1 of DNA Barcode-only species being returned in sensible positions (eg. *Myoleja sinensis*), but more testing is necessary. Optimizing substitution models may also help conserve phylogenetic signal in these single-gene species, but this remains to be tested.

The trade-off between maximizing taxon coverage and minimizing missing data in large-scale phylogenetic analyses like this study has been extensively debated in the literature (Pollock et al. 2002, Hillis et al. 2003, Hedtke et al. 2006, Heath et al. 2008, Lemmon et al. 2009, Simmons 2012, Roure et al. 2013, Brower and Garzón-Orduña 2017). In this dataset, missing data cannot be presumed to be randomly distributed by gene or species, a factor that has been shown to introduce artifacts in likelihood-based phylogenetic analyses (Xi et al. 2016). Here, when comparing between treatments in which taxon coverage is sacrificed for data coverage (eg. Fig. 2 versus Fig. 8, Fig. 3 versus Fig. 9, etc.; see Table 2 for comparative dataset statistics), tree topologies are observed to be significantly incongruent when taxon sampling is reduced. While superfamily composition is generally similarly conserved between comparative trees, the relationships between superfamilies differ dramatically, sometimes with the polarity of the entire tree being reversed (Fig. 7 versus Fig. 11). This phenomenon begs the question of which of each pair of phylogenies represents the "correct" topology. As previously noted, the ALL+2 analyses with optimized substitution models show the greatest congruency with previous hypotheses, and so, in this study, increased taxon sampling has been valued more heavily than reduced missing data, a conclusion supported by more extensive tests of empirical data (Roure et al. 2013, Jiang et al. 2014). Ultimately, more data from more species is desirable, and thus future projects contributing to this supermatrix approach should sequence as many genes from as many taxa as possible, however if a choice must be made between more genes or more taxa, taxon sampling should be prioritized over gene sampling.

Finally, tree topology changed significantly between treatments with a GTR+G substitution model applied to all partitions and treatments with substitution models and partitions optimized by ModelFinder (eg. Fig. 2 versus Fig. 3, Fig. 5 versus Fig. 6, etc.). This more advanced application of substitution models may partially explain why this study was better able to resolve Schizophora relationships compared to Wiegmann et al. (2011), in which the authors did not partition their data by codon position, and applied a GTR+G substitution model to all genes. The importance of partitioning and substitution model optimization are now recognized as two of the most important

factors in large-scale phylogenetic analyses when using likelihood methods (Sullivan and Joyce 2005, Frandsen et al. 2015). While standard substitution models and *a priori* partitioning are increasingly being found to be inadequate for dealing with the complexity of real-world nucleotide evolution (see further discussion under *Phylogenetic Signal, Homoplasy, and Phylogenetic Progress*), the results of this study demonstrate that making the best of an imperfect substitution model is better than foregoing their potential all together.

Relationships of Schizophora; DNA support for morphological classifications

The relationships of Schizophora have proven difficult to elucidate using DNA data, with little consistency with morphological relationships, or even between molecular analyses (Gibson et al. 2010, Winkler et al. 2010, Wiegmann et al. 2011). Here, for the first time for several higher-level taxa, we demonstrate that molecules can agree with morphology, provided taxon sampling is high and analytical methods are optimized. Nerioidea, Ephydroidea, Calyptratae, Lauxanioidea, and Conopoidea were each recovered as monophyletic units in at least one of the preferred trees (Fig. 3, 6), with Tephritoidea and Diopsoidea also being recovered as individual clades in the 3rds-included preferred tree, save for small clusters of intruding taxa which are proposed to be a result of homoplasy rather than any indication of true relationship (discussed in more detail below), while Sciomyzoidea was also consistently resolved, albeit always with the exclusion of Sepsidae and Ropalomeridae and the inclusion of Conopoidea (a classification henceforth referred to as Sciomyzoidea*). The only superfamilies that were consistently recovered as non-monophyletic were Sphaeroceroidea, Carnoidea, and Opomyzoidea, of which the latter two appear to be closely entwined with one another, as proposed by McAlpine (1989). Taxon availability is of particular concern for Sphaeroceroidea and Carnoidea, which is proposed as the primary obstacle to recovering these taxa as monophyletic and for increasing phylogenetic stability across Schizophora. The relationships between superfamilies vary in their stability, with Lauxanioidea and Sciomyzoidea* always being recovered as sister taxa, while Nerioidea, Tephritoidea, and Diopsoidea are consistently found to be related. Ephydroidea and Calyptratae are consistently recovered as individually monophyletic, but their relationships with the remaining Schizophora remain unstable. Carnoidea, Opomyzoidea, and Sphaeroceroidea remain too unstable presently to draw any conclusions regarding their monophyly and/or relationships to other Schizophora.

The relationship between Calyptratae and other Schizophora was highly dependent on phylogenetic signal contained within 3rd codon positions (Fig. 3,6). With 3rd codon positions excluded, Calyptratae was returned deep within Schizophora and the sister taxon of Ephydroidea, although

Calyptratae was not monophyletic, with several Hippoboscoidea taxa being recovered elsewhere. This relationship, rendering acalyptrate Schizophora paraphyletic, has been recovered by several modern molecular studies (Gibson et al. 2010, Han and Ro 2016, Junqueira et al. 2016), including the FlyTree of Life molecular data (Wiegmann et al. 2011; Fig. 1). However, all of these studies either excluded 3rd codon positions in their analysis (Gibson et al. 2010, Wiegmann et al. 2011), or were based entirely on mitochondrial data (Han et al. 2016, Junqueira et al. 2016). In this study, when 3rd codon positions for all protein coding genes were included and both nuclear and mitochondrial data were concatenated, Calyptratae were recovered near the base of Schizophora, and sister to a largely monophyletic Acalyptratae (save for a few disparate and unrelated acalyptrate taxa that were recovered with their expected families and/or superfamilies with high support when 3rd positions were excluded). A monophyletic Acalyptratae was proposed by both Hennig (1973) and McAlpine (1989) based on their respective qualitative morphological analyses, and while the monophyly of Acalyptratae was not supported by Griffiths (1972) or modern quantitative morphological analyses (Lambkin et al. 2013), Caravas and Friedrich (2013) did find support for a monophyletic Acalyptratae when nuclear data from Wiegmann et al. (2011) were analyzed independently from mitochondrial data, with and without 3rd codon positions (Caravas et al. 2013; Fig. S1). Caution is necessary in assessing a basal Calyptratae and monophyletic Acalyptratae however, as long branch attraction is known to draw highly homoplasious lineages towards basal positions as saturated nucleotide sites become randomly associated with outgroups in phylogenies (Bergsten 2005). Because the monophyly of Schizophora can be reasonably assumed on the basis of the ptilinum and other morphological characters, future analyses should experiment with analyzing schizophoran datasets *with* and *without* the inclusion of non-schizophoran Diptera as outgroups. By comparing the unrooted trees resulting from both approaches, long-branch attraction artifacts can be detected following Bergsten (2005). Increasing taxon sampling for Calyptratae will also help reduce multiple substitution saturation, further reducing apparent homoplasy by providing greater evolutionary context for unique sequences, which will also minimize the influence of long branch attraction and random associations. However, the results of the present study indicate it is still too early to conclusively say how Calyptratae and acalyptrate Diptera are related to one another, and future efforts to disentangle the relationships of Schizophora should remain open to the possibility of a monophyletic Acalyptratae.

While the relationships between schizophoran superfamilies remain unsettled, several previously proposed superfamily classifications were returned and supported in one or both of the preferred analyses. Despite the economic and biomedical importance of Drosophilidae, Ephydroidea, have

received very little phylogenetic attention, particularly above the family level. In addition to McAlpine (1989), morphological phylogenies have been proposed by Chandler (1987) and Grimaldi (1990), while Wiegmann et al. (2011) provided the first molecular evidence for the relationships of the superfamily, which was at odds with all morphological hypotheses. While Curtonotidae, Camillidae, Drosophilidae, Diastatidae, and Ephydridae have constituted Ephydroidea since Hennig (1958), the FlyTree of Life project revealed the enigmatic Braulidae and Cryptochetidae also belonged within Ephydroidea (Wiegmann et al. 2011). Unpublished data referred to by Kirk-Spriggs and Wiegmann (2013) and Wiegmann and Yeates (2017) suggests the equally enigmatic family Mormotomyiidae may also belong in Ephydroidea. Here, Ephydroidea as considered by Wiegmann et al. (2011), is returned as monophyletic when 3rd codon positions are excluded (Fig. 6), and sister to Calyptratae with weak support. When 3rd codon positions are included, the poorly sampled Ephydridae is recovered as paraphyletic, with three taxa forming an unsupported sister relationship with Calyptratae, and the remaining Ephydroidea sister to Sepsidae plus errant Opomyzoidea, Carnoidea, and Sphaeroceroidea (Fig. 3). The family relationships recovered here largely match those in Wiegmann et al., although here Braulidae is returned as sister to Curtonotidae, not Cryptochetidae, which is itself recovered as either sister to Drosophilidae (Fig. 3), or within Drosophilidae (Steganinae) (Fig. 6). The novel positioning of Braulidae as sister to Curtonotidae is of particular note. Establishing morphological synapomorphies between Braulidae and other Schizophora, including Ephydroidea will likely continue to prove particularly challenging due to the extreme autapomorphic morphology of Braulidae resulting from their phoretic lifestyle on bees of the genus *Apis*. Kleptoparasitism of aculeate Hymenoptera has also been recorded in Drosophilidae (Coutin and de Chenon 1983), and more tenuously, Curtonotidae (Klymko and Marshall 2011), observations which may prove fruitful for establishing relationships between Braulidae and Ephydroidea. This behaviour is also recorded from Carnoidea however (Chloropidae; Sivinski et al. 1999), where J.F. McAlpine (1989) placed Braulidae. The only molecular data currently available for Mormotomyiidae are COI Barcodes which place the monotypic family within Sepsidae (Fig. 1); conclusions on the phylogenetic placement of this family are best held until more data are available.

Internal relationships of Nerioidea were recovered largely as in Chapter 2, with Micropezidae confidently returned as sister to Neriidae, Cypselosomatidae, and Pseudopomyzidae. The relationships among these three latter taxa were unstable and unsupported, with Cypselosomatidae being returned as sister to Neriidae when 3rd codon positions were included, and Pseudopomyzidae+Neriidae being returned when 3rd codon positions were removed. Relationships

between Micropezidae subfamilies were stable, consistently matching the relationships recovered in Chapter 2. The relationship between Nerioidea and the remaining Schizophora remain unstable, although previously proposed relationships remain plausible. Nerioidea was never recovered basally within Schizophora as proposed by D. K. McAlpine (1966), Shatalkin (1994), and D. K. McAlpine and Shatalkin (1998). In all analyses, various combinations of opomyzoid and carnoid taxa were returned as immediate sister to Nerioidea, as well as a frequently returned but unsupported relationship between Ropalomeridae+Neminidae and Nerioidea. The relationships between Nerioidea and Ropalomeridae+Neminidae are likely artifactual, as no association between these three taxa has ever been proposed previously, and there is little biological or biogeographical evidence to suggest an evolutionary connection. However, other relationships here recovered bear further examination. Both Hennig (1948, 1952, 1958) and D.K. McAlpine (1996) tentatively noted Nerioidea and Clusiidae share similarities in their morphology, both as larvae and as adults (head, wing, and aedeagal characters in particular) and a sister relationship between Clusiidae (plus Ropalomeridae+Neminidae) and Nerioidea was here recovered in the preferred tree including 3rd positions (Fig. 3), albeit without support. Other suggested relationships of Nerioidea include Tephritoidea and Diopsoidea (J.F. McAlpine 1989), both of which were recovered near Nerioidea in analyses presented here, as were Sphaeroceroidea, which supports D.K. McAlpine's ultimate conclusion on the higher relationships of Nerioidea (1996). Unfortunately, until the backbone phylogeny of Schizophora can be further stabilized, the higher relationships of Nerioidea remain a mystery.

Relationships within Tephritoidea have been well-explored since McAlpine (1989), using both morphological evidence (V. A. Korneyev 2000) and mitochondrial sequence data (Han et al. 2002, Han and Ro 2005, 2009, Han et al. 2016). The relationships returned here largely support the work of Korneyev and Han et al., although the analyses were split on whether the Lower Tephritoidea were monophyletic (3rd codon positions included), or whether Piophilidae was more closely related to the remaining Tephritoidea (3rd codon positions excluded). In both analyses, Pallopteridae was strongly and consistently recovered as belonging to Tephritoidea in contradiction of Wiegmann et al. (2011) and Pape et al. (2011), although Eurygnathomyiinae was never recovered with the remaining Pallopteridae. Relationships of Higher Tephritoidea are largely unremarkable, although the relationships between a clade consisting of Ctenostylidae, Pyrgotidae, Tachiniscidae, and, Blepharoneurinae (Tephritidae) as sister to the remaining Tephritidae are potentially significant, albeit weakly supported with the data available. Blepharoneurinae are generally considered to be the sister group to the remaining Tephritidae (Norrbom and Condon 2000), and are well-known

for their hyper-specialization and extreme niche partitioning of host plants (Condon et al. 2014); their being returned exterior to the remaining phytophagous Tephritidae and more closely to the parasitic lineage (Korneyev 2014) is worth further examination in the future.

The clade containing Lauxanioidea, Conopoidea, and Sciomyzoidea* was among the most stable and highly supported relationship recovered across all analyses. Chamaemyiidae was routinely returned as sister to the Lauxanioidea+Conopoidea+Sciomyzoidea* clade, while Celyphidae was consistently returned within Lauxaniidae, albeit in a relatively basal position and with only moderate to weak support. Natalimyzidae, which was assuredly allied with Sciomyzoidea+Lauxanioidea when initially described (Barraclough and McAlpine 2006), but which could not be assigned a sister group therein, is here recovered as of uncertain relationship; when 3rd codon positions are included, Natalimyzidae is returned as sister to Lauxanioidea (Fig. 3), but when 3rd codon positions are excluded, Natalimyzidae is recovered as sister to Heterocheilidae (Fig. 6). Both topologies are statistically unsupported, and so the position of Natalimyzidae will remain uncertain until additional species can be sequenced. Within Sciomyzoidea*, the largely saprophagous lineages (Dryomyzidae, Heterocheilidae, Helcomyzidae, Huttoninidae, Helosciomyzidae, and Coelopidae) were consistently recovered as a supported clade, while the parasitoid lineages were also recovered together. Conopidae was strongly and reliably returned within Sciomyzoidea, as proposed by Wiegmann et al. (2011), although *Stylogaster* was nearly always returned with strong support as the basal lineage of Conopidae here (the sole exception being the ALL+5 analysis using GTR+R and excluding 3rd codon positions, Fig. 10), contradicting the paraphyletic Conopidae returned by Wiegmann et al. (2011). Gibson et al. (2010) recovered Lauxaniidae as sister to Conopidae in their analysis of molecular data, while morphological data from Gibson and Skevington (2013), which also included Lauxaniidae but not Sciomyzoidea in the outgroup, were unable to ascertain relationships with other Schizophora. The putative relationship between Sciomyzoidea and Conopidae has yet to be fully accepted by some Conopidae specialists (Stuke 2017), although there is little doubt based on the molecular evidence available at this time. The preferred trees place Conopidae as either sister to Sciomyzidae+Phaeomyiidae when 3rd codon positions are included (Fig. 3), or sister to Phaeomyiidae when 3rd codon positions excluded (Fig. 6), albeit with much weaker support. This relationship between Conopidae, Sciomyzidae, and Phaeomyiidae is entirely satisfying from a natural history perspective, and brings the major acalyptrate lineages of invertebrate parasitoids together as a single evolutionary unit, necessitating a re-examination of the evolution of parasitoidism and host partitioning and transitions among sciomyzoid flies (Knutson and Vala 2011). It now seems that Conopidae should be classified as belonging to Sciomyzoidea,

but morphological synapomorphies supporting this new definition of Sciomyzoidea are lacking. This restructuring of Sciomyzoidea to include Conopidae should also provide ample opportunity to exclude Sepsidae and Ropalomeridae, for which no evidence of relationship, either with Sciomyzoidea or even with each other, could be found among any of the analyses in this study. This lack of relationship between Sepsidae, Ropalomeridae, and Sciomyzoidea was first reported in Wiegmann et al. (2011), although, like most acalyptrate results in that study, branch supports were low throughout, and there was seemingly little reason to doubt the decades of morphological assurance that Sepsidae and Ropalomeridae formed a strong clade within Sciomyzoidea. Now, with greatly increased taxon selection and rigorous phylogenetic testing continuing to fail to recover any relationship between these groups, it's worth examining how this potential issue could have been overlooked for so long.

The relationship between Ropalomeridae, Sepsidae and the remaining Sciomyzoidea has, as per Meier (1995b), "never been seriously questioned" (pg. 100), with Hennig (1973), Griffiths (1972), and J.F. McAlpine (1989) all agreeing with J.F. McAlpine's earlier work denoting putative synapomorphies for Ropalomeridae+Sepsidae tying them to Sciomyzoidea and Lauxanioidea (J.F. McAlpine 1963) although Speight (1969) clearly rejected both families' inclusion in Sciomyzoidea. Meier would go on to repeatedly question both the placement of these families in Sciomyzoidea and their supposed sister relationship upon studying egg (Meier 1995a), larval (Meier 1995b), and adult morphological characters (Meier 1996), however the higher relationships of these taxa were never significantly challenged through expanded outgroup sampling, even as molecular sequence data were employed (Meier and Wiegmann 2002, Su et al. 2008). Only after Wiegmann et al. (2011) revealed the potential non-monophyly of Sciomyzoidea were outgroups from beyond Sciomyzoidea included (Zhao et al. 2013), but relationships between Sepsidae and the remaining Schizophora went unreported. While the results reported in this chapter have largely returned support for the work of Hennig and J.F. McAlpine, their treatment of Sepsidae and Ropalomeridae within Sciomyzoidea can no longer be regarded with the same confidence, necessitating a significant reassessment of the relationships and evolutionary history of these groups. For example, Feng-Yi Su et al. (2008), in trying to explain the unanticipated relationship between the putatively plesiomorphic and Coelopidae-like Sepsidae genus *Orygma* and the more ant-like Sepsidae genus *Ortalischema*, proposed that the ant-like habitus had evolved twice in Sepsidae (Fig. 3 in Su et al. 2008). If, however, we look at the relationship between *Orygma* and *Ortalischema* (a relationship that was recovered with high confidence in all analyses presented here) without assuming an ancestral relationship with the Sciomyzoidea, it can be argued that the ancestral habitus of

Sepsidae was ant-like, and that *Orygma* is a basal sepsid with an apomorphic habitus that has been converged upon several times by unrelated wrack-associated acalyptrate Diptera (e.g. Canacidae and Coelopidae). While Sepsidae was never recovered with Sciomyzoidea in the current analyses, the relationships between Sepsidae and the remaining Schizophora were also unstable and poorly supported in the preferred trees, although Acartophthalmidae is almost always returned as sister to Sepsidae. Ropalomeridae was similarly never returned with Sciomyzoidea, instead grouping almost exclusively with Neminidae. This theoretical relationship between the strictly New World Ropalomeridae and the strictly Old World Neminidae is difficult to fathom, particularly given the general disparity in their forms and known biologies (Marshall 2012), and may be an artifact of the single exemplar representation of both families. It is clear that the relationships of Sepsidae and Ropalomeridae must be reconsidered in light of the accumulating molecular evidence, including a critical reassessment and comparison of morphological characters and synapomorphies across the broader Schizophora.

Diopsoidea has received considerable phylogenetic attention since J.F. McAlpine, although most attempts at phylogeny reconstruction have focused on single families with few, and occasionally no, outgroup taxa (Meier and Hilger 2000, Baker et al. 2001, Meier and Baker 2002, Marshall et al. 2009), although quantitative work by Lonsdale (2013) on the relationships of Tanypezidae, and D.K. McAlpine's qualitative analysis of Diopsoidea to ascertain the placement of Gobryidae (1997), include numerous Diopsoidea representatives for comparison. Here, Diopsoidea as classified by J.F. McAlpine is mostly recovered, although the internal relationships include some significant divergences from his topology. Tanypezidae and Nerioidea have frequently been considered to be related, with Aczél treating Nerioidea as a part of his "Tanypezidiformes" (1949, 1951, 1954), and D.K. McAlpine (1997) suggesting Tanypezidae (including Strongylopthalmyiidae) should be assigned to Nerioidea on the basis of multiple morphological characters. Hennig appears to have considered a relationship between Tanypezidae and Nerioidea as early as 1944 (see Fig. 61 on page 127 of Schmitt 2013, in which an unpublished cladogram appears with "Tany."+an illegible name drawn as the sister group to Nerioidea), although he would only mention this potential relationship when considering larval characters (1952), and would eventually treat Tanypezidae as part of his Nothyboidea alongside Diopsoidea, Nothybidae, Megamerinidae, and Psilidae. In both preferred trees recovered here, Tanypezidae (including Strongylophthalmyiinae) was returned outside of Diopsoidea, being recovered as the sister group to a clade consisting of Diopsoidea+Opomyzoidea, Nerioidea, Sphaeroceroidea, Ephydroidea and Calyptratae when 3rd positions were excluded (Fig. 6), and as sister to Diopsoidea+Opomyzoidea alongside Psilidae when 3rd codon positions

were included (Fig. 3). A sister relationship between Psilidae and Tanypezidae was recovered by Lonsdale (2013) and supported by numerous morphological synapomorphies, while J.F. McAlpine (1989) considered Tanypezidae+Strongylopthalmyiidae to be sister to the remaining Diopsoidea (themselves the sister group of Nerioidea). While there does not appear to be a relationship between Nerioidea and Tanypezidae, the relationships and affinities of Tanypezidae remain unresolved, and their inclusion within Diopsoidea seems tentative at best. The higher relationships of Psilidae have been little tested, although their placement within Diopsoidea/Tanypezoidea/Nothyboidea has never been doubted (Table 1), with J.F. McAlpine (1989) considering them to be sister to Somatiidae. Here, neither preferred tree recovered Psilidae with Diopsoidea, instead placing it with Tanypezidae as sister to Diopsoidea+Opomyzoidea when 3rd codon positions were included (Fig. 3), or within the lower Tephritoidea when 3rd codon positions were excluded (Fig. 6). Both preferred trees revealed Chylizinae renders Psilinae paraphyletic, perhaps unsurprisingly given the defining characters provided for each subfamily by Buck (2010).

Diopsidae (including the basal Centrioncinae) was recovered as monophyletic, and sister to a strongly supported clade containing Somatiidae, Gobryidae, and Syringogastridae being consistently recovered with or without the inclusion of 3 codon positions (Fig. 3, 6). This contradicts D.K. McAlpine (1997), who considered Syringogastridae and Diopsidae ("Diopsoinea") as assuredly sister taxa, while concluding there was little evidence to support Somatiidae as even being considered in Diopsoidea, and that evidence for a relationship between Gobryidae and Syringogastridae+Diopsidae was "weak and ambiguous". The relationship recovered between Somatiidae, Gobryidae and Syringogastridae has biogeographical implications given Somatiidae and Syringogastridae are thus far only known from New World tropics, while Gobryidae is restricted to Australia and the Oriental region (Pape et al. 2009).

The remaining two families classified within Diopsoidea by J.F. McAlpine (1989), Nothybidae and Megamerinidae, present somewhat more complicated scenarios, as might be expected given the conflicting classifications for each family proposed by earlier workers. Nothybidae has confounded generations of schizophoran systematists, being treated as belonging to numerous families, superfamilies, and positions within Schizophora along the way (reviewed by Lonsdale and Marshall 2016), with Aczél (1955) going so far as to treat Nothybidae as a "missing-link" at the root of Acalyptratae due to the symmetrical nature of the male postabdomen. Although Nothybidae was maintained within Diopsoidea by Pape et al. (2011), its placement within Schizophora remains uncertain (Lonsdale et al. 2016), with COI sequence data analysed by Galinskaya et al. (2016)

failing to provide any reliable evidence of relationships. The relationships of Nothybidae remain obscure and unstable in the presented analyses, although they are never recovered basally within Schizophora or Acalyptratae *sensu* Aczél, with the family instead being returned as related to Sphaeroceroidea, Anthomyzidae, and Periscelididae (Fig. 3), or Acartophthalmidae+Sepsidae (Fig. 6). As will be discussed later, the groups Nothybidae are allied with in the preferred trees are among the most unstable taxa across all analyses, and thus it is impossible at this time to confidently assess the relationships of Nothybidae.

Megamerinidae, known only from the Palaearctic and Oriental regions, was placed within Nothyboidea by Hennig (1958, 1973) and Diopsoidea by J.F. McAlpine (1989), but was considered by Griffiths (1972) to be better placed within Sciomyzoidea. D.K. McAlpine long held that Megamerinidae belonged to Nerioidea (1966, 1997), while Pape et al. (2011) transferred the family to Opomyzoidea on the basis of Wiegmann et al. (2011), which placed Megamerinidae as the basal sister taxon to most of the remaining acalyptrate Schizophora. Here, Megamerinidae is only represented by two species with no overlapping data, resulting in the family being recovered as paraphyletic in all analyses, relationships which are almost certainly an artifact given the strong morphological synapomorphies linking these taxa. *Texara* was particularly unstable, being recovered as sister to Fergusoninidae without support when 3rd codon positions were included (Fig. 3), but sister to Psilidae (together within the Lower Tephritoidea) when 3rd codon positions were excluded (Fig. 6). *Megamerina* was recovered as sister to Teratomyzidae with strong support, and together returned within Diopsoidea regardless of 3rd codon positions, appearing to be allied with Diopsidae, Somatiidae, Gobryidae, and Syringogastridae (Fig. 3, 6). In their work on Syringogastridae, Marshall et al. (2009) provide a brief discussion regarding the relationships of Megamerinidae, with the authors citing unpublished work from Buck et al. that not only places Megamerinidae within Diopsoidea, but as the sister group to Syringogastridae+Diopsidae on the basis of "several newly discovered synapomorphies" (Marshall et al. 2009: pg 5), which would appear to find support in this analysis. Whether the relationship between Megamerinidae and Teratomyzidae recovered here is an artifact or whether it reflects a previously unrecognized evolutionary association is unknown. Hennig (1971), J.F. McAlpine (1989) and Colless and D.K. McAlpine (1991) considered Teratomyzidae to be affiliated with Asteiidae and related families, but Griffiths (1972) interpreted the male genitalia of Teratomyzidae as being related to the families Nothybidae, Psilidae, Periscelididae, and Somatiidae (which he treated as a subfamily of Periscelididae), suggesting there may be characters that tie Teratomyzidae to Diopsoidea, although these characters as interpreted by Griffiths may themselves homoplasious artifacts. The relationships

between Diopsoidea, Megamerinidae, and Teratomyzidae thus remain contentious, and potentially complicated further by the recovered relationship linking Diopsoidea with Opomyzoidea and, tentatively given the poor taxon availability, Carnoidea.

Opomyzoidea have presented a challenge to schizophoran systematics since Hennig (1958), who struggled to find uniting characters, ultimately considering them "Familien mit unklaren Verwandtschaftsbeziehunge" (= "Families with confused or vague affinities", G.H. Harder translation for the Entomology Research Institute, Ottawa, Canada). Hennig would eventually settle on grouping these difficult families into a pair of superfamilies (Agromyzoidea and Anthomyzoidea) (Hennig 1973) that would be expanded upon, further divided, and applied to alternative classifications and concepts by Griffiths (1972), Colless and D.K. McAlpine (1970, 1991), and J.F. McAlpine (1989) (reviewed by Winkler et al. 2010). Even with DNA sequence data, support for Opomyzoidea has remained elusive, leading Winkler et al. (2010) to conclude that McAlpine's concept of Opomyzoidea was intangible and non-monophyletic on the basis of their broad sampling of opomyzoid representatives and other Schizophora. Here, by incorporating additional data from several subsequent molecular studies focused on opomyzoid families (Scheffer et al. 2007, Winkler et al. 2009, Lonsdale et al. 2010, Roháček et al. 2014, Scheffer et al. 2017), and optimizing partitioning schemes and substitution models with and without 3rd codon positions, Opomyzoidea is recovered in large part as a single clade. Within the major Opomyzoidea clade recovered in the preferred tree (Fig. 6), there is varying evidence supporting the subgroups proposed by J.F. McAlpine (1989): Clusiidae was recovered with *Stenomicra* (Periscelididae), which together formed the sister group to a clade reminiscent of McAlpine's Asteioinea, consisting of Asteiidae, *Cyamops* (Periscelididae), Aulacigastridae, and Fergusoninidae. The other major clade consists of Neminidae (and Ropalomeridae), Periscelididae s.s., Odiniidae, and Agromyzidae, along with several likely unrelated taxa artifactually drawn into the Opomyzoidea. Opomyzoidea remain paraphyletic in the preferred tree, with Anthomyzidae, Opomyzidae, Marginidae, Asteiidae, and Xenasteiidae (as well as tentatively Teratomyzidae and/or Megamerinidae as previously discussed) remaining outside the core opomyzoid clade. Xenasteiidae was always recovered with very high support as sister to Australimyzidae, a relationship also recovered by Winkler et al. (2010) and Wiegmann et al. (2011). Winkler et al. (2010) go on to suggest Xenasteiidae may be better transferred to Carnoidea, should Carnoidea be a recognizable superfamily (see below), although no additional evidence has thus far been presented supporting or refuting this relationship or classification. The other opomyzoid families returned outside the Opomyzoidea clade had much

more varied relationships between analyses, but were often associated with Carnoidea, the most poorly sampled and unstable superfamily in all analyses.

Carnoidea has been a contentious and confusing superfamily for systematists to place using morphological characters, with widely varying differences in opinion between classifications (see Buck 2006 for a detailed review of changes and theories regarding relationships of Carnoidea). To date, no molecular studies have been published testing the relationships of Carnoidea, or any of the families proposed as belonging to this superfamily. The only Carnoidea available for inclusion are exemplars from Winkler et al. (2010) and/or Wiegmann et al. (2011), which are found to be insufficient to resolve the relationships of Carnoidea other than confirming a close and consistently returned relationship between Chloropidae and Milichiidae, supporting Brake (2000). The remaining Carnoidea are scattered across Schizophora, independently returned as sister to a wide array of taxa that change between analyses and data perturbations, suggesting that these arrangements are a result of random association rather than relation. These random associations are most likely an artifact of multiple substitution saturation resulting in unrelated sequences sharing common elements by chance that the analysis software is unable to recognize as homoplasy (Philippe et al. 2011, Roure et al. 2013). This phenomenon can be corrected for by adding more taxa related to the problem species, which helps break long branches by providing additional evolutionary context for the origins of these seemingly unique sequences (Zwickl and Hillis 2002). In the case of Carnoidea, less than 0.5% of the total species diversity of the superfamily is represented in this study, with only 0.3% being represented in the preferred trees (species totals based on Pape et al. 2009). This exemplar sampling approach results in each of the independent carnoid lineages appearing as islands of apomorphic sequences that are misinterpreted by computer algorithms as being related to non-carnoid taxa due to the random accumulation of homoplasious mutations rather than via shared evolutionary changes. By increasing taxon sampling, these islands of apomorphy begin to form archipelagos of synapomorphy, connecting sequences and allowing algorithms to better model the ways in which these islands are connected. In the preferred analyses, Carnoidea are frequently returned in association with various Opomyzoidea; whether this is a manifestation of the largely exemplar taxon sampling for both Carnoidea and Opomyzoidea or evidence of relationship between these subfamilies *sensu* J.F. McAlpine (1989) is unknown at this time.

One additional consequence of this poor taxon sampling that results in weak, randomly assorted relationships is a general lack of branch supports from tip to backbone for clades containing Carnoidea. As the resampling mechanism of the bootstrap disrupts the tenuous phylogenetic

placement of each carnoid species, they are returned in different places along the tree with every iteration, resulting in lowered support values for clades that include these unstable, or rogue, taxa (Sanderson and Shaffer 2002, Aberer et al. 2013).

The final acalyptrate superfamily, Sphaeroceroidea, is nearly as poorly represented taxonomically as Carnoidea save for the work of Kits et al. (2013), and as such, relationships within and between the superfamily are unstable, with sphaeroceroids being recovered across Schizophora in most analyses. While Sphaeroceroidea was recovered within a single clade in the ALL+2-No3rds-GTR+R analysis (Fig. 5), the taxon availability for Sphaeroceroidea is too limited to make any conclusions about higher classification. Like Carnoidea, significantly more sampling from across Sphaeroceroidea will be required to confidently assess the relationships of this group.

Phylogenetic Signal, Homoplasy, and Phylogenetic Progress

Using a molecular supermatrix including data from a broad sample of taxa, a molecular phylogeny of Schizophora was demonstrated to exhibit significant congruence with morphological hypotheses of higher relationships. However, even with the progress demonstrated here, the phylogeny of Schizophora remains fragmented across two preferred trees recovered from non-synonymous datasets because uneven phylogenetic signal in 3rd codon positions could not be reconciled using currently available analytical methods. Ideally, a hybrid variant of the two preferred trees returned in this study could be produced, but this hybrid will almost certainly require advanced phylogenetic methods that are computationally prohibitive or impossible given available resources and software packages, even with a dataset of relatively few characters such as this one.

The seemingly uneven distribution of phylogenetic signal across sites and lineages, particularly within 3rd codon positions, is likely a result of differing rates of evolution resulting in differential substitution saturation of sites at varying points in the dataset and/or phylogeny. Namely, some groups, like Diopsoidea, are better recovered when 3rd codon positions are included than when they're excluded (Fig. 3 vs Fig. 6), while other groups, like Opomyzoidea, are the reverse, and better recovered when 3rd codon positions are excluded than when they're included. This suggests that for the genes included in this analysis, Diopsoidea may be evolving more slowly, resulting in lower saturation of 3rd codon positions (and thus less homoplasy and greater phylogenetic signal being retained), while Opomyzoidea may have evolved more rapidly, resulting in higher saturation in 3rd codon positions (and thus more homoplasy and non-phylogenetic signal being present). While it may seem obvious to assume that species and lineages evolve at different rates, this variable

rate actually violates a central assumption of substitution model-based likelihood analyses: that evolution proceeds at a standard rate from root to tip within partitions. Variable evolutionary rates between sites or lineages, or heterotachy (Lopez et al. 2002, Kolaczkowski and Thornton 2008) can cause the detrimental effects and potentially artifactual results in phylogenetics due to the use of models that are oversimplified for real-world data. Unfortunately, there are no easy solutions currently available to properly deal with heterotachy in molecular phylogenetic analyses, although three alternative strategies are currently available for dealing with complex molecular data: amino acid analysis, codon analysis, and the application of heterogenous mixture models.

If multiple substitution saturation is considered a significant concern when analyzing nucleotide data for protein coding genes, nucleotide codons can be translated to amino acids and analysed instead. Due to the translational nature of amino acids, whereby the 64 possible permutations of 3-nucleotide codons translate to only 20 amino acids, numerous nucleotide sequences can code for the same amino acid, allowing for nucleotides to evolve at synonymous sites without being exposed to natural selection by changing the amino acid they are translated into. Therefore amino acid analysis, by design, negates all phylogenetic signal in these synonymous codon positions, which are most prevalent among third codon positions. This also means that substitution models for amino acids need to account for more variables to account for the increased number of translation outcomes from a single changed nucleotide, making amino acid analyses computationally more expensive; a 20x20 substitution matrix introduces 25 times more transition options that need to be tested than a 4x4 substitution matrix does (Felsenstein 2004). However, there is increasing evidence that available amino acid substitution models poorly fit arthropod molecular sequence data and return results that may include more phylogenetic artifacts than nucleotide analyses (Zwick et al. 2012, Gillung et al. 2018). Amino acid evolution should also be just as likely to exhibit heterotachy as nucleotide evolution, so even more complex amino acid mixture models will need to be developed and deployed (see Le et al. 2008).

Moving beyond amino acid analysis, codon analysis is an as-yet still emerging technique, in which each nucleotide triplet is analyzed as a single character, rather than the individual nucleotide positions or subsequent translation. This codon method, in theory, would recognize all of the phylogenetic signal (or non-signal) available in codon positions that might be overlooked in an amino acid analysis, while also maintaining the biological context of the entire codon, potentially making the best of both worlds in exploring the different evolutionary pressures at work between sites and codons. In practice, codon analyses do appear to return superior results when compared

to nucleotide and amino acid analyses (Seo and Kishino 2009), and seem to resolve both recent and deep evolutionary divergences (Ren et al. 2005). However, codon analyses are only beginning to be explored, and as such there is little supporting research or available resources for implementing these types of analyses. For example, the only available codon models implemented in IQ-TREE are either limited in their available parameters (Goldman and Yang 1994, Kosiol et al. 2007), were designed for use in plants (Muse and Gaut 1994) or are based on empirical data from vertebrates (Schneider et al. 2005). The latter two models are likely of limited use for invertebrates given the known differences in codon translation and evolution unique to invertebrates, particularly when analyzing data using the invertebrate mitochondrial code which will require a 62x62 matrix rather than the standard 61x61 matrix developed for use in standard genetic code. Even if there were appropriate models available, datasets that include data from different genetic codes such as the one presented here are currently impossible to analyze using IQ-TREE due to a bug that does not allow for different genetic codes to be concatenated and analyzed using codons (Minh, pers. comm.), although the computational effort required for a analyzing a such a dataset are well beyond what is currently possible; Felsenstein (2004) calculates that a standard codon analysis would require 3,547 times the computational power of a regular nucleotide analysis. Barring major advances in computational power, analytical efficacies, and codon substitution model development, codon analysis appears to be out of reach for large datasets for the foreseeable future, despite potentially being the best available option for drawing phylogenetic signal out of homoplasy.

The final and most promising method for resolving molecular data in the face of heterotachy are mixture models, complex substitution models that allow for model parameters to be optimized independent of sites, lineages, and time (Kolaczkowski et al. 2008). These models are relatively new in phylogenetics and necessarily complex, requiring a level of theoretical and statistical awareness beyond that required to run more traditional phylogenetic analyses. Mixture models are also computationally expensive, with Kolaczkowski et al. (2008) projecting that mixture model optimization and analysis could take upwards of 100 times as long as for a simple model depending on the complexity of the data and the number of model classes needed to account for that complexity. Nucleotide mixture models designed to deal with heterotachy are in their infancy (Boussau et al. 2014), and only just beginning to be tested in maximum likelihood analysis using programs such as IQ-TREE (Crotty et al. 2017). Mixture models represent the best option for dealing with heterotachy in phylogenetic data, but their application to large datasets such as the one presented here will likely remain unattainable for some time.

More taxa vs more genes, and the future of Schizophora systematics

60 years after Willi Hennig published his first cladistic consideration of Schizophora relationships (1958), and with decades of methodological, technological, and philosophical advances, we find ourselves essentially back where we began when it comes to relationships of Schizophora. While we can agree with increasing confidence on the limits and concepts of most schizophoran superfamilies, others have maintained their confused or vague affinities, stubbornly resisting our best efforts at elucidating their evolutionary history. However, much as Hennig's concepts and conclusions changed over the course of his career as he was exposed to additional material, it should come as no surprise that attempts to reconcile relationships based on different character sets (in this case morphology and DNA) are similarly improved with increased taxon sampling. While those invested in phylogenomic studies continue to argue that more genes and more data will be required to solve the higher relationships among Schizophora (Wiegmann et al. 2017), the present study would suggest there remain other routes to resolving the higher relationships of schizophoran Diptera while simultaneously raising methodological concerns that could be further exaggerated by genome-sized datasets.

The current study, despite averaging just over 3,000 base pairs of nucleotide data per included species (Table 2), has recovered most superfamilies of acalyptrate Diptera as previously proposed on the basis of morphology. All indications from this study would seem to suggest that there remains enough phylogenetic signal in the relatively small amount of data analyzed here to ascertain broad relationships across Schizophora. New Schizophora genomic data presented by Bayless et al. (2018) at the 9th International Congress of Dipterology appears to be converging on a topology similar to that recovered in the optimized ALL+5 analysis with 3rd codon positions excluded presented here (Fig. 11). This suggest that although the internal backbone branches of the preferred phylogenies presented here are recovered as short and without statistical support, they may still represent a valid topology. The short internal branches and lack of statistical support for deep relationships among Schizophora are a recurring "problem" in recent molecular studies (Kutty et al. 2008, Kutty et al. 2010, Winkler et al. 2010, Wiegmann et al. 2011, Cerretti et al. 2017) and is often attributed to a deficiency of phylogenetic signal remaining in sequences (Wiegmann et al. 2011) and the rapid radiation of schizophoran diversity following the Cretaceous-Paleogene mass extinction event (Grimaldi and Engel 2005, Wiegmann et al. 2011). This rapid radiation hypothesis is primarily founded on the absence of schizophoran fossils from the Cretaceous period (145-65 mya) despite a large proportion of modern acalyptrate family-level fauna being represented in Baltic amber (~42 mya; von Tschirnhaus and Hoffeins 2009, Grimaldi 2018), along with molecular

clock dating analyses in Wiegmann et al. (2011), which put the origins of Schizophora at or around 70 mya. However, Cerretti et al. (2017) have recently pushed back the estimated origin of Calyptratae to 70 mya, making the phylogenetic position of this clade all the more important in understanding the evolution of Schizophora. If Calyptratae and a monophyletic Acalyptratae are sister groups as indicated in the preferred tree including 3rd position data recovered here (Fig. 3), then the acalyptrate lineage should be roughly the same age as calyptrates (~70mya), supporting the rapid radiation theory. However, if Calyptratae are a more derived lineage, as inferred from the preferred tree excluding 3rd position data (Fig. 6), the age of Schizophora and basal acalyptrate lineages could be older than currently believed, and potentially extending back into the Cretaceous. Molecular dating analyses are highly dependent both on taxon selection and substitution models (Bromham et al. 2018), and given the conclusions of the present study that taxon sampling and substitution model parameters remain lacking to fully resolve and address the evolutionary history of Schizophora, any conclusions regarding their geological origin based on molecular clock analysis should be considered tentative. While it's possible that genomic-sized datasets may be able to "brute force" a phylogeny of Schizophora that has improved support among internal nodes, it seems just as likely that the relative phylogenetic signal distributed across internal and terminal branches will remain similar, if not constant, across a genomic scale, theoretically resulting in little appreciable gain in phylogenetic signal over homoplasy and multiple substitution saturation as that observed here. Thus, it seems likely that genomic datasets will need to employ mixture models as is concluded for the data reported here, and so it's important to estimate the computational obstacles facing schizophoran systematists moving forward and assess whether genomic datasets of suitable taxon diversity could even be analyzed.

In this study, the 1740 taxon ALL+2 dataset (with 3rd codon positions included) that resulted in one of the preferred trees (Fig. 3) took 148 wall-clock hours (407 CPU hours), including parametrization (52% of the total CPU hours), using 24 processors running on the CIPRES cluster (M. A. Miller et al. 2010). Following Kolaczkowski et al.'s (2008) estimation of increased computational requirements of optimizing molecular datasets for maximum likelihood mixture models, ranging from 5 to (at least) 100 times longer depending on the complexity of the dataset (from Figure S5 of Kolaczkowski et al.), the wall-clock time required to analyze the ALL+2 dataset using a hypothetical mixture model would be between 30 days (2,035 CPU hours) and 616 days (40,700 CPU hours) depending on the number of mixture classes required. Comparing to Bayless (2016), in which he reports his 70 taxon genomic dataset took 50 days just to parametrize (using computationally comparable software), we could estimate a total analysis time using mixture

models of anywhere from 500 days to 10,000 days, generously assuming that the computational effort for a maximum likelihood analysis is comparable between a 1740 taxon x 23,000 character dataset and a 70 taxon x 1.5 million character dataset, and that only 70 transcriptomes from across the nearly 90 families of schizophoran Diptera will recover acceptable relationships. In practice, taxon sampling for genomic research will almost certainly need to be expanded to include many more taxa, further increasing computational burden and time. Given that CIPRES policy limits users to a maximum of 50,000 CPU hours (30,000 CPU hours for researchers outside the US) (M. Miller n.d.), mixture model analyses of genomes are computationally inaccessible for the foreseeable future.

As we stand at the "Dawn of the Postgenomic Era" (Trautwein et al. 2012) and look towards a future of new technologies granting access to near limitless molecular data, it's imperative that we ask whether we are ready to enter that future, or whether we're better served looking back at the data that we as a community have spent decades accumulating to ensure that we have exhausted our ability to understand all that it can tell us. While medical science is driving the price of genomic data per sample down rapidly (van Nimwegen et al. 2016), a future of schizophoran systematics billed on the necessity of genomics is a future that excludes those who don't have access to the million dollar machinery and institutional infrastructure necessary to sequence and build high-quality genomes (Glenn 2011). This study demonstrates that genomic data are not necessarily the only way forwards for Schizophora systematics, showing that small sections of DNA, when considered across a wide diversity of taxa, can arrive at solutions congruent with both morphology and genomics. As sequencing technologies capable of recovering data applicable to this supermatrix analysis become available at a fraction of the total and per-sequence cost of other methods (Srivathsan et al. 2018), and that can be reliably taken anywhere a laptop can go (Krehenwinkel et al. 2018, Pomerantz et al. 2018), taxon deficiencies identified herein can be successively filled by more people from more places who wish to independently contribute to the testing and resolution of dipteran systematics. Advances in sequencing technologies are also allowing us to recover short regions of DNA from small, old, and potentially rare museum specimens (Kanda et al. 2015, Sproul and Maddison 2017), while enabling us to explore the relationship genotype and phenotype, and how subtle changes to the former can have significant implications on the latter (LeVasseur-Viens and Moehring 2014, Xu et al. 2017, Tanaka et al. 2018), and to trace physiological adaptations tied to life-history characteristics through time and species (Maczkowiak and Lage 2006, Da Lage 2018). We are undoubtedly on the dawn of a new era in Diptera systematics, but in order to fully

realize its potential we must keep our minds and eyes open to all available methods, even those some would have us believe we have left behind.

Conclusions

By combining thousands of Schizophora DNA sequences accumulated over the past 30 years, and optimizing substitution models and partitioning strategies, a pair of phylogenies that are strongly congruent with morphological classifications are recovered. These phylogenies provide evidence that with additional taxon sampling a resolved phylogeny of Schizophora may be within grasp, and without necessarily requiring genome-sized datasets. Evidence that Calyptratae and Acalyptratae may be reciprocally monophyletic as originally proposed by Hennig (1973) and J.F. McAlpine (1989) was recovered, but much more testing using multiple character sets will be required before these relationships can be confidently determined. Relationships between acalyptrate superfamilies, while weakly supported in this study, show some consistency between analyses and data perturbations, suggesting that unravelling the evolutionary history of these important and highly diverse flies remains feasible. Notably, Conopidae is returned with high consistency and support as belonging to Sciomyzoidea, likely forming a sister relationship with Sciomyzidae, while Sepsidae and Ropalomeridae were never returned as related to either Sciomyzoidea or each other. Current concepts of Nerioidea, Tephritoidea, and Lauxanioidea are supported as monophyletic by this analysis, while some support was found supporting the monophyly of Opomyzoidea and Diopsoidea. Sphaeroceroidea and Carnoidea remain too poorly sampled to assess monophyly and relationships of either superfamily at this time. Future studies focusing on targeted PCR sequencing of genes included in this study are encouraged, while the continued development and application of novel phylogenetic methods and analytical strategies is identified as being necessary to fully resolve the relationships of Schizophora.

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Table 1: Classification schemes of Schizophora across time and by various authors. ºWiegmann et al 2011 recovered a paraphyletic Conopidae within the Sciomyzoidea. ‡Wiegmann et al 2011 recovered a paraphyletic Conopidae within the Sciomyzoidea, and Sepsidae & Ropalomeridae were not recovered within Sciomyzoidea.

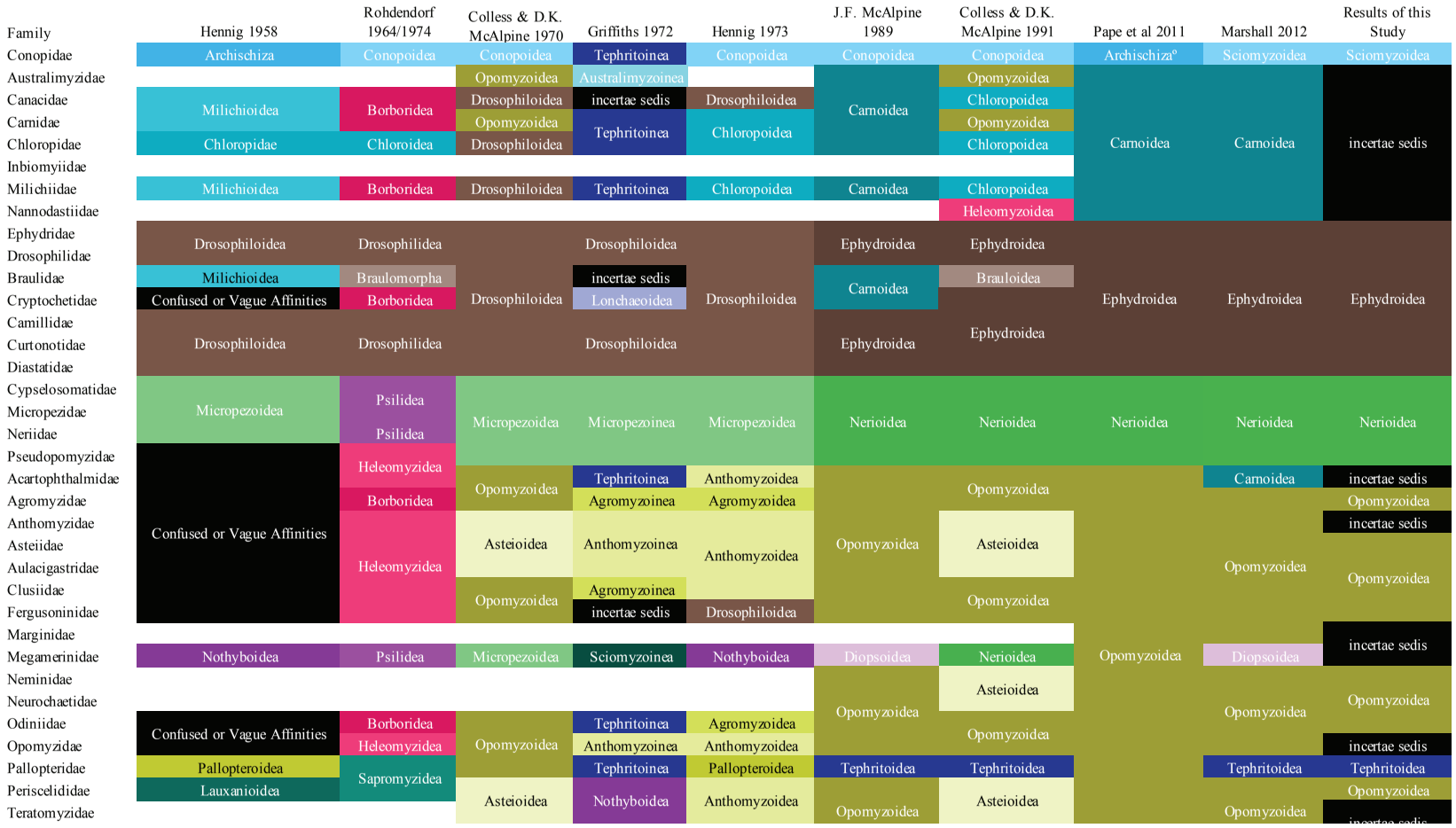

Table 1, continied: Classification schemes of Schizophora across time and by various authors. ºWiegmann et al 2011 recovered a paraphyletic Conopidae within the Sciomyzoidea. ‡Wiegmann et al 2011 recovered a paraphyletic Conopidae within the Sciomyzoidea, and Sepsidae & Ropalomeridae were not recovered within Sciomyzoidea.

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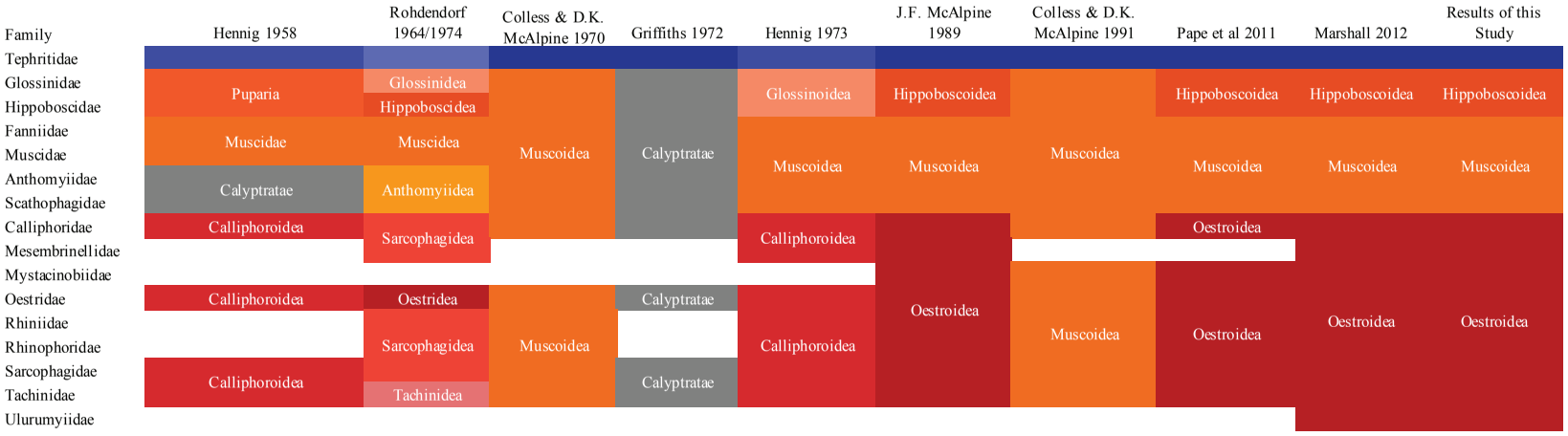


Table 2: Matrix statistics for each of the datasets analyzed. “Total missing data” represents the number of cells in the matrix represented by “?”.

	ALL+ALL	ALL+2	ALL+2 (no 3rds)	ALL+5	ALL+5 (no 3rds)
Number of taxa	2,301	1,740	1,740	412	412
Total alignment length	26,923	26,764	20,983	26,764	20,983
Total missing data	90%	88%	84%	76%	75%
Average sequence length/ taxon (bps)	2,687	3,302	2,646	6,338	5,170
Average number of loci/taxon	3	4	4	7	7

Table 3: ALL+2 model and partition data recovered from ModelFinder. These partitions were applied to the ALL+2 dataset with and without 3rd codon positions included, as well as the ALL+5 dataset with and without 3rd codon positions included.

12S	GTR+F+R8	G6PD_pos1	SYM+G4
16S	GTR+F+R8	G6PD_pos2_PEPCK_pos2_PUG_pos2	TIM3+F+I+G4
28S	TVM+F+R10	G6PD_pos3	TN+F+G4
AATS_pos1	SYM+R5	ND3_pos1	TIM3+F+G4
AATS_pos2	TVMe+R5	ND3_pos2_SYX1A_pos1	TIM3e+R2
AATS_pos3	SYM+R5	ND3_pos3	TIM2+F+R3
ATP6_pos1	TIM2+F+G4	ND4L_pos1	TVM+F+G4
ATP6_pos2	TPM3u+F+R2	ND4L_pos2	HKY+F+R2
ATP6_pos3	TPM2+F+R4	ND4L_pos3	TIM3+F+R3
ATP8_pos1	TIM3+F+I	ND6_pos1	TIM2+F+R5
ATP8_pos2	F81+F+G4	ND6_pos2	TVM+F+I+G4
ATP8_pos3	HKY+F+G4	ND6_pos3	TIM2+F+R5
CAD_pos1	SYM+R7	PEPCK_pos1	JC+G4
CAD_pos2	GTR+F+R7	PEPCK_pos3	TPM2+F+G4
CAD_pos3	GTR+F+R7	PER_pos1	TIM2e+G4
COI_pos1	GTR+F+R8	PER_pos2	K2P+G4
COI_pos2	TVM+F+R6	PER_pos3	TN+F+R3
COI_pos3	GTR+F+R9	PGD_pos1	SYM+R5
COII_pos1	GTR+F+R7	PGD_pos2	GTR+F+R5
COII_pos2	TVM+F+R5	PGD_pos3	TIM2+F+R6
COII_pos3	TPM2+F+R9	PUG_pos1	TIM2+F+G4
COIII_pos1	TIM2+F+I+G4	PUG_pos3	TIM2+F+G4
COIII_pos2	K3Pu+F+R2	SINA_pos1	TIM2e+G4
COIII_pos3	TPM3u+F+R4	SINA_pos2	JC+G4
CYTB_pos1	GTR+F+R7	SINA_pos3	TVM+F+G4
CYTB_pos2	GTR+F+R6	SYX1A_pos2	F81+F
CYTB_pos3	GTR+F+R6	SYX1A_pos3	K2P+R3
EF1A_pos1	GTR+F+R4	TPI_pos1	TVMe+I+G4
EF1A_pos2	GTR+F+R4	TPI_pos2	TVM+F+I+G4
EF1A_pos3	TIM2e+R5	TPI_pos3	TVMe+R5

Figure 1
ALL+ALL
3rds incl.
GTR+G

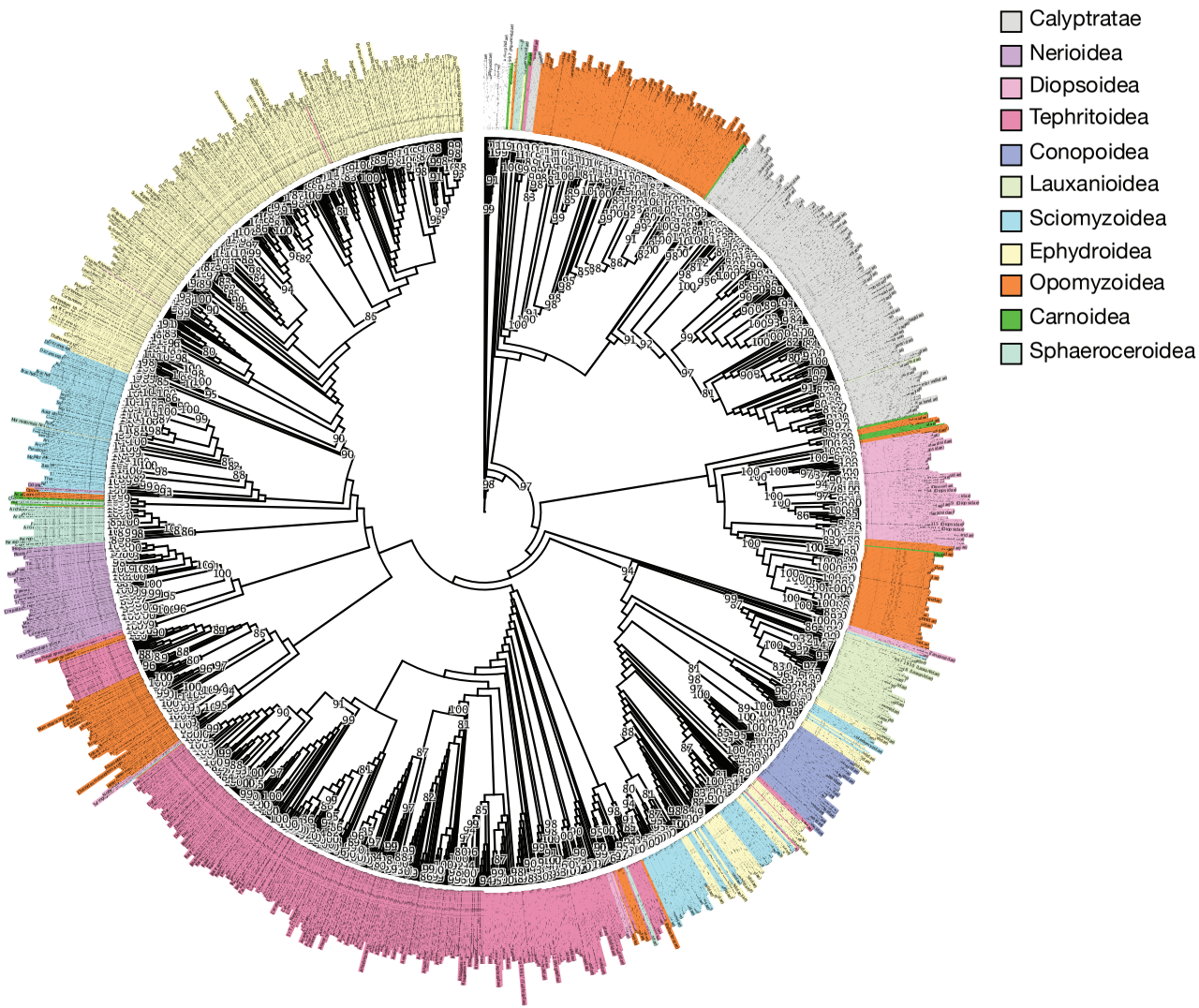


Figure 1: Maximum likelihood phylogeny recovered from the ALL+ALL dataset with third codon positions included and a GTR+G substitution model applied to all data partitions. Numbers at nodes represent Ultrafast Bootstrap supports above 80. Taxon labels are coloured according to superfamily classification. Branches are not proportional to their length.

Figure 2
ALL+2
3rds incl.
GTR+G

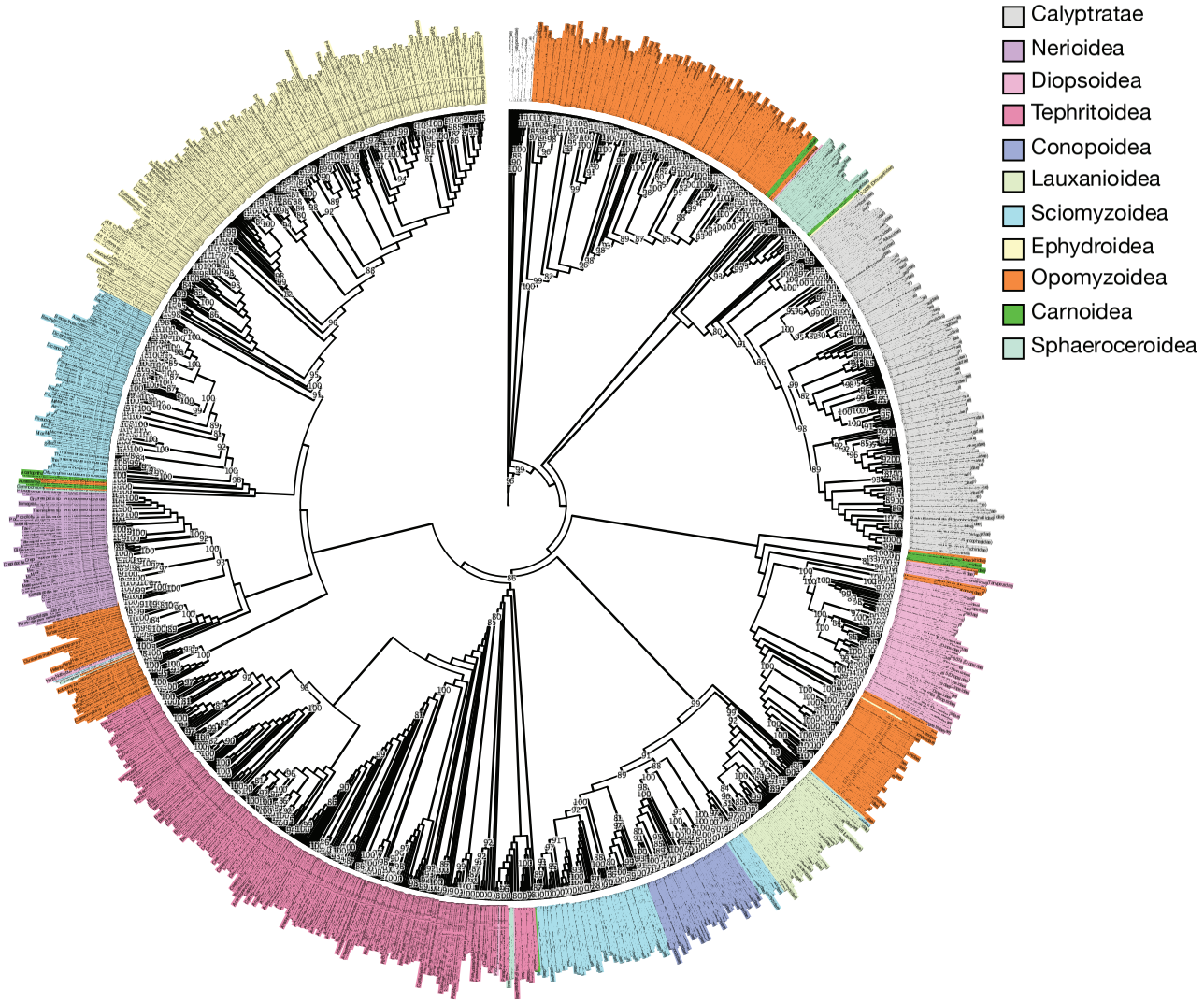


Figure 2: Maximum likelihood phylogeny recovered from the ALL+2 dataset with third codon positions included and a GTR+G substitution model applied to all data partitions. Numbers at nodes represent Ultrafast Bootstrap supports above 80. Taxon labels are coloured according to superfamily classification. Branches are not proportional to their length.

Figure 3
ALL+2
3rds incl.
Various Models

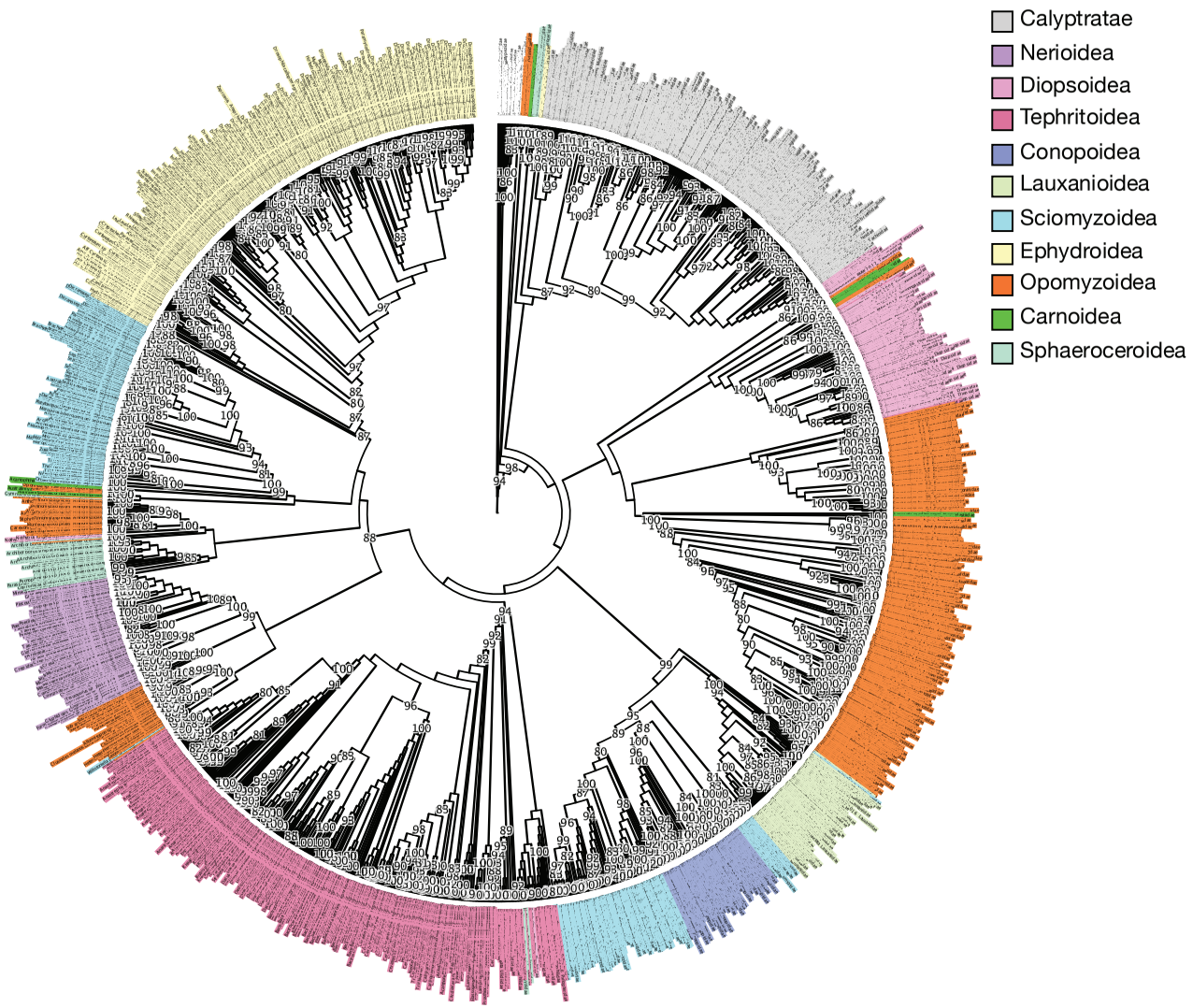


Figure 3: Preferred maximum likelihood phylogeny recovered from the ALL+2 dataset with third codon positions included and substitution models and partitions optimized by ModelFinder (see Table 3). Numbers at nodes represent Ultrafast Bootstrap supports above 80. Taxon labels are coloured according to superfamily classification. Branches are not proportional to their length.

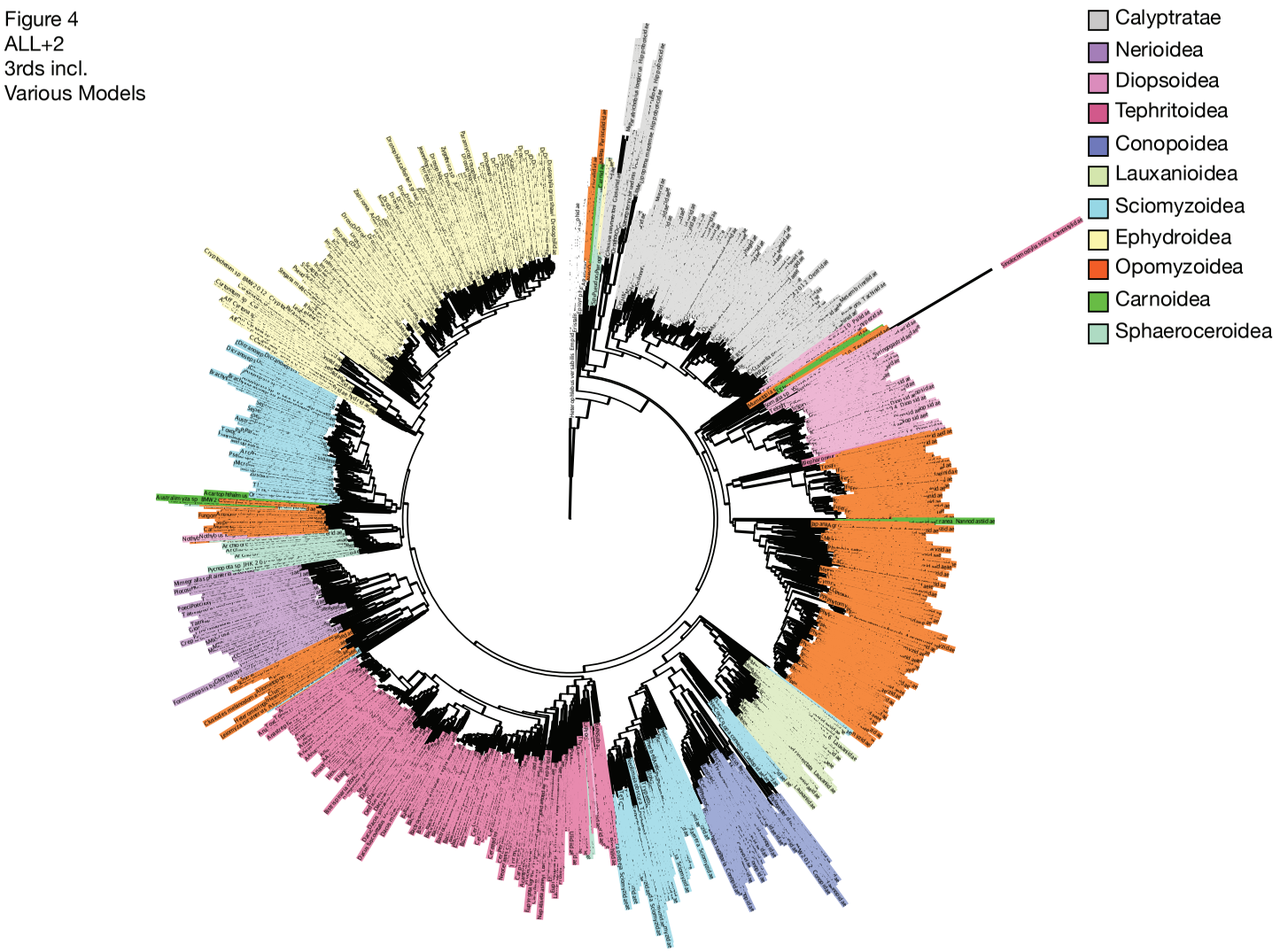


Figure 4: Preferered maximum likelihood phylogeny recovered from the ALL+2 dataset with third codon positions included and substitution models and partitions optimized by ModelFinder (see Table 3). Taxon labels are coloured according to superfamily classification. Branches are proportional to their length.

Figure 5
ALL+2
3rds excl.
GTR+G

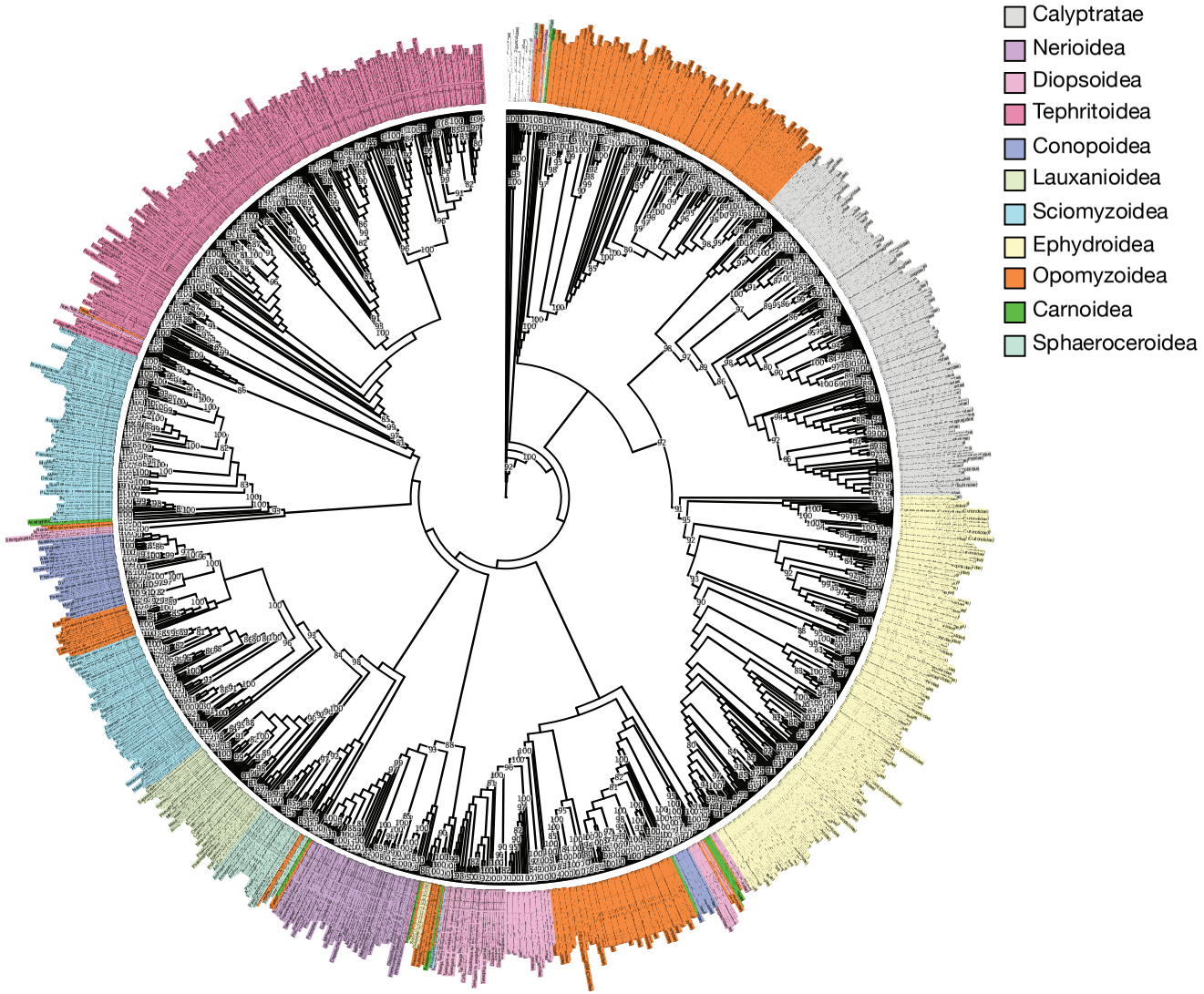


Figure 5: Maximum likelihood phylogeny recovered from the ALL+2 dataset with third codon positions excluded and a GTR+G substitution model applied to all data partitions. Numbers at nodes represent Ultrafast Bootstrap supports above 80. Taxon labels are coloured according to superfamily classification. Branches are not proportional to their length.

Figure 6
ALL+2
3rds excl.
Various Models

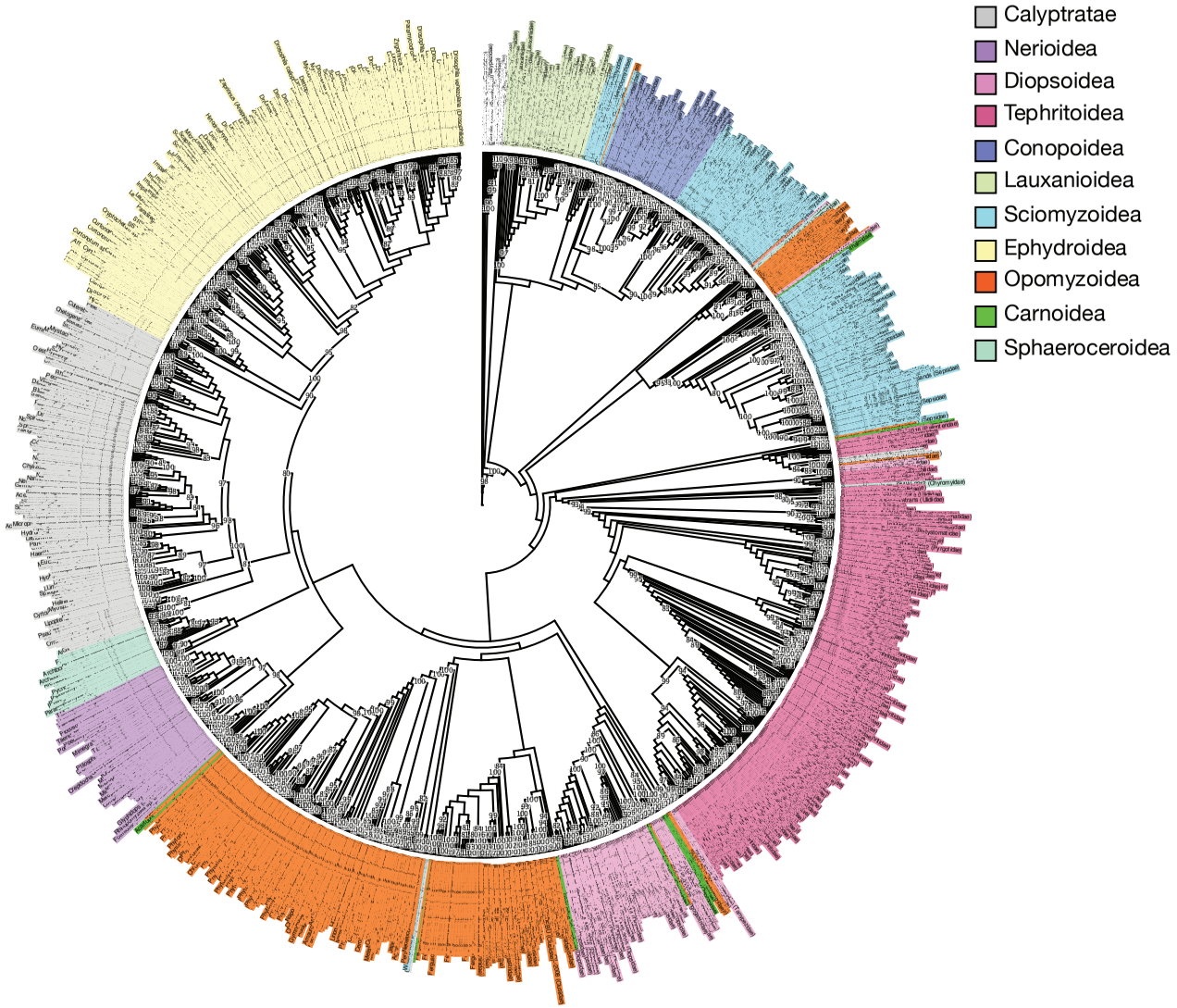


Figure 6: Preferred maximum likelihood phylogeny recovered from the ALL+2 dataset with third codon positions excluded and substitution models and partitions optimized by ModelFinder (see Table 3). Numbers at nodes represent Ultrafast Bootstrap supports above 80. Taxon labels are coloured according to superfamily classification. Branches are not proportional to their length.

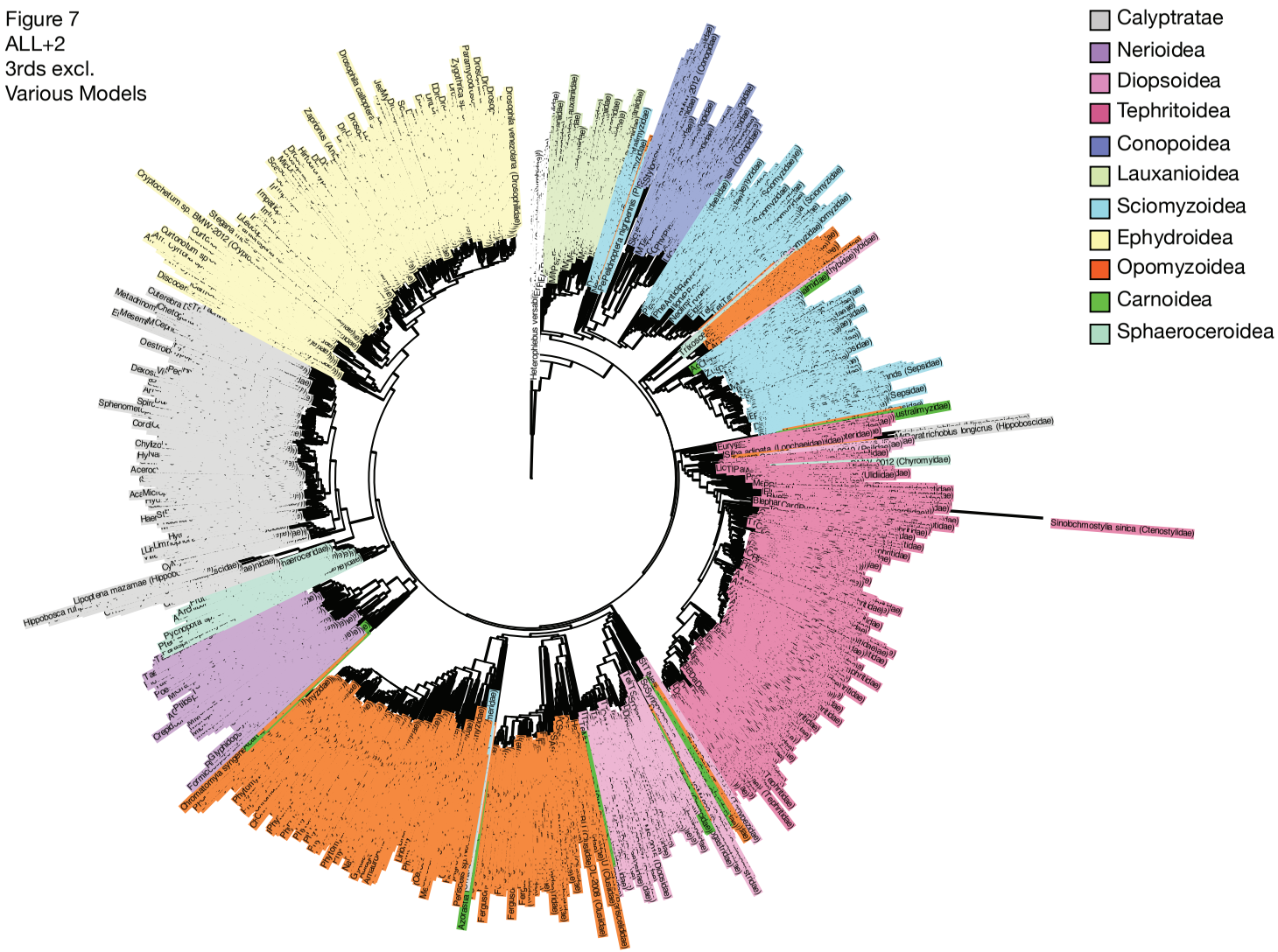


Figure 7: Preferred maximum likelihood phylogeny recovered from the ALL+2 dataset with third codon positions included and substitution models and partitions optimized by ModelFinder (see Table 3). Taxon labels are coloured according to superfamily classification. Branches are proportional to their length.

Figure 8
ALL+5
3rds incl.
GTR+G

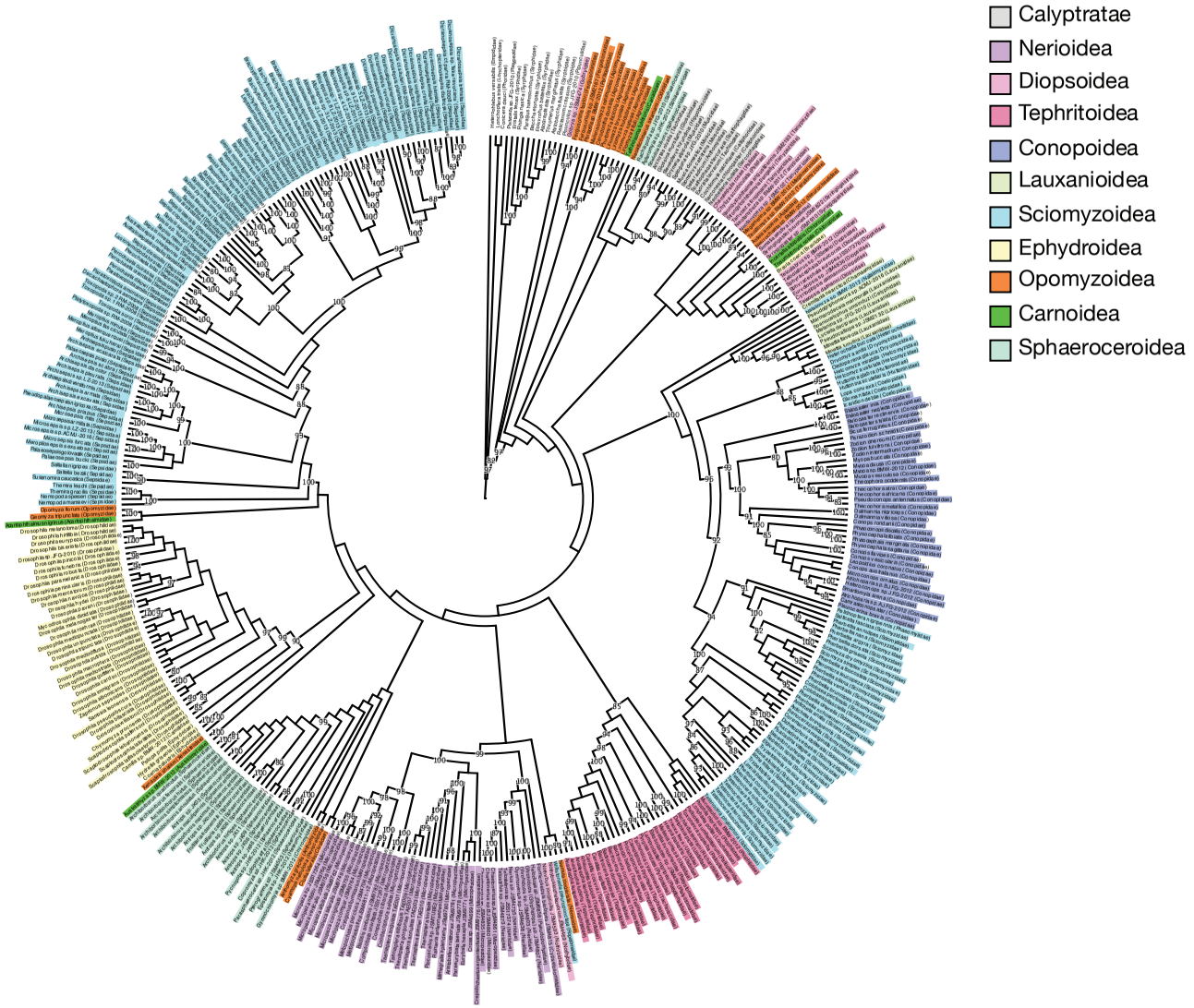


Figure 8: Maximum likelihood phylogeny recovered from the ALL+5 dataset with third codon positions included and a GTR+G substitution model applied to all data partitions. Numbers at nodes represent Ultrafast Bootstrap supports above 80. Taxon labels are coloured according to superfamily classification. Branches are not proportional to their length.

Figure 9
ALL+5
3rds incl.
Various Models

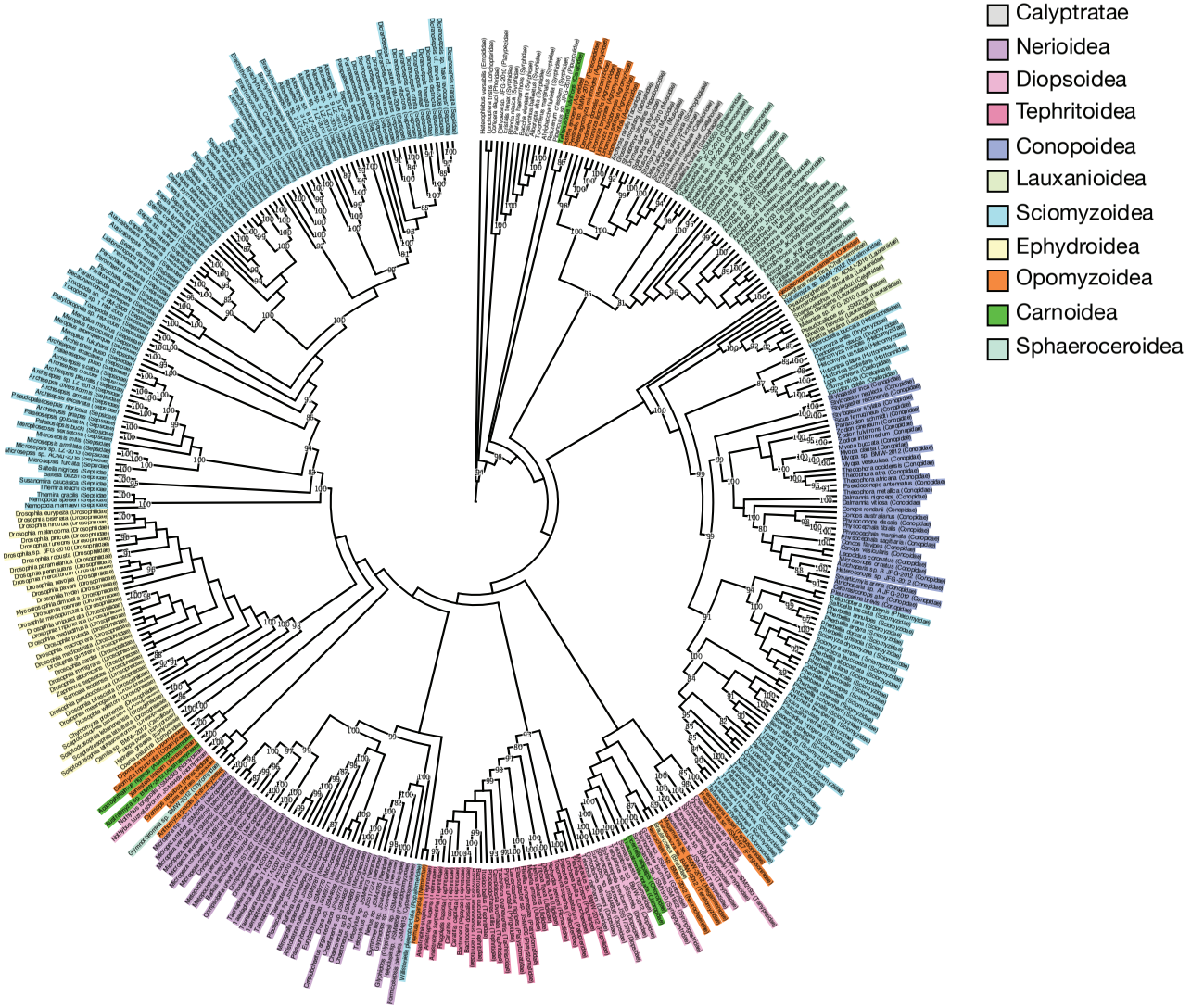


Figure 9: Maximum likelihood phylogenies recovered from the ALL+5 dataset with third codon positions included and substitution models and partitions optimized by ModelFinder (see Table 3). Numbers at nodes represent Ultrafast Bootstrap supports above 80. Taxon labels are coloured according to superfamily classification. Branches are not proportional to their length.

Figure 10
ALL+5
3rds excl.
GTR+G

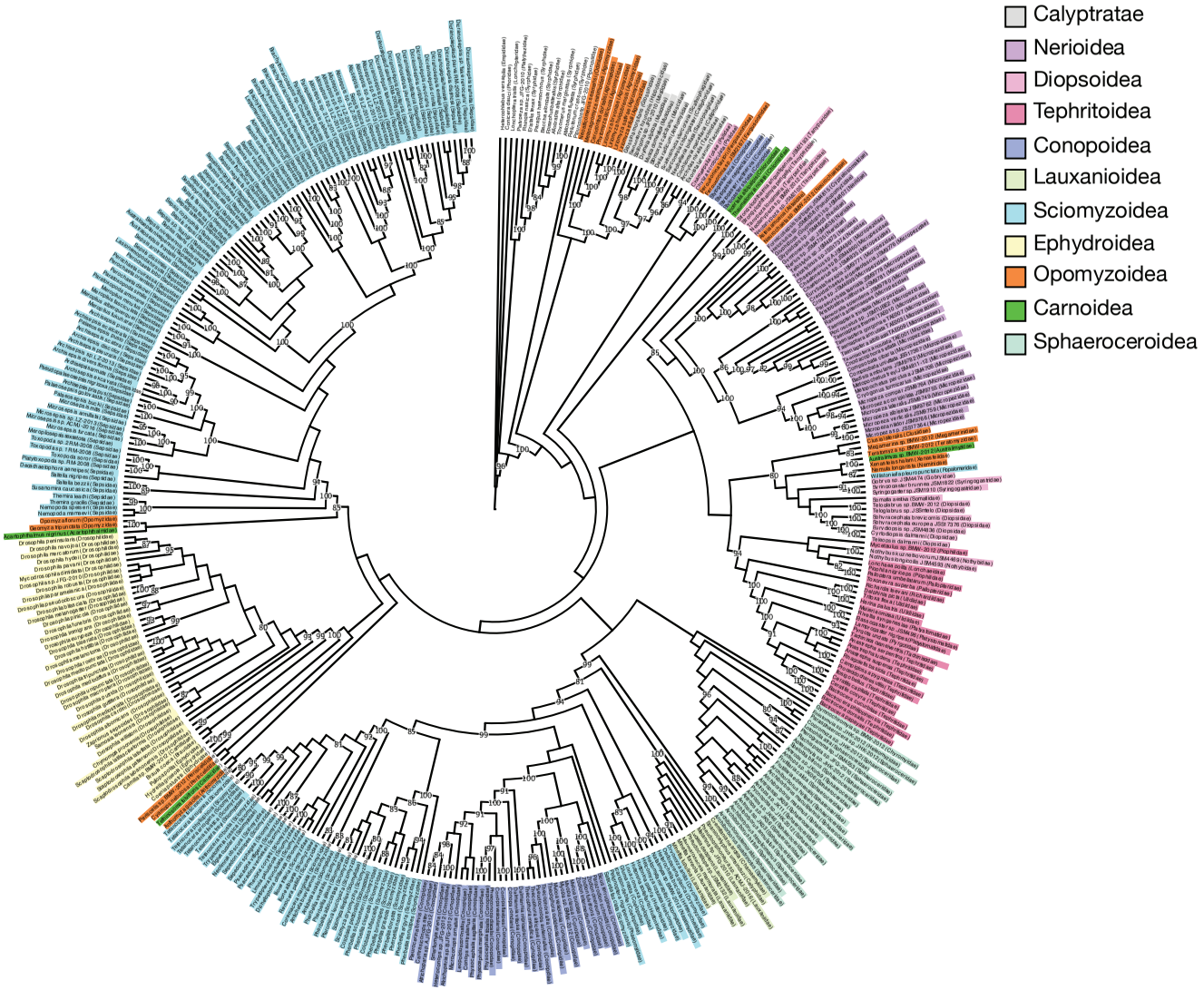


Figure 10: Maximum likelihood phylogeny recovered from the ALL+5 dataset with third codon positions excluded and a GTR+G substitution model applied to all data partitions. Numbers at nodes represent Ultrafast Bootstrap supports above 80. Taxon labels are coloured according to superfamily classification. Branches are not proportional to their length.

Figure 11
ALL+5
3rds excl.
Various Models

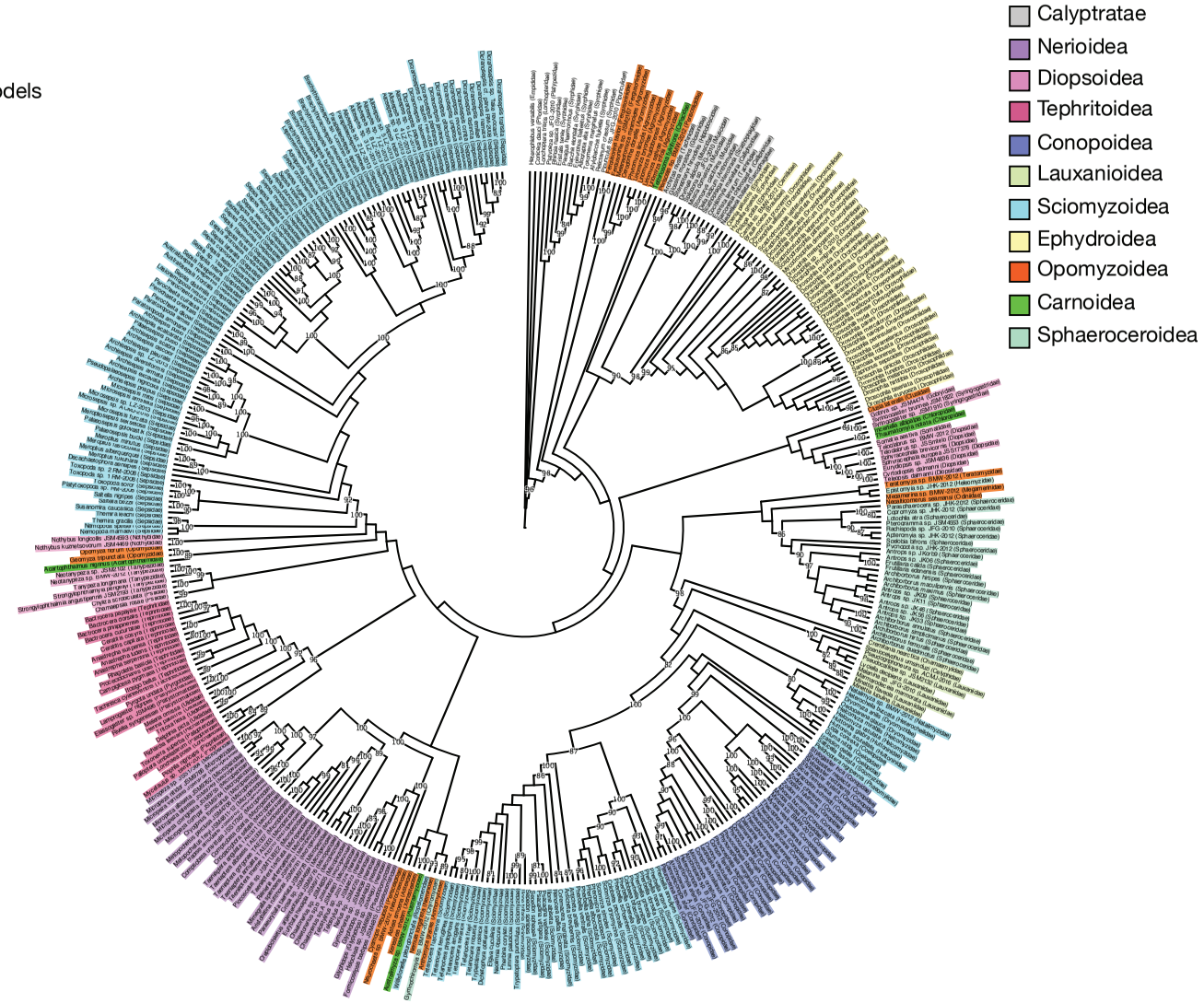
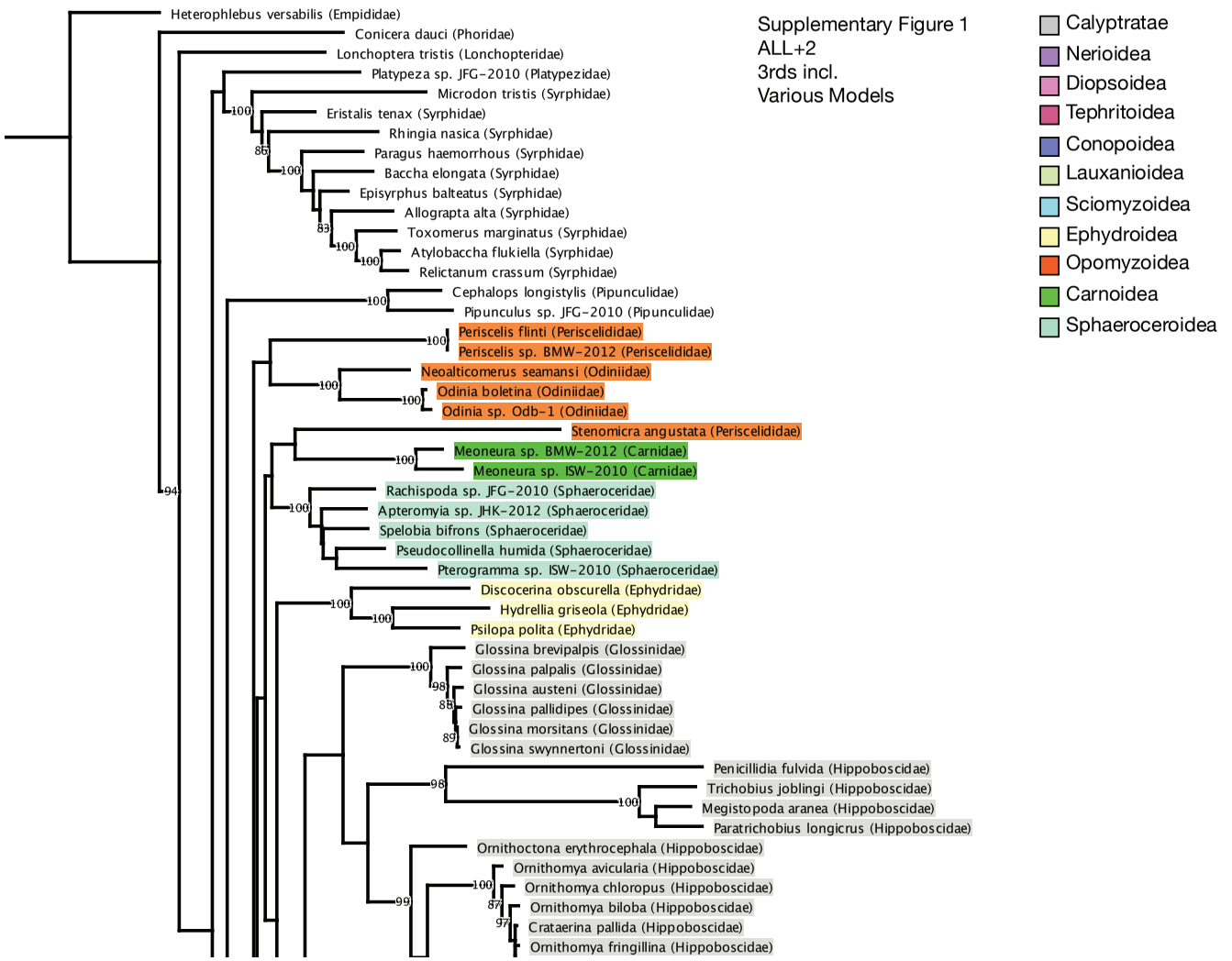
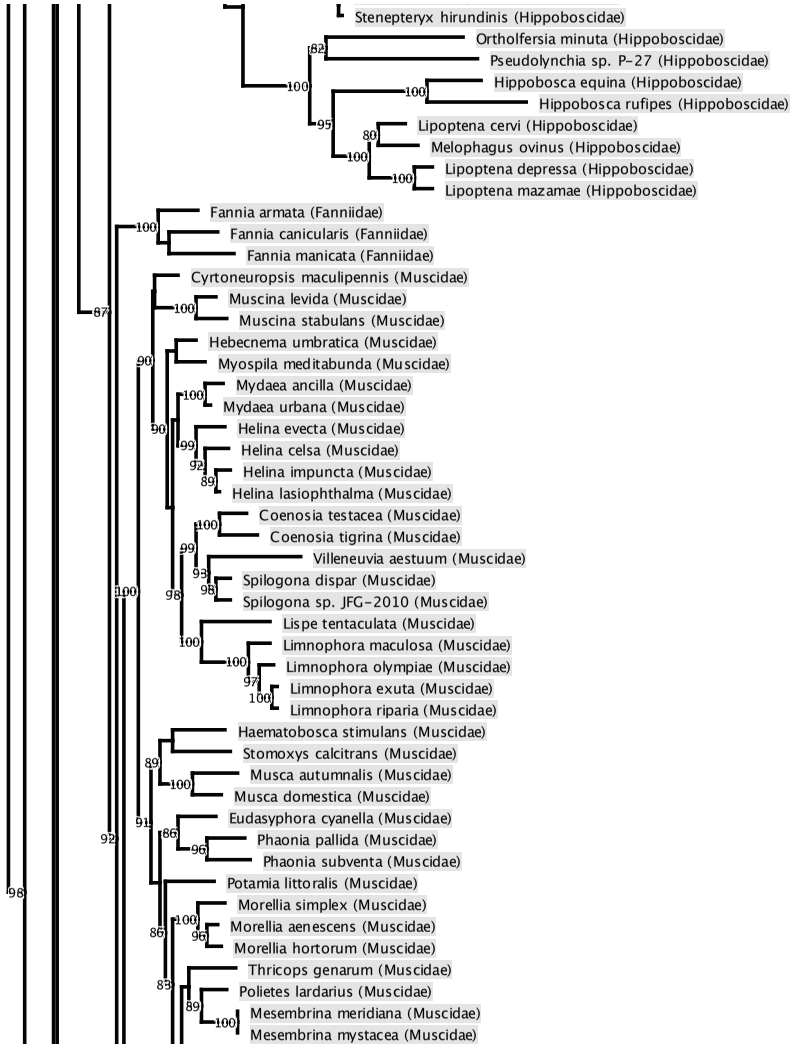


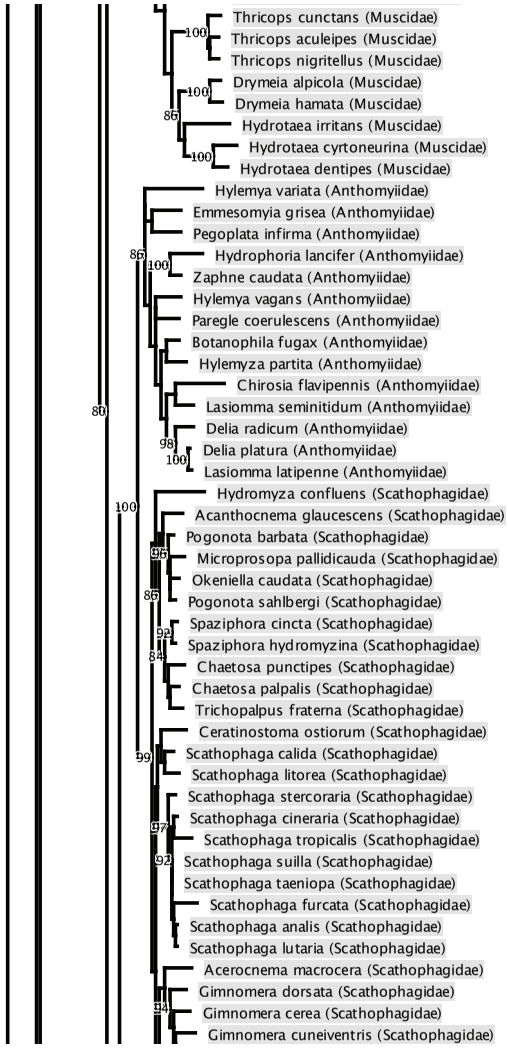
Figure 11: Maximum likelihood phylogenies recovered from the ALL+5 dataset with third codon positions excluded and substitution models and partitions optimized by ModelFinder (see Table 3). Numbers at nodes represent Ultrafast Bootstrap supports above 80. Taxon labels are coloured according to superfamily classification. Branches are not proportional to their length.



Supplemental Figure 1: Preferred maximum likelihood phylogeny recovered from the ALL+2 dataset with third codon positions included and substitution models and partitions optimized by ModelFinder (see Table 3). Numbers at nodes represent Ultrafast Bootstrap supports above 80. Taxon labels are coloured according to superfamily classification. Branches are proportional to their length.



Supplemental Figure 1, continued: Preferred maximum likelihood phylogeny recovered from the ALL+2 dataset with third codon positions included and substitution models and partitions optimized by ModelFinder (see Table 3). Numbers at nodes represent Ultrafast Bootstrap supports above 80. Taxon labels are coloured according to superfamily classification. Branches are proportional to their length.



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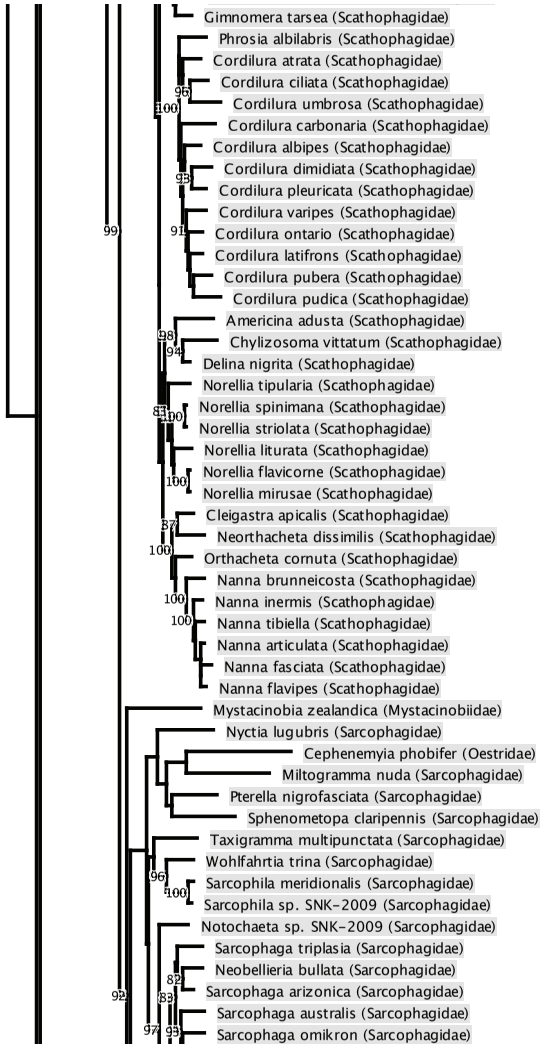

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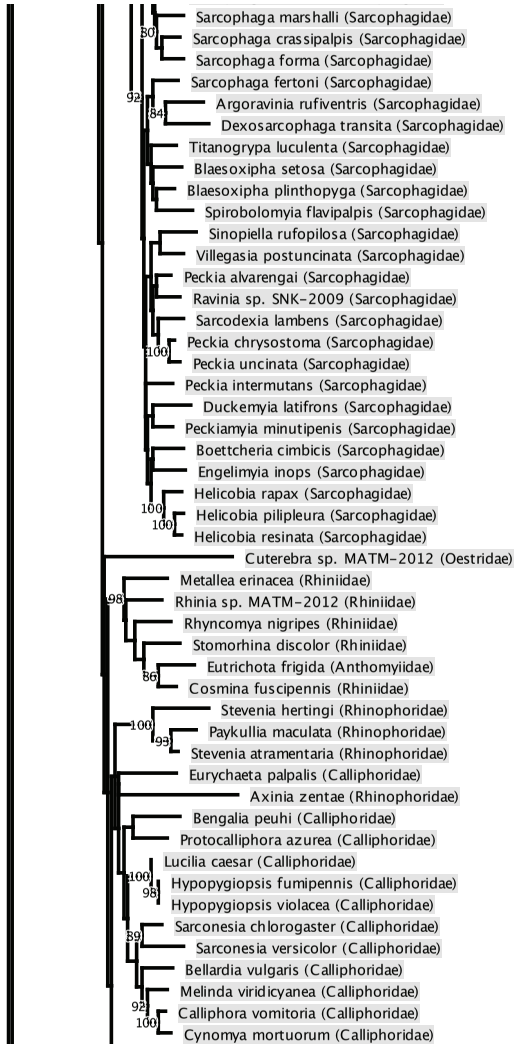

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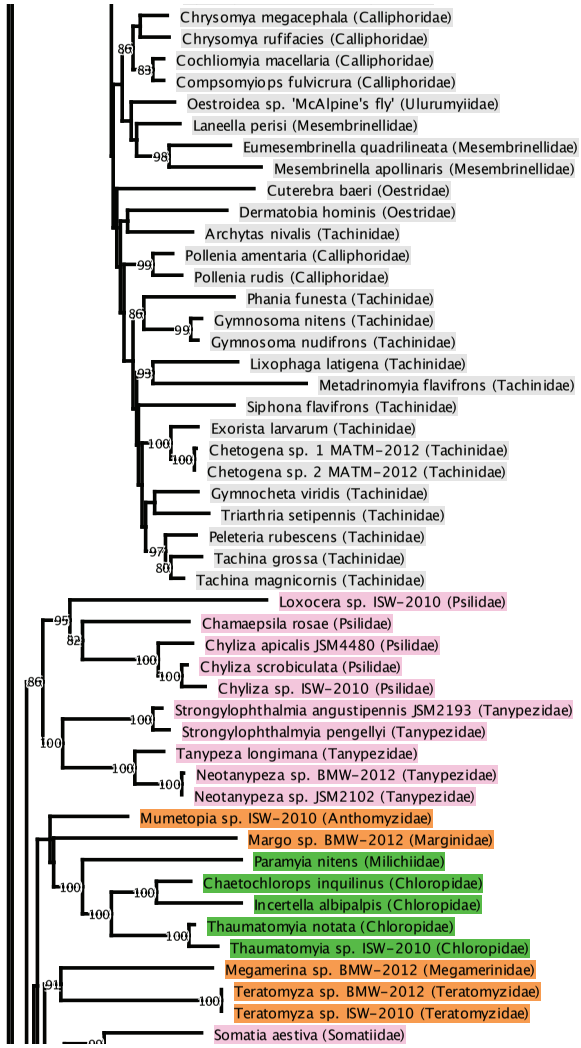

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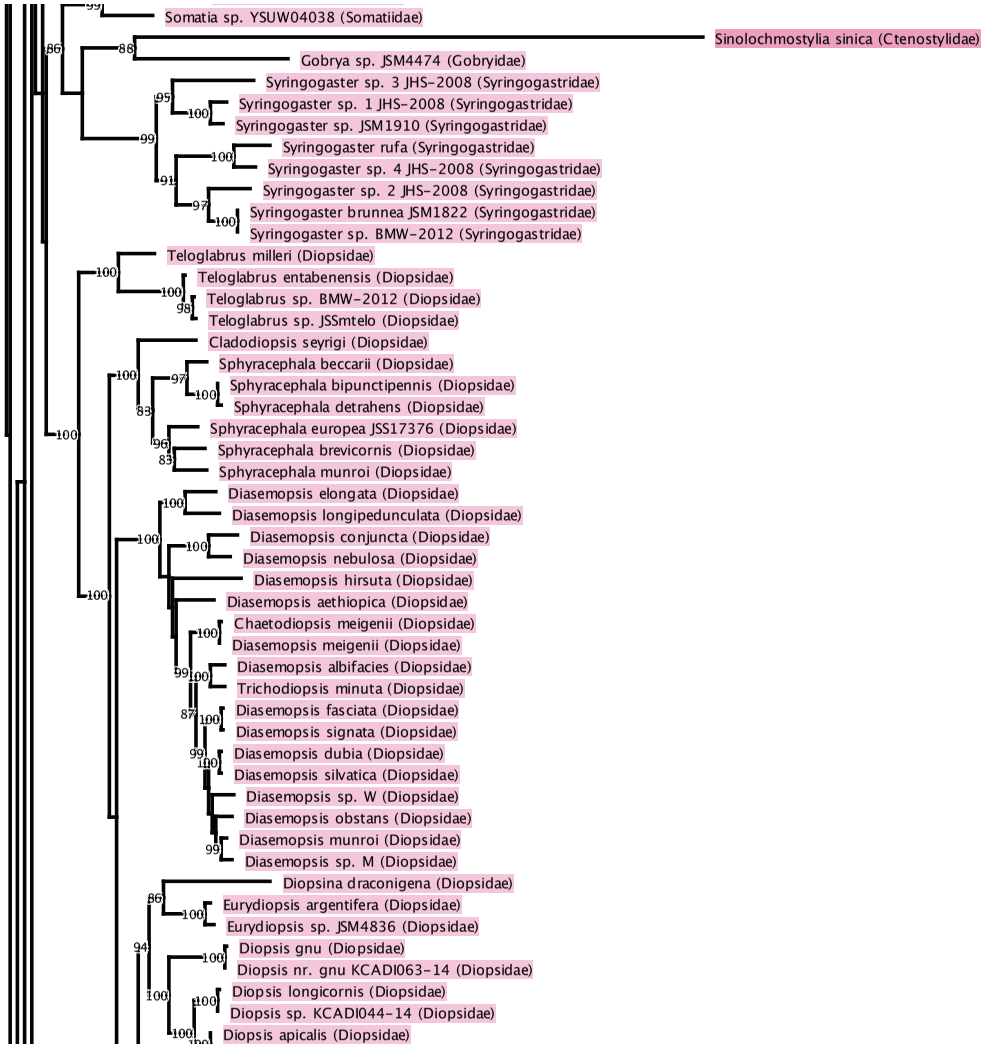

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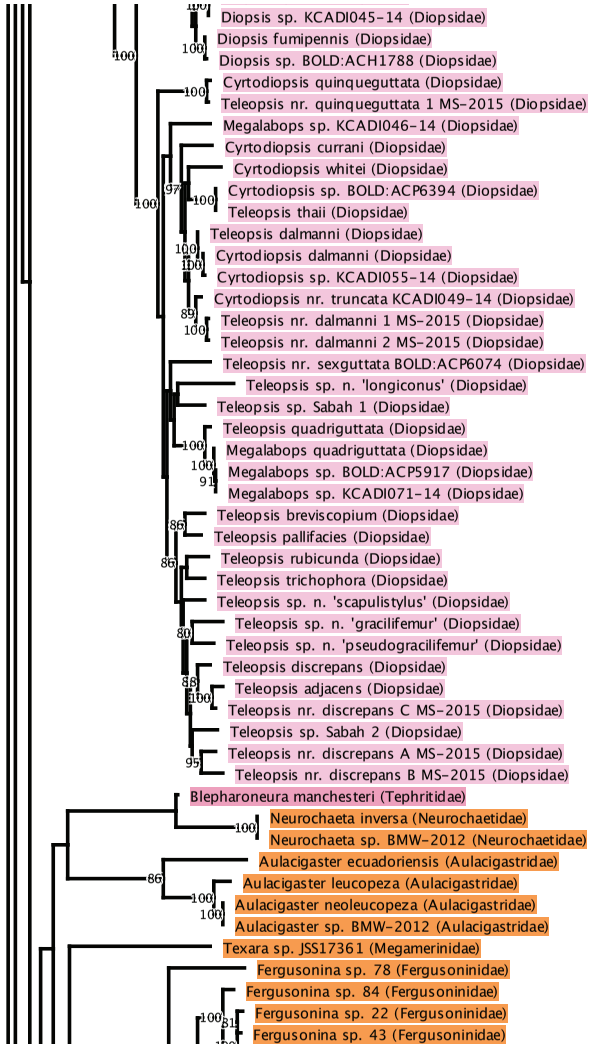

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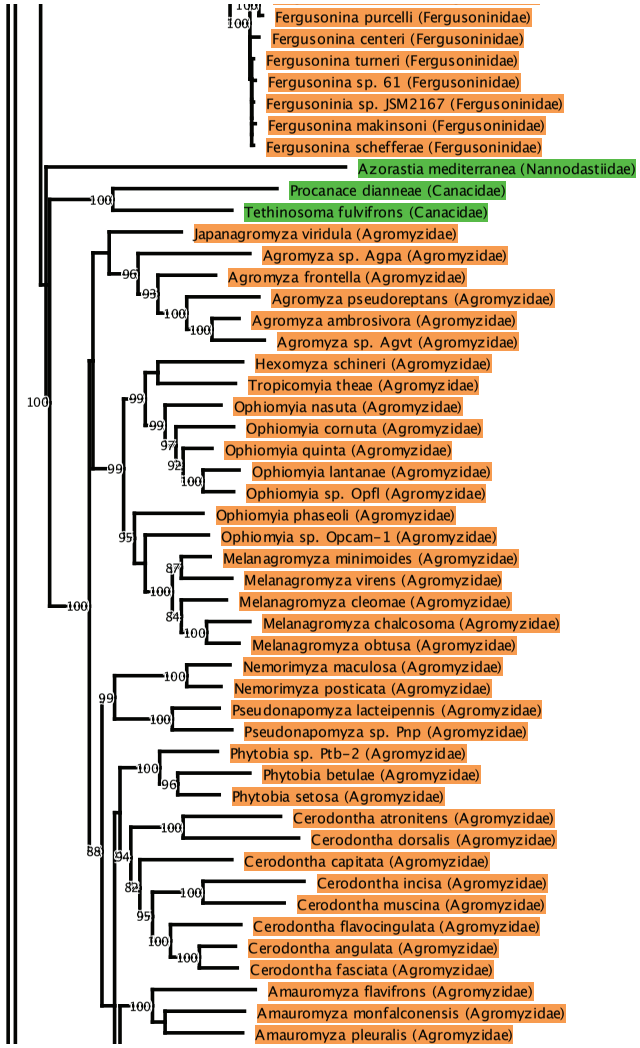

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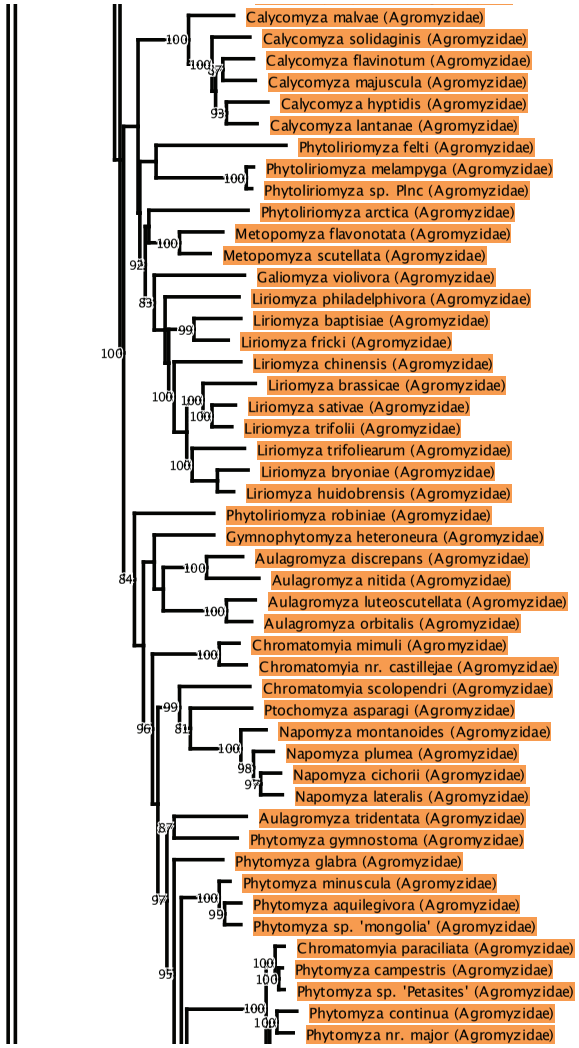

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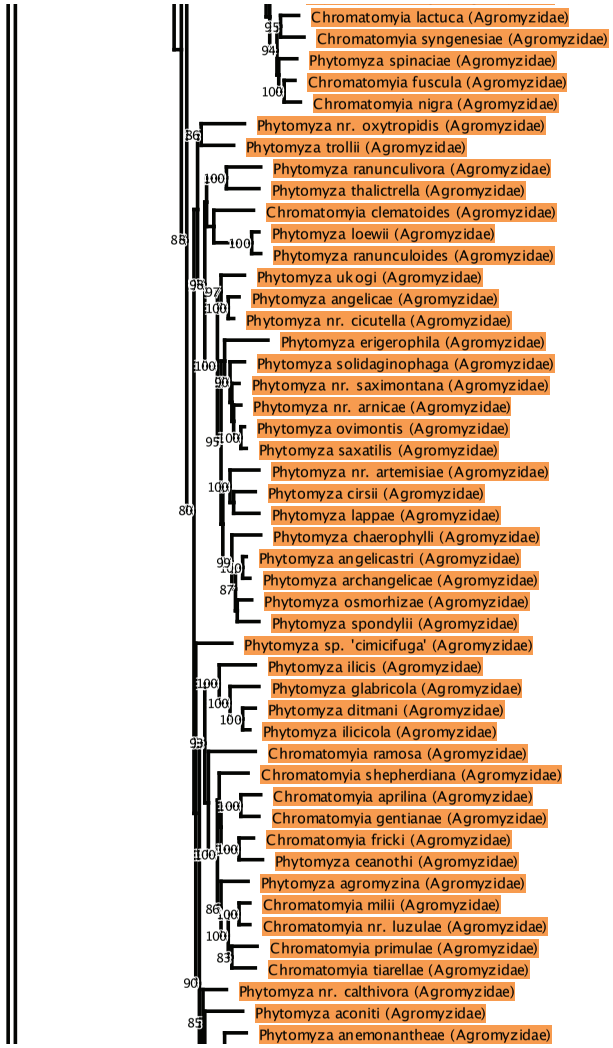

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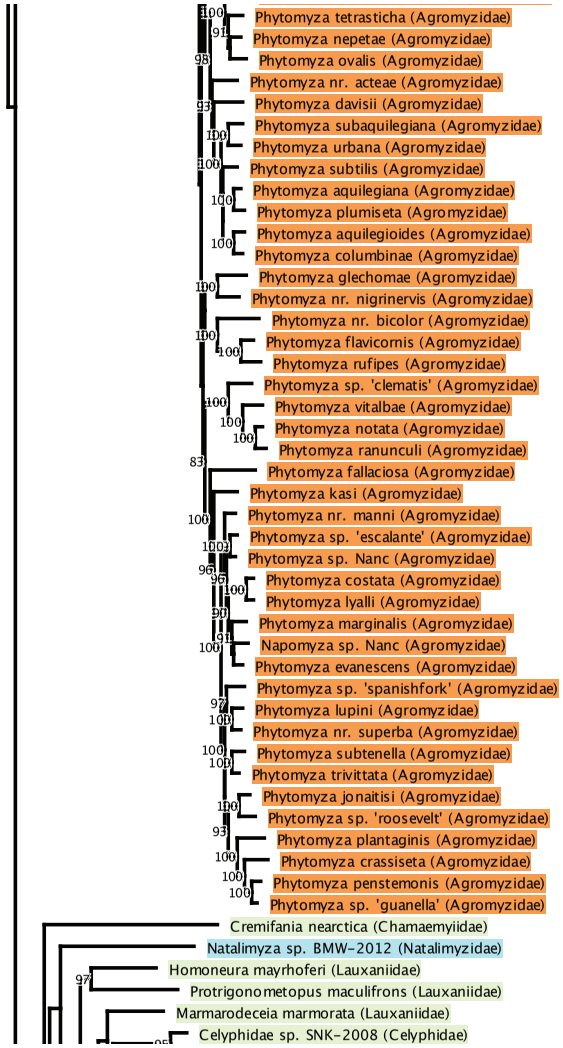

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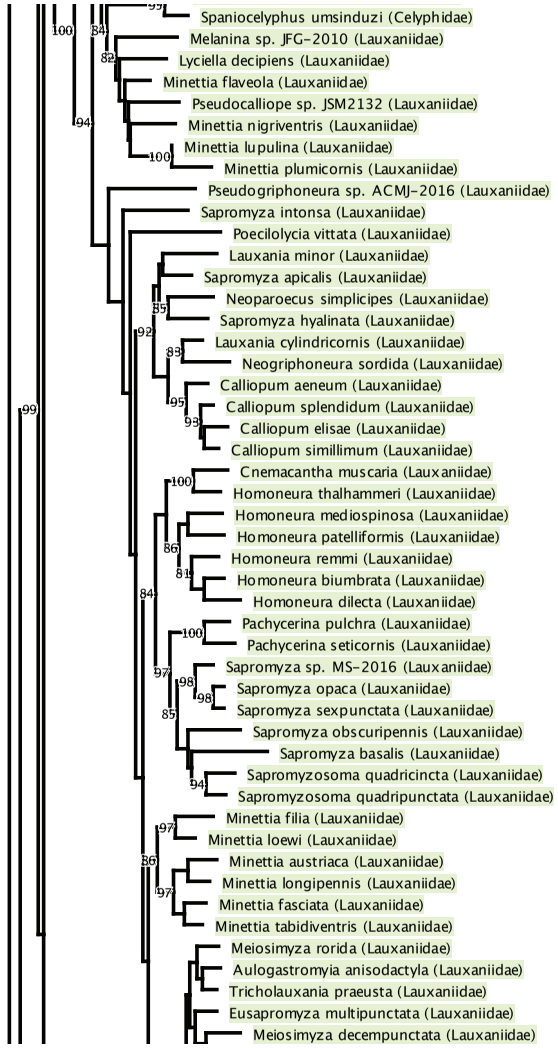

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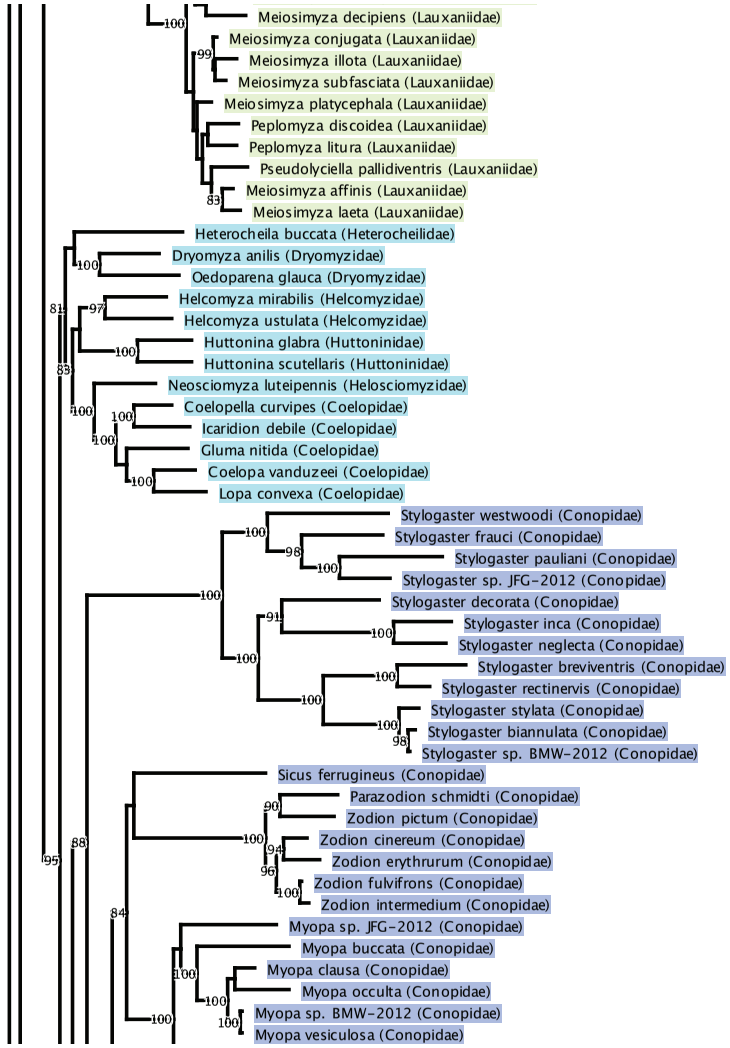

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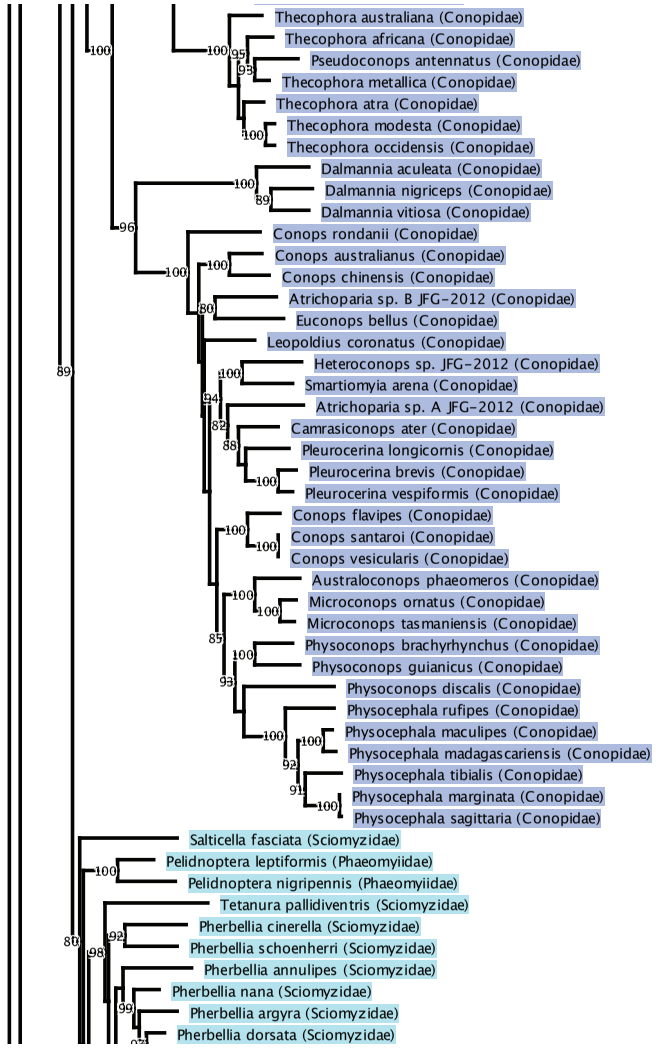

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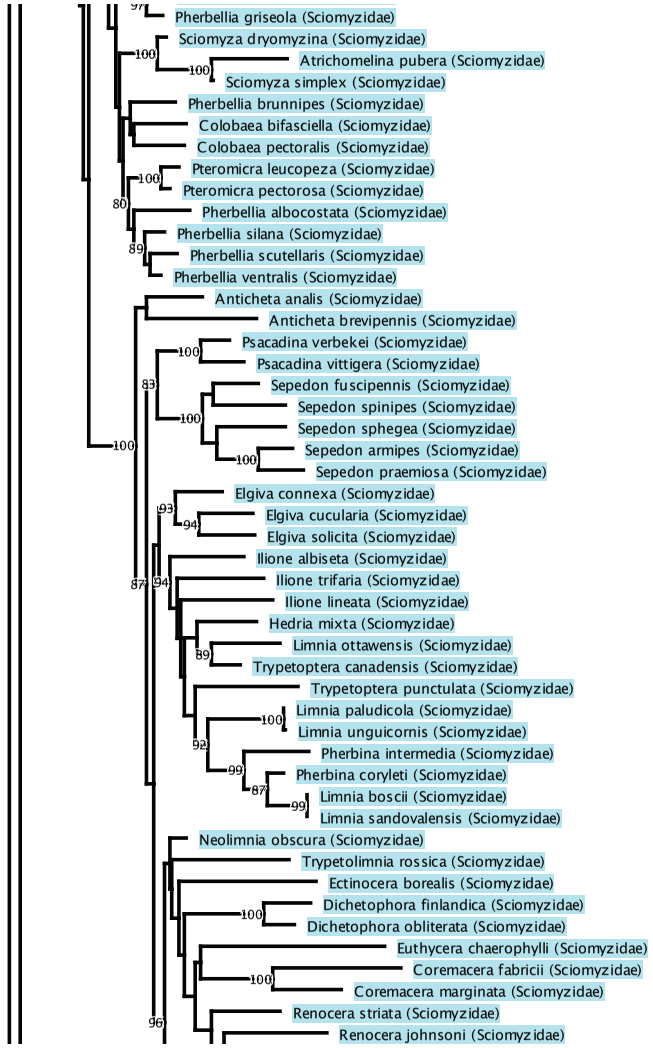

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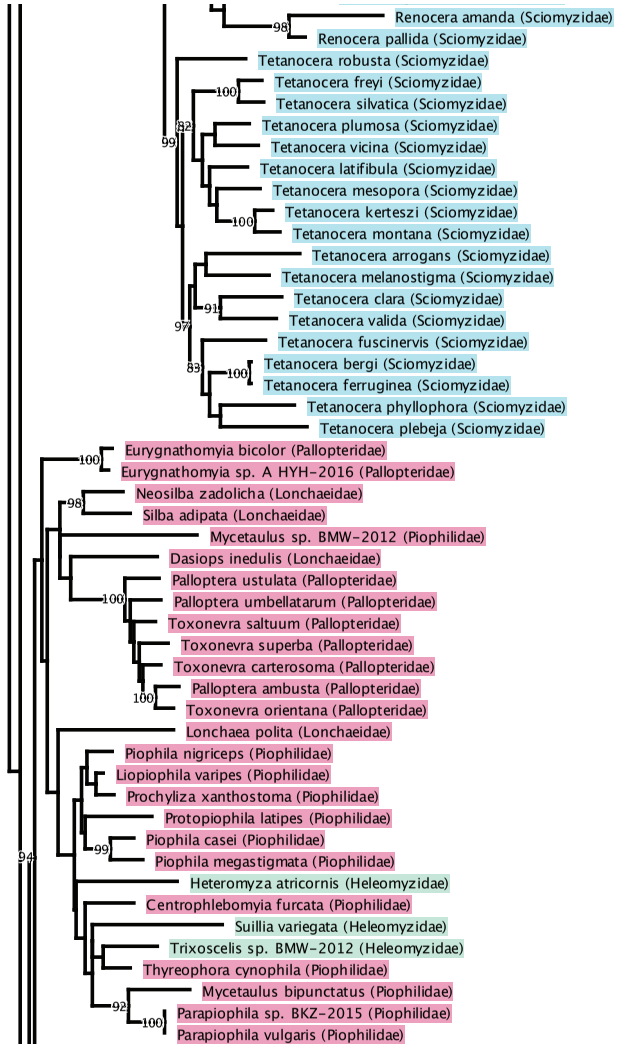

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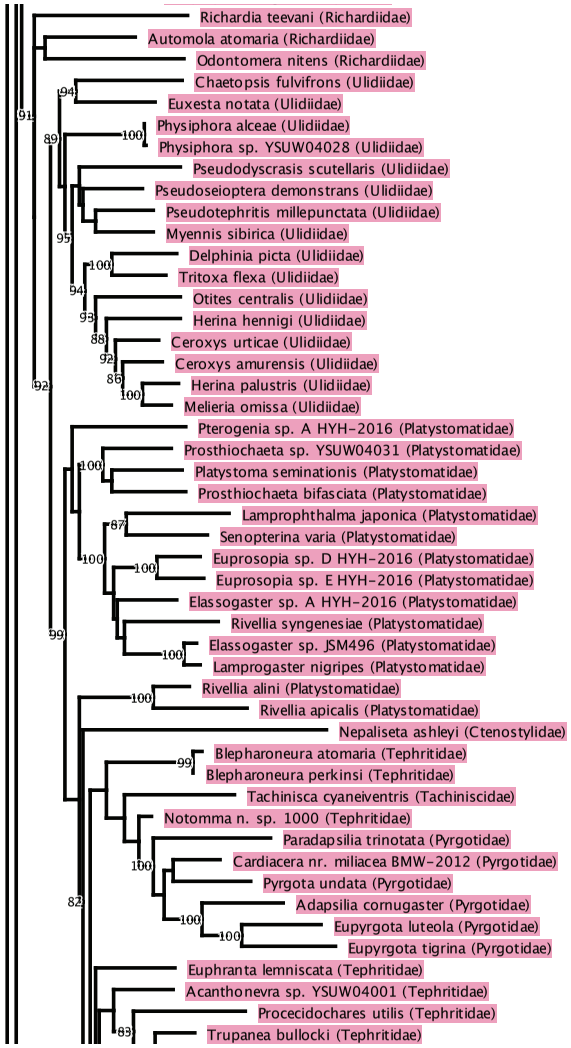

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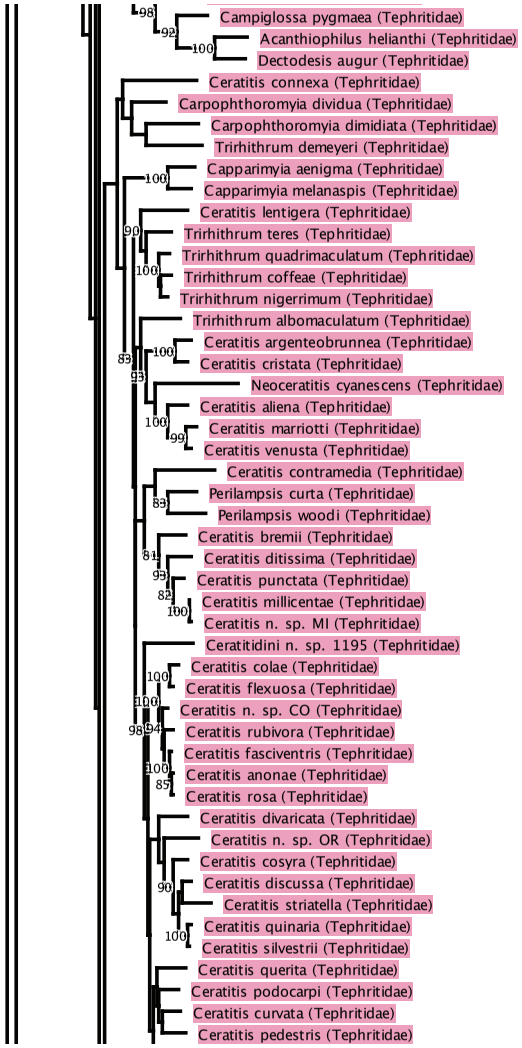

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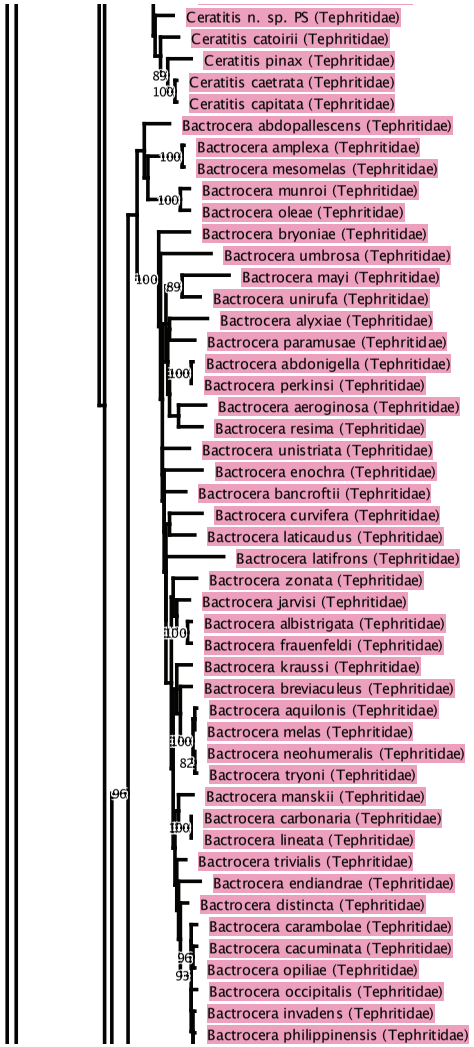

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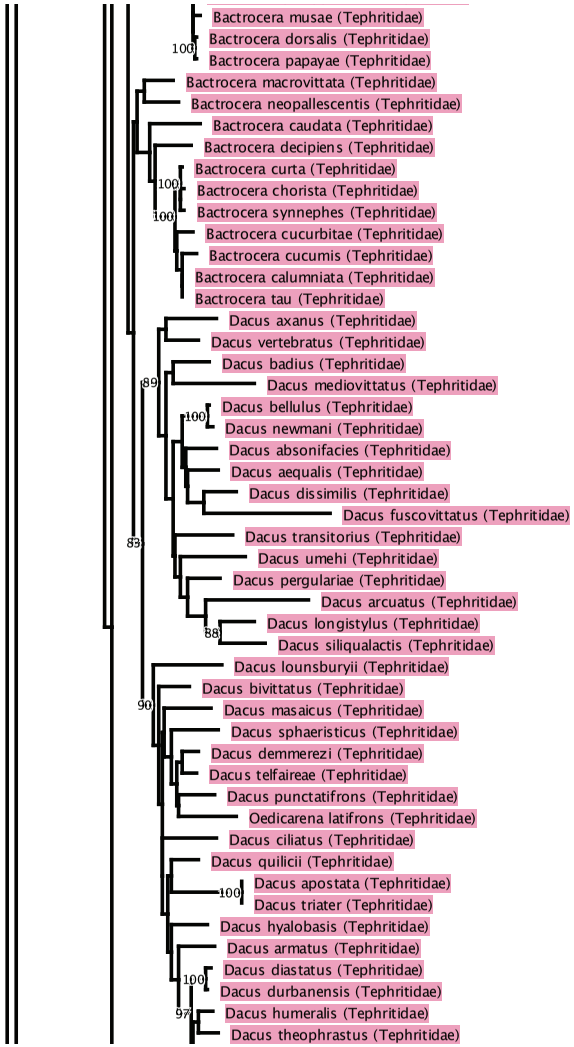

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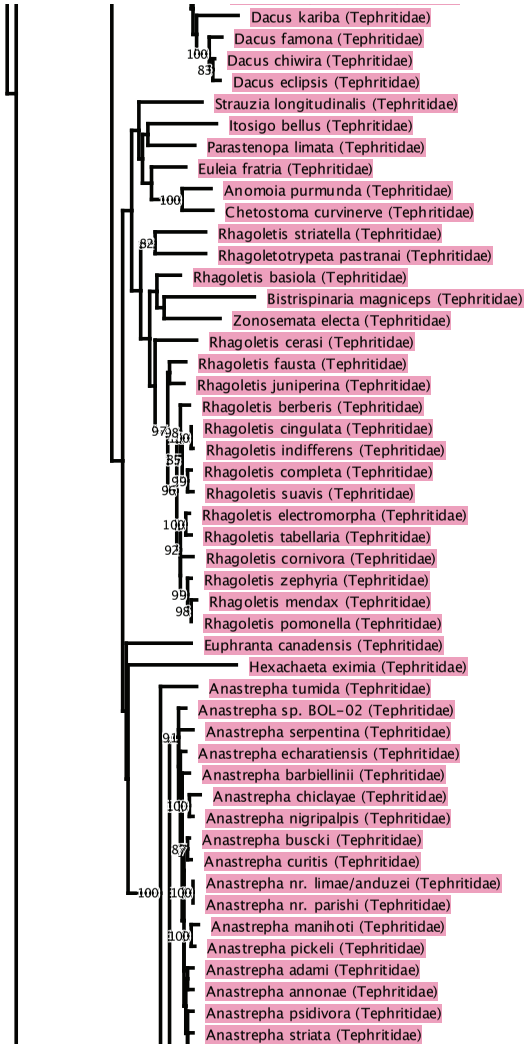

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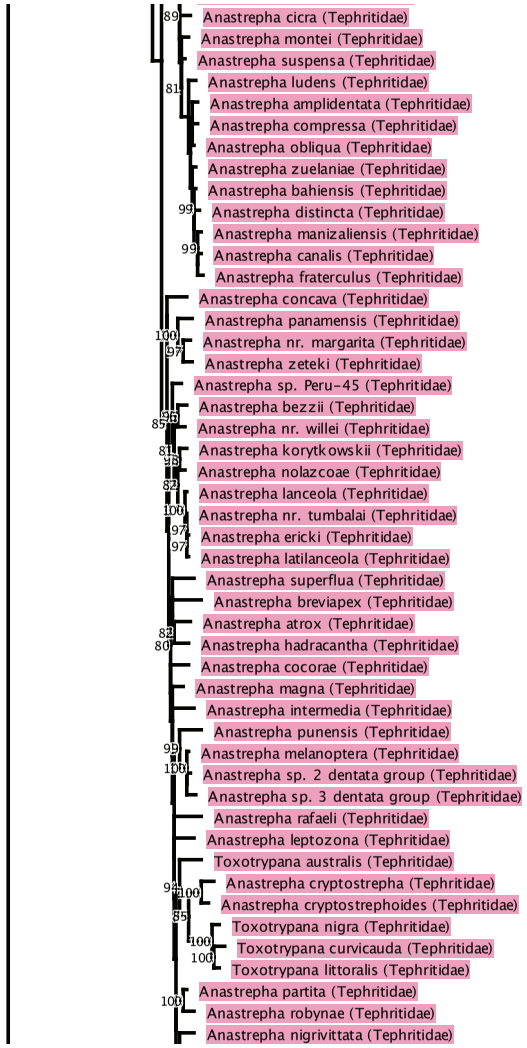

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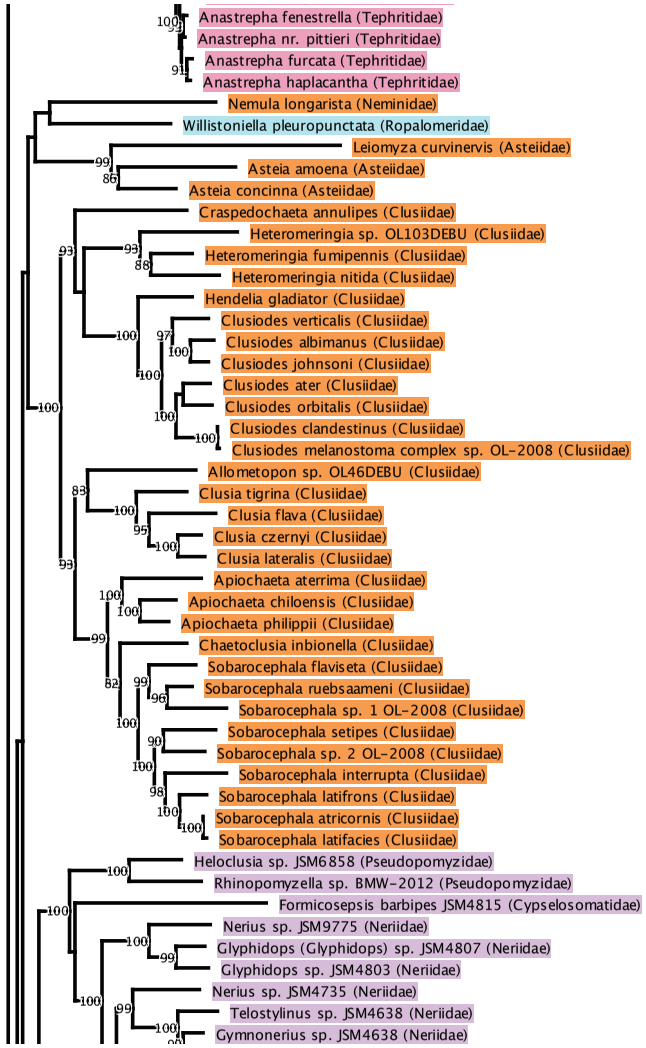

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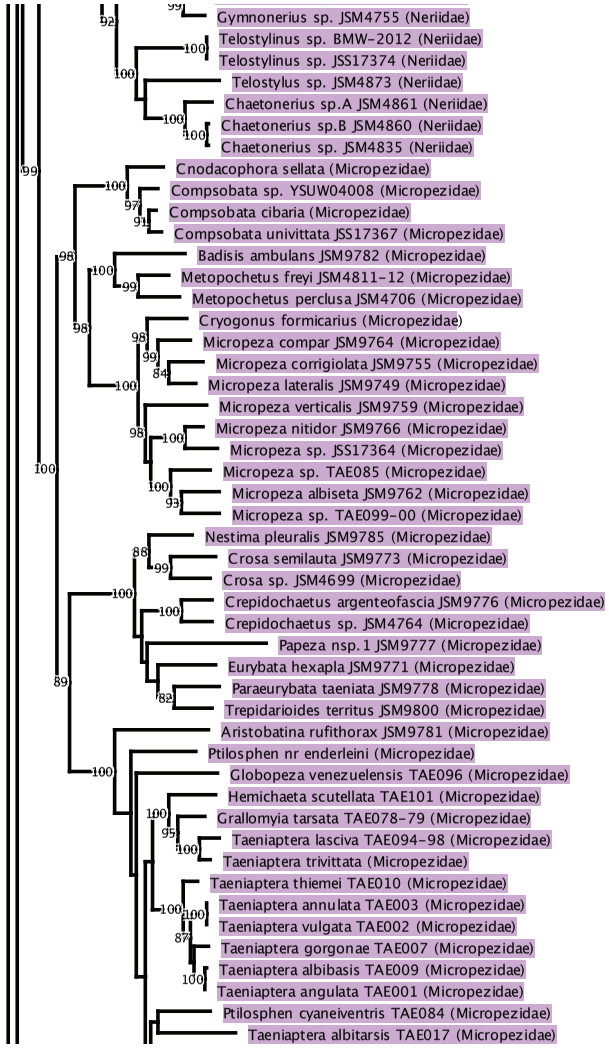

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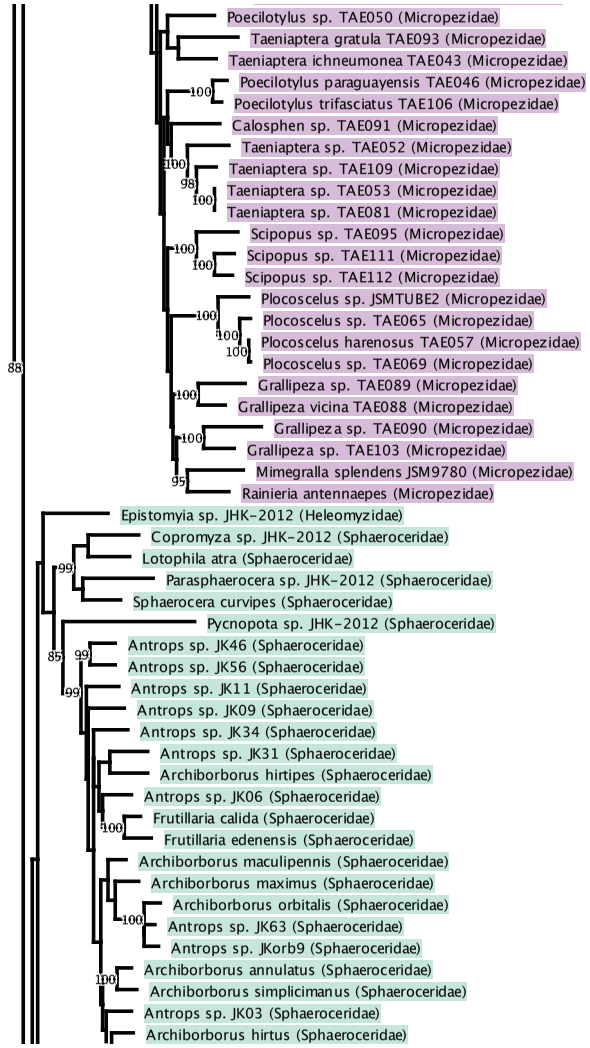

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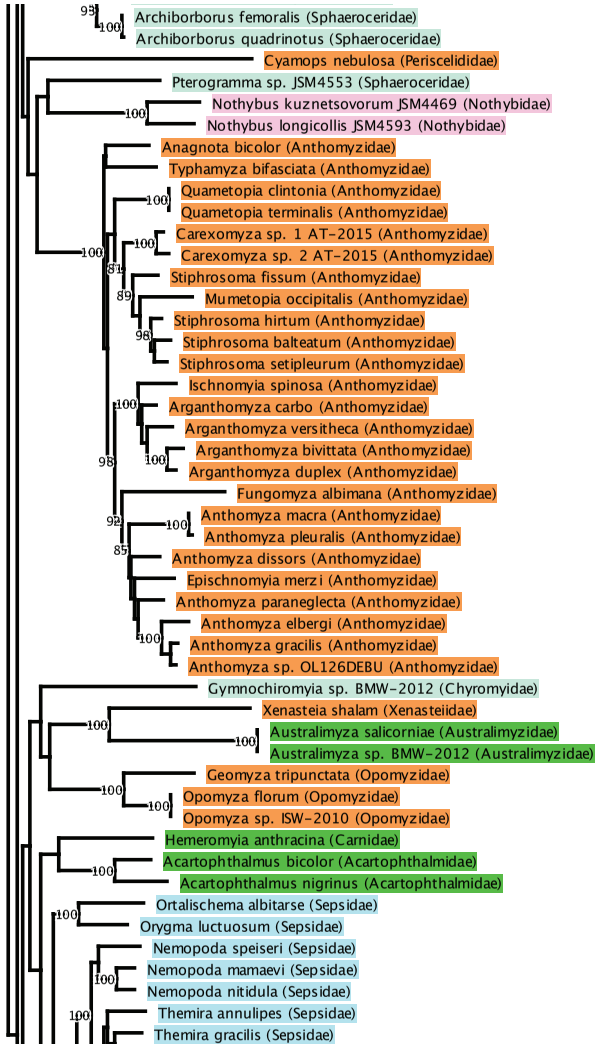

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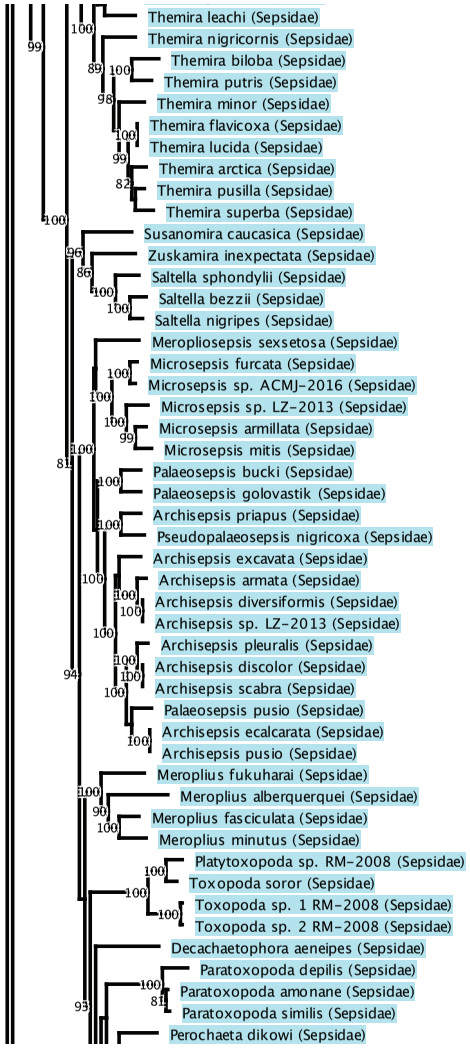

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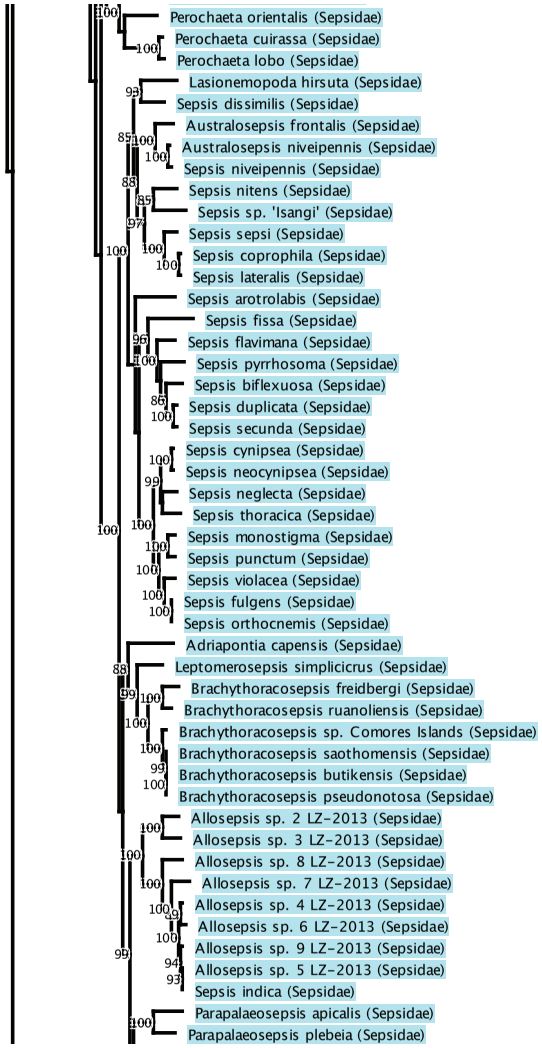

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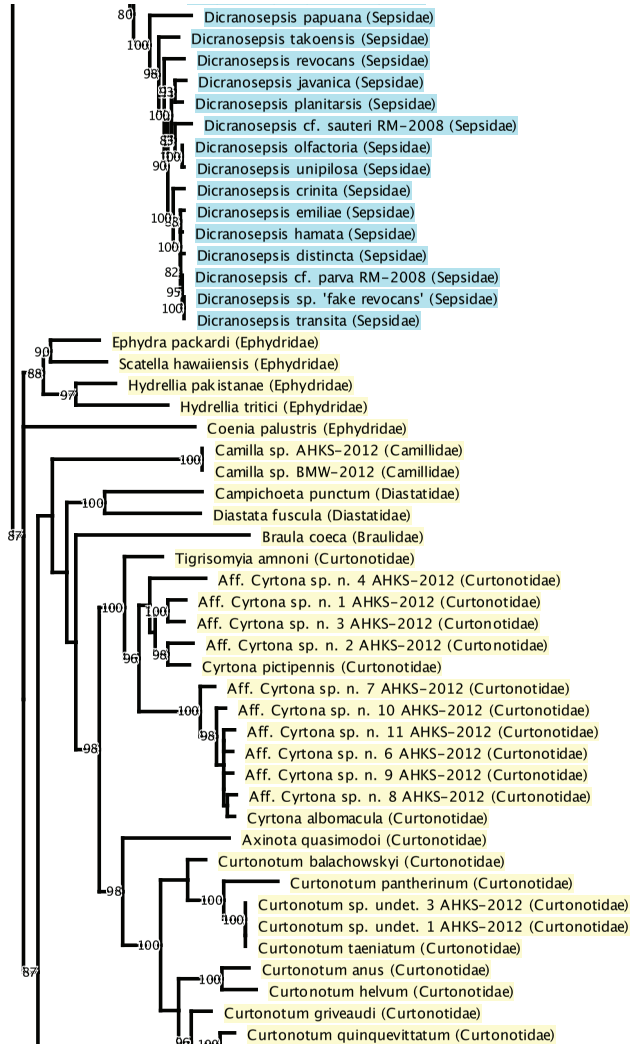

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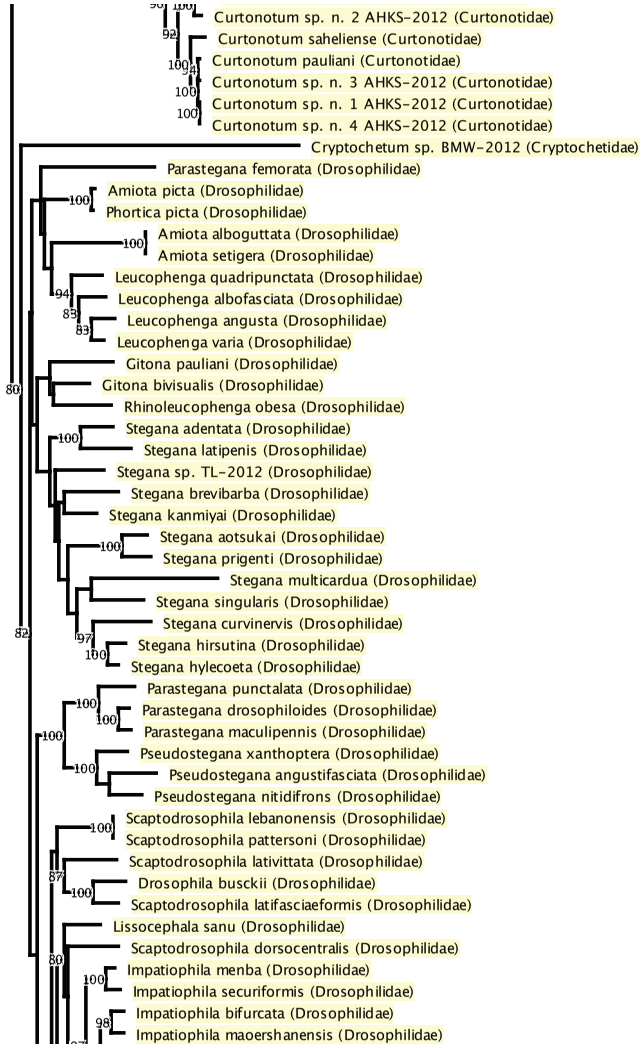

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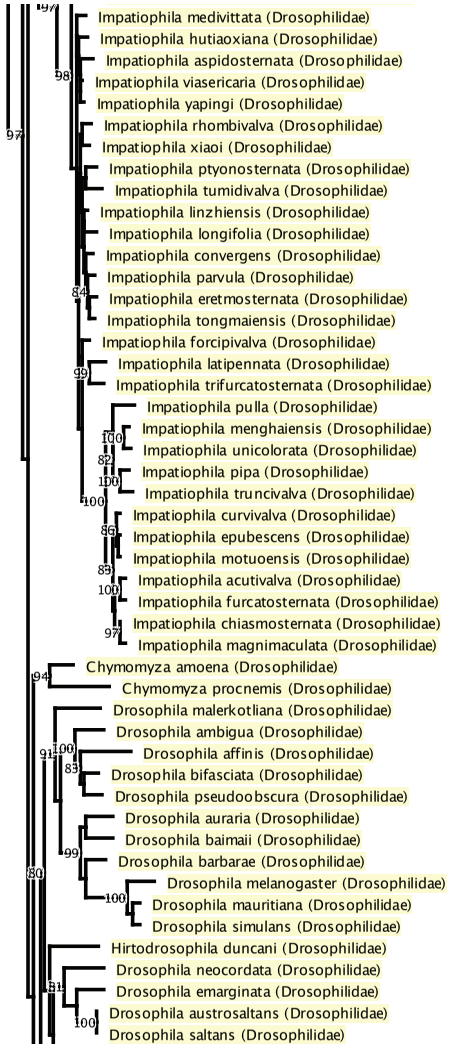

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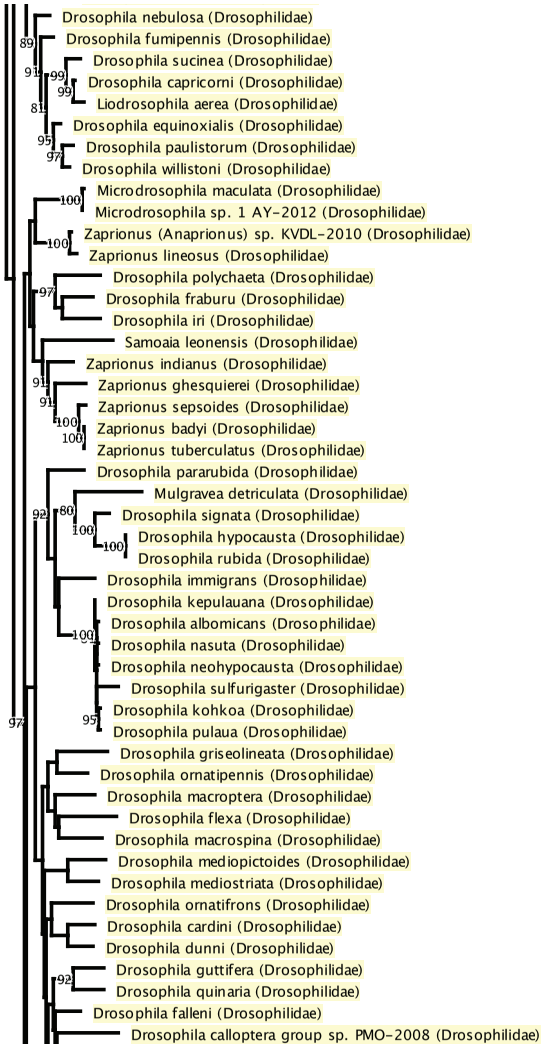

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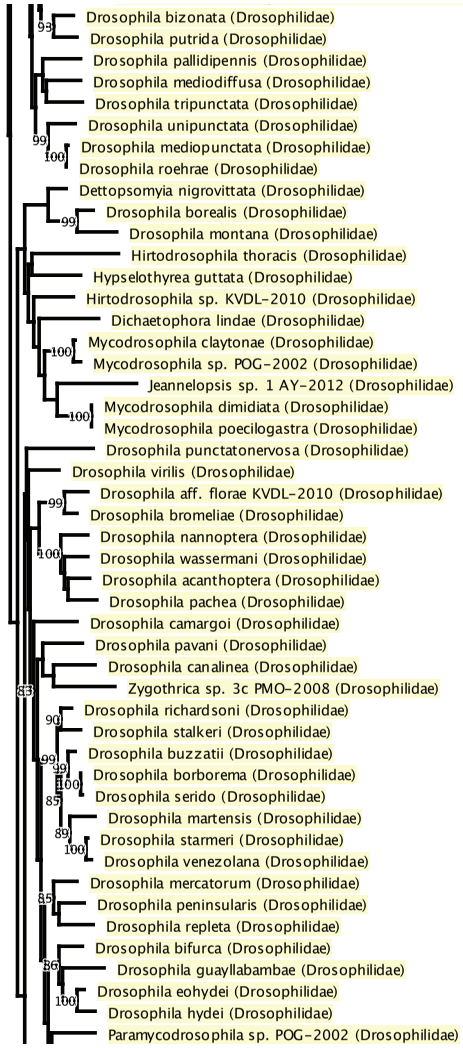

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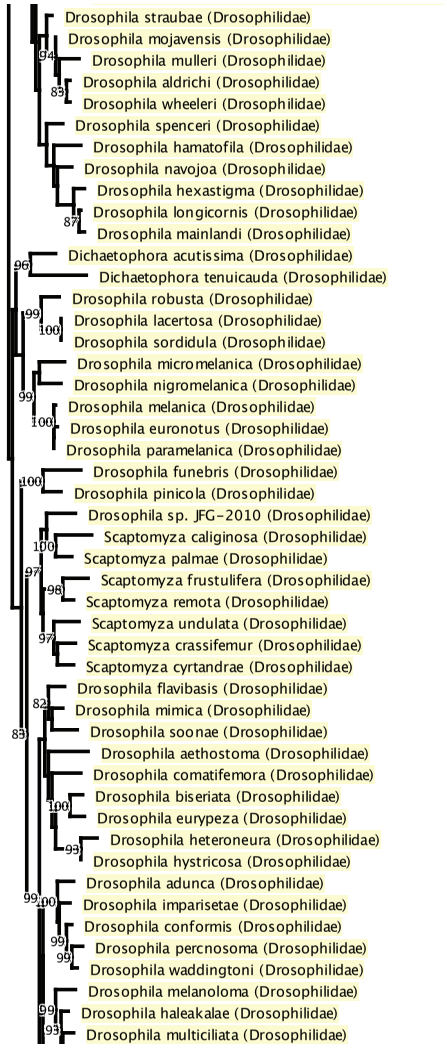

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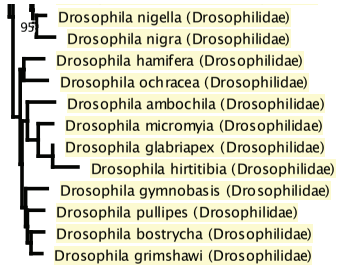

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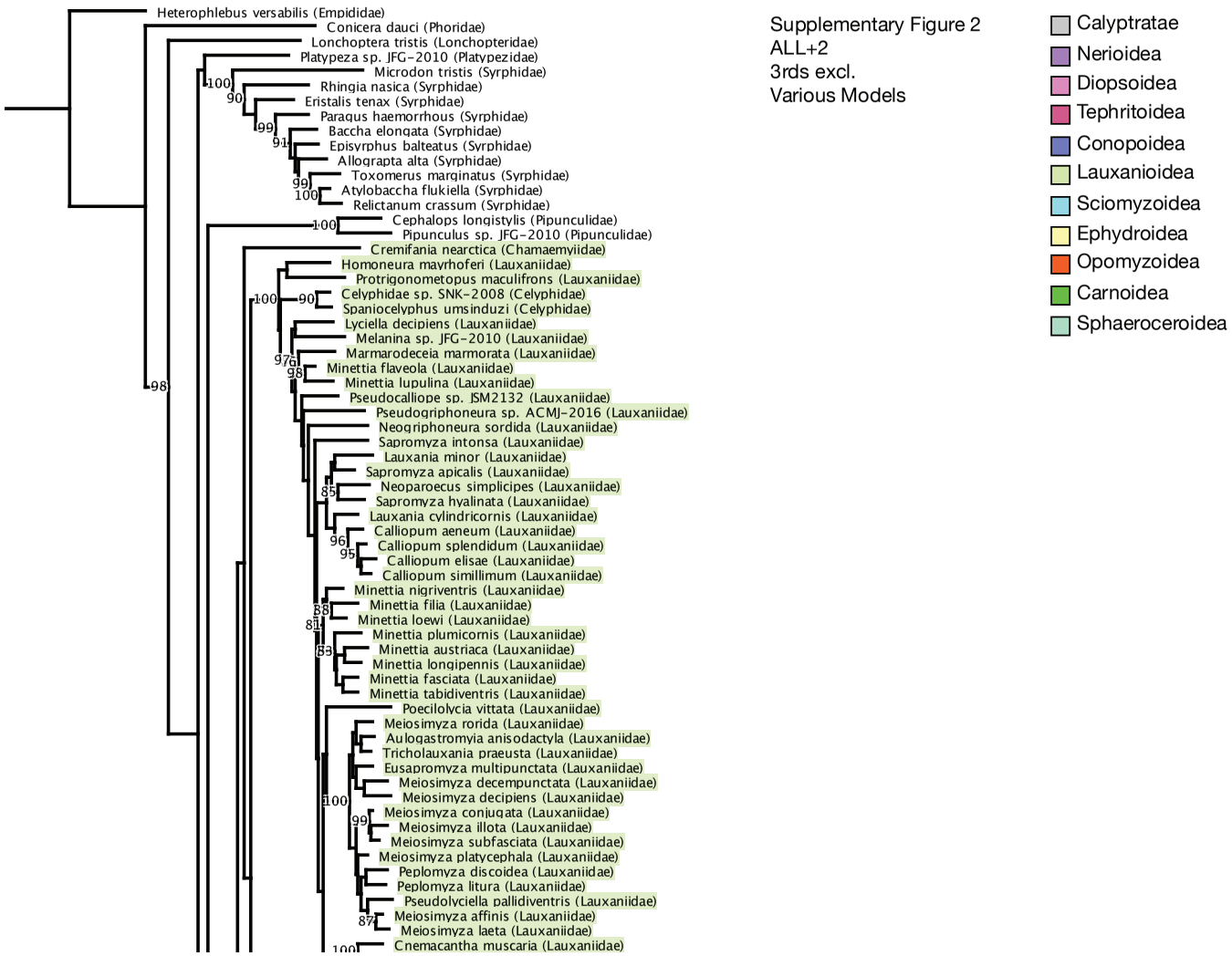

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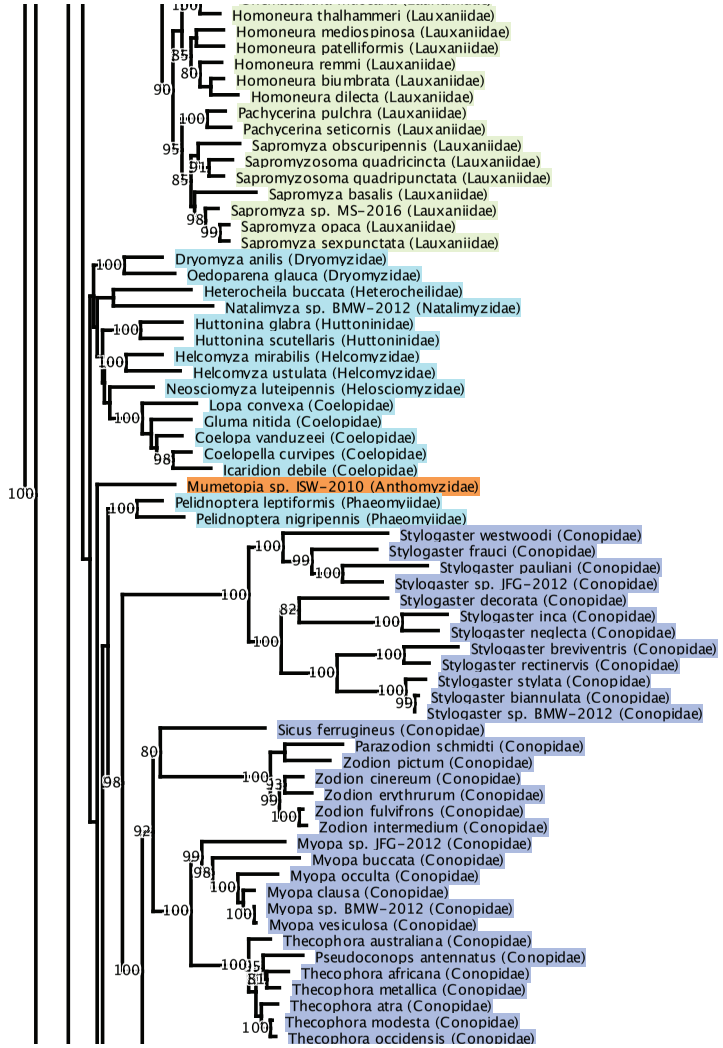

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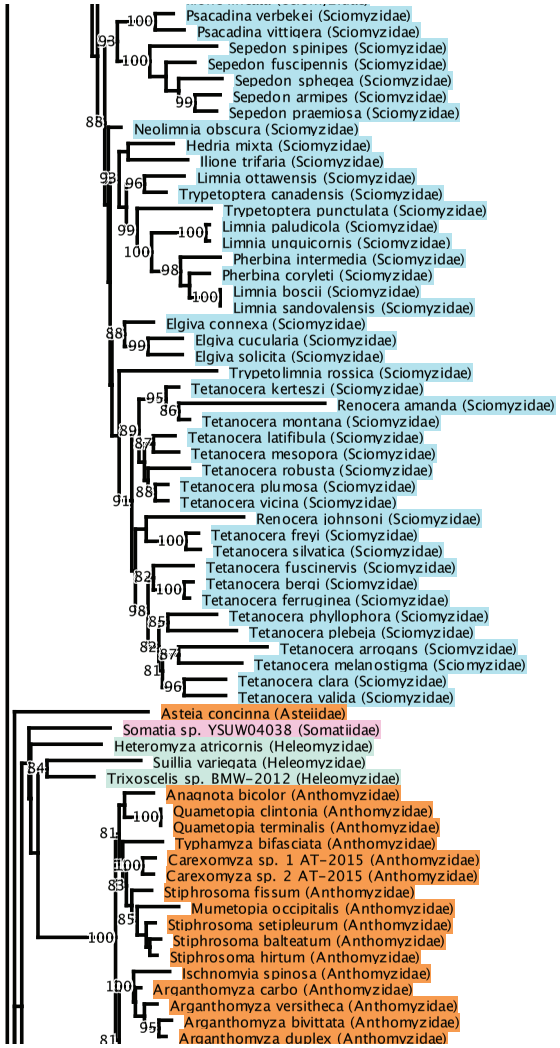

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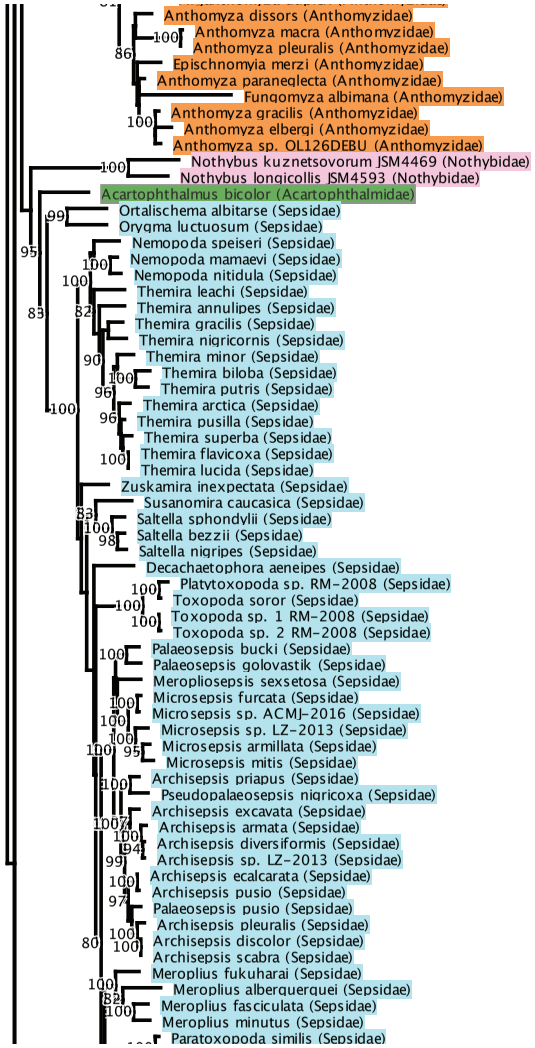

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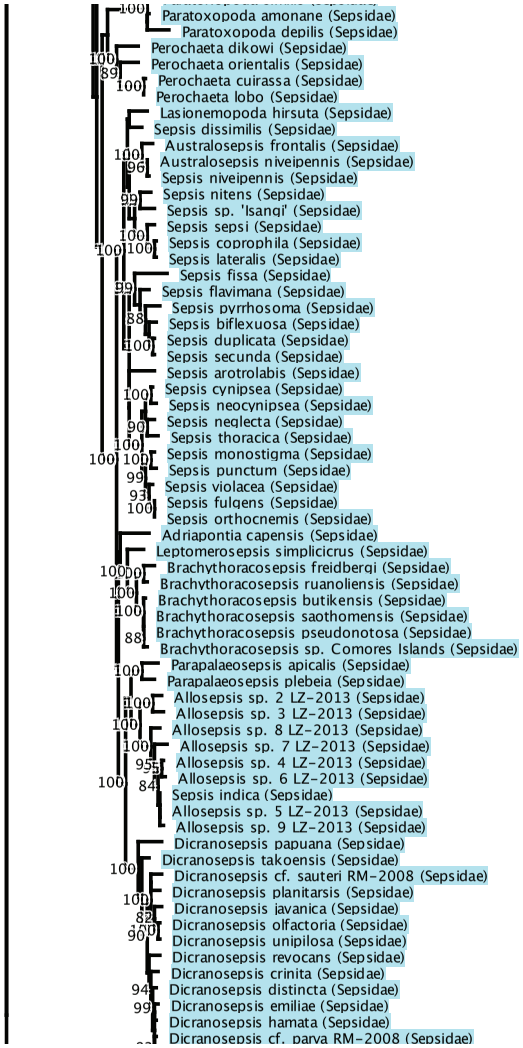

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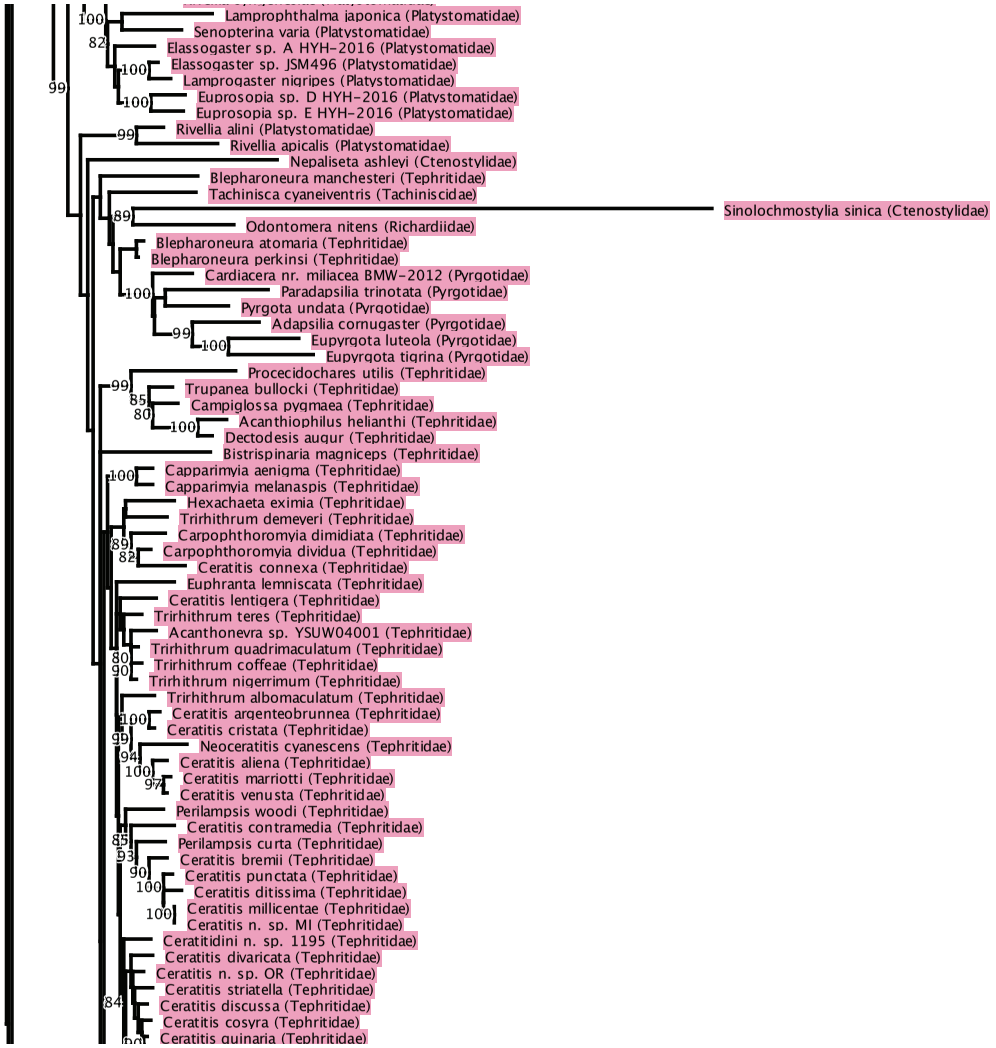

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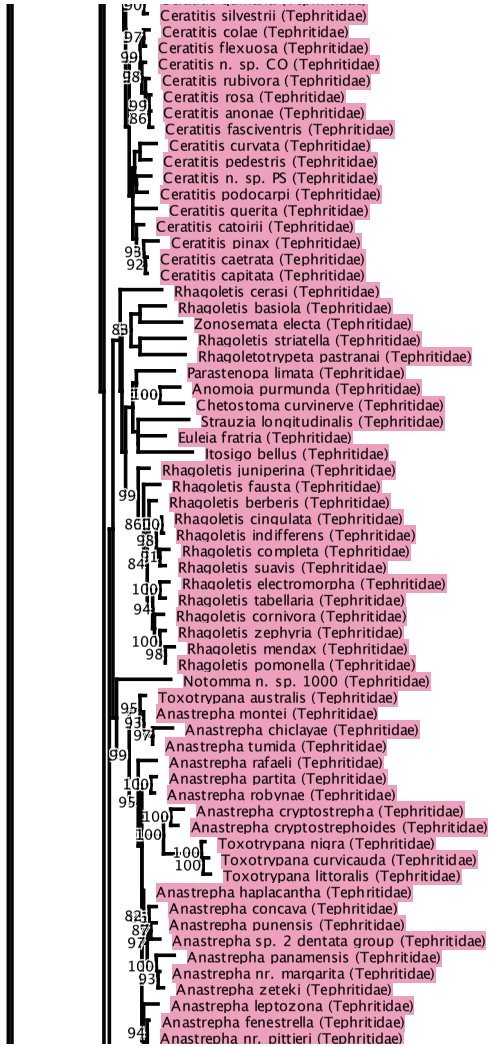

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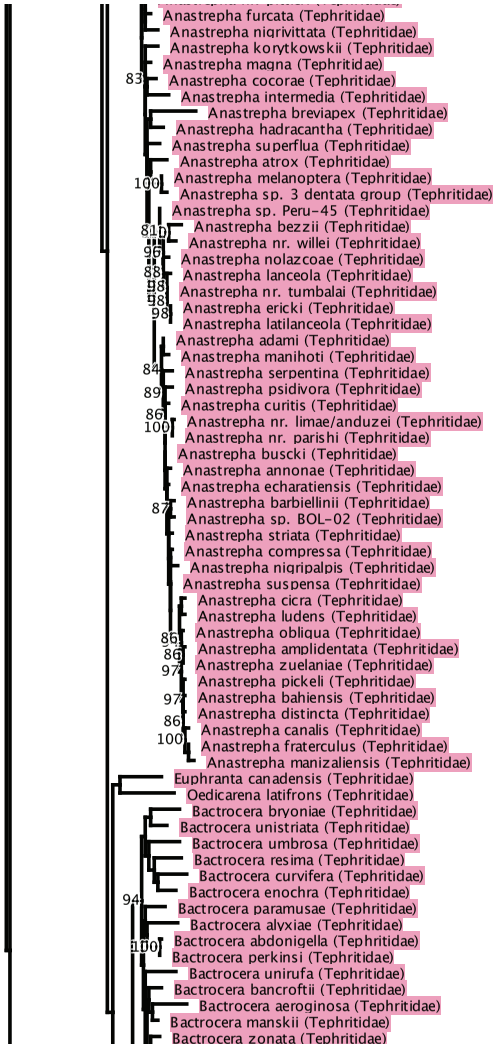

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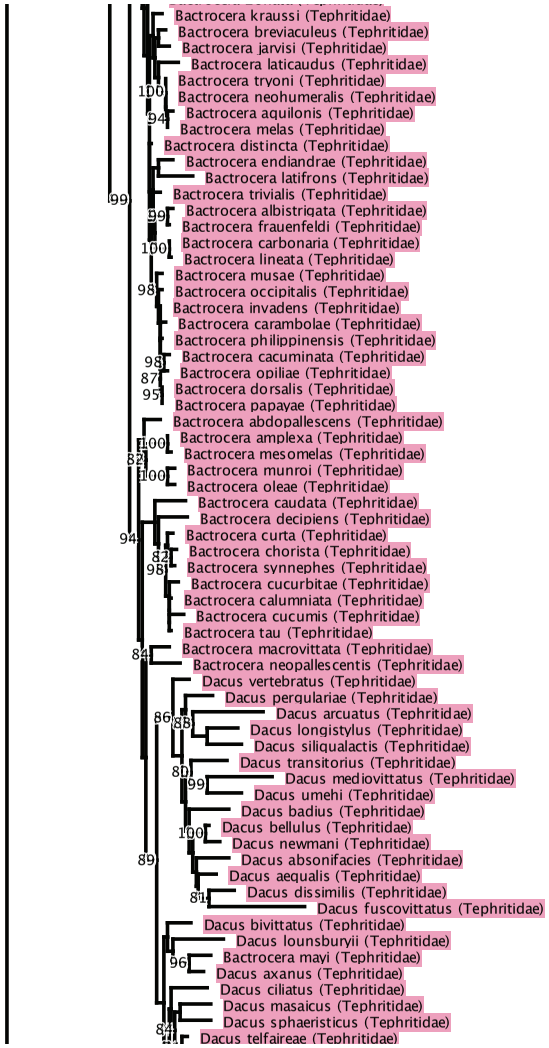

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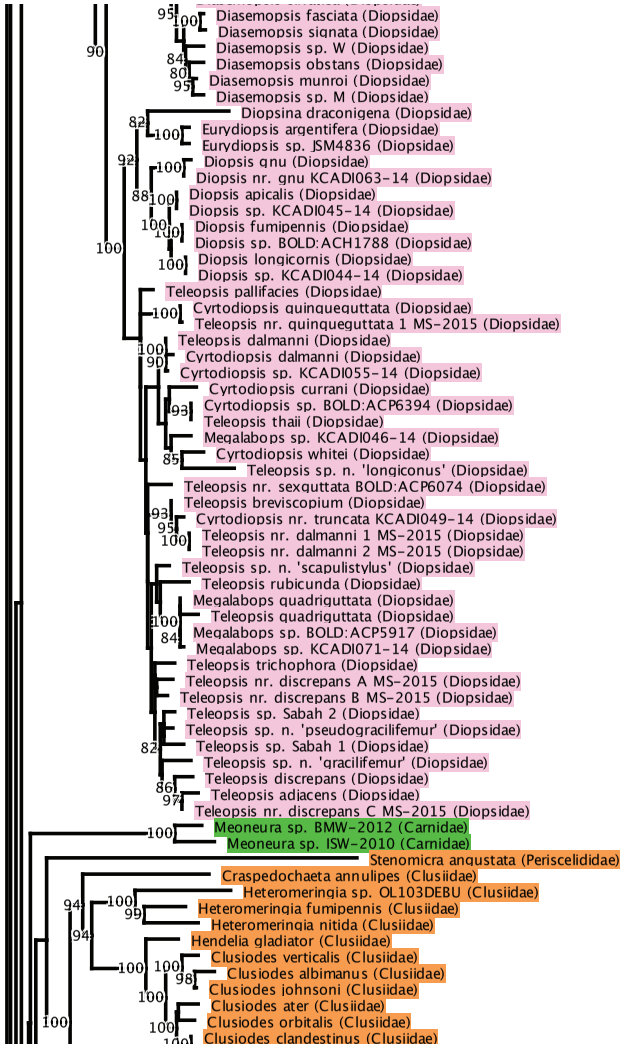

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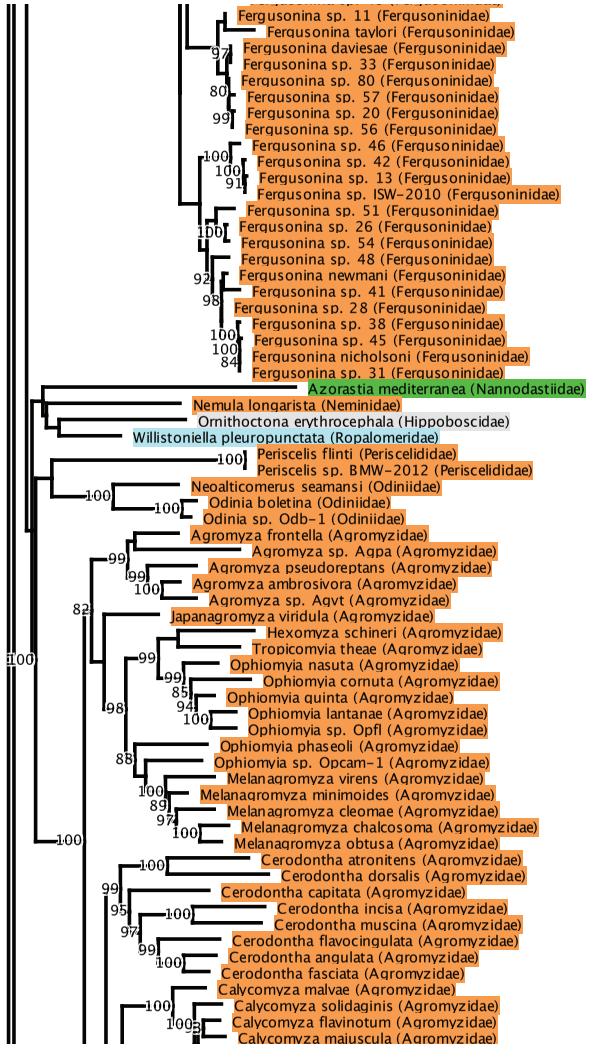

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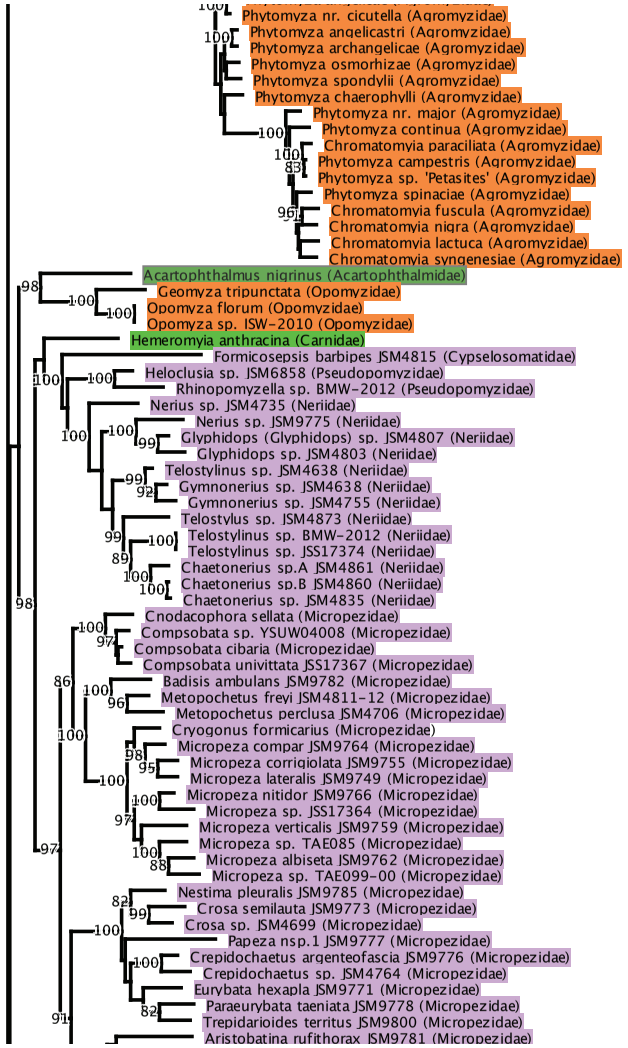

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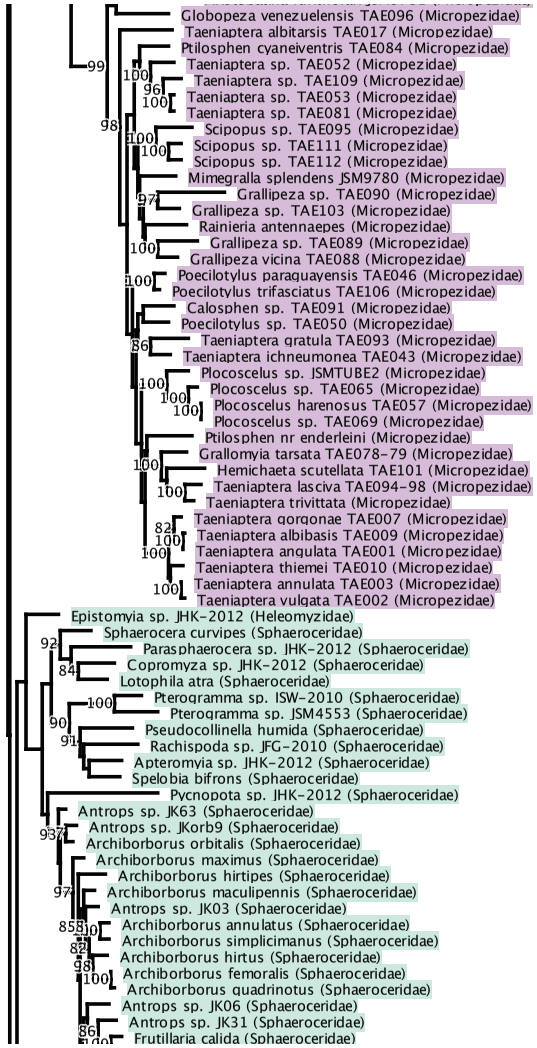

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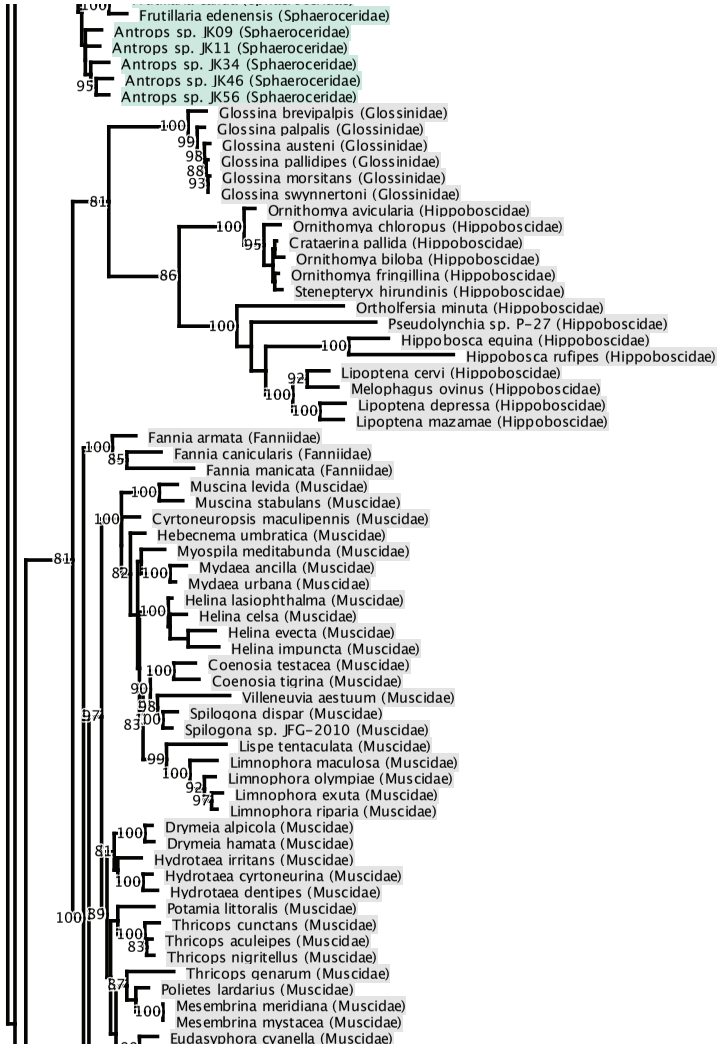

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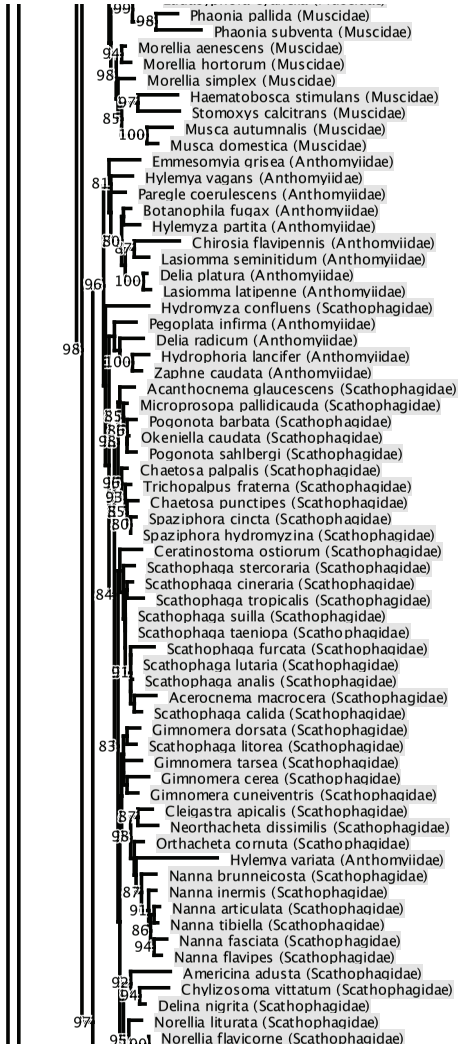

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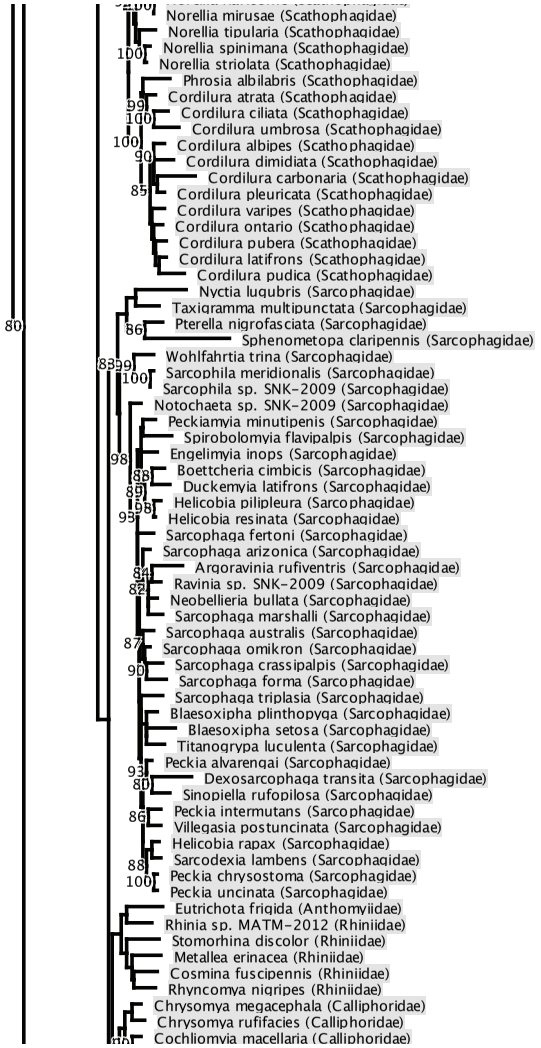

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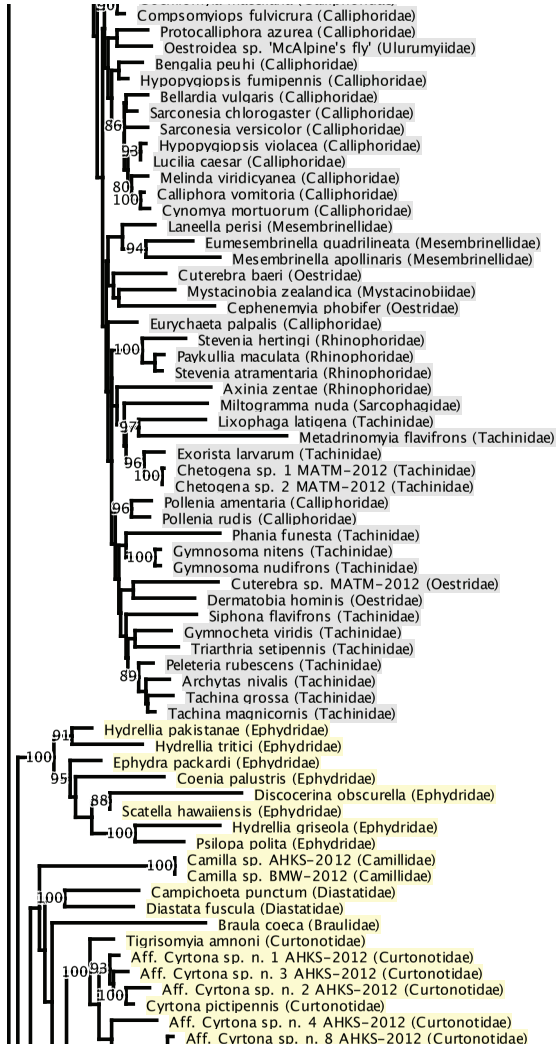

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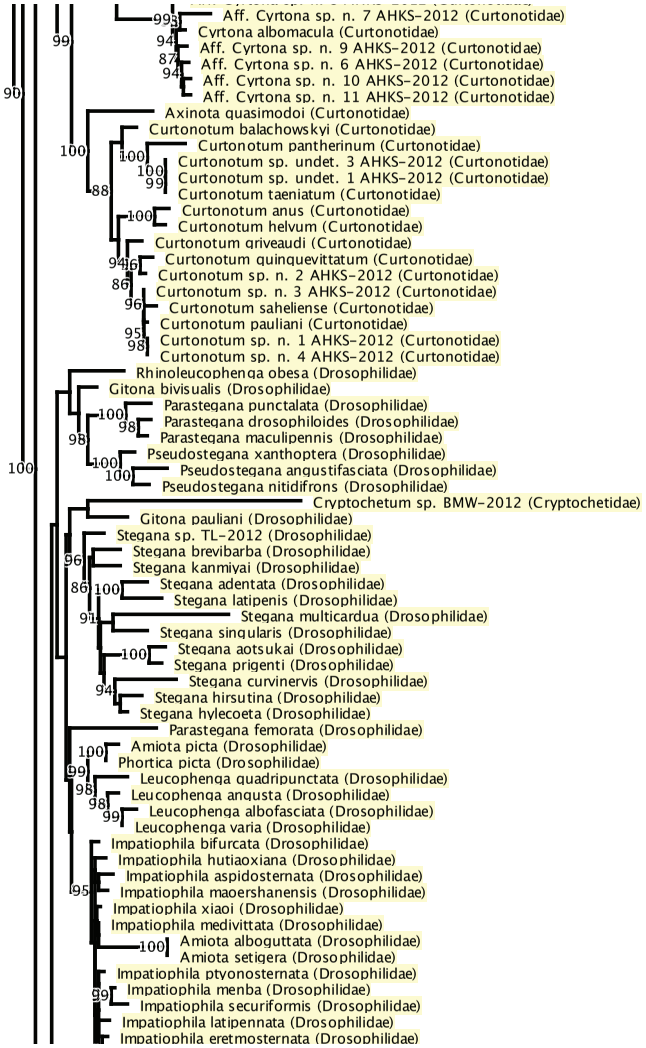

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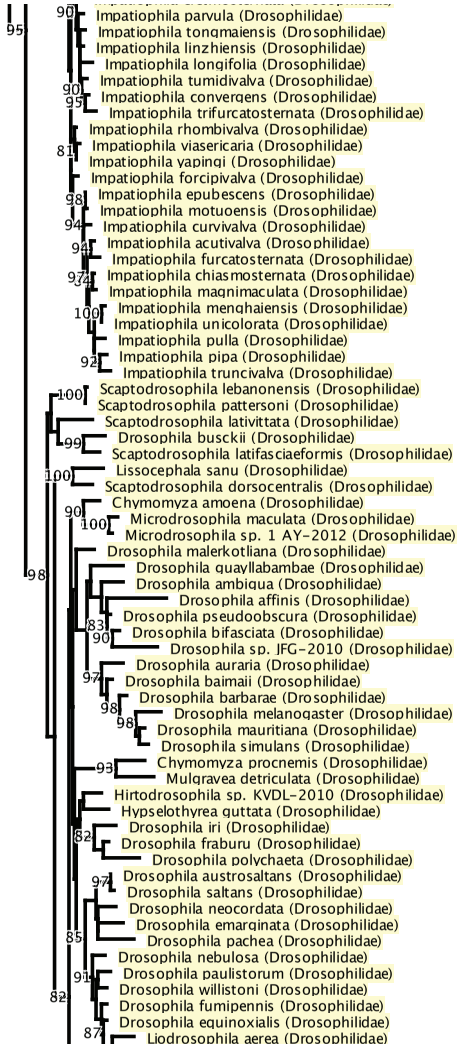

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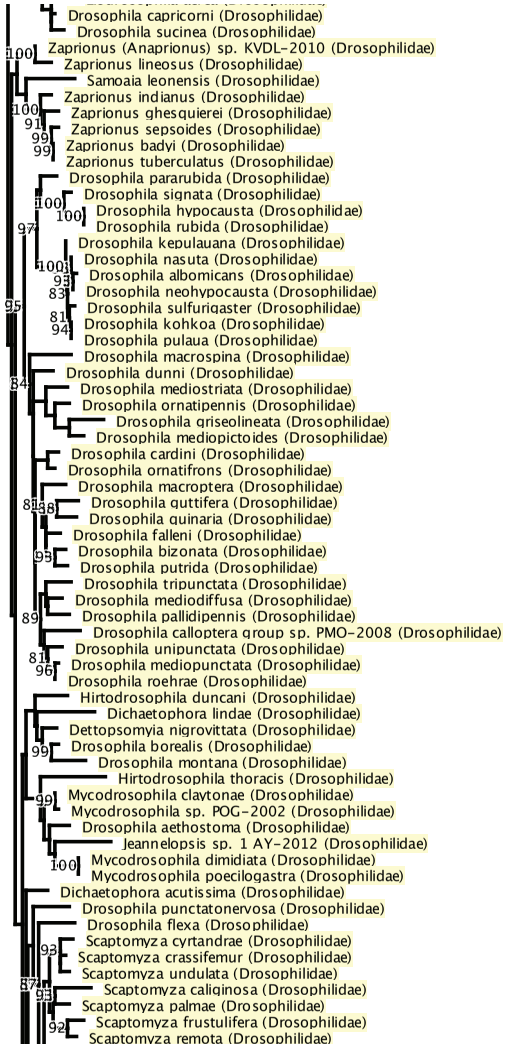

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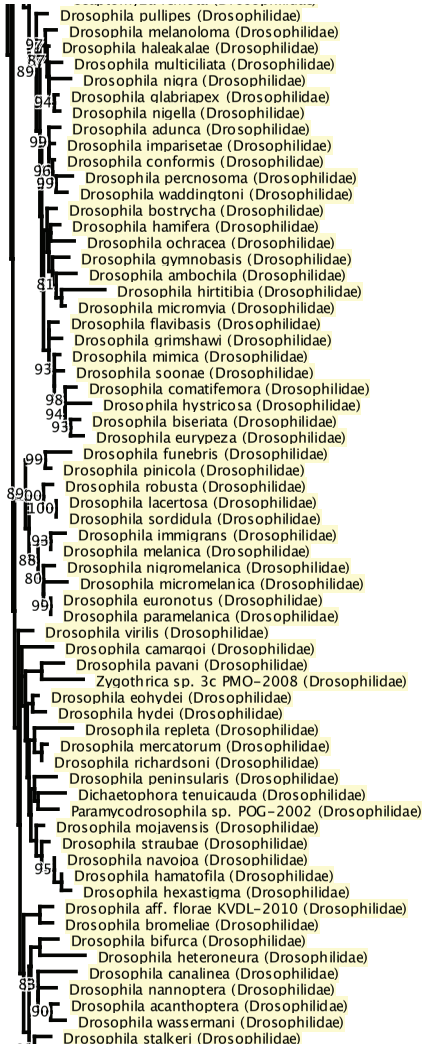

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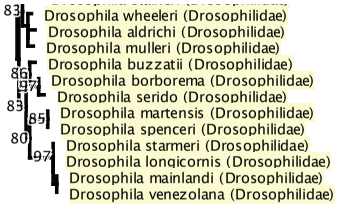

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CONCLUSIONS AND FUTURE DIRECTIONS

The higher relationships of Micropezinae recovered in Chapters One and Two using DNA data are largely in agreeance with previously proposed morphological hypotheses, and are well-supported with the data analyzed here. In Chapter One, Micropezinae is recovered as the sister group to the newly elevated subfamily Metopochetinae, together forming a sister group relationship with Calobatinae. Additionally, Micropezidae are recovered as sister to the remaining Nerioidea, itself recovered in Chapter Two as most likely related to a clade of still unstable composition, but which consistently features Sphaeroceroidea at its root. These relationships were all previously proposed by D.K. McAlpine throughout his career, and finding molecular support for his morphological hypotheses lends additional credibility to the data and methods employed here. However, much work remains to fully expand and contextualize the results of this thesis.

The higher relationships of Schizophora recovered using DNA data are found to largely agree with previous morphological classifications and hypotheses. However, several important issues are identified which will need to be resolved before the phylogeny of Schizophora is phylogenetically stabilized. Taxon sampling remains a weakness, particularly among the diverse Carnoidea and Sphaeroceroidea. This lack of available data may explain the inability for model-based phylogenetic methods to consistently recover higher relationships of Schizophora using molecular data as they are unable to adequately assess homoplasy and substitution saturation within swiftly evolving positions, resulting in unstable and random assignments of these undersampled taxa within the phylogeny. Increasing the diversity of taxa sampled within and across these superfamilies will hopefully help assess the monophyly and relationships of these superfamilies moving forwards, while also stabilizing the deeper relationships of Schizophora. In concert with increased taxon sampling, significant research into the development and deployment of more realistic and complex models of DNA evolution will be necessary that include parameters for differential rates of evolution within and between lineages. In addition, advances in computational efficiency of phylogenetics software will continue to be required to handle ever-larger and more diverse datasets. Traditional single-gene sequencing methods will continue to play an important role in the future of Schizophora systematics, while comparison with new genomic data and the continued exploration and interpretation of morphological data will be vital for finding congruence and stability in schizophoran systematics.

The relationships of Nerioidea, including an increasingly likely sister group relationship with a clade including Sphaeroceroidea, are recovered here with high support. The relationships of Cypselosomatidae, Pseudopomyzidae, and Neriidae remain less confidently resolved, with different

datasets and analysis methods recovering slight variations in the relationships between them. These relationships will benefit from greater taxon sampling, particularly within Pseudopomyzidae and Cypselosomatidae, as well as the further study and inclusion of other character sets such as adult and larval morphology. The Micropezidae, recovered as a monophyletic group and sister to the remaining Nerioidea, featured strong support for five distinct major clades aligning with previous morphological classifications. The primarily New World Micropezinae was recovered as sister to the Australian Metopochetinae, together related to the Holarctic Calobatinae. This arrangement, tentatively predicted previously by D.K. McAlpine, presents an interesting biogeographic challenge under current views of the geological age of Schizophora. Moving forward, a detailed examination and analysis of morphological data will be required to place fossil Micropezidae to properly calibrate molecular clock analyses for assessing the evolution and radiation of these clades. Currently, a hypothetical evolutionary transition from a Laurasian distribution to a chiefly Gondwanan distribution seems plausible and would appear to mirror the evolution and dispersal of the similarly aged marsupial mammal radiation, which are believed to have also transitioned from Laurasia to South America and eventually across Antarctica to Australia prior to the separation and isolation of the southern continents[1]. Further taxon sampling within Metopochetinae and Micropezinae will be imperative to further refine the relationships and biogeographical history of these flies moving forward.

Now, with a well-supported phylogeny establishing the ancestral relationships of Micropezinae, the way is clear for a full taxonomic revision of the subfamily. Preliminary revisionary work has revealed dozens of new species awaiting description, particularly within the northern Nearctic region of Mexico, while the relationships recovered here using molecular data reveal a pattern of evolution and diversification that defies prior generic concepts. In addition to the description of new taxa, there remains much to be learned regarding the natural history of these flies that will likely prove extremely useful in establishing the relationships between species and groups. From mating behaviours and copulation positions to larval habitats and host associations, increased field work and natural history observation will be critical for understanding the evolution and diversification of Micropezinae beyond what can be learned simply from preserved specimens or their DNA. In an age of rapidly advancing technologies and techniques, taking time to observe and reflect on the natural history of species should remain a priority moving forward, and will undoubtedly provide much-needed biological context for the relationships, characters, and hypotheses conceived within the lab.

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1 Kemp, T.S. 2005. *The origin and evolution of mammals*. Oxford: Oxford University Press. 330 pp.

APPENDIX 1: ANNOTATED CATALOG OF FAMILY-GROUP NAMES IN THE NERIOIDEA

Unlike genus- and species-group names, which are often formally referred to with proper authorship and attribution, family-group names are frequently used with little acknowledgement of the nomenclatural origins and history behind them. This can result in the incorrect application of names, which in turn can have ramifications on the stability of nomenclature and classifications. Here, a comprehensive review of family-group names erected for the Nerioidea and included families is provided, along with annotations documenting the historical application of family-group names, and recommendations for future usage.

According to the International Code on Zoological Nomenclature, Fourth Edition (hereafter "the Code"; International Commission On Zoological Nomenclature 1999), Family-group names encompass "all nominal taxa at the ranks of superfamily, family, subfamily, tribe, subtribe, and any other rank below superfamily and above genus that may be desired" (Article 35). These names are governed in most ways under the same guidance as genus- and species-level names, however there are several exemptions specified for historical situations to encourage stability of nomenclature.

This catalog is formatted in the same style as, and indeed draws heavily upon, Sabrosky's Family-Group Names in Diptera (1999), with supplemental information from Systema Dipterorum version 1.5 (Thompson and Pape 2013). This catalog would not be possible were it not for the careful curation of nomenclature by these authors. While most of the information provided in this catalog can be found among those two works, this catalog provides additional context for the status of names, as well as some nomenclatural updates and corrections. Other spellings for family-group names noted by Sabrosky (1999) are not republished, although additional misspellings and variations not mentioned by Sabrosky are here included.

This Catalog is arranged alphabetically by current usage of family and subfamily names, and subsequently alphabetically by stem genus within; a catalog of unavailable family-group names of significance has been included at the end for completeness and reference. Although authorship of names in the family-group are fixed regardless of rank at which they are used (Article 50.3.1), this catalog will document the first usage of a name at each rank with full citation because authorship has been misattributed for some names due to misapplication of this Article. Annotations providing context and explanations regarding the status of names are provided where necessary, relevant, or interesting.

Names in **Bold** have priority in either the genus-group or family-group; underlined names are those used in contemporary publications (McAlpine 1998, Buck 2010, Buck and McAlpine 2010, Marshall 2010).

CYPSELOSOMATIDAE Hendel 1931

Cypselosoma Hendel 1913: 105 [stem Cypselosomat-]. Type, *C. gephyrae* Hendel 1913: 105 (by monotypy).

Cypselosominae Hendel 1931: 5.

Cypselosomatinae Brues et al. 1954: 377.

1) This name is also used by Rohdendorf 1964: 108, and the subsequent English translation of this work (1974)

Cypselosomatidae Hennig 1958: 551.

Formicosepsis Meijere 1916: 199 [stem Formicoseps-]. Type, *F. tinctipennis* Meijere 1916: 200 (by monotypy).

Formicosepsidinae Rohdendorf 1964: 108. *nomen nudum*

2) Sabrosky (1999) corrects the spelling based on the stem (=Formicosepsinae) if it should need to be applied in the future.
3) This name also appears in the English translation of Rohdendorf 1964 (1974, page 110).

MICROPEZIDAE Blanchard 1840

CALOBATINAE Bigot 1853

Calobata Meigen 1803: 276. Type, *Musca petronella* Linnaeus 1758 (by monotypy).

Calobatidae Bigot 1853: 303, 312

Calobatinae Schiner 1868: 229

Calobatini Cresson 1930: 317

Calobatoidea Crampton 1944a: 9, 1944b: 154

Trepidaria (Meigen 1800): 35. No named species; fixed by subsequent designation and subsequent monotypy by Hendel 1908: 63 (a second name therein is a nomen nudum). Type, *Musca petronella* Linnaeus 1758 (subsequent monotypy). *Trepidaria* and all derivitives from its stem are unavailable by suppression of the Meigen 1800 work (ICZN 1963).

Trepidariinae Czerny 1930: 2

Trepidariini Hennig 1938: 2

Trepidariidae Crampton 1944b: 154

CALYCOPTERYGINAE McAlpine 1974

Calycopteryx Eaton 1875: 59 [stem Calycopteryg-]. Type, *C. mosleyi* Eaton 1875: 59 (by monotypy).

Calycopteryginae McAlpine 1975: 237

4) Other spellings: Caleopteryginae McAlpine 1989: 1432

EURYBATINAE Aczél 1955

Eurybata Osten-Sacken et al. 1882: 204. Type, *Eurybata hexapla* Osten-Sacken et al. 1882 (original designation).

Eurybatini Aczél 1955: 4

5) Sabrosky (1999) attributes Eurybatini to Frey (1958): 45, however Aczél uses the name while discussing morphological similarities of his newly established family Nothybidae. Azcél does not provide a differential description, which would make this a *nomen nudum*, except for Article 13.2.1. which states

> *"A family-group name first published after 1930 and before 1961 which does not satisfy the provisions of Article 13.1 is available from its original publication only if it was used as valid before 2000, and also was not rejected by an author who, after 1960 and before 2000, expressly applied Article 13 of the then current editions of the Code."*
>
> (International Commission On Zoological Nomenclature 1999).

The use of this name as valid by Frey (1958), albeit under the pretense of it being the first such use, and subsequently other authors after 1960 makes this name available from its original publication.

Eurybatinae McAlpine 1975: 233

6) While the attribution of this name is largely academic given the priority established by Aczél, there is some confusion over the date of first appearance of this name at the subfamily rank. Sabrosky (1999) attributes the name to Colless and McAlpine 1975: 95, but in the associated bibliography to Sabrosky (Thompson et al. 1999), the dates for both publications are recorded as being 1975, with McAlpine 1975

listed as being published January 7, 1975 (note the journal issue is dated 1974), while Colless and McAlpine 1975 was published in March 1975 (note the front matter of the book lists its publication and copyright date as 1974). Additionally, Colless & McAlpine 1975 would be treated as a *nomen nudum* because it fails to meet the requirements of Article 13.1.

Metopochetus Enderlein 1922: 164, 171. Type, *Metopochetus ralumensis* Enderlein 1922 (original designation).

Metopochetini McAlpine 1975: 235.

MICROPEZINAE Blanchard 1840

Micropeza Meigen 1803: 276. Type, *Musca corrigiolata* Linnaeus 1767 (as Fabricius) (by monotypy).

Micropézites Blanchard 1840: 622.

7) Sabrosky (1999) lists this name as being "vernacular", and thus does not consider it applicable for priority. However, Article 11.7.2 of the Code states:

> *"If a family-group name was published before 1900, in accordance with the above provisions of this Article but not in latinized form, it is available with its original author and date only if it has been latinized by later authors and has been generally accepted as valid by authors interested in the group concerned and as dating from that first publication in vernacular form." (International Commission On Zoological Nomenclature 1999)*

This name has been latinized and widely adopted by authors interested in the group, and thus has priority over later latinized iteratations that attribution of availability has been previously made (e.g. Desmarest 1860, Loew 1861, 1862).

8) A consequence of Sabrosky's rejection of this vernacular name's availability was his assertion that Calobatidae Bigot 1853 had priority over Micropezidae, and that formal action by the ICZN was needed to suppress Calobatidae and maintain stability. This is no longer an issue given the provisions of Article 11.7.2 establishing the priority of Blanchard 1840.

Micropezitae Desmarest 1860: 39.

Micropezidae Loew 1861: 89.

Micropezinae Hendel 1903: 201.
Micropezini Müller 1957: 247.
Micropezoidea Hennig 1958: 550, 554.
Micropezoinea Griffiths 1972: 156 (pre-family).

Tylos Meigen 1800: 31 [stem Tyl-]. No named species; fixed by subsequent designation by Coquillet (1910). Type, *Musca corrigiolata* Linnaeus 1767 (subsequent monotypy). *Tylos* was suppressed (ICZN 1955) for both priority and homonymy (*Tylos* Audouin 1820; Isopoda), and was later also made unavailable by suppression of the Meigen 1800 work (ICZN 1963).

9) Hendel (1908) included *Tylos* when he republished Meigen (1800), however his association of this name with *Micropeza* appears to be less definitive than other taxa, with Sabrosky (1999) calling attention to Hendel's inclusion of "?" in reference to the association and typological formatting differences that suggest Hendel did not fully accept this association. When Aczél (1950) petitioned ICZN to suppress *Micropeza* in favour of *Tylos*, he was unaware of the priority and homonymy issues related to either the isopod genus *Tylos* Audouin 1820 (and family; Tylidae Dana 1852) or the arachnid genus *Tylos* Heyden 1826, both of which came to light following publication of Aczél's letter, and which ultimately lead to the suppression of *Tylos*.

Tylinae Hendel 1910: 312.
Tylidae Bezzi 1914: 311.
Tyloidea Hendel 1916: 298.
Tylini Hennig 1936: 162, 164.

TAENIAPTERINAE Cresson 1930 (=**Rainieriinae Czerny 1930 (1927))**

Cardiacephala Macquart 1843: 399 [stem Cardiacephal-]. Type, *Musca longipes* Fabricius 1787 (preoccupied: *Musca longipes* Scopoli 1763) = *Cardiacephala guttata* (Walker 1852) [*Calobata*] (original designation as "*Nerius longipes*, Fab.").

Cardiacephalina Albuquerque 1990: 19.

Grallipeza Rondani 1850: 180 [stem Grallipez-]. Type, *Calobata unimaculata* Macquart 1846 (original designation).

Grallipezini Aczél 1951: 483 and following pages, 533.

10) This name has been formally used three times in the published literature since Aczél established it; once by Albuquerque (1991) in discussing Brazilian micropezids, once by Marshall (2004) in discussing the affinities of his newly described genus *Globopeza* Marshall 2004, and once by Jackson et al. (2015), in which it was correctly noted (but by incorrectly citing Cresson (1938) rather than Czerny (1930); see *Rainieria*) that Rainiierini has priority.

11) Aczél (1959) treated Grallipezini as a junior subjective synonym of Rainieriini.

Rainieria Rondani 1843: 40 [stem Rainieri-]. Type, *Calobata calceata* Fallén (original designation)

Rainieriinae Czerny 1930: 2 (priority from 1927, Tanipodinae Frey).

12) In a turn of cosmological coincidence, Czerny and Cresson both published family-group names for the same group but with different generic stems within days of one another. According to Sabrosky (1999) and Thompson et al. (1999), Czerny's name (Rainieriinae) was published September 15, 1930, while Cresson's name (Taeniapterinae) was published September 19, 1930. However, both were preceded by Frey's Tanypodinae (1927) which has priority (see *Tanipoda*). Following these two publications, we see a divide in the application of names, with some authors following Czerny and using Rainiierinae (e.g. Czerny 1932, Hendel 1936) while others follow Cresson and use Taeniapterinae (e.g. Hennig 1935a, 1935b, 1935c, 1936c, 1936b, 1936a), until Cresson (1938) acknowledges the issue, and incorrectly asserts that his name has priority as it was published in September and Czerny's in October (p. 346). From this point onward, the subfamily is referred to as the Taeniapterinae.

Taeniapterinae has been nearly universally adopted in the literature since 1936 (save for an unpublished doctoral thesis which maintains Rainieriinae; Hamrum (1957)), and is included in nearly all major works relating to Micropezidae taxonomy, systematics, and identification, raising a question of stability vs. priority in the family-group. The relevant language of The Code can be found in Article 35.5:

> *"***35.5. Precedence for names in use at higher rank.** *If after 1999 a name in use for a family-group taxon (e.g. for a subfamily) is found to be older than a name in prevailing usage for a taxon at higher rank in the same family-group taxon (e.g. for the family*

within which the older name is the name of a subfamily) the older name is not to displace the younger name."

(International Commission On Zoological Nomenclature 1999)

Cresson (1938) not only incorrectly asserted priority for his Taeniapterinae, but also erected a new tribal classification for the Taeniapterinae, consisting of the Taeniapterini and the Rainieriini. This appears to allow the application of Art. 35.5 to continue using the younger Taeniapterinae despite there being a senior synonym available because it is being used at a lower taxonomic rank. However, the issue of priority between Taeniapterinae, Rainieriinae, and Tanipodinae was recognized by Sabrosky (1999), thus making Rainieriinae the senior subjective synonym.

Article 23.9 of the Code discusses the reversal of precedence:

"23.9. **Reversal of precedence.** *In accordance with the purpose of the Principle of Priority [Art. 23.2], its application is moderated as follows:*

23.9.1. prevailing usage must be maintained when the following conditions are both met:

23.9.1.1. the senior synonym or homonym has not been used as a valid name after 1899, and

23.9.1.2. the junior synonym or homonym has been used for a particular taxon, as its presumed valid name, in at least 25 works, published by at least 10 authors in the immediately preceding 50 years and encompassing a span of not less than 10 years."

(International Commission On Zoological Nomenclature 1999)

While the conditions of Article 23.9.1.2 are met by the wide acceptance and usage of Taeniapterinae in the literature for more than the 50 years necessary, those of Article 23.9.1.1 are not, in which circumstance Article 23.9.3 applies:

"23.9.3. If the conditions of 23.9.1 are not met but nevertheless an author considers that the use of the older synonym or homonym would threaten stability or universality or cause confusion, and so wishes to maintain use of the younger synonym or homonym, he or she must refer the matter to the Commission for a ruling under the plenary power [Art. 81]. While the case is under consideration use of the junior name is to be maintained [Art. 82]."

(International Commission On Zoological Nomenclature 1999)

Thus, it will be necessary to petition the ICZN to conserve Taeniapterinae Cresson 1930 (1927) by giving it precedence over the senior subjective synonym Rainieriinae Czerny 1930 (1927).

Rainierinii Cresson 1938: 347.

Taeniaptera Macquart 1835: 491 [stem Taeniapter-]. Type, *Taeniaptera trivittata* Macquart 1835 (by monotypy).

Taeniapterinae Cresson 1930: 317.

13) See discussion under *Rainieria* for current status and conflicts of this commonly used name.

Taeniapterini Cresson 1938: 347.

Taeniapteridae Hennig 1950: 243.

14) Hennig's first usage of this name at the family rank came in his original German work on phylogenetic systematics, and not in his Micropezidae research. The name does not appear in the English translation (Hennig 1966).

Taeniapterina Albuquerque 1990: 19. *nomen nudum*

Tanipoda Rondani 1856: 116 (unnecessary new name for *Rainieria* Rondani 1843) [stem Tanipod-]. Type, *Calobata calceata* Fallén 1820 (automatic fixation) = *Rainieria calceata* (Fallén). Junior subjective synonym of *Rainieria* Rondani 1843.

Tanypoda (error) Schiner 1864: 191.

Tanypodinae Frey 1927: 65-67.

15) While the genus *Tanipoda* was considered by both Czerny (1930) and Cresson (1930) to be a junior synonym of *Rainieria*, it's usage in the family-group should not have been affected (Article 40). However, as per Article 40.2., because both Czerny and Cresson did synonymize it, and erected new, junior synonym family-group names in its place (see discussion under *Rainieria*) prior to 1961, and that these names are in prevailing usage, the junior synonym with priority (Rainieriinae) is to be maintained, which will retain authorship of the name, but gain priority of the replaced name.

16) Due to the spelling error made by Schiner and propagated by subsequent authors, the usage of Tanypod- in the family group are all junior homonyms of Chironomidae family-group names built on the stem of *Tanypus* Meigen 1803.

17) Sabrosky (1999) fixed the spelling of this name in the family-group (Tanipodinae, Tanipodidae).

Tanypodidae Frey 1958: 37.

NERIIDAE Westwood 1840

NERIINAE Westwood 1840

Longina Wiedemann 1830: 553 [stem Longin-]. Type, *Longina abdominalis* (by monotypy).

Longinidii Bigot 1852: 482.

Longinidi Bigot 1858: 595.

Nerius Fabricius 1805: 264 [stem Neri-]. Type, *Nerius pilifer* Fabricius 1805 (designated by Coquillett 1910: 575).

Neriades Westwood 1840: 148.

18) This name is almost certainly based on the genus *Neria* Robineau-Desvoidy 1830, which has been used extensively in the Micropezidae literature to refer to a small genus of Calobatinae (Type = *Musca ephippium* Fabricius, 1794), in theory making this the name with priority for Micropezidae. However, *Neria* was deemed an unjustified emendation of *Nerius* by Evenhuis et al. (2010) and treated as a junior synonym, with *Paracalobata* Hendel 1922 being resurrected for the Micropezidae genus.

19) Thompson et al. (1999) list the publication date of Westwood (1840) as June 6, 1840, and Blanchard (1840), the establishing publication for Micropezidae in the family-group, as published December 26, 1840. Thus, Nerioidea has priority over Micropezoidea, as noted by J.F. McAlpine (1989).

Nerioidae Agassiz 1846: 248.

Neriinae Hendel 1903: 202.

Neriidae Hendel 1916: 297.

20) Despite having priority in the family-group since 1840, Hendel (1916) is the first instance of Neriidae being recognized as an independent family-level taxon from Micropezidae.

Neriini Enderlein 1922: 141.

Nerioidea McAlpine et al. 1981: 3.

21) Other spellings: Neriddae Cresson 1938: 303, Nereidae Meier 1995: 425.

Telostylinae Enderlein 1922

Telostylus Bigot 1859: 306 [stem Telostyl-]. Type, *Telostylus binotatus* Bigot 1859 (by monotypy).

Telostylini Enderlein 1922: 141.

Telostylinae Czerny 1932: 296.

PSEUDOPOMYZIDAE McAlpine 1966

Latheticomyia Wheeler 1956: 306 [stem Latheticomy-]. Type, *Latheticomyia tricolor* Wheeler 1956 (original designation).

Latheticomyiidae Commonwealth Institute of Entomology 1958: 462. *nomen nudum*

Latheticomyiinae Krivosheina 1979: 186, 167 (111-112).

Pseudopomyza Strobl 1893: 284 [stem Pseudopomyz-]. Type, *Pseudopomyza nitidissima* Strobl 1893 (by monotypy) = *Pseudopomyza atrimana* (Meigen 1830).

Pseudopomyzidae Frey 1941: 29. *nomen nudum*

Pseudopomyzidae McAlpine 1966: 683.

Pseudopomyzinae McAlpine 1987: 760.

UNAVAILABLE NAMES

These names are unavailable because they are not based on a valid genus. They are included here because they are used in important early works on the taxonomy of Nerioidea.

Leptopodites Latreille 1829: 530. Division for micropezid genera.

22) This name was used to refer to what would eventually become the Micropezidae in many early taxonomic works, particularly by Macquart (1835, 1843, 1846, 1848, 1851, 1855), as Leptopoditae.

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