IDENTIFYING DRYINIDAE (HYMENOPTERA) - AUCHENORRHYNCHA (HEMIPTERA) HOST ASSOCIATIONS USING PHYLOGENETICS

BY

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ABSTRACT

Dryinidae is a family of ectoparasitoid wasps with cosmopolitan distribution that exclusively preys on and parasitizes members of the suborder Auchenorrhyncha (Hemiptera). Host records of these important biocontrol agents are fragmentary because previous records have been based on tedious laboratory rearing of parasitized individuals requiring environmental control and long waiting periods, usually with limited success. Molecular phylogenetic methods provide an alternative to expand knowledge of dryinid host breadth by DNA sequencing of host attached parasitoid larvae. For this study, 142 late-stage dryinid larvae were removed from parasitized individuals of Auchenorrhyncha (Hemiptera), mostly from a wet insect collection at the Illinois Natural History Survey representing all major biogeographic regions. The 28S D2-D3 nuclear ribosomal gene region was amplified using PCR and sequenced. Attempts to sequence Cytochrome c oxidase subunit 1, Cytochrome B and 18S DNA regions were unsuccessful due to contamination with host DNA. Sequence data were combined with data from a previous phylogenetic study based on adults and a maximum likelihood tree search was performed in the IQ-Tree webserver. The best tree was used to explore the significance of natural history traits including distribution, host taxonomy and habitat, for explaining host association patterns. The number of species represented by larval samples was conservatively estimated by integrating genetic distances with natural history data. Host identification revealed 70 new dryinid-host associations, adding Eurybrachidae as a host planthopper family. The resulting phylogeny provided good resolution at the subfamily level, except in Anteoninae, which was divided in two non-contiguous clades. Host attached larvae formed part of at least four subfamilies, an unidentified lineage inferred to represent Bocchinae based on host associations, Anteoninae, Aphelopinae and Gonatopodinae, the latter having the highest diversity among sampled larvae. Biogeography and host associations at the level of family-group taxa explained some phylogenetic patterns among clades, but habitat was less significant. Overall, this study, corroborates previous studies indicating that dryinids are generalist parasitoids of Auchenorrhyncha, but it also provides evidence supporting cryptic species complexes.

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INTRODUCTION

Diversity and Distribution

The pincer wasp family (Fig. 1), Dryinidae, is a small group of hymenopteran parasitoids (approximately 1,800 species) all parasitizing and often preying on nymphs and adults of the hemipteran suborder Auchenorrhyncha (leafhoppers, planthoppers and relatives; hoppers hereafter), except Cercopoidea and Cicadoidea (Guglielmino et al. 2013; Olmi & Xu 2015). Along with their hosts, dryinids have a cosmopolitan distribution, except Antarctica, and fossil evidence indicates they evolved at least 128 Mya during the Early Cretaceous (Olmi et al. 2014). The major contribution to the study of Dryinidae was the monograph of the world fauna by Olmi (1984), who revised the taxonomy of the family and provided keys ranging from the subfamily to the species level (Tribull 2015). There is no current classification at the tribe level, with the last account of the tribes in a major taxonomic work being Richards (1939). The tribes were later recognized as subfamilies and the abandonment of the tribal classification may have followed due to the physical similarity among and lack of general knowledge about the males (Richards 1953).

The family Dryinidae is composed of 12 extant subfamilies (Olmi 1984; Olmi 2007; Olmi & Virla 2014; Olmi & Xu 2015; Xu et al. 2013) and four extinct subfamilies (Olmi et al. 2010, 2014) (Table 1). Anteoninae, Aphelopinae, Bocchinae, Dryininae and Gonatopodinae have a worldwide distribution encompassing over 90% of the known Dryinidae diversity (Olmi and Virla 2014, Xu et al. 2013) with the genera *Anteon* Jurine, 1807, *Dryinus* Latreille, 1804, and *Gonatopus* Ljungh, 1810, containing more half of the described species (Tribull 2015). Other subfamilies have a more limited distribution. Apoaphelopinae is only known from the Afrotropics with two species, Apodryininae from Gondwana with 13 species, Conganteoninae from the Palearctic, Afrotropical and Oriental region with 15 species, Erwiniinae from Ecuador with 1 species, Plesiodryininae from Florida with one species, Thaumatodryininae with a worldwide distribution except the Palearctic with 31 species and Transdryininae from Australia

with two species (Tribull 2015). There are two additional species from Brazil without an assigned subfamily, *Chelogynus brasiliensis* Arlé, 1935 and *Prodryinus affinis* Arlé, 1935 (Olmi & Virla 2014).

Behavior and Life History

The life cycle of Dryinidae begins at oviposition with a female laying an egg inside the hopper host. The larval stage has five instars. The first instar emerges by dissolving the chorion and then feeding on the host to complete its development. In most cases, this feeding results in host death (Olmi 1994). The development is initially internal, but by the third stadium the larva emerges from the body of the host, continuing to cling to it; hence, dryinids are referred as ectoparasitoids (Olmi 1994). The exuviae from the second and following instars form an external cyst or sac, called a thylacium, which may protect the larva from external damage (Guglielmino & Olmi 2015). The fifth instar devours the remaining contents of the host, splits open the thylacium and crawls out to pupate in the soil or on a plant inside a silk cocoon (Jervis 1980). Overwintering often occurs inside the cocoon as a mature larva or prepupa. Developmental time from oviposition to adult can last approximately 40 days, but it can extend to 74 days or longer in diapausing generations (Waloff and Jervis 1987).

The adult emerges after pupation to look for a mate if it is a male, or search for potential host or prey if it is a female. Waloff (1974) described the foraging behavior of various species providing evidence of the utility of the cheliform forelegs for capturing prey and hosts that will jump when disturbed (Dietrich 2002; Olmi 1984, 1994). Generally, when searching for a host, dryinids appear to randomly walk on the substrate until reaching a distance of a few millimeters from a potential host. Some species drum their antenna on the substrate or wave and vibrate their antenna when a host is near (Waloff 1974). After a brief pause, they pursue or jump on their host and capture it with the chelae. They grasp the hind legs or abdomen and often cling to it with the mandibles, keeping the body of the hopper at a right angle relative to the longitudinal axis of the wasp. The host may be lifted up from the substrate and, within a few seconds, the dryinid stings and paralyzes it. Subsequently the dryinid feeds, oviposits, or engages in both behaviors. Two species, *Gonatopus sepsoides* Weswood, 1883, and

Pseudogonatopus distinctus (Kieffer, 1906) drag their prey with their chelae along the substrate, a behavior reminiscent of hymenopterans that provision their nests (Askew 1971). The females of Aphelopinae, which do not possess chelae, approach the host quickly and capture it for oviposition using their front and middle legs (Olmi 1994). On the other hand, all of the males of the family lack raptorial forelegs and have a shorter adult lifespan, mostly spent searching for females. Most of these searches are unsuccessful, but females are capable of parthogenetic reproduction (Guglielmino 2002).

Host Associations

Parasitoid-host associations have a major impact in natural and anthropogenic landscapes. These relationships help regulate population dynamics of hosts, which are usually herbivores, and release plants from pressures imposed by feeding damage and pathogen transmission (Quicke 1997). Dryinid wasps are generalist parasitoids of hoppers, with approximately 1,800 species, often attacking several distantly related species occupying the same habitat (Guglielmino et al. 2013; Olmi 1994, 1984; Olmi & Virla 2014; Olmi & Xu 2015; Xu et al. 2013). With over 40,000 species, hoppers are among the most numerous and diverse insect groups in the biosphere (Bartlett et al. 2014; Dietrich 2002), especially in prairie and grassland ecosystems, where their numbers can reach densities higher than 1000 individuals per m^2 (Waloff 1980). Plant damage results from oviposition, feeding and the transmission of a wide array of pathogens, such that hoppers can have devastating effects on crops (Nickel & Hildebrandt 2003; Redak et al. 2004). One extreme example is the leafhopper-transmitted bacterium *Xylella fastidiosa*, a plant pathogen with strains affecting grapes, citrus, almond, alfalfa, stone fruits, landscape ornamentals and native hardwoods that usually causes death of the plant and for which there is no cure (Hopkins 1989; Hopkins & Purcell 2002; Purcell 1990, 1979). Thus, the study of the natural enemies of these herbivores can provide the basis for future conservation and biological control programs.

Dryinids kill their hosts through parasitism and predation helping regulate hopper population dynamics, although their effect varies by species or location (Chua et al. 1984; Moya-Raygoza et al. 2004; Quicke 1997; Waloff 1975; Waloff & Thompson 1980). This positive

impact as parasitoids has driven successful biological control programs and studies, but only a limited number. In rice fields in China, combined attack on the small brown planthopper *Laodelphax striatellus* (Fallén, 1826) by the dryinids *Haplogonatopus apicalis* Perkins, 1905, *H. oratorius* (Westwood, 1833), *Gonatopus flavifemur* (Esaki & Hashimoto, 1932), *Gonatopus nigricans* (Perkins, 1905) and *Pseudogonatopus sp.* results in almost 50% parasitism rates (Ôtake et al. 1976). Similarly, *Haplogonatopus hernandezae* Olmi, 1984, killed 73.5% of *Tagosodes orizicolus* (Muir, 1926) feeding on rice in a greenhouse setting (Mora-Kepfer & Espinoza 2009). In Hawaii introduction of *Haplogonatopus vittensis* Perkins, 1906, from Fiji, successfully controlled *Perkinsiella saccharicida* Kirkaldy, 1903, populations on sugar cane (Swezey, 1928). Most of these studies on parasitism rates are restricted to agricultural ecosystems and laboratory settings, with minor efforts to study these associations in natural landscapes. However, Waloff (1975) found *Gonatopus bicolor* (Haliday, 1828) and *Gonatopus clavipes* (Thunberg, 1827) to be the dominant parasitoids of delphacids and cicadellids, respectively, in English acidic grasslands.

Studying the effect of host taxonomy, morphology and ecology, e.g., diet, habitat, geographic location, will reveal how dryinid-hopper associations are shaped in ecological and evolutionary time and space (Smith et al. 2008, 2007 2006; Stireman & Singer 2003). Unfortunately, the extent of dryinid-hopper associations remains largely unknown, with hosts recorded only for 300 or approximately 17% of dryinid species (Guglielmino et al. 2013). Hosts are known only for 16 of the 51 genera representing only six subfamilies, Anteoninae, Aphelopinae, Bocchinae, Dryininae, Gonatopodinae and Thaumatodryininae (Table 2). From these, Gonatopodinae has the broadest host range, attacking 11 planthopper families and 8 subfamilies of leafhoppers. The most host-restricted groups are Thaumatodryininae, attacking only Flatidae, and Bocchinae, attacking Caliscelidae, Tropiduchidae and Deltocephalinae. Aphelopinae presents an interesting case as, besides attacking Typhlocybinae, it is the only dryinid group recorded to attack Membracidae. Nevertheless, more studies in host associations could broaden these records (Guglielmino et al. 2013).

This gap in knowledge is likely a result of the difficulty and constraints of current methodology as host records are only obtained by rearing wasps to the adult stage from

parasitized hosts (Guglielmino et al. 2013). This method requires careful control of environmental parameters and long waiting periods for adult emergence, often resulting in high mortality rates (Giordano et al. 2002). Additionally, collecting and rearing in the tropics is harder due to climatic or environmental conditions and/or transportation of living specimens. These factors emphasize the need for new methods that will provide a more complete host record.

Phylogeny and Evolution

The larvae of parasitoids are completely dependent upon their hosts to reach adulthood (Heraty 2009). This intimate relationship results in two major lifestyles that reflect different evolutionary patterns: specialists and generalists. Specialists are predicted to co-speciate with their hosts (Farenholz's rule) so closely related hosts will have a set of closely related parasitoid species (Eichler 1948). Eichler's rule states that highly diverse host groups will harbor highly diverse groups of parasitoids as they track host speciation events (Brooks 1979). In contrast, generalist parasitoids fail to co-speciate with their hosts and parasitzation is the result of more opportunistic tracking of available hosts that share certain traits in a habitat, as predicted by ecological fitting (Agosta & Klemmens 2008). Ecological fitting differs from coevolution in that the required traits conferring the ability to use novel environments or hosts evolved prior to the interaction (Janzen, 1980).

Dryinids are generalist parasitoids, with many species able to develop in hosts belonging to different families (Guglielmino et al. 2013; Olmi 1984). According to Olmi (1994) the main evolutionary pressures on Dryinidae are host/prey capture and reaction. Thus, with females being the only individuals having contact with the host, selection has favored features that increase their ability to seize and manipulate hoppers, resulting in the strong sexual dimorphism seen in many species (Mita & Matsumoto 2012) whereby adult females have morphological features adaptive for particular microhabitats or for distinctive foraging strategies. By contrast, males are short-lived and more sedentary than females, moving primarily to search for mates and to feed occasionally on pollen, nectar and honeydew (Olmi 1994).

Three characters, present only in females, support the adaptation of females for preying upon and parasitizing hoppers: wing reduction, ant-like appearance and raptorial forelegs. Wing reduction and aptery are usually accompanied by the reduction of the meso- and metathorax, improving mobility and foraging efforts in dense vegetation where the hosts are often found, e.g., grasslands and similar plant communities (Olmi 1994; Waloff & Jervis 1987). According to these authors, females mainly walk around plants, even when fully winged. Many dryinid females appear to be ant mimics, especially the apterous species, allowing them to capture unsuspecting hosts and prey that often form mutualistic associations with ants (Donisthorpe 1927; Moya-Raygoza & Truijllo-Arriaga 1993; Waloff & Jervis 1987). Perkins (1905) even suggested *Anteon myrmecophilum* (Perkins, 1905) to be a true myrmecophile as its females parasitize leafhoppers frequently tended by ants, have an external ant-like appearance and display behavior that resembles food soliciting in ants. Richards (1939) disputed this hypothesis as there are no records of this species living within ant nests.

Perhaps the most important character for prey/host capture is the modification of the forelegs into a pair of chelae, accompanied by enlarged femora and/or coxae and trochanters (Olmi 1994). These pincer-like structures are formed by the fifth tarsal segment and an enlarged opposable claw, the second claw being reduced and rudimentary (Guglielmino et al. 2002; Olmi 1984, 1999). The fifth tarsal segment or inner side of the tarsal claw can bear lamellae, bristles, teeth, hairs or peg-like hairs (Olmi 1994, 2007; Xu et al. 2006). The lamellae and some hairs have blunted and rounded tips that may facilitate grasping the host without damaging it (O'Neill 2001). This structure is absent in the males of all species and in the non-predatory females of Aphelopinae, suggesting that predatory feeding behavior is associated with the possession of chelae (Olmi 1994). Chelate forelegs are also lacking in the female of *Erwinius prognatus* Olmi & Guglielmino, 2010, the only known species of the Neotropical subfamily Erwiniinae (Olmi & Virla 2014). The behavior of this species is still unknown as specimens have been captured only through insecticidal fogging of rainforest canopy. Furthermore, the females of Anteoninae do not possess an enlarged trochanter and coxa and their chelae are scarcely mobile when compared to those of Gonatopodinae and Dryininae (Olmi 1994).

Based on these morphological and behavioral features, Olmi (1994) and other authors (Moya-Raygoza and Trujillo-Arriaga 1993; Waloff & Jervis 1987) concluded that Aphelopinae and Anteoninae are less specialized subfamilies owing to their simpler forelegs. Olmi (1994) performed the first morphology-based phylogenetic analysis of the family, finding Aphelopinae as the sister clade to the remanining Dryinidae and Anteoninae as a sister clade of Dryininae + Gonatopodinae. His analysis was incomplete, including female characters only from the four subfamilies found in Fennoscandia and Denmark, Anteoninae, Aphelopinae, Dryininae and Gonatopodinae. The cladogram presented by Carpenter (1999) expanded the taxon sample to include ten subfamilies using 32 morphological characters from both sexes based on the available literature. The tree was poorly resolved, including many polytomies. Tribull (2015) published the first molecular phylogeny of the family containing information for 77 adult specimens in 8 subfamilies from four genes, 28S D2-D3, 18S, COI and CytB. She revalidated Thaumatodryininae, a subfamily Olmi (1993) synonymized with Dryininae on the basis of the similar male mandible. More novel is the placement of Apodryininae as the sister group to the rest of the family. Although Tribull's analysis did not include all the subfamilies and Aphelopinae is situated on a long branch, her results suggest that chelae were acquired early in the evolution of Dryinidae and that loss of the chelae is a derived character in this group.

Overall, Dryinidae presents a series of morphological adaptations beneficial for a parasitoid lifestyle. However, it is difficult to infer how these affected the evolution and the host selection strategies given the largely incomplete host record. Recent molecular phylogenetic studies incorporating DNA sequences of 77 adults (Tribull 2015) raise the possibility of using DNA to identify host-attached larvae, or at least place them within the existing phylogenetic framework. Unlike dryinids, auchenorrhynchans are easily collected in vast numbers employing the same techniques used for adult dryinids, even when parasitized (personal observations). There are no taxonomic keys for identification of dryinid larvae, but the late-stage larvae are easily seen on parasitized hosts as a prominent dark sac (Olmi 1984; Waloff & Jervis 1987). Given the availability of a well-resolved molecular phylogeny of known Dryinidae, I utilized DNA sequencing methods coupled with phylogenetic analysis to identify and associate late-stage larvae attached to field-collected auchenorrhynchans with identified

adult dryinids. This is the first attempt to use molecular data to identify larvae of Dryinidae taxonomically. The project had three main goals: 1) to expand available Dryinidae host records by morphologically identifying host Auchenorrhyncha specimens with dryinid larvae attached, 2) to obtain molecular data from the host-attached larvae and field-captured adults and 3) to use this information to supplement the previously published Dryinidae phylogeny, place the larvae in a phylogenetic framework and identify them taxonomically. In this way, I provided an enriched database of host associations based on a phylogenetic framework that could be used in future studies to infer the main evolutionary drivers of dryinid parasitoids and choose potential biological control agents.

MATERIALS AND METHODS

Sampling

The material examined came from the Illinois Natural History Survey (INHS) collection of cold ethanol-preserved hoppers collected through various methods, e.g., vacuum, pan traps, Malaise traps, and aspirators. Specimens were available from all ecoregions of the world with a preliminary inspection revealing a parasitization rate of 0.03–4.70% parasitized specimens per sample. I identified 175 host-attached larvae, with hosts belonging to Membracoidea and Fulguroidea some individuals having multiple attached parasitoid larvae, indicating multiparasitism or superparasitism. Before host identification, the dryinid larva was removed by carefully holding the parasitized Auchenorrhyncha with forceps and pulling the larva out with another set of forceps or steel insect pin. Forceps and pins were washed in ethanol and flamed before handling specimens to prevent contamination. Dryinid larvae were stored in 95% ethanol at –20°C for later molecular analysis. After host identification, a provisional name, based on host identity, was assigned to the dryinid larvae with the following format: sampleID|Genus|species|Tribe|Subfamily (Smith et al. 2008). Vouchers and DNA extracts are deposited at INHS.

DNA extraction, amplification and sequencing

Dryinidae larvae lack distinctive features for morphological identification (Olmi 1984), so a molecular analysis of larval DNA provides a mechanism to associate these characters with molecular and morphologically identified adult dryinids. For this analysis, I extracted genomic DNA from whole larvae using the DNeasy Blood & Tissue Kit (Qiagen, Inc.) with a modified protocol: specimens were incubated at 56°C for 24 hours in the Buffer ATL and proteinase K solution.

I used PCR to amplify the 28S D2-D3 region using the primer pairs For28SVesp and Rev28SVesp (Hines et al. 2007; Tables 3-4) in a total reaction volume of 25 µl with Taq Polymerase (Promega Corp.). Additionally, I used TD-PCR on a subset of samples that did not yield clear bands under normal PCR conditions. PCR protocols are presented in Tables 5 and 6. Efforts to obtain sequence data from the genes COI, CytB and 18S yielded poor results. PCR products were visualized under a 1% agarose gel and amplification positive products were purified using QIAquick PCR Purification Kit (Qiagen, Inc.). The forward and reverse strands were sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) for 15% of the samples. Sequencing products were read on an ABI 3730XL (Applied Biosystems) at the Roy J. Carver Biotechnology Center (RCBC) at the University of Illinois at Urbana-Champaign.

For the remaining samples, comprising 85% of total samples, I first measured DNA concentration using the Qubit dsDNA HS Assay Kit (Life Technologies Corp.) on a Qubit 3.0 Fluorometer (Life Technologies Corp.) and submitted DNA for sequencing at the RCBC using the same protocol described above.

Phylogenetic analysis

Forward and reverse sequences were assembled and edited in Geneious 10.1 (Kearse et al. 2012). The 28S sequences were aligned on the MAAFT (Katoh et al. 2017) web server (http://mafft.cbrc.jp/alignment/server/) using the E-INS-i algorithm (Katoh et al. 2005) with the default parameters. This algorithm has provided an accurate alignment for Dryinidae (Tribull 2015) and Hymenoptera (Klopfstein et al. 2013) and has a higher accuracy for difficult

alignments than other methods (Morrison 2009; Notredame 2007). The resulting alignment was edited by hand as needed.

Molecular evolution models were selected using ModelFinder (Kalyaanamoorthy et al. 2017). I did three separate runs to verify the precision of the selected evolutionary model as model selection with preliminary data was not consistent. I conducted a maximum likelihood (ML) analysis of these sequences on IQ-Tree webserver v1.5.5 (Trifinopoulos et al. 2016) with the initial numbers of trees set to default, 100; user-defined molecular substitution model, GTR+I+G4; and state frequencies determined by empirical counts from alignment. Outgroup taxa selection followed Tribull (2015) and outgroup sequences were accessed through GenBank. To assess branch support, we performed an ultrafast bootstrap analysis (UFB) (Minh et al. 2013) with 1000 replicates and a SH-like approximate likelihood ratio test (SH-aLRT) (Guindon et al. 2010).

Genetic distance

Genetic distances of 28S D2-D3 region were calculated in MEGA7 using p-distance default parameters (Kumar et al. 2016). First, the minimum genetic distance was calculated within genera to obtain the baseline variation at this taxonomic level using only sequences derived from adults. For Anteoninae and Gonatopodinae, minimum distance was calculated at the subfamily level because many of their genera are not monophyletic, as revealed by phylogenetic analysis in this study or Tribull (2015). If the baseline variation was not zero, distances were calculated for larvae and adult specimens in the same clades. These calculations were also performed for the unknown Dryinidae clade for which no adult data were available.

Biogeographic analysis

To assess the importance of biogeography on dryinid host usage, I performed a biogeographic analysis in RASP v3.2 (Yu et al. 2015) using a Bayesian Binary Model (BBM). We inputted the resulting 28S phylogeny and used GPS coordinates to map and categorize the location of each specimen according to its biogeographic realm (Olson et al. 2001): Afrotropic, Australasia, Indo-Malay, Nearctic, Neotropical, Oceania or Palearctic. By using GPS coordinates I

avoided ambiguous categories in countries where ecoregion transitions occur. I ran 10 MCMC chains in two independent analysis under the F81 + G model for 50000 generations, sampling every 100 generations (Ye et al. 2016).

Ancestral host and habitat reconstruction

To determine the effects of host taxonomy and habitat, I reconstructed ancestral dryinid hosts in Mesquite v3.31 (Maddison & Maddison 2017) using ML under the Mk1 model and default parameters. I obtained host subfamily data from this study and literature records (Guglielmino, Olmi & Bückle 2013). Host information for many taxa used by Tribull (2015) were unavailable as they were not identified to species or the hosts were unknown. I determined habitat type by inputting sample geographic latitude and longitude in the online data viewer of the Map of Global Ecological Land Units (Sayre et al. 2014; https://rmgsc.cr.usgs.gov/ecosystems/dataviewer.shtml), excluding taxa from Tribull (2015) for which locality data were not provided. Based on land cover, I was able to determine two broad categories: (1) forest or (2) grass, scrub or shrubland. A subset of the samples fell within a cropland habitat. This is attributed to an anthropogenic landscape change as collection dates extend to more than 10 years ago. Hence, as samples were never taken in crop fields, I determined the predominant land cover of adjacent areas in the four cardinal directions for localities currently categorized as croplands.

RESULTS

DNA extraction, amplification and sequencing

Initial attempts to amplify and sequence the COI barcode region, CytB and 18S from DNA extracted from dryinid larvae were unsuccessful using primers pairs previously reported to amplify these gene regions in adult dryinids (Table 3; Tribull 2015; Vrijenhoek 1994; Simon et al. 1994). This is likely a consequence of contamination with DNA from the hopper host as review of sequence data revealed overlapping peaks in many of the chromatograms. Due to the utility of COI in delimiting species, new primers more specific to hymenopteran DNA were developed

using LCO and HCO and C1-J1-859 and TL2-N-3014 (Simon et al. 1994) primer pairs as templates or identifying non-variable regions within the sequences provided by Tribull (2015). These primers were chosen for their efficacy in sequencing dryinid specimens belonging to different groups (Mita and Matsumoto 2012; Tribull 2015). Only one primer pair was produced (Table 4), but PCR was unsuccessful. Dryinid COI sequences possessed many indels and substitutions, hindering the possibility of developing a universal dryinid-specific primer. Additionally, searching in Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) revealed that some potential primers perfectly aligned to the COI region of hoppers. Nevertheless, DNA from dryinid larvae was successfully sequenced using the primer pairs For28SVesp and Rev28SVesp, targeting the 28S D2-D3 region (Table 3).

Phylogenetic analysis

A total of 143 specimens, including 142 unidentified dryinid larvae removed from hoppers and the single adult G. *elongatus*, provided high-quality sequence data from the stock of 175 specimens, an 81.7% success rate. Sequence length ranged from 734-818 bp after assembly and editing. Alignment yielded a matrix of 1101 bp, which was used for phylogenetic analysis. The best ML tree from 100 independent runs had a score of -12941.41 with a BIC score of 28971.59 and 70 near-zero-length internal branches; hence, the tree was transformed into a cladogram (Fig. 2, 3. 4, 5, 6, 7, 8, 9, 10 and 11; Appendix A). Although UFB and SH-aLRT indicate high support for most subfamilies, Anteoninae is broken into two separate clades, (*Anteon* + *Lonchodryinus*) and (*Deinodryinus* + Aphelopinae), the latter clade with low support. Using a four-gene phylogenetic analysis, Tribull (2015) recovered these three genera as members of Anteoninae. The same study recovered Aphelopinae as a sister group to Bocchinae + Conganteoninae in a ML analysis, with the three subfamilies forming a clade sister to the rest of the subfamilies except Aprodryininae. Most genera were not monophyletic, consistent with a previous phylogenetic study where *Anteon*, *Deinodryinus*, *Gonatopus* and *Echthrodelphax* were not monophyletic (Tribull 2015). However, some taxa formed clades with high support allowing placement of unidentified dryinid larvae within the current dryinid classification.

Because of these inconsistencies with the previous dryinid phylogeny, including

presence of a long branch joining Aphelopinae to *Deinodryinus sp.* I ran an additional ML analysis in IQ-Tree constraining all Anteoninae taxa to form one clade: *Deinodryinus* + (*Anteon* + *Lonchodryinus*) (Appendix B-C). The constrained and unconstrained trees were subjected to an approximately unbiased test (AU) (Shimodaira 2002). The best ML tree still recovered *Deinodryinus* spp. as separate monophyletic group from Anteoninae and had a lower likelihood and BIC score, -12951.53 and 28921.77, respectively, than the unconstrained tree but the AU test found no significant differences between the two ($p = 0.36$). Thus, the conflicting relationship of Anteoninae and Aphelopinae is not well supported in this study and the split of Anteoninae can be ignored regarding the classification and taxonomy of Dryinidae (Tribull 2015). Including additional genes, including faster evolving genes, should improve phylogenetic resolution and may provide improved discrimination of dryinid species in the larval stage.

Genetic distance

Adult dryinid mean pairwise genetic distance within the seven main dryinid clades for the sequenced 28S region ranged from 0.00 – 5.63, with the minimum distance ranging from 0.00 – 4.03 (Table 7). The minimum genetic distance within most subfamilies and genera was zero, indicating that this locus may not be sufficiently variable to differentiate some species within these clades. Based on Tribull's (2015) data, the minimum distance among sampled species of Aphelopinae for which 28S sequences are available is 2.28%. When larval data were added, minimum divergences were higher than 2.00%, except for the following combinations: ARI-04, CAL-04 and CAL-05; SAF-38 and SAF-39; *Aphelopus* sp.3 and SAF-39; *Aphelopus* sp.3 and THA-02; and *Aphelopus* sp.3 and ARI-06. Other groups with non-zero distances were *Dryinus* spp. and *Thaumatodryinus* spp., but no larval samples grouped with adults of these genera in the phylogeny. Based on available host records, these two genera attack only fulgoroid planthoppers, with *Thaumatodryinus* only parasitizing Flatidae (Guglielmino et al. 2013), a family not represented in this study.

Host and parasitoid identification

Most parasitized hopper specimens were identified to the species level, revealing a

diverse set of hosts belonging to 12 hopper subfamilies, with most specimens assigned to the leafhopper (Cicadellidae) subfamily Deltocephalinae (Tables 8, 9, 10, 11, 12 and 13). Hosts were from six different biogeographic regions (Olson et al. 2001), with most of them obtained from the Nearctic, Neotropical and Palearctic (Tables 8, 9, 10 and 11). The combination of genitalia atrophy due to parasitism and an absence of taxonomic keys and samples of adult males (upon which most species-level hopper taxonomy is based) prevented species level identification in a few host samples. I also captured an adult *Gonatopus elongatus* Olmi, 1984, specimen from Illinois, ILI-25, extending northward the distribution of this species, previously known only from Tallahassee, Florida (Olmi 1994).

After phylogenetic analysis, three criteria were used to determine new host records: 1) the hopper represented a species, genus or subfamily previously not reported as a host, 2) the dryinid larva was inferred to represent a genus or subfamily for which the host has not been recorded and/or 3), the host record was new for the country of origin. This phase yielded positive results with 8 dryinids belonging to an unidentified clade, 25 to Anteoninae, 11 to Aphelopinae and 98 to Gonatopodinae, with a subset of Anteoninae classified to the genus level according to their phylogenetic relationship to identified Dryinidae adults (Fig. 12 and Tables 12, 13 and 14). This effort resulted in a total of 70 new host records, 49% of sequenced samples, in the leafhopper subfamilies Cicadellinae, Coelidiinae, Deltocephalinae, Eurymelinae, Megophthalminae, Neocoelidiinae and Typhlocybinae, and planthopper families Caliscelidae, Delphacidae and Eurybrachidae (Tables 12, 13 and 14). These records include 1 new genus and 4 new species of Deltocephalinae and 1 new species of Typhlocybinae. Genetic distance could not be used to differentiate some species as the minimum genetic distance was 0.00 for some pairs of the adults identified as different species in most clades, except Aphelopinae. If 28S D2-D3 is consistently variable between species of this subfamily, then most larvae in this clade represent species different from the adult species included by Tribull (2015) although ARI-06, SAF-39 and THA-02 may be the same species as Tribull's *Aphelopus* sp3.

Biogeographic analysis

The phylogeny produced in this analysis was used to reconstruct the biogeography, ancestral host and ancestral habitat of dryinids. These results should be considered preliminary and interpreted with caution as the phylogeny is based on a single gene, several internal nodes have low branch support, taxon sampling is uneven, and not all dryinid subfamilies are represented. The reconstruction of ancestral geographic distributions is shown in Figs. 13, 14, 15, 16 and 17 with the two independent runs showing similar results for major dryinid clades. Dryinidae was recovered as having a Neotropical ancestral distribution, with a 75.91% probability, with a Nearctic ancestry being less favored, with a 15.50% probability (Table 15). Ancestral ranges for the subfamilies and other clades were in three of the major biogeographic zones: Nearctic, Neotropical or Palearctic. Other major clades were not considered due to low branch support for the clades involved.

Ancestral host and habitat reconstruction

Ancestral host reconstruction in Mesquite had a marginal probability of 127.71 under the Mk1 model and strongly suggested Deltocephalinae leafhoppers as the ancestral host of Dryinidae, with a 96.11% likelihood (Fig. 18, Table 16 and Appendix D). Most subfamilies had an unresolved ancestral host reconstruction, with the exception of some clades within subfamilies. As in the biogeographic analysis, other major clades were not considered due to the incorrect phylogenetic placement of *Deinodryinus*.

Ancestral habitat reconstruction in Mesquite had a marginal probability of 91.35 under the Mk1 model, but the ancestral habitat of Dryinidae was equivocal, with forest versus grass, scrub or shrubland (grass-scrub-scrub hereafter) having likelihoods of 55.84% and 44.16%, respectively (Fig. 19, Table 17 and Appendix E). The unknown clade had a forest ancestral distribution with likelihood of 90.14%. Other major clades had equivocal or unresolved reconstructions or were not considered due to the incorrect phylogenetic placement of *Deinodryinus*.

DISCUSSION

The gene region 28S D2-D3 provided good phylogenetic resolution for most of the subfamilies in the present study. Anteoninae is divided into two clades, *Anteon* + *Lonchodryinus* and *Deinodryinus*, but both groups are phylogenetically differentiated from other subfamilies or clades enabling the taxonomic classification of the larvae at least to the subfamily level. The placement of Aphelopinae, Bocchinae and Conganteoninae remains controversial as their phylogenetic relationships were not resolved by 28S alone and were inconsistent across Tribull's (2015) analysis. Adding more genes to the analysis, especially faster evolving mitochondrial genes, would provide a more accurate estimate of the true species number and relationships between the dryinid species of this study (Derocles et al. 2016). Moreover, including a wider range of genetic markers would provide a robust phylogeny needed for more accurately testing the effects of host taxonomy, geographic range and habitat on the diversification of Dryinidae (Stireman & Singer 2003).

Although the ribosomal gene 28S is among the most conserved markers used in insect phylogenetic studies, it can still provide insights to the evolutionary history of parasitoids (this study; Derocles et al. 2016). As generalists, dryinid species are known to attack distantly related hoppers (Guglielmino 2002; Guglielmino et al. 2013), which in addition to morphologically similar larvae, limits the ability to discern parasitoid species at the larval stage. To overcome this difficulty, biogeographical and ecological data can be used in combination to available 28S sequence data to identify species tentatively or at least differentiate complexes of closely related dryinids. Several studies confirm that morphology alone is frequently insufficient for delimiting species among insects with parasitoid lifestyles. Instead, a better approach to define species in these groups without morphological differentiation is by integration of morphology, host associations, natural history, geographic range and genetic markers (Derocles et al. 2016; Smith et al. 2006, 2007 & 2008).

Evolutionary history

Although considerable progress has been made to catalogue the world Dryinidae taxa through regional monographs (Olmi 1984, 1994, 2014; Olmi & Virla 2014; Olmi & Xu 2015; Xu et

al. 2013), the developmental biology, ecology and host associations of these parasitoids are poorly known. To date, only one molecular phylogeny including seven of the twelve extant subfamilies has been published, yielding Thaumatodryininae as a resurrected subfamily and multiple paraphyletic genera and genus groups in three subfamilies (Tribull 2015). These findings attest to the difficult taxonomy of the group, which, after recent revisions, resulted in multiple genera being synonymized (Olmi & Virla 2014; Xu et al. 2013). This study adds 70 new host records for Dryinidae, expanding these to an unrecorded planthopper family, Eurybrachidae, and to several species of planthoppers and leafhoppers previously unrecorded as dryinid hosts. Some new geographical records are reported as well.

I found host-attached larvae in samples from all biogeographic zones where Dryinidae are present, except Oceania. Biogeographic analysis revealed an ancestral distribution coinciding with the present Neotropics, likely in Gondwana, with subsequent vicariance and dispersal explaining the current cosmopolitan distribution. Although no divergence time analysis was done in this study, Branstetter et al. (2017) found Dryinidae to have originated between 125-120 Ma (millions of years ago) in the Lower/Early Cretaceous, agreeing with the earliest known fossil, *Aphelopus palaeophoenicius* Olmi, 2000, from Lebanese amber (120-136 Ma) (Olmi et al. 2014). However, Branstetter et al. only included one species of Anteoninae in their analysis. Moreover, Tribull (2015) found Apodryininae as an earlier diverging clade than Anteoninae, indicating a possible earlier origin of the family. The same study found that Sclerogibbidae + (Embolemidae + Dryinidae) split from the rest of Aculeata around 165-160 Ma, forming their own parasitoid superfamily, with Embolemidae + Dryinidae splitting from Sclerogibbidae around 145 Ma, during the Jurassic to Cretaceous transition (Branstetter et al. 2017).

The ancestral host for the groups in this study was recovered as Deltocephalinae, a leafhopper subfamily originating around 151 Ma. Although in agreement with divergence time analysis and previous host records (Branstetter et al. 2017; Dietrich et al. 2017; Guglielmino et al 2013), this is unlikely to be the case. Embolemidae and Dryinidae both comprise parasitoids of Auchenorrhyncha, and, with Dryinidae having a broad host niche, I interpret this pattern to mean that the ancestral host of this clade and Dryinidae was an ancestor of Auchenorrhycha

(Guglielmino et al. 2013; Olmi 1984; Olmi & Copeland 2011). Reconstruction of Deltocephalinae as the ancestral host may be a consequence of sampling bias. Deltocephalinae leafhoppers are 4.9 times more abundant than the second most abundant leafhopper subfamily in the included samples, Megophthalminae. Examination of label data revealed that many samples were collected by vacuum sampling, a method commonly used in dense, grassy vegetation in which Deltocephalinae are the most abundant Auchenorrhyncha (Buntin 1989; Novikov et al. 2006).

With respect to the host associations for the subfamilies of Dryinidae as found by the phylogenetic analysis of host-attached larvae, I found that, although no data could be obtained from the COI gene, my results allow a conservative estimate of the number of larval species using pairwise distance comparisons within clades. Using 2.00% divergence as the cutoff value, i.e., samples less than 2.00% divergent were classified as the same species, provided a conservative estimate of species diversity, but the 28S distances among some species included by Tribull (2015) were less divergent from each other indicating that faster evolving genes would provide a more precise estimate of the species number among the larvae of this study.

Unknown Dryinidae

The clade recovered in the present analysis as sister to the remaining Dryinidae did not include any sequence data from adults, preventing confident determination of the subfamily or genera forming this clade. However, host taxonomy provides some insights. Members of the clade were exclusively associated with fulgoroid planthoppers, consisting of three species of Caliscelidae from the New World, *Brucomorpha jocosa*, *B. abrupta* and *Plagiopsis* sp., and one species of Eurybrachidae from Australia, *Platybrachys* sp. Dryinid subfamilies with known caliscelid and other fulgoroid hosts are Bocchinae, Dryininae and Gonatopodinae (Guglielmino et al. 2013). Based on its phylogenetic placement, I infer members of this clade belong to the subfamily Bocchinae, possibly the genus *Bocchus*. Although previous phylogenetic analysis included a representative of *Bocchus*, 28S sequence data were not obtained for this species (Tribull 2015), so I was unable to include adult Bocchinae in this study. This genus has a worldwide distribution with about 104 species and matches the distribution of the hosts (Xu et al. 2013). Previous host records of Bocchinae are from the Afrotropics, Nearctic and Palearctic,

but no records exist from Australia or Australasia (Guglielmino et al. 2013). Additionally, this is the first report of Dryinidae attacking Eurybrachidae, a planthopper family restricted to the Old World tropics in habitats ranging from tropical wet forests to arid areas (Constant 2005). Some species in this family have filamentous wing tails, false eye-spots and or a mimetic resemblance to jumping spiders (Constant 2005b), and likely have unique adaptations for avoiding predation and/or parasitization.

Ecology and genetic distances confirm the presence of at least 3 species among the larvae grouped in this clade, one per locality within three biogeographic regions, Australasia (Australia, samples AUS1.1-1.4), Nearctic (Illinois, United States, samples ILI-21-22) and Neotropical (Argentina, samples ARG-78 and 92). This clade is reconstructed as having an ancestral range coinciding with the present Neotropics, indicating that geographical distribution may have a strong effect on dryinid host/prey selection and divergence. Its subsequent dispersal and vicariance suggest an ancestral Gondwanan distribution, but more analysis is needed, such as divergence time estimation, to confirm this suggestion. Ancestral habitat of the clade was reconstructed as forest, possibly indicating a landscape role in shaping these dryinid-hopper associations. Habitat reconstruction failed for the other clades, possibly because I was unable to obtain this information for the adult-derived sequences.

Anteoninae (*Anteon* + *Lonchodryinus*) and *Deinodryinus* spp.

Anteoninae included a total of 16 new host records distributed among the leafhopper subfamilies Cicadellinae, Coelidiinae, Deltocephalinae and Eurymelinae. Cicadellinae and Coelidiinae, THA-12 and ILI-15, respectively, are recorded for the first time as hosts of Anteoninae (Guglielmino et al. 2013). This subfamily is also reconstructed to have an origin in the present Neotropics, with dispersal and vicariance responsible for further distribution into the Palearctic and the Indo-Malasian regions. These events likely played a role in shaping the relationships within this subfamily as taxa are mostly separated in two major clades, one containing mostly New World specimens and the other mostly Old World specimens. The ancestral host was reconstructed as Deltocephalinae, likely having an impact on current host

use patterns across the subfamily, which are mostly in the tribes Deltocephalini and Paralimnini.

Distributional data and genetic distances indicate the presence of at least 9 species among included larvae. In the New World clade, most samples from the Nearctic grouped together with samples from Neotropical Mexico. In this clade, only samples from Zambia, Illinois and Taiwan (ZAM-01, ILI-12 and TAI-29, respectively) are likely to be different species owing to their disjunct geographic locations. Locality and habitat data reveal that larvae from Mexico could belong to the same species. The Illinois samples ILI-24, 34-35 likely belong to the same dryinid species. Surprisingly, the Old World samples from Mongolia (MON-07-09, 11, 14-15 and 19) were collected in the same locality but MON-15 is part of a separate clade, suggesting it is a different species from the rest. Phylogenetic placement and genetic distance indicate that additional samples from Thailand (THA-12) and Illinois (ILI-15) both represent separate species from the rest of the clade.

Genetic distance indicates the sample from Madagascar (MAD-01) is a different species from any others in the genus *Deonidryinus*. MAD-01 is likely to form part of this genus as it is more related to *Deinodryinus* sp.3 than to *Fiorianteon junonium*, although branch support for the clade is low. Only one species of *Fiorianteon*, F. *sulcatum* Guglielmino, Olmi, Marletta & Speranza, 2016, is known from Madagascar (Guglielmino et al. 2016). In contrast, 13 species of *Deinodryinus* are known from Madagascar, many of which are endemic (Guglielmino & Olmi 2015), strongly suggesting that this specimen belongs to this genus. If so, Acostemmini is recorded as a new host tribe utilized by this dryinid genus. Madagascar holds the largest diversity of Acostemmini, with several endemic species (Zahniser & Dietrich 2013), hence more associations unique to Madagascar await discovery. With about 600 species, Anteoninae is the most speciose dryinid subfamily, with over half of the species distributed in the Neotropical and Oriental regions (Olmi & Virla 2014; Xu et al. 2013). These findings suggest that more associations await discovery, possibly extending across more cicadellid subfamilies. Additional inferences on the evolution of *Deinodryinus* are not attempted due the small sample size and low support for this clade.

Aphelopinae

Strikingly, the potato leafhopper, *Empoasca fabae* (ILI-18), an important agricultural pest, is here recorded for the first time as a host for this subfamily, as well as for Dryinidae (Guglielmino et al. 2013), highlighting the generally incomplete understanding of dryinid host associations. *E. fabae* is a widespread, polyphagous species that causes yield losses in crops through feeding damage and transmission of plant pathogens (Barnes & Shaeffer 1985; Hunter & Backus 1989; Pastore et al. 2004). I also found a Gonatopodinae wasp, ARI-15, attacking this leafhopper species; due to its wide geographic range and diet, this leafhopper is likely attacked by other dryinid species. This is the only confirmed new host record found for Aphelopinae in this study. Other host hoppers for members of this clade were either unidentified females or males with atrophied genitalia that prevented positive species identification.

The ancestral host for most of the clade was reconstructed as Typhlocybinae, supporting Olmi's (1994) remarks that this subfamily is restricted to Membracidae (not represented in my dataset) and nymphal Typhlocybinae. Several of the larvae were found on adult Typhlocybinae, but it was not possible to determine the life stage when the hosts represented in my samples were attacked. More sampling and host preference studies are needed to determine the host range for this subfamily.

Examination of this clade reveals a minimum of 8 species among the included larvae. All Nearctic larvae, except an Arizona sample (ARI-06), are part of a highly supported clade. The long branch and genetic distance between ILI-18 and the rest of this clade indicates that it is a different dryinid species from that represented by the Arizona (ARI-24) and California (CAL-04 and 05) samples. Samples from South Africa (SAF-38 and 39) are likely to be the same species as they are genetically similar and were captured in the same locality. Phylogenetic placement and distance between the larvae from Thailand and Taiwan indicates that each specimen is a different species.

Gonatopodinae

Phylogenetic analysis revealed that my sampling was strongly biased toward Gonatopodinae, with 98 of 143 sequenced samples belonging to this clade. Most of these had

Deltocephalinae hosts commonly captured in prairies and other grasslands, observations supporting patterns found in other studies (Moya-Raygoza et al. 2004; Waloff 1975; Waloff & Jervis 1987). As a result, 30 of the 40 new host records in this subfamily are from this group of leafhoppers. Other relevant host records originate from Argentina. Similar to the case of *E. fabae*, *Hortensia similis*, ARG-60, is another widespread hopper species reported for the first time as a host of this subfamily and Dryinidae. *H. similis* is one of the most common hopper species in the New World, occurring in South America, Caribbean islands and the southern United States (Wilson & Claridge 1986; Young 1977). This species is also a vector of *Xylella fastidiosa*, making it an economically important pest of citrus and other crops (Coll et al. 2000; Redak et all. 2004). Additionally, *Coelidiana brasiliensis* from Argentina (ARG-59) is attacked by Gonatopodinae, making this the first report of Neocoelidiinae as a host of Dryinidae. This subfamily is restricted to the New World, where it is collected primarily in Neotropical forest habitats (Dietrich 2003). This specimen was collected by vacuum sampling forest understory, supporting observations that some species are associated with forbs (DeLong 1953).

This clade is reconstructed as having an ancestral range coinciding with the present Nearctic with subsequent dispersal and vicariance events responsible for colonization of other geographic zones. The ancestral host for most of the subfamily was reconstructed as Deltocephalinae, a possible explanation for the widespread use of this group of hosts across this subfamily of dryinids.

Inspection of this clade reveals some phylogenetic structuring by geographic location. There is an early diverging clade formed by Palearctic larvae (hypothesized to be at least one dryinid species) that parasitizes mostly leafhoppers of the tribe Paralimnini (Deltocephalinae) in Mongolia and France. The specimen from France (FRA-02) might represent an additional species in this group. Another clade was formed by Nearctic larvae parasitizing two species, *Ceratagallia gillettei* and C. *agricola*; this clade is likely composed of the same dryinid species or a complex of cryptic species, inasmuch as C. *gillettei* was found in different localities of Arizona that included forest and grass-shrub-scrub habitats and C. *agricola* in a prairie of Illinois.

There are other smaller clades with the same characteristics, i.e., clustering by geographic range and host taxonomy, composed of specimens captured in Argentina, Mexico,

Illinois and Arizona, collectively representing about 30 species. Within these, larvae attacking *Athysanella wardi* in Mexico seem to form at least two separate species that also parasitize other deltocephaline leafhoppers. Dryinids attacking *Haldorus* spp. in Argentina present a similar case, with larval specimens forming part of three separate clades. While larvae on *H. sexpunctatus*, ARG-84 and 88, clustered together and were from the same locality, other larvae on the same host species were in different clades, i.e., ARG-34 and 98, ARG-85 and 87. This suggests that they are separate species, most likely for ARG-34 and 98 as they were collected in separate localities. Genetic differences separate two Arizona (ARI-14) and California (CAL-01) larvae as separate species from other included Dryinidae, but observations across other specimens were limited due to the large number of comparisons, more than 5,000.

Evolution of host use patterns

The results of this study indicate that dryinid host associations extend to a wider range of Auchenorrhycha taxa than previous host records indicate. Although use of a slowly evolving genetic marker that has not been used previously for molecular taxonomy prevented confident resolution of species boundaries, host use patterns support the observations of previous authors suggesting that a generalist host preferences are widespread among species of Dryinidae (present study; Guglielmino et al. 2013). These associations are likely to result through ecological fitting, a process by which traits relevant to a given biological interaction evolved under different conditions and were co-opted in ecological time to form such associations, differing from exaptation which occurs over evolutionary time (Janzen 1985; Agosta & Klemens 2008). This process can be facilitated by two non-mutually exclusive mechanisms: (1) resource tracking, by which a species will shift to a new resource similar to the ancestral one; and (2) sloppy fitness space (Agosta & Klemens 2008), in which species use a completely novel resource representing different conditions from the ancestral one. These two are representative of the preexisting phenotypic plasticity of individual genotypes allowing species to colonize, use, and/or disperse to novel environments or hosts (Janzen 1985).

For dryinids, different hopper species found in different habitats represent a host range that can contribute similar gains in fitness (Agosta et al. 2010), i.e., successful oviposition and

larval development. Supporting evidence indicates that host associations for a given dryinid species can be temporally variable. Becerra-Chiron et al. (2017) found dryinid species parasitizing different leafhopper species of different tribes during the winter season, January-May, in edges of maize fields in Mexico. Additionally, dryinids have a functional response to their hosts, utilizing grassland leafhopper species in proportion to their abundance which, when combined with a polyphagous diet, reduces risks of local extinction (Waloff 1975, 1980). Similarly, these traits promote dispersal and gene flow between populations. Passive migration as host-attached larvae is likely the primary dispersal mechanism as suggested by the poor flying ability or aptery of adult females, host capture by aerial traps and genetic structuring between otherwise isolated populations in East Asia (Mita et al. 2012, 2013; Olmi 1994; Waloff 1973). Hence, these life history traits maintain or increase the fitness of dryinid species because they allow utilization of different hosts while maintaining gene flow within a species.

Nevertheless, the possibility of cryptic species complexes cannot be ruled out. Several studies of morphologically similar parasitoid species have shown that they represent species complexes with narrow host ranges (Smith et al. 2006, 2007, 2008), with geographic isolation also playing a role in differentiation (Derocles et al. 2016). This is especially the case for tropical zones. Studies in the Area de Conservación Guanacaste (ACG), Costa Rica, found the presence of several cryptic, genetically distinct tachinid parasitoids with narrow host ranges within single morphospecies previously thought to be host generalists (Smith et al. 2006, 2007). Perhaps this phenomenon is best illustrated by parasitoid wasps of the subfamily Microgastrinae (Braconidae). A study in ACG integrating taxonomy, DNA barcoding and natural history found more than 313 provisional species to be specialists (Smith et al. 2008). Among these, the supposed generalist, *Apanteles leucostigmus*, was discovered to be a complex of 36 cryptic species each attacking one or two closely related host species.

For Dryinidae, the knowledge gap with respect to host associations combined with the existence of morphologically similar species, some of them with wide host ranges, suggest the presence of cryptic, specialist species complexes cannot be ruled out (Guglielmino et al. 2013; Olmi 1984). Current species numbers (Tribull 2015) suggest that dryinids are more likely to form a mosaic of generalist and specialist species, like tachinid flies, but not to the extent found in

Microgastrinae. Specialist dryinids might evolve by undergoing a complete host shift after a host range expansion event, followed by genetic isolation and local adaptation of the population in the novel host (Agosta & Klemmens 2008). Much more genetic data incorporating more rapidly evolving markers will be needed to elucidate the actual species diversity of Dryinidae.

Relevance

To my knowledge this study is the first attempt to use phylogenetics to identify host-attached dryinid larvae taxonomically. Although several previous studies have attempted to define host associations of parasitoids using molecular markers and network analysis, these studies relied on sequencing adult parasitoids after rearing them from field-captured hosts (Smith et al. 2006, 2007, 2008). Laboratory rearing of parasitized hosts is often a time-consuming process that can result in high mortality rates in the parasitoid of interest (Giordano et al. 2002). To overcome this obstacle, several investigators have attempted to obtain parasitoid molecular sequences directly from the host. Varennes et al. (2014) successfully retrieved parasitoid DNA from aphid mummies, but the study was restricted to laboratory colonies and DNA could not be amplified after three weeks. Another study determined Pentatomidae-Scelionidae associations from laboratory- and field-collected egg masses (Garyepi et al. 2013). In this study, I demonstrate that insect collections, combined with molecular phylogenetic methods, can circumvent the difficulties associated with host rearing to expand knowledge of parasitoid-host associations.

Samples for this study were obtained from a wet insect collection where 81.7% of the specimens provided high-quality 28S sequence data enabling the placement of all dryinid host-attached larvae to the subfamily level and many to genus level. My ability to identify species from larval specimens was limited, owing to this conservative molecular marker and low availability of molecular data from previous studies. Future studies should focus on more rapidly evolving genes amplified with dryinid-specific primers (Gariepy et al. 2013) or next-generation sequencing (Vacher et al. 2016) that allows the capture and separation of parasitoid DNA from that of the host.

Parasitoid wasps constitute 70% of the diversity in Hymenoptera and are important regulators of their host populations (Heraty 2009; Ulrich 1999) in natural and agricultural landscapes. Knowledge of host-parasitoid associations is important for understanding the specific ecosystem services provided by particular species, but remains highly incomplete. This study provides an example of the active role of insect collections in advancing understanding of the ecology of natural and managed systems. Research incorporating alcohol-preserved material can yield discoveries of biological interactions that have not been previously documented through traditional field studies (Pérez-Lachaud & Lachaud 2017). Because they house a significant fraction of undiscovered arthropod diversity (Green 1998), entomological collections may provide the basis not only for studies of biological diversity but also for studies of ecological interactions. Such studies are essential for rapidly disappearing biodiversity hotspots threatened by anthropogenic land transformation and climate change (Scheffers et al. 2012). One example is Madagascar, a country suffering from accelerated biodiversity loss (Harper et al. 2007; Lenzen et al. 2012), where I discovered the first interaction between *Deinodryinus* sp. and Acostemmini (Deltocephalinae: Cicadellidae) (MAD-01). The presence of endemic species for both groups indicate that more interactions unique to Madagascar are yet to be discovered. Because entomological collections studies provide strong evidence for a dense network of ecological interactions among the species present, such studies could be used to support and enhance conservation efforts in these areas.

TABLES AND FIGURES

Figure 1. Life stages and morphology of Dryinidae. A) *Gonatopus elongatus* (Illinois, USA, ILI-25) dorsal view; B) same lateral; C) same, ventral; D) same, left foreleg chela; E) dryinid larva on *Bruchomorpha* sp. (Caliscelinae: Caliscelidae) nymph from Ford Co., Illinois, USA; F) same on Gyponini (Iassinae: Cicadellidae), probably *Gypona* sp., from Serra do Tepequem, Roraima, Brazil; G) larvae of an Anteoninae (Dryinidae) on *Sorhoanus xanthoneurus* (Cicadellidae: Deltocephalinae: Paralimnini) (Mongolia, MON-07). Scale bar equals 1 mm.

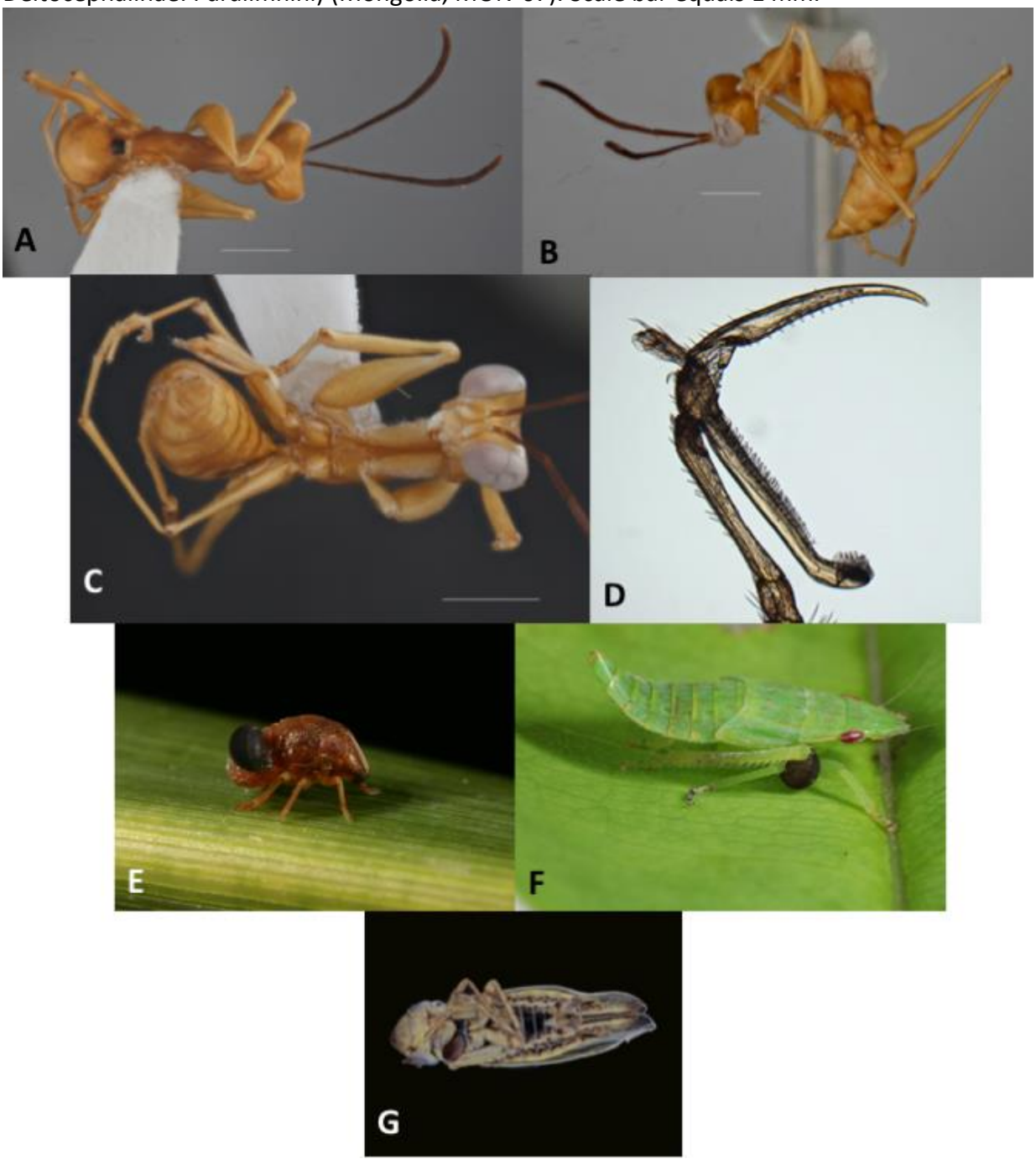

Figure 2. Dryinidae 28S D2-D3 gene region ML analysis summary. Clades follow the same color scheme of the full phylogeny.

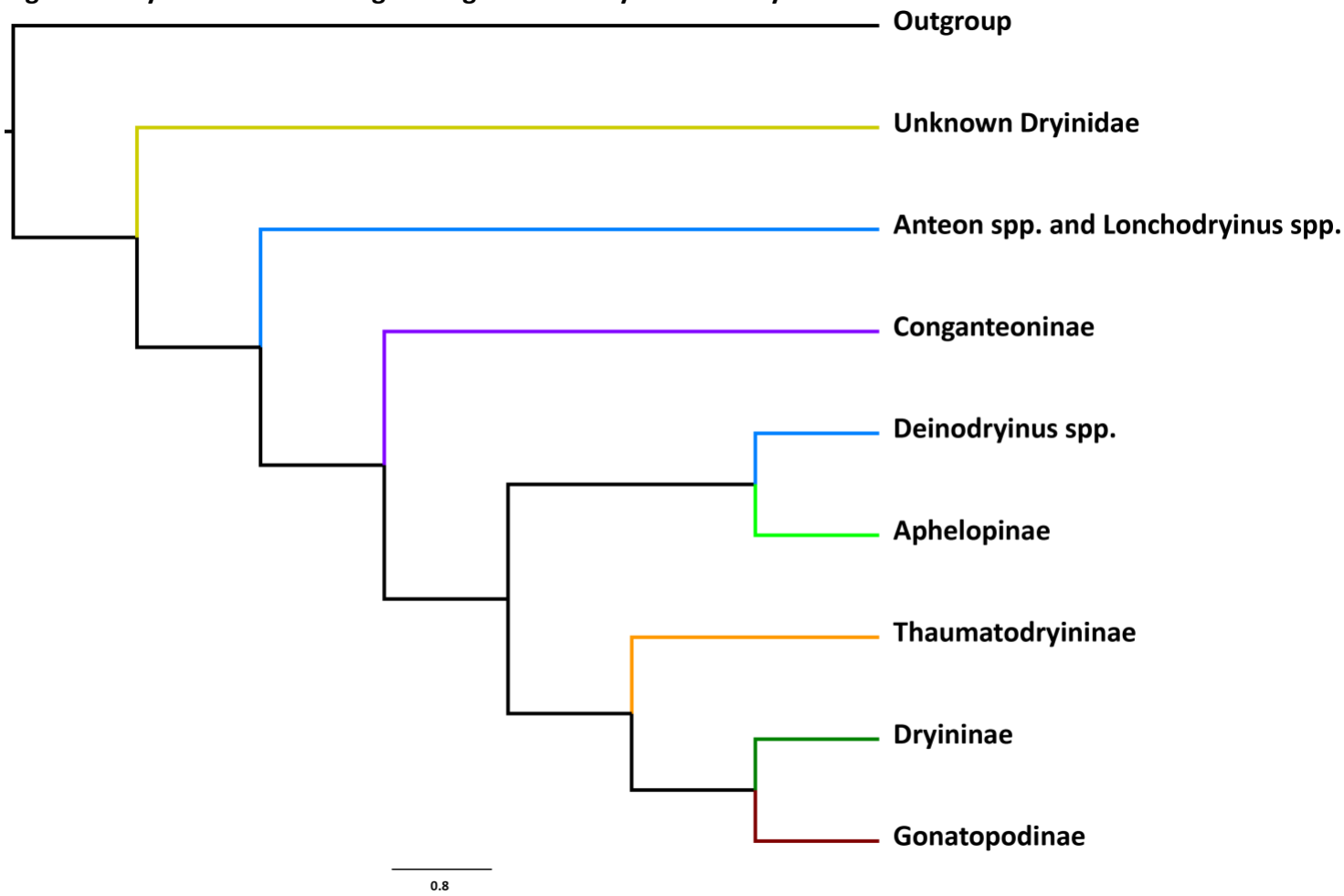

Figure 3. Tribull (2015) ML analysis summary. Topology resulting from four genes: *CO1, CytB, 18S* and *28S* D2-D3 region.

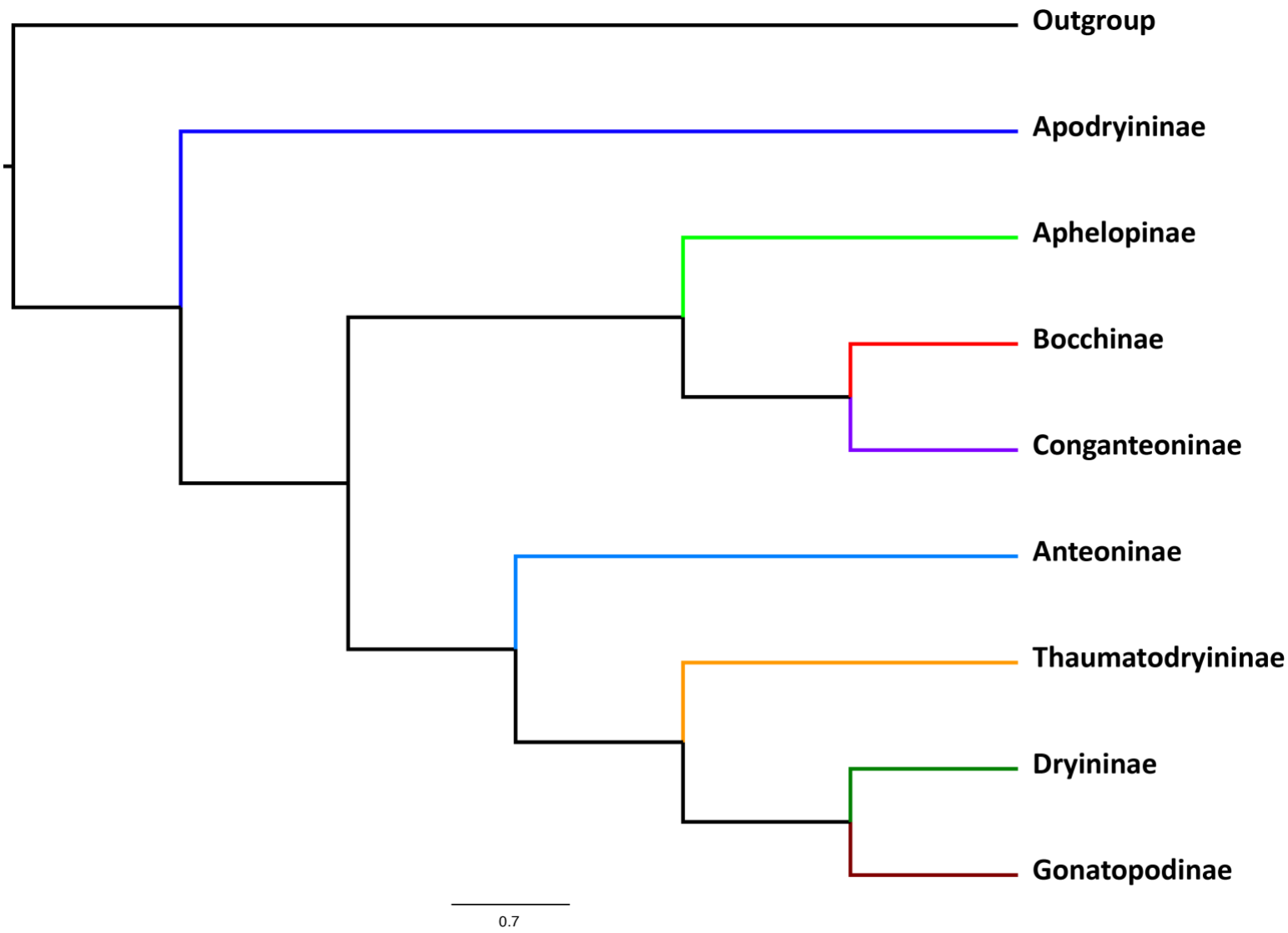

Figure 4. Tribull (2015) Bayesian analysis summary. Topology resulting from four genes: *CO1, CytB, 18S* and *28S* D2-D3 region.

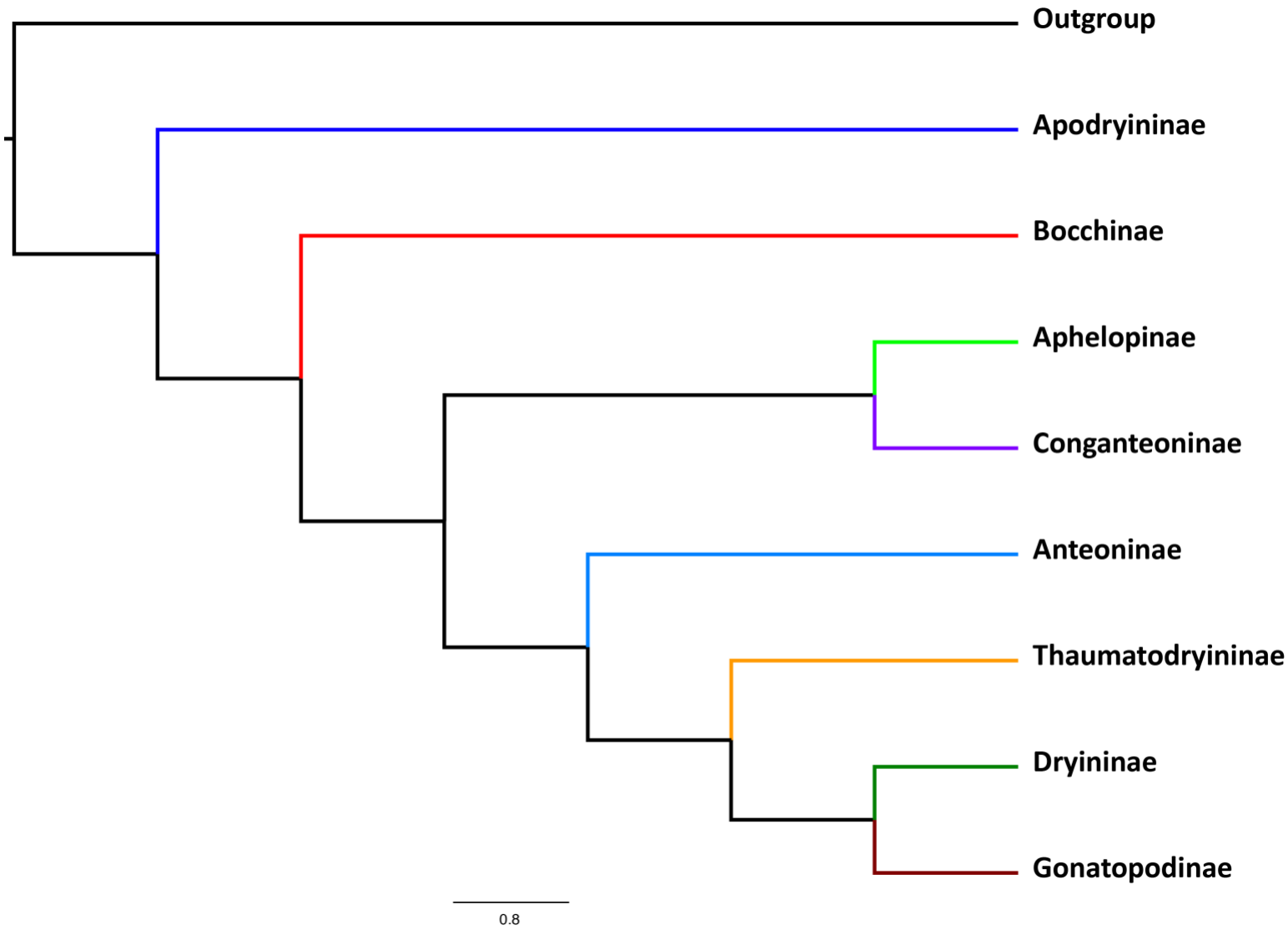

Figure 5. 28S ML cladogram. Taxon names in the format sampleID|Genus|species|Tribe|Subfamily. Circles on branches indicate clade is supported by: black, UFB and SH-aLRT; gray, SH-aLRT; white UFB. Clades without circles received low support.

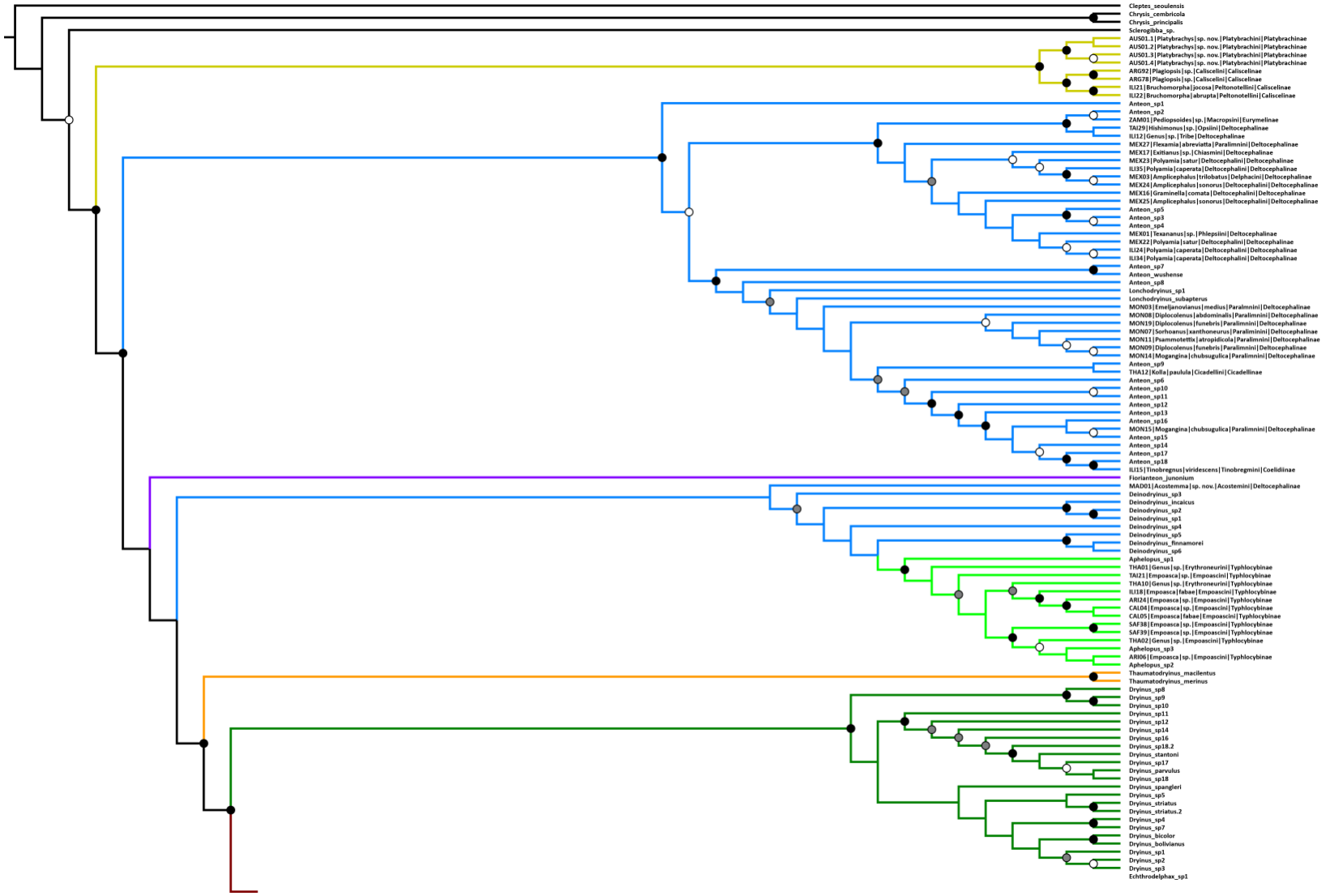

Figure 5 (continued)

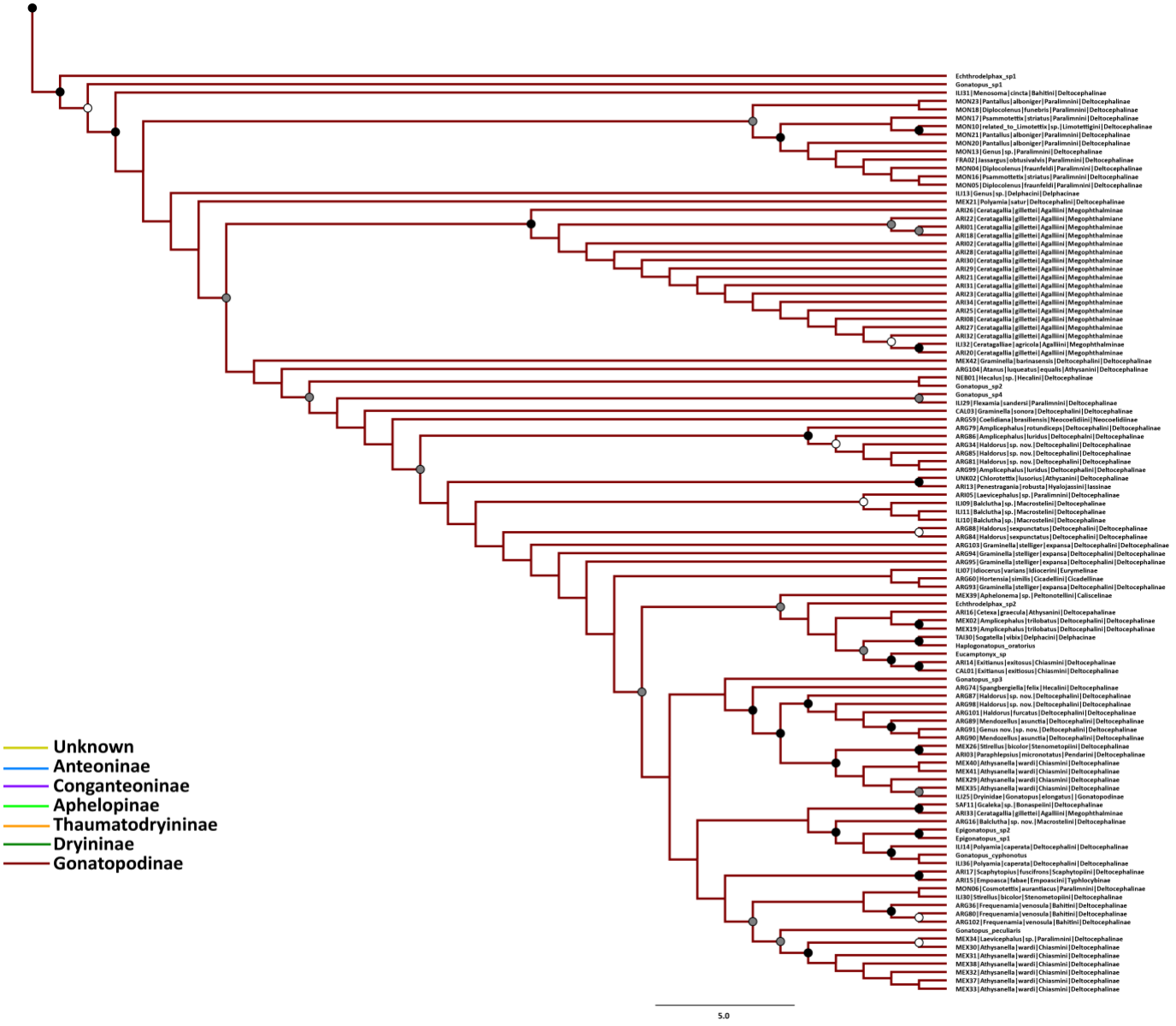

Figure 6. Unknown clade as in the 28S phylogeny. Taxon names as in Fig. 2. Branch support is not presented for easier visualization od short branches.

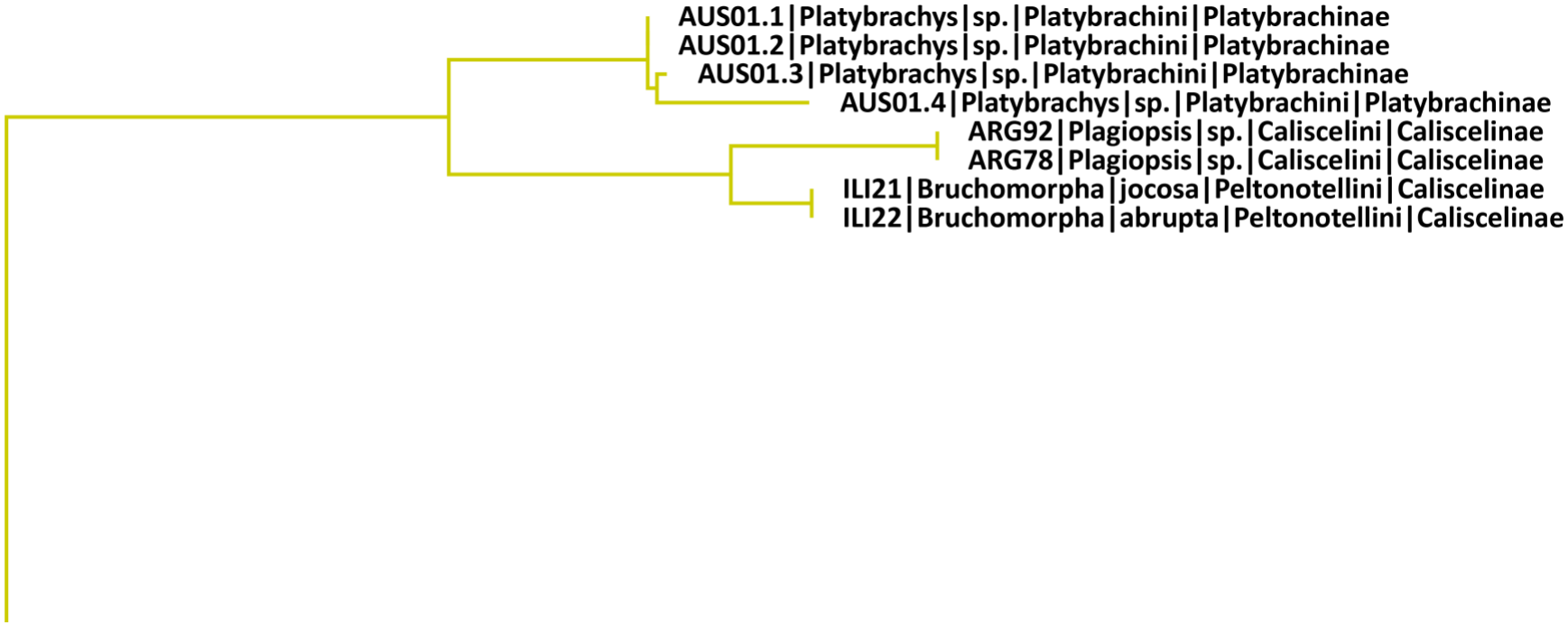

Figure 7. Anteoninae (*Anteon* + *Lonchodryinus*) as in the 28S phylogeny. Taxon names as in Fig. 2. Branch support is not presented for easier visualization od short branches.

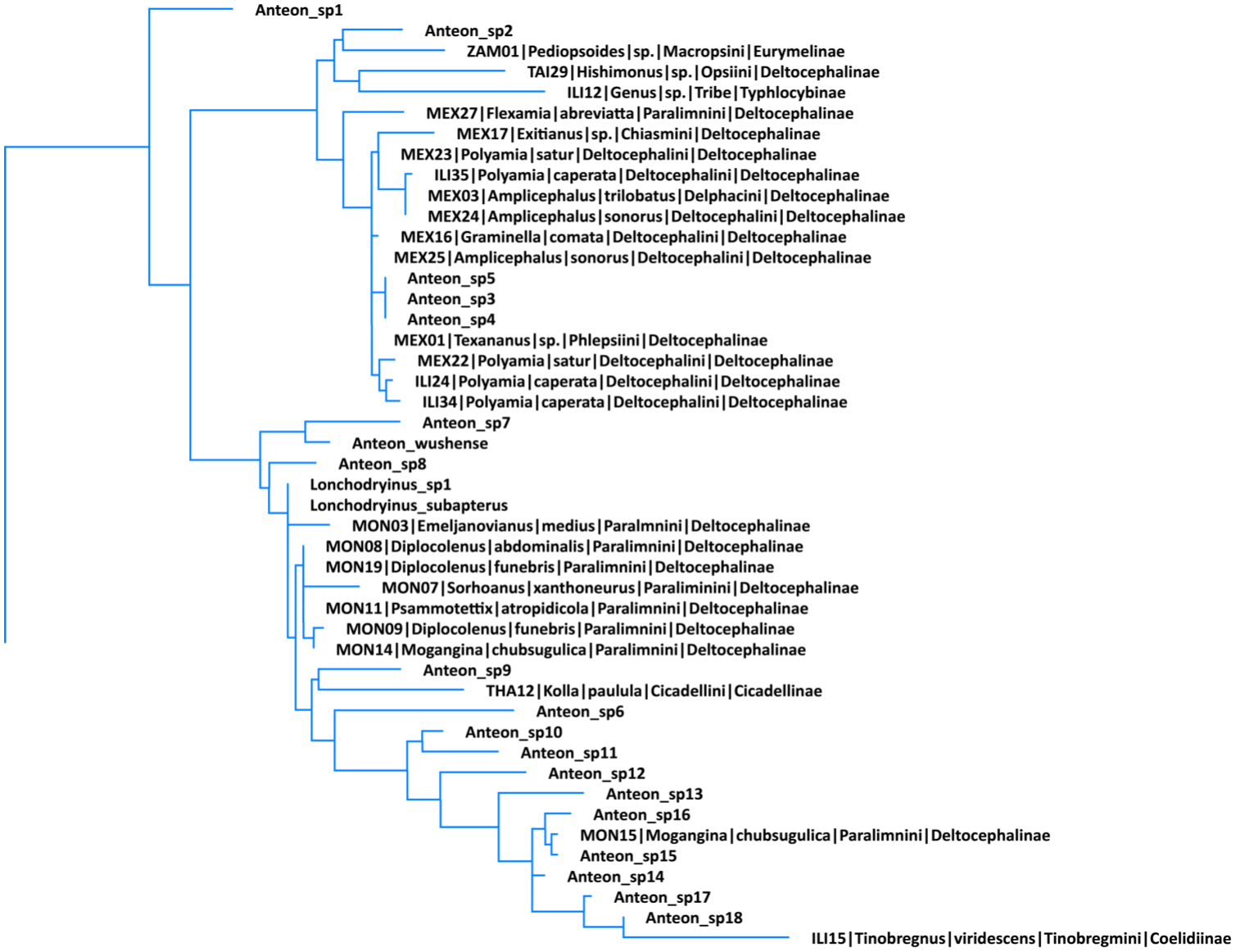

Figure 8. Conganteoninae and *Deinodryinus* as in the 28S phylogeny. Taxon names as in Fig. 2. Branch support is not presented for easier visualization od short branches.

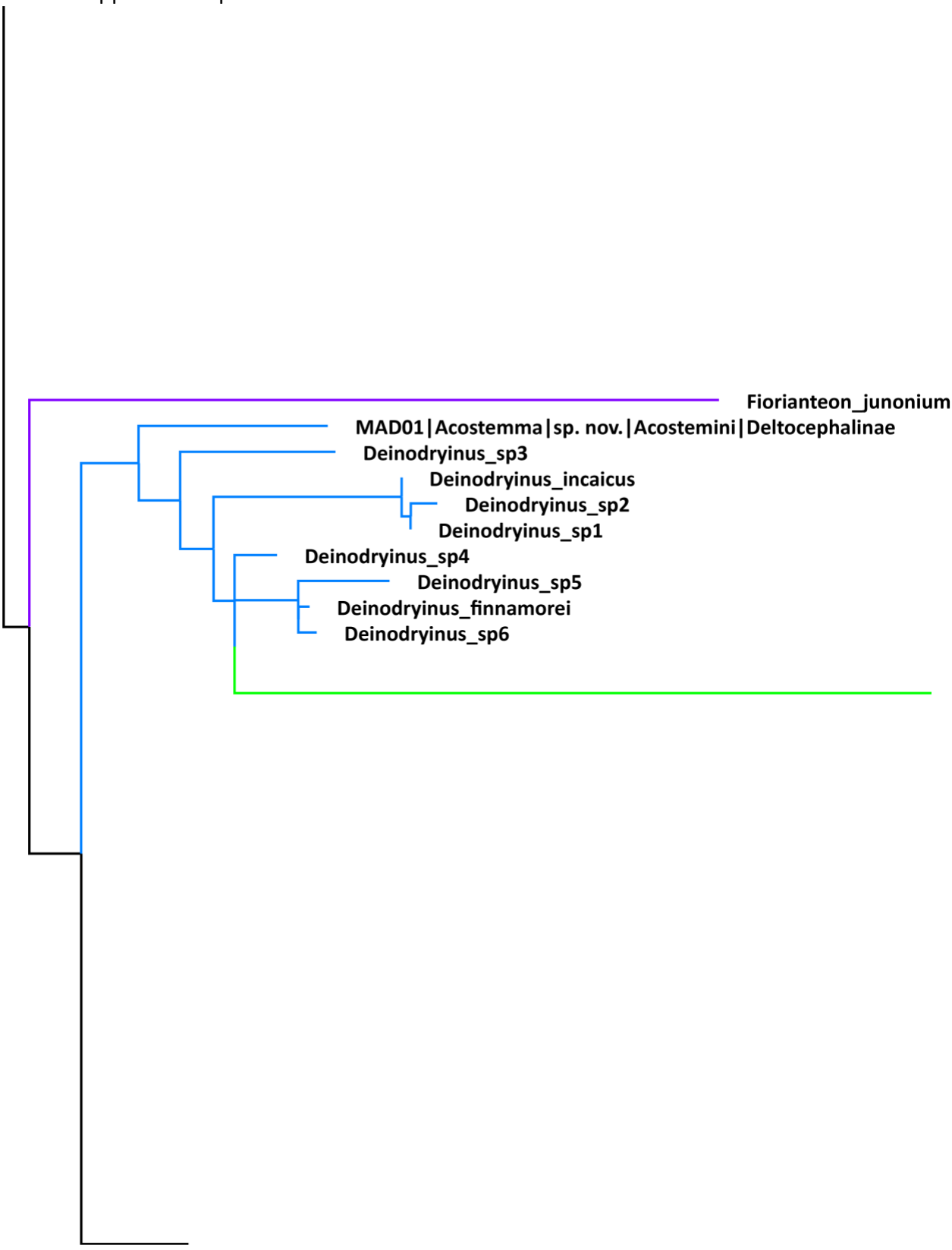

Figure 9. Aphelopinae as in the 28S phylogeny. Taxon names as in Fig. 2. Branch support is not presented for easier visualization od short branches.

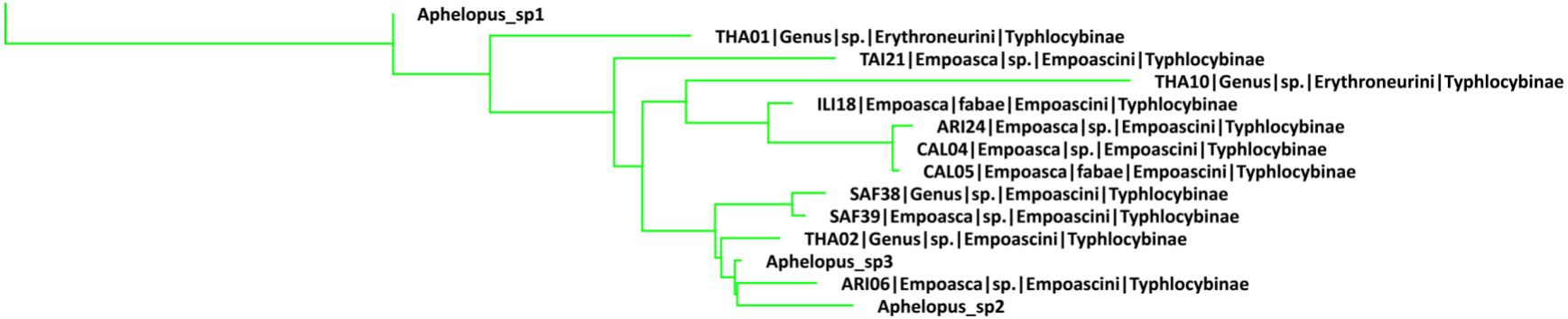

Figure 10. Thaumatodryininae and Dryininae as in the 28S phylogeny. Taxon names as in Fig. 2. Branch support is not presented for easier visualization od short branches.

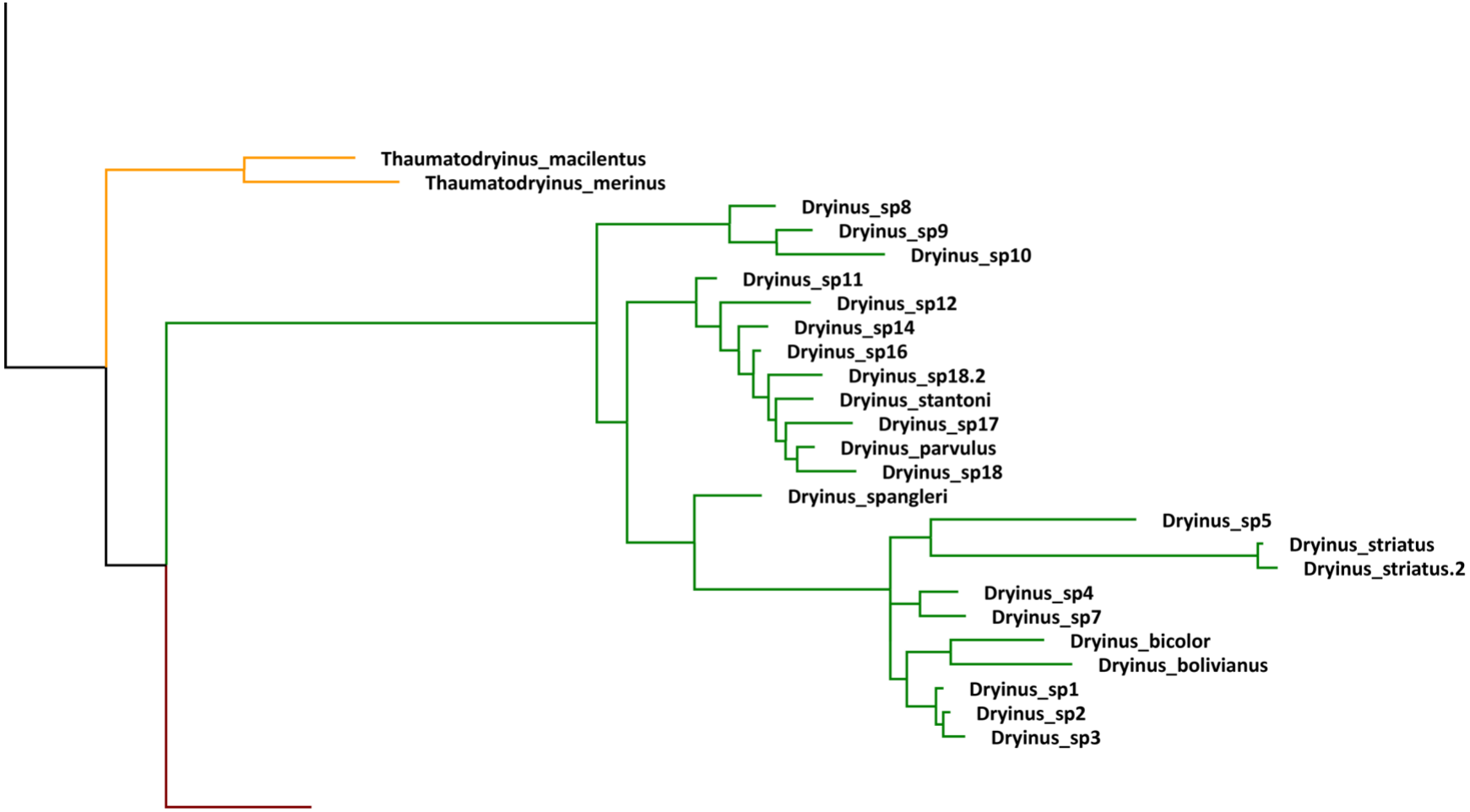

Figure 11. Gonatopodinae as in the 28S phylogeny. Taxon names as in Fig. 2. Branch support is not presented for easier visualization od short branches.

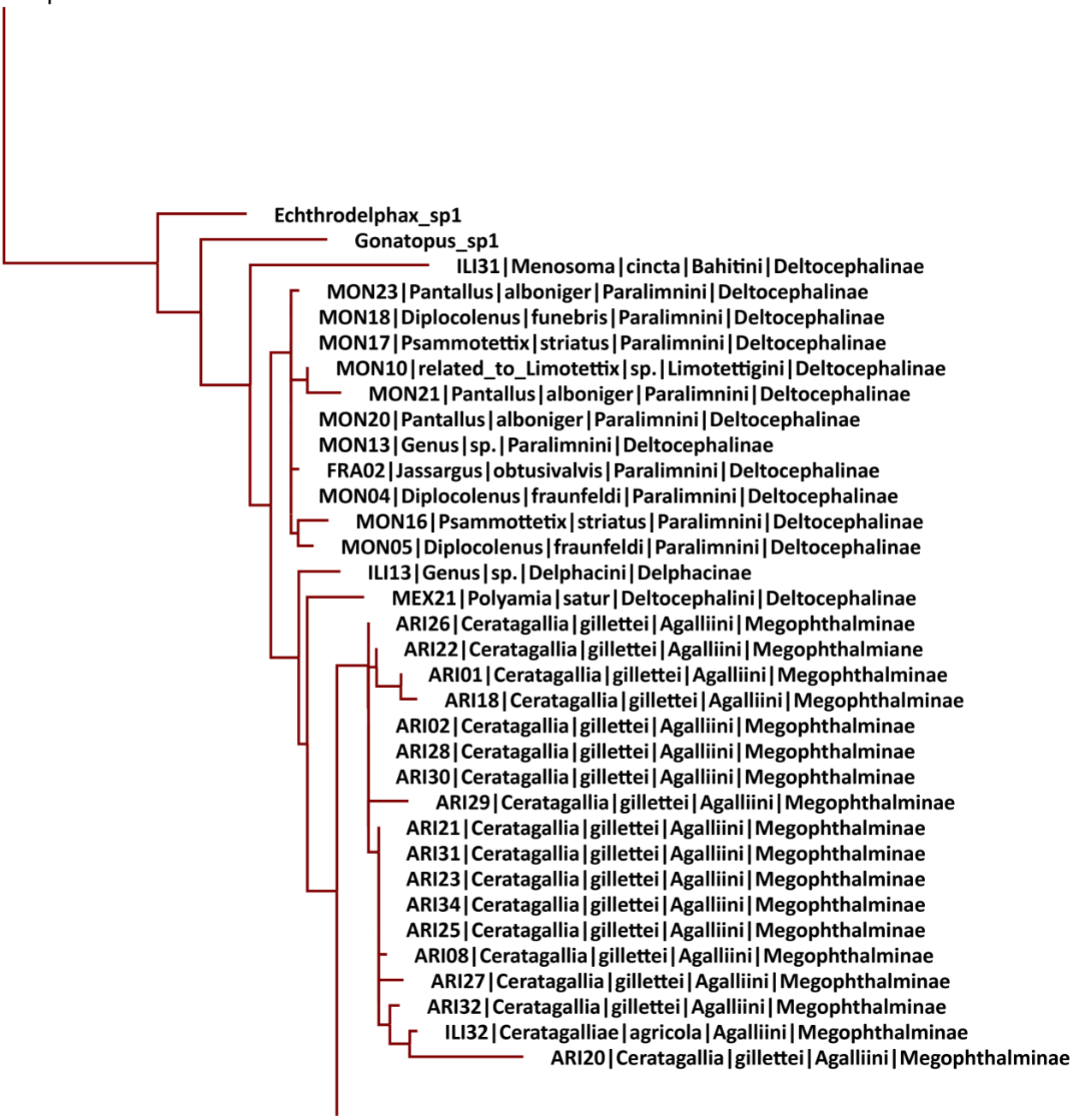

Figure 11 (continued)

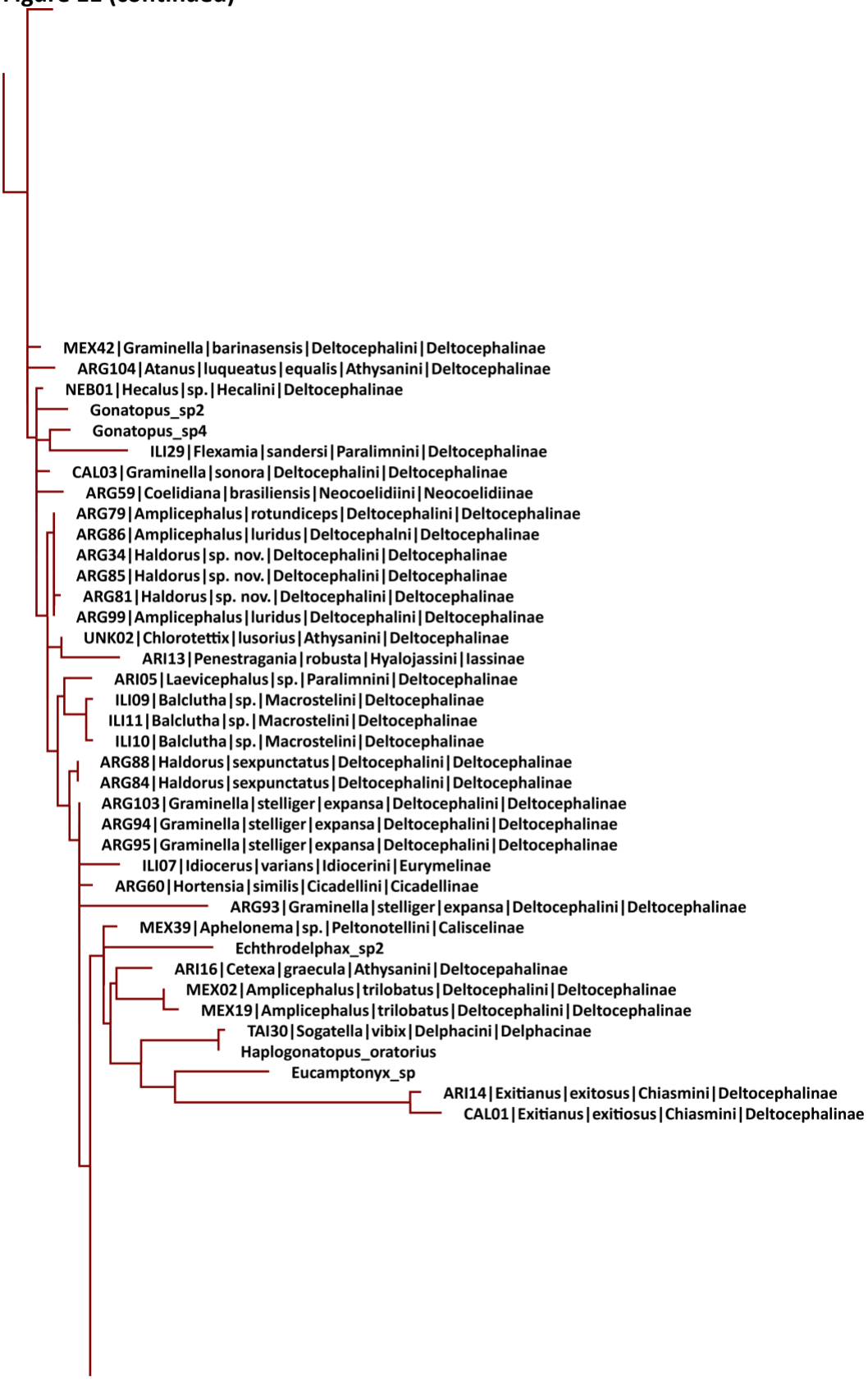

Figure 11 (continued)

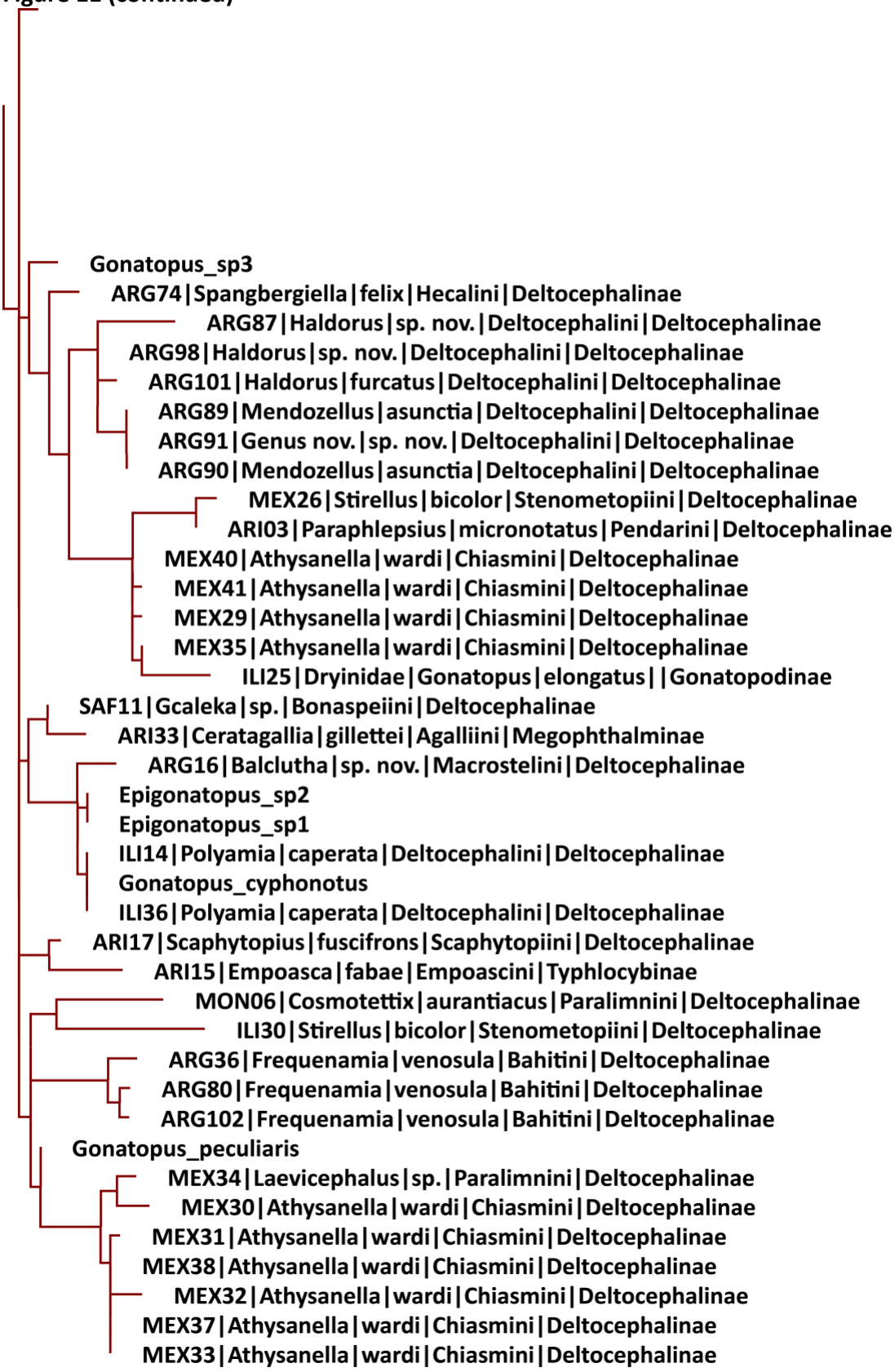

Figure 12. Summary of Classification of Dryinid Larvae. Different colors represent different subfamilies, following the same color scheme as in the phylogeny. Anteoninae is followed by the subset of samples identified to genera.

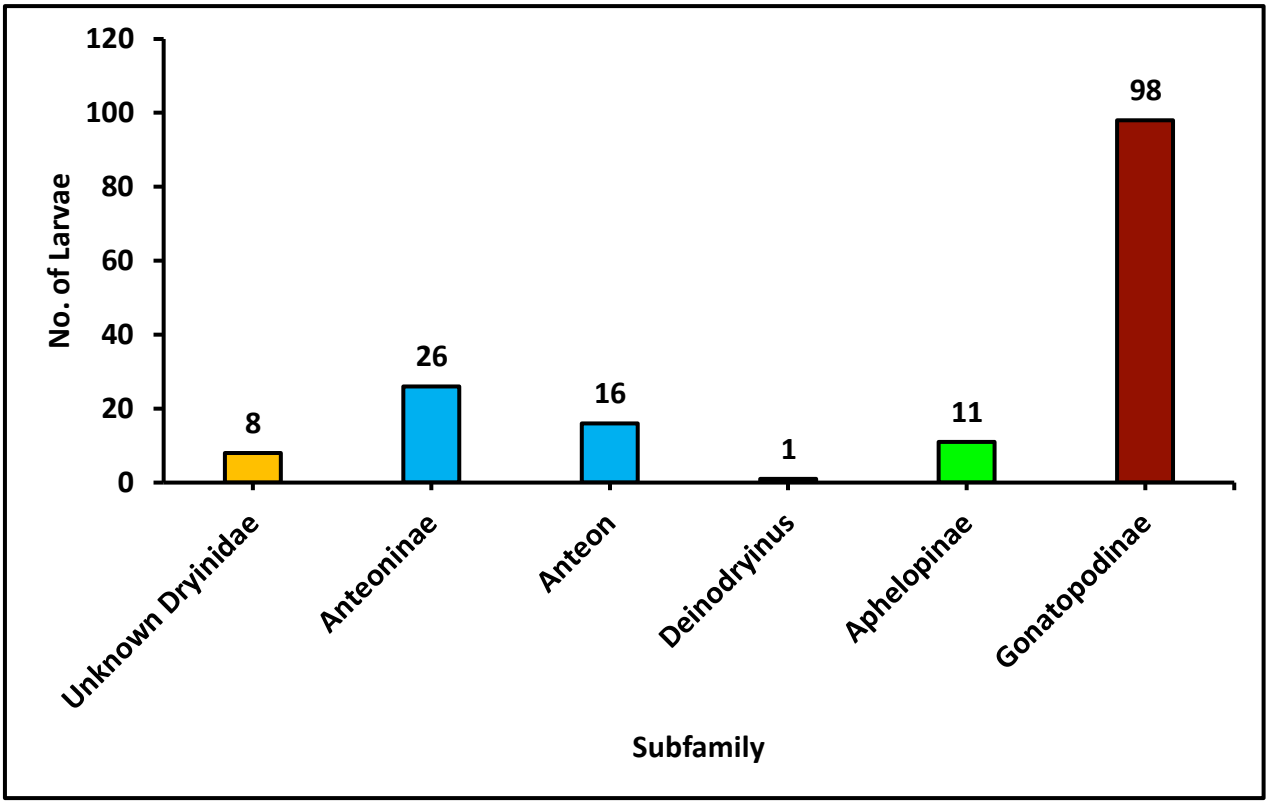

Figure 13. Biogeographic analysis under BBM showing outgroup and unknown Dryinidae. Ancestral distributions at each node are presented as pie charts with the most likely reconstruction designated by the letter in the middle. Dispersal and vicariance events are indicated by green and blue circles, respectively. Legend shown in Fig. 17.

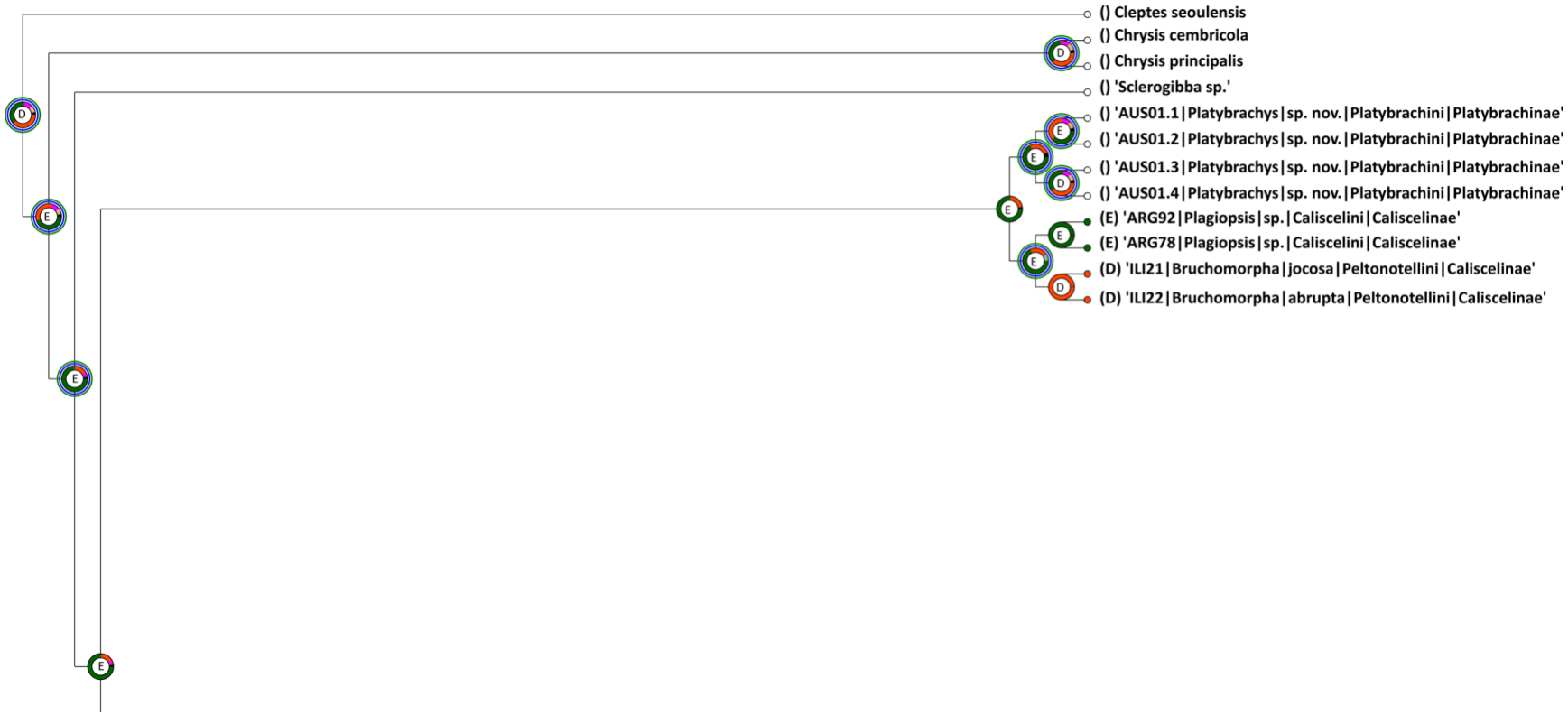

Figure 14. Biogeographic analysis under BBM showing Anteoninae (*Anteon* + *Lonchodryinus*). Details as in Fig. 13.

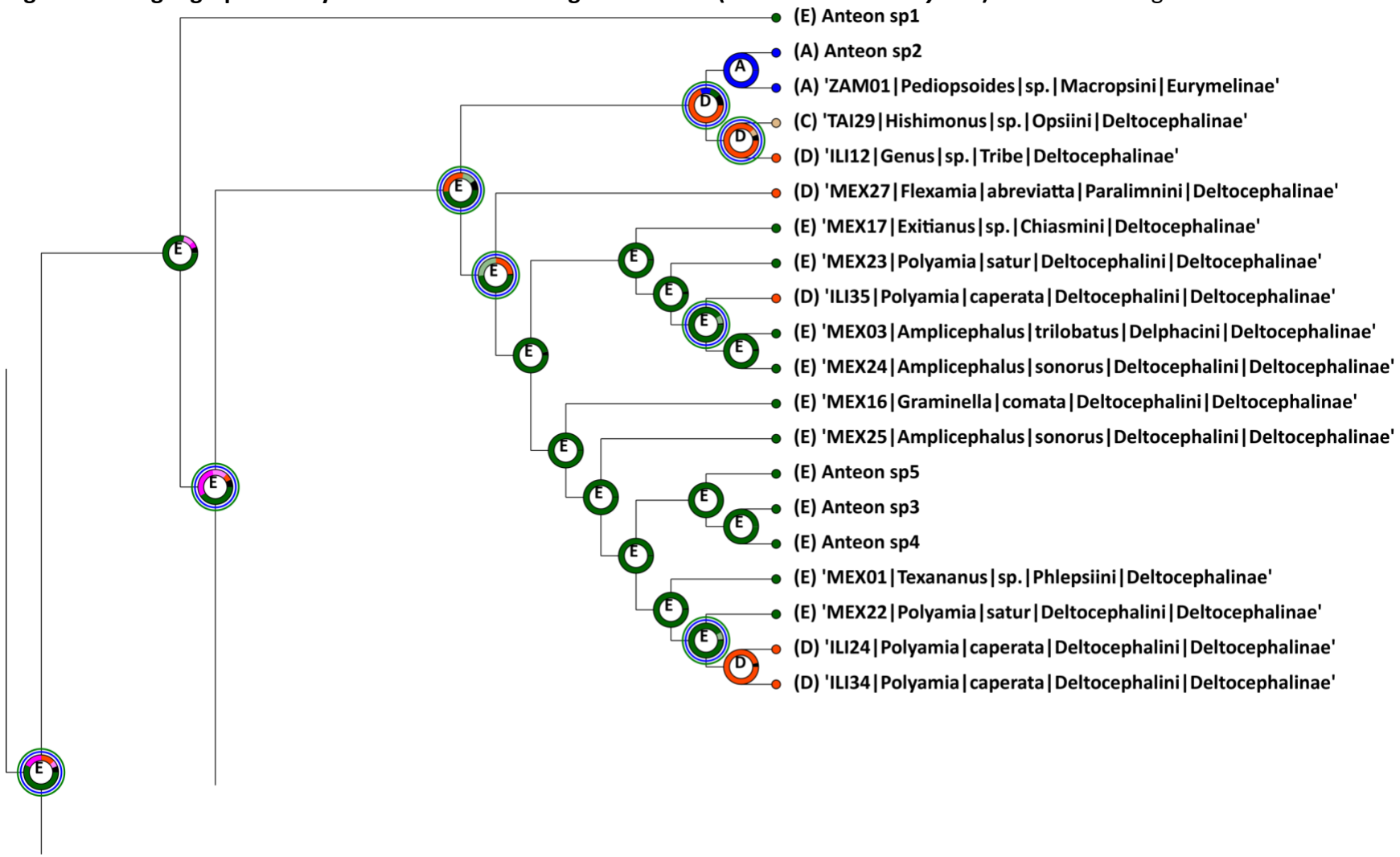

Figure 14 (continued)

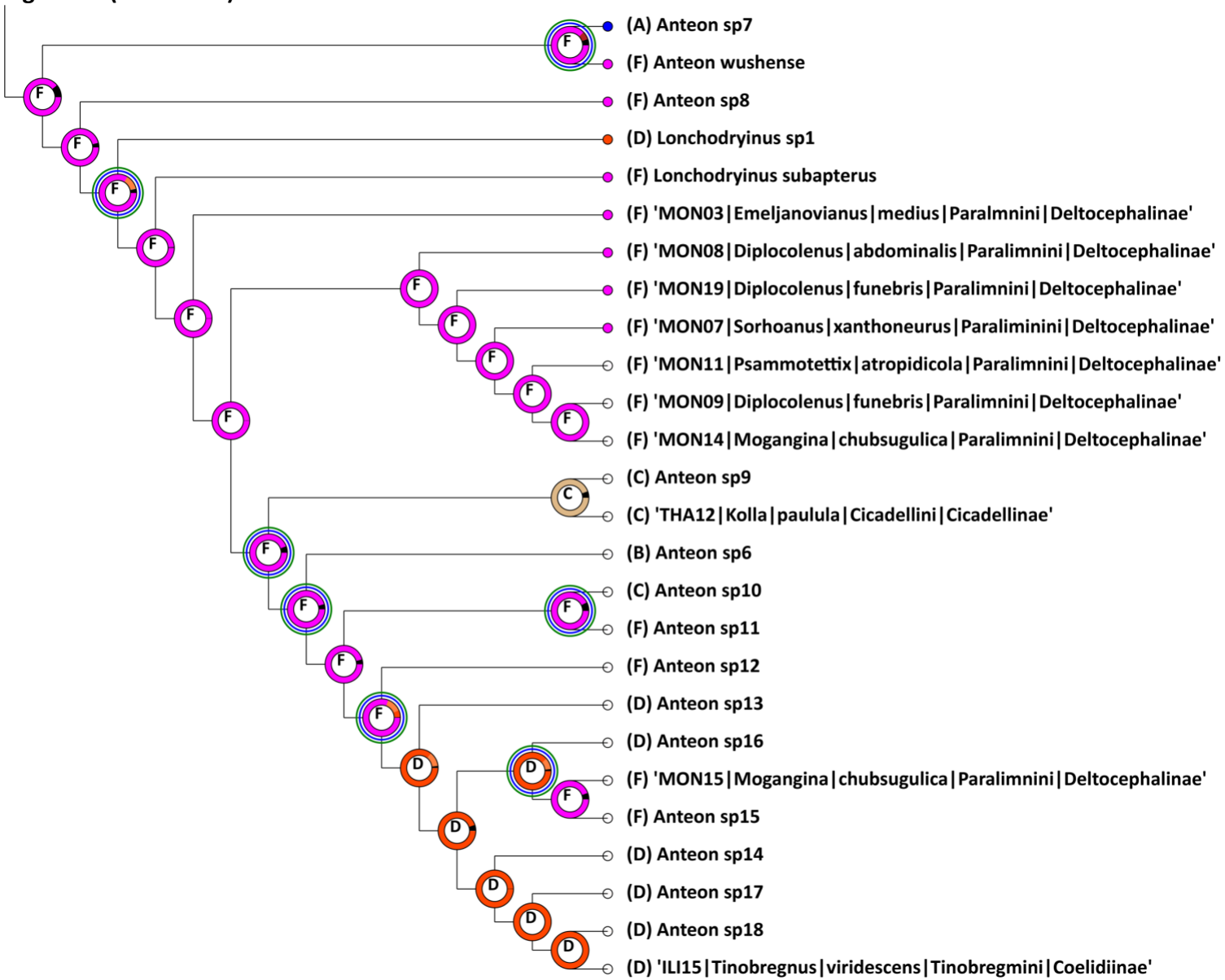

Figure 15. Biogeographic analysis under BBM showing Conganteoninae, *Deinodryinus* and Aphelopinae. Details as in Fig. 13.

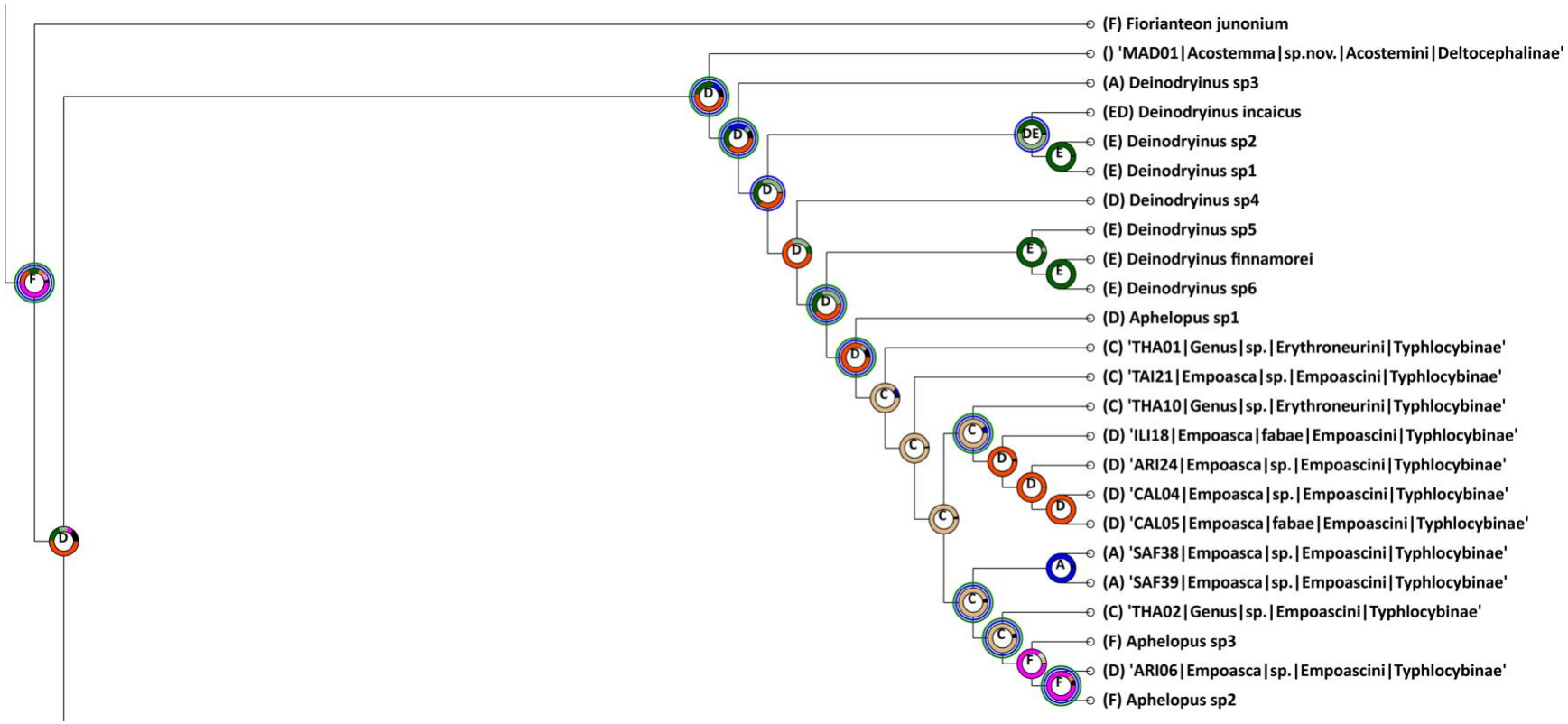

Figure 16. Biogeographic analysis under BBM showing Thaumatodryininae and Dryininae. Details as in Fig. 13.

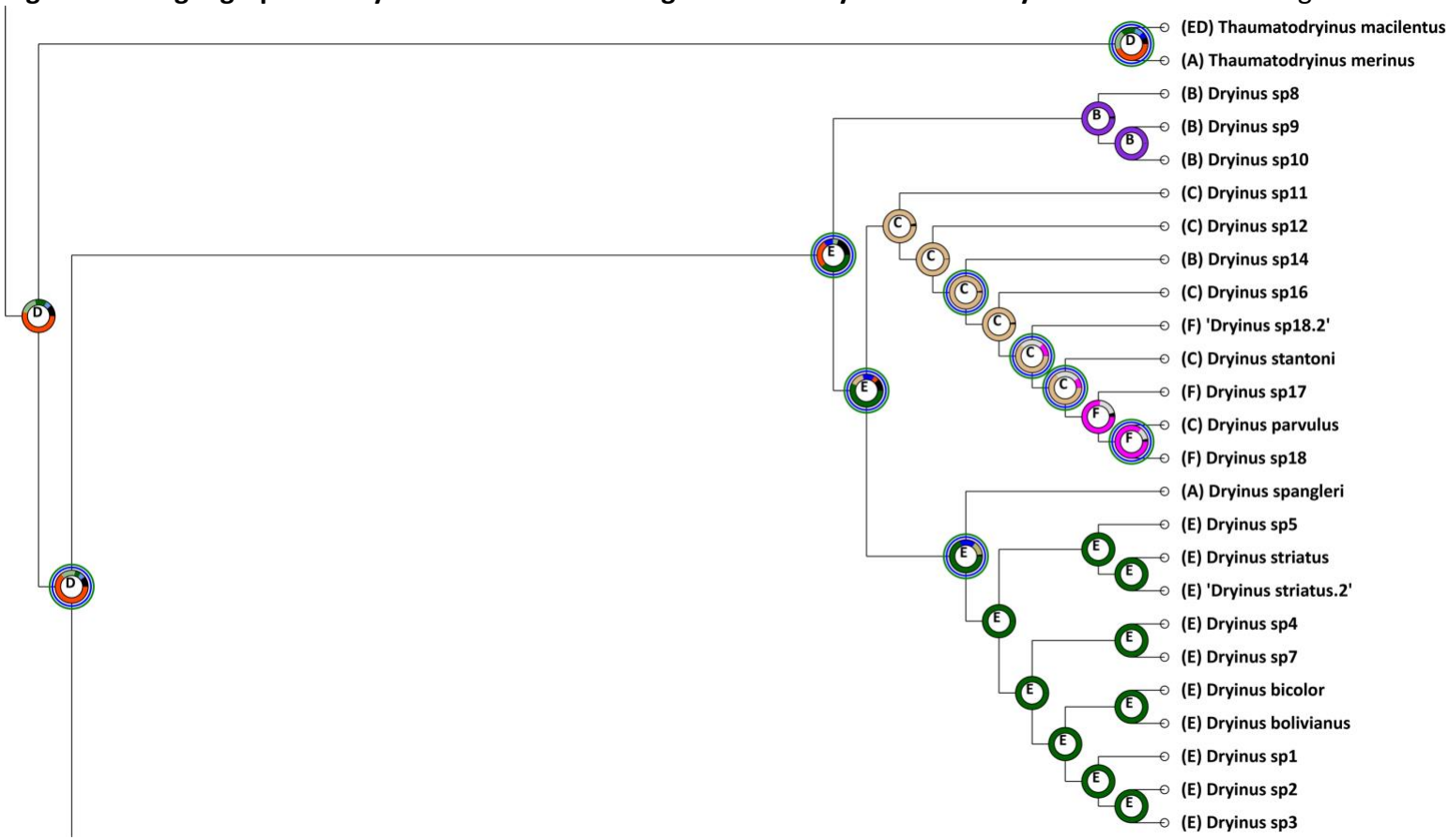

Figure 17. Biogeographic analysis under BBM showing Gonatopodinae. Details as in Fig. 13.

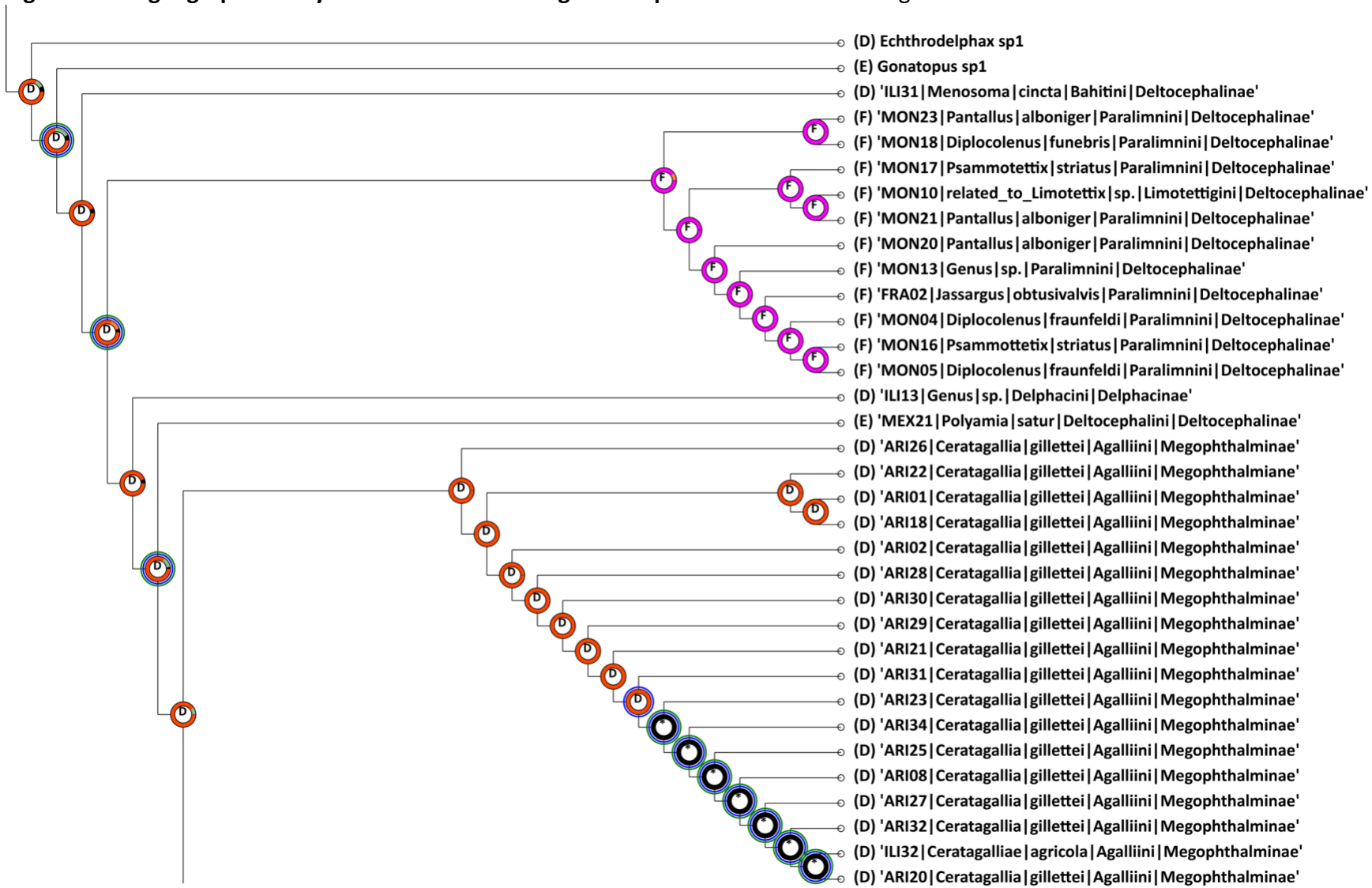

Figure 17 (continued)

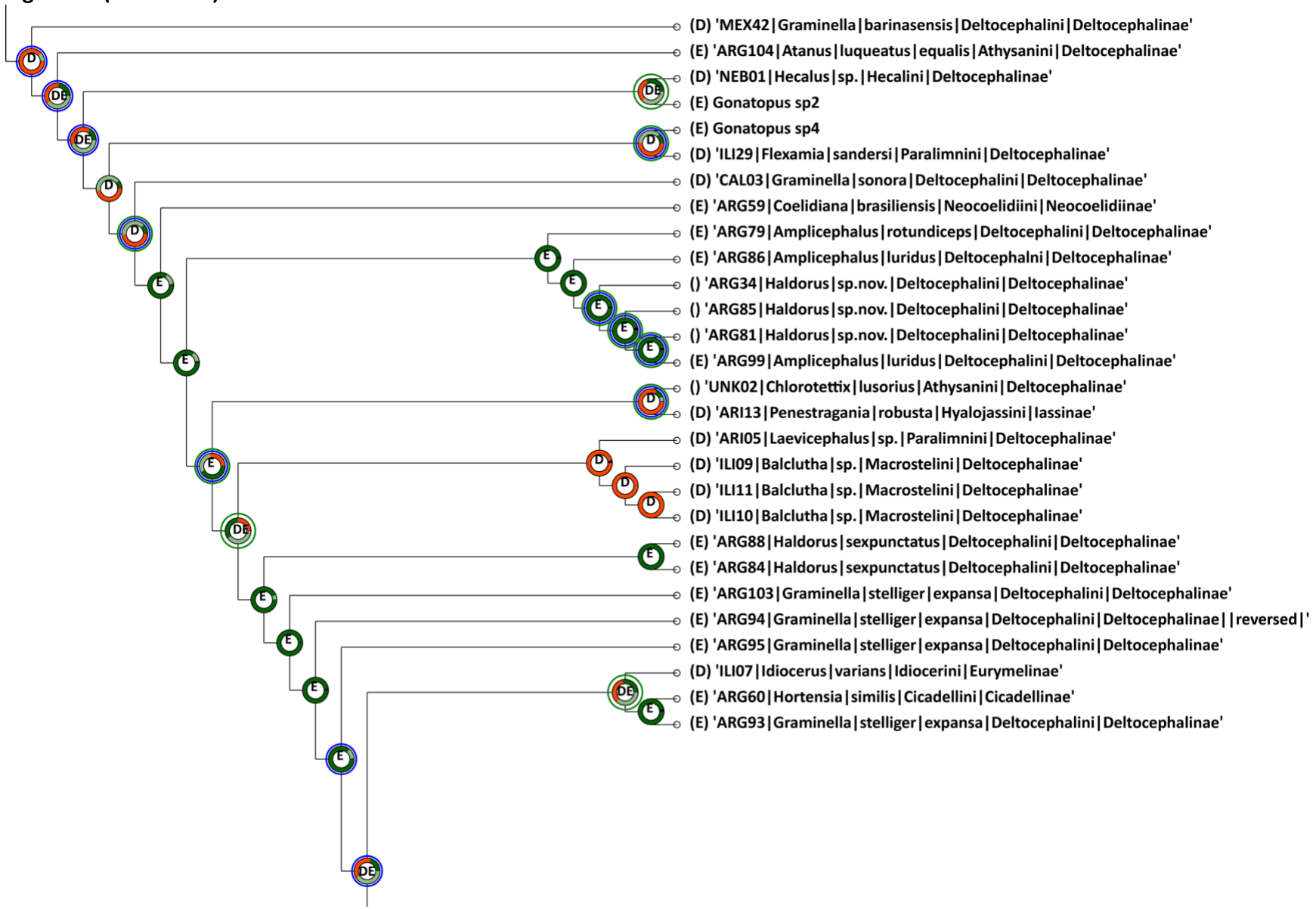

Figure 17 (continued)

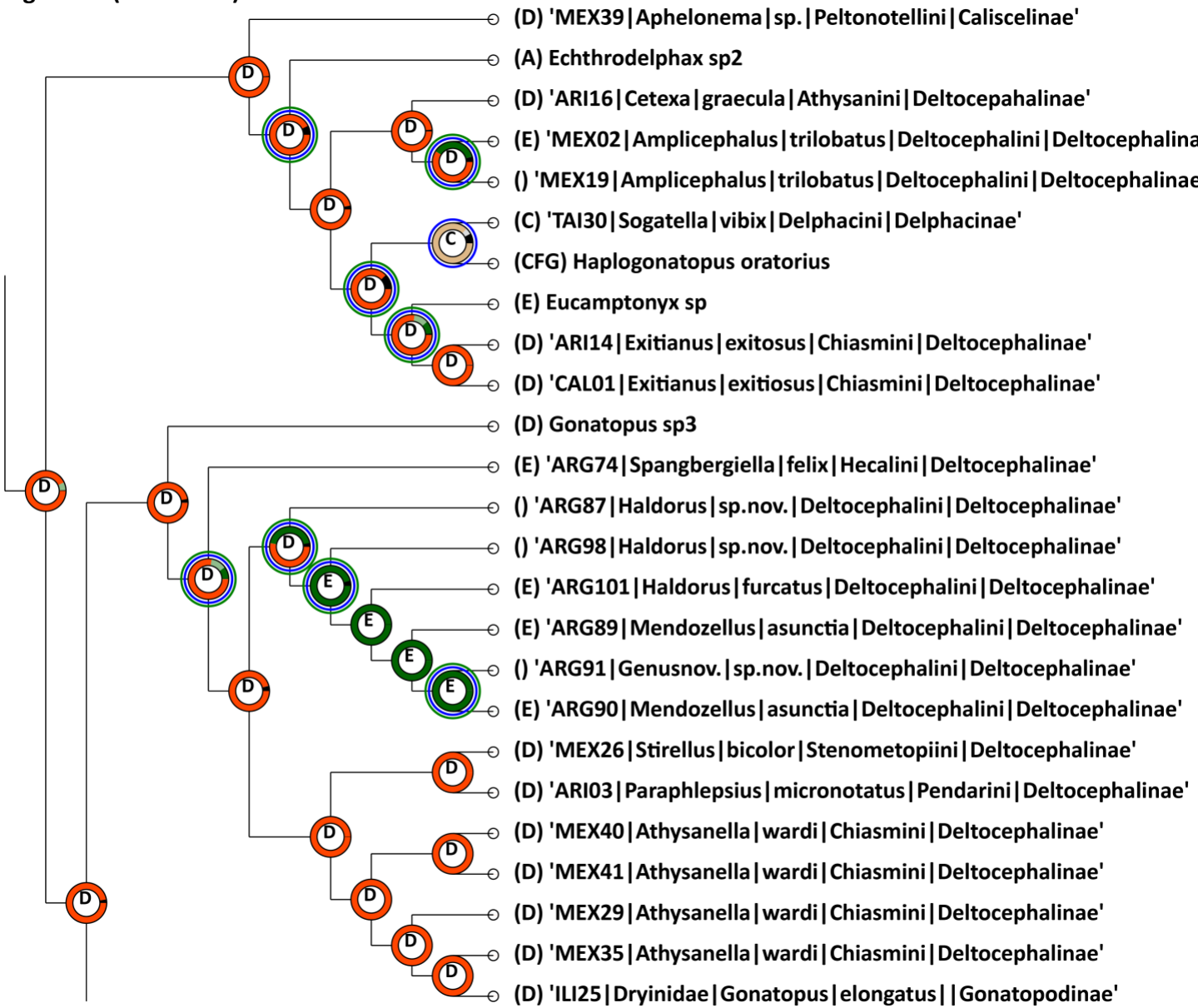

Figure 17 (continued)

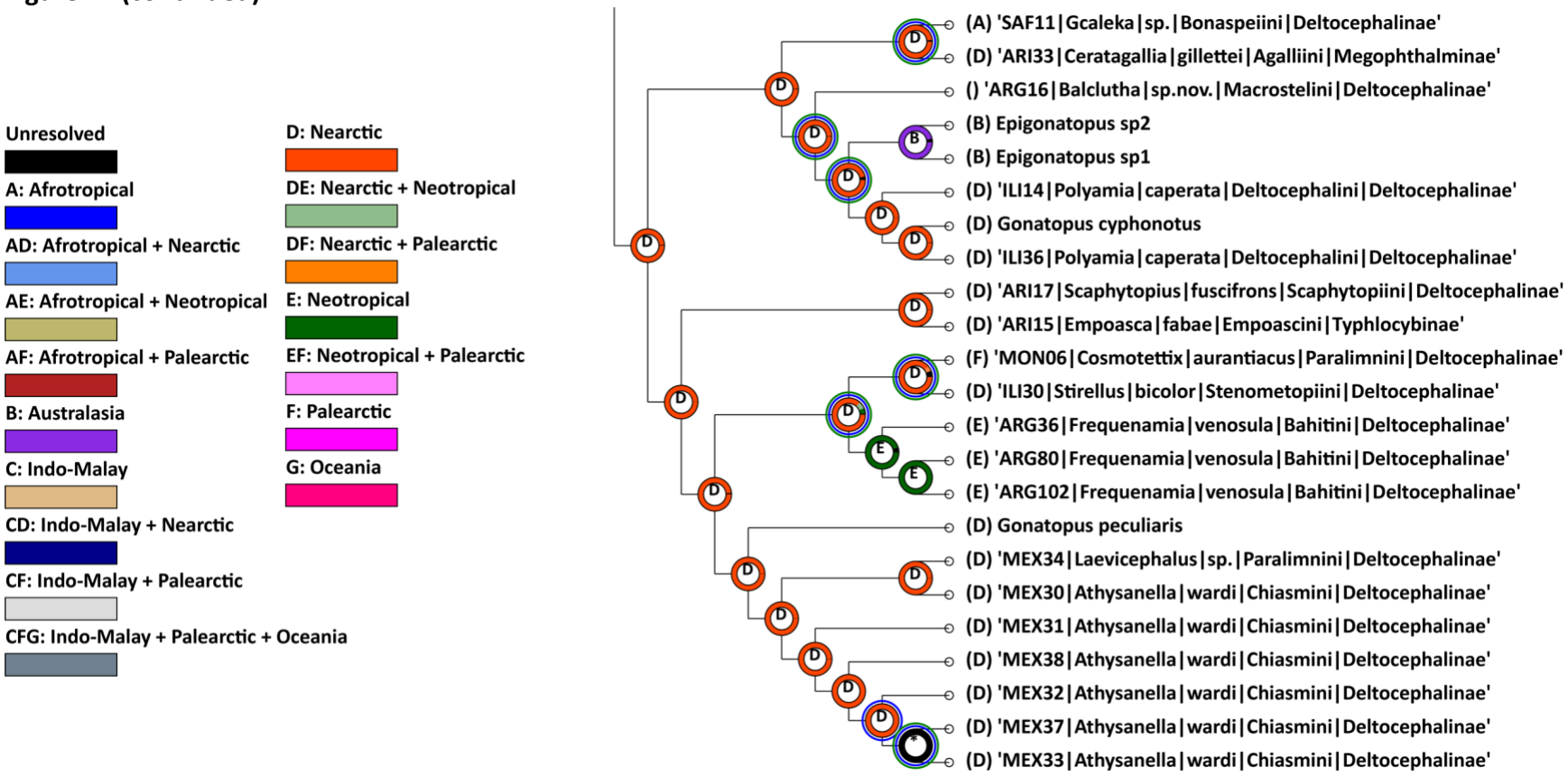

Figure 18. Summary of ancestral host reconstruction using ML under the Mk1 model. Significant results labeled in figure.

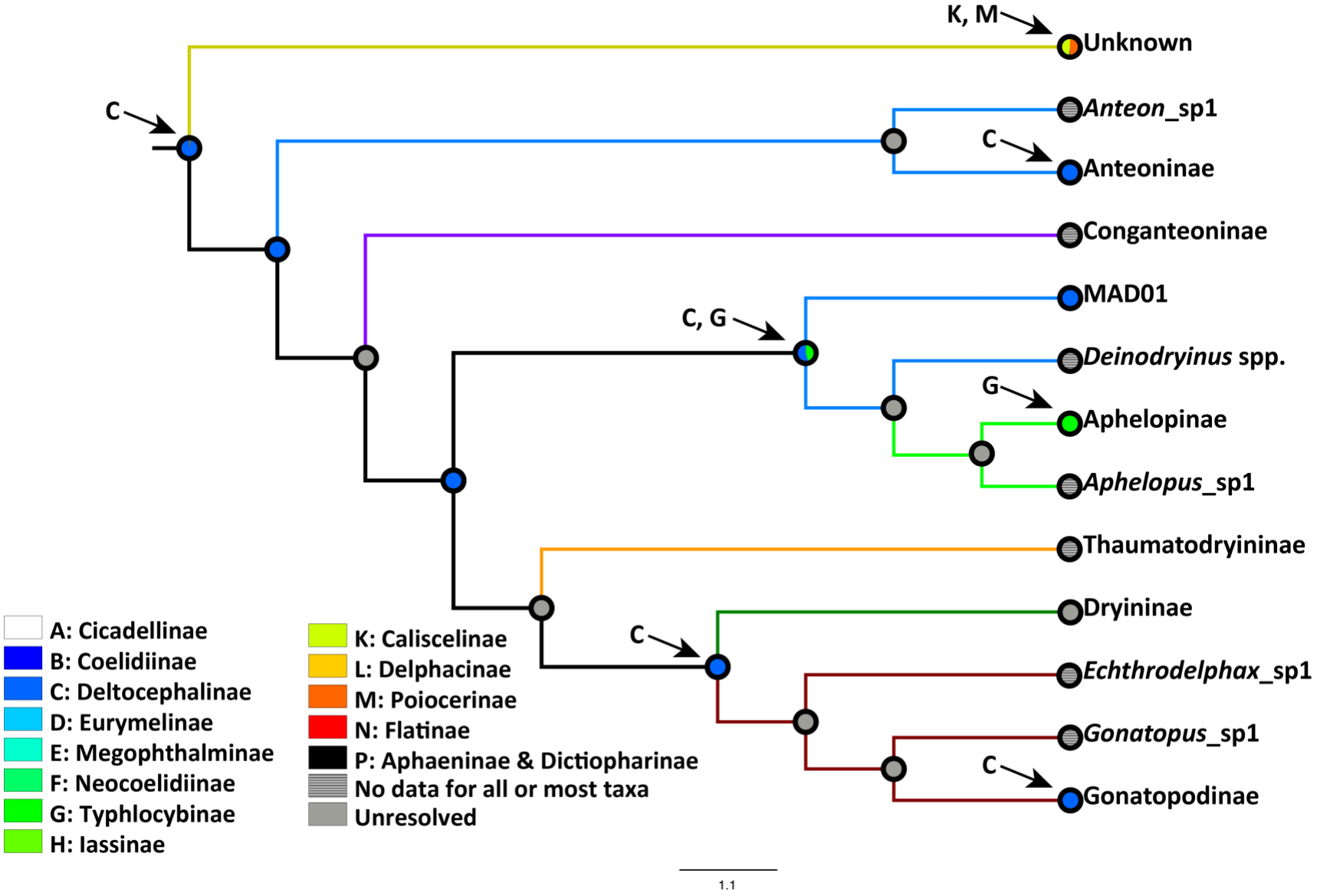

Figure 19. Summary of ancestral environment reconstruction using ML under the Mk1 model. Significant results labeled in figure.

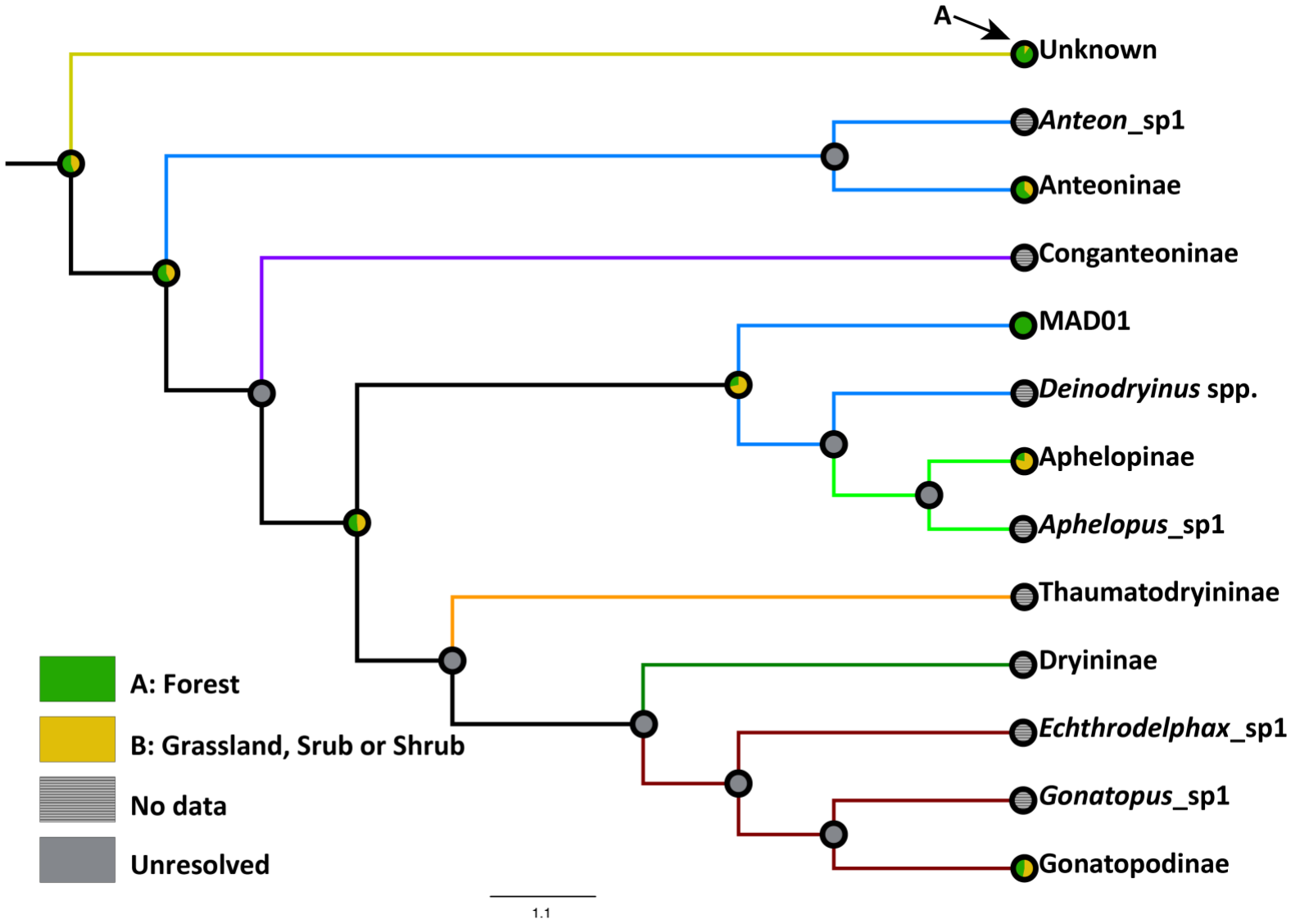

Table 1. Subfamily and genera of Dryinidae. Approximate species numbers shown only for subfamilies.

Subfamily	Distribution	Species
Burmadryininae	**Burmese amber (Myanmar)**	**1**
Burmadryinus Olmi, Xu and Guglielmino, 2014	Burmese amber (Myanmar)	
Palaeoanteoninae	**Baltic amber**	**1**
Palaeoanteon Olmi, 2000	Baltic amber	
Ponomarenkoinae	**Baltic amber**	**1**
Ponomarenkoa (N. Ponomarenko, 1988)	Baltic amber	
Protodryininae	**Baltic amber**	**1**
Protodryinus Guglielmino & Olmi, 2012	Baltic amber	
Anteoninae	**Worldwide**	**603**
Anteon Jurine, 1807	Worldwide	
Anteonopsis Olmi, Rasnitsyn & Guglielmino, 2010	Obeshchayushchiyi marl (Siberia)	
Burmanteon Engel, 2003	Burmese amber (Myanmar)	
Deinodryinus R. Perkins, 1907	Worldwide	
Janzeniola (Olmi, 2000)	Baltic amber	
Lonchodryinus Kieffer, 1905	Worldwide	
Metanteon Olmi, 1984	Neotropical	
Aphelopinae	**Worldwide**	**92**
Aphelopus Dalman, 1823	Worldwide	
Covettia Olmi, 1984	Oriental, Nearctic, Neotropical, Australia and Indo-Malay	
Apoaphelopinae	**Afrotropical**	**2**
Apoaphelopus Olmi, 2007	Afrotropical	
Apodryininae	**Gondwanian (Argentina, Chile, Galapagos Islands (Ecuador), Australia; Madagascar; South Africa)**	**13**
Apodryinus Olmi, 1984	Neotropical	
Apogonatopus Olmi 2007	Afrotropical	
Bocchopsis Olmi 1991	Australia	
Gondwanadryinus Olmi 2007	Afrotropical	
Madecadryinus Olmi 2007	Afrotropical	
Peckius Olmi & Virla, 2014	Neotropical	
Vannoortia Olmi 2007	Afrotropical	
Bocchinae	**Worldwide**	**106**
Bocchus Ashmead, 1893	Worldwide	

Table 1 (continued)

Subfamily	Distribution	Species
Myrodryinus Ponomarenko, 1972	Paleartic	
Mystrophorus Förster, 1856	Paleartic	
Conganteoninae	**Palaearctic, Afrotropical, Oriental**	**17**
Conganteon Benoit, 1951	Afrotropical & Oriental (including Nepal)	
Fiorianteon Olmi, 1984	Palaearctic and Oriental regions	
Dryininae	**Worlwide**	**346**
Cretodryinus Ponomarenko 1975	fossil, Taimyr amber	
Dryinus Latreille 1804	Worldwide	
Harpactosphecion Haupt, 1944	Palaearctic, Neotropical	
Hybristodryinus Engel, 2005	fossil, Burmese amber	
Gonadryinus Olmi, 1991	Neotropical	
Megadryinus Richards, 1953	Neotropical	
Palaeodryinus Olmi & Bechly, 2001	fossil, Baltic amber	
Pseudodryinus Olmi 1991	Palearctic, Afrotropical, Oriental and Austrlia	
Erwiniinae	**Neotropical**	**1**
Erwinius Olmi & Guglielmino, 2010	Neotropical	
Gonatopodinae	**Worldwide**	**560**
Adryinus Olmi, 1984	Afrotropical, Oriental, Nearctic, Neotropical	
Echthrodelphax Perkins, 1903	Worldwide	
Epigonatopus (R. Perkins 1905)	Australia	
Esagonatopus Olmi, 1984	Nearctic, Neotropical	
Eucamptonyx R. Perkins, 1907	Australian, Nearctic, Neotropical	
Gonatopus Ljungh, 1810	Worldwide	
Gynochelys Brues 1906	Afrotropical	
Haplogonatopus R. Perkins, 1905	Worldwide	
Neodryinus Perkins, 1905	Worldwide	
Pareucamptonyx Olmi 1991	Neotropical	
Pentagonatopus Olmi 1984	Australian, Nearctic	
Plesiodryininae	**Nearctic**	**1**
Plesiodryinus Olmi, 1987	Nearctic	
Thaumatodryininae	**Worldwide except Palearctic**	**33**
Thaumatodryinus R. Perkins, 1905	Worldwide except Palearctic	
Transdryininae	**Australia**	**2**
Transdryinus	Australia	
Incerate sedis	**Neotropical**	**2**

Table 1 (continued)

Subfamily	Distribution	Species
Chelogynus brasiliensis Arlé, 1935	Neotropical (Brazil)	
Prodryius affinis Arlé, 1935	Neotropical (Brazil)	

Table 2. Summary of Dryinidae host records. Guglielmino et al. (2013) categorized as erroneous all records of Anteoninae parasitizing Typhlocybinae.

	Anteoninae	Aphelopinae	Bocchinae	Dryininae	Gonatopodinae	Thaumatodryininae
Fulgoromorpha					**X**	
Acanaloniidae				**X**	**X**	
Caliscelidae			**X**		**X**	
Cixiidae				**X**	**X**	
Delphacidae					**X**	
Dictyopharidae				**X**	**X**	
Flatidae				**X**	**X**	**X**
Fulgoridae				**X**		
Issidae				**X**	**X**	
Lophopidae				**X**	**X**	
Meenoplidae				**X**	**X**	
Ricaniidae				**X**	**X**	
Tropiduchidae			**X**	**X**	**X**	
Cicadomorpha						
Aphrodinae					**X**	
Cicadellinae					**X**	
Coelidiinae					**X**	
Deltocephalinae	**X**		**X**		**X**	
Eurymelinae	**X**					
Iassinae	**X**				**X**	
Ledrinae	**X**					
Megophthalminae					**X**	
Tartessinae	**X**				**X**	
Typhlocybinae	**X**	**X**			**X**	
Membracidae		**X**				

Table 3. Primers developed by previous authors used for PCR of Dryinidae larvae. Only the 28S gene was successfully and consistently amplified and used for phylogenetic analysis.

Gene	Primer name	Sequence	Reference
COI	LCO1490	5'-GGTCAACAAATCATAAAGATATTGG-3'	Vrijenhoek 1994
	HCO2198	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'	
	C1-J-1859	5'-GGTACAGGTTGAACTGTTTACCCTCC-3'	Simon et al. 1994
	TL2-N-3014	5'-TCCAATGCACTAATCTGCCATATTA-3'	
CytB	CB1	5'-TATGTACTACCATGAGGACAAATATC-3'	Simon et al. 1994
	CB2	5'-ATTACACCTCCTAATTTATTAGGAAT-3'	
18S	18SF2	5'-CTACCACATCCAAGGAAGGCAG-3'	Rokas et al. 2002
	18SR2	5'-AGAGTCTCGTTCGTTATCGGA-3'	
28S	For28SVesp	5'-AGAGAGAGTTCAAGAGTACGTG-3'	Hines et al. 2007
	Rev28SVesp	5'-GGA ACC AGC TAC TAG ATG G-3'	

Table 4. Developed primers for COI gene amplification. Efforts to develop a primer for Dryinidae COI gene amplification did not yield consistent results for the amplification of this gene.

Primer name	Sequence
C1-J-1859mod	5’-GGAACAGGATGAACAGTTTACCCTCC-3’
TL2-N-3014insert	5’-TCCAATGCACTATATCTGCCATATTA-3’

Table 5. PCR protocols for each gene. Only the 28S ribosomal gene was sequenced successfully with primer pair For28SVesp/Rev28SVesp.

Primer Pair	Step	Temperature	Time	Cycles
HCO2198/ LCO1490	Heat Lid	110°C		
	Initial Temp.	95°C	5:00	
	Denaturation	95°C	1:00	
	Annealing	40°C	1:00	35
	Extension	72°C	1:30	
	Final Extension	72°C	10:00	
	Hold	10°C	Until removal	
C1-J-1859/ TL2-N-3014	Heat Lid	110°C		
	Initial Temp.	94°C	5:00	
	Denaturation	94°C	0:30	
	Annealing	50°C	1:00	35
	Extension	72°C	1:00	
	Final Extension	72°C	5:00	
	Hold	10°C	Until removal	
C1-J-1859mod/ TL2-N-3014insert	Heat Lid	110°C		
	Initial Temp.	94°C	5:00	
	Denaturation	94°C	0:30	
	Annealing	50°C	1:00	35
	Extension	72°C	1:00	
	Final Extension	72°C	5:00	
	Hold	10°C	Until removal	
CB1/CB2	Heat Lid	110°C		
	Initial Temp.	95°C	5:00	
	Denaturation	95°C	1:00	
	Annealing	42°C	1:00	40
	Extension	72°C	1:00	
	Final Extension	72°C	10:00	
	Hold	10°C	Until removal	
18SF2/18SR2	Heat Lid	110°C		
	Initial Temp.	95°C	5:00	
	Denaturation	95°C	0:30	

Table 5 (continued)

Primer Pair	Step	Temperature	Time	Cycles
	Annealing	56°C	0:40	34
	Extension	72°C	0:40	
	Final Extension	72°C	10:00	
	Hold	10°C	Until removal	
For28SVesp/ Rev28SVesp	Heat Lid	110°C		
	Initial Temp.	94°C	5:00	
	Denaturation	94°C	1:00	35
	Annealing	50°C	1:00	
	Extension	72°C	1:00	
	Final Extension	72°C	5:00	
	Hold	10°C	Until removal	

Table 6. TD-PCR protocols for For28SVesp/Rev28SVesp. The touchdown PCR is performed in the initial 10 cycles starting at 58°C and decreasing 1°C each cycle until reaching 48°C. Only a subset of samples that did not amplified in normal PCR conditions was chosen for this method.

Primer Pair	Step	Temperature	Time	Cycles
For28SVesp/ Rev28SVesp	Heat Lid	110°C		
	Initial Temp.	94°C	5:00	
	Denaturation	94°C	0:40	
	Annealing	58-48°C	0:40	10
	Extension	72°C	1:00	
	Denaturation	94°C	0:40	
	Annealing	48°C	0:40	35
	Extension	72°C	1:00	
	Final Extension	72°C	5:00	
	Hold	10°C	Until removal	

Table 7. P-distances of 28S D2-D3 region. Larvae were included when the minimum p-distance of sequences from adults (Tribul 2015) was higher than zero.

Genus	Mean	Max	Min
Anteoninae	4.45	7.12	0.00
Aphelopinae w. larvae	5.63	9.89	0.00
Aphelopinae	4.42	6.43	2.28
Deinodryinus spp.	3.15	5.16	0.00
Dryinus spp.	5.25	9.45	0.44
Gonatopodinae	2.68	4.82	0.00
Thaumatodryinus spp.	4.03	4.03	4.03
Unknown Dryinidae	4.99	8.91	0.00

Table 8. Number of sequenced samples by biogeographic location. The Australasia sample AUS-01 provided one hopper specimen from which 4 dryinid larvae were extracted. Hoppers No., N=140; Larvae No., N=142; Adult No., N=1.

Location	Hoppers No.	Larvae No.	Adult No.
Afotropical	5	5	0
Australasia	1	4	0
Indo-Malay	7	7	0
Nearctic	67	66	1
Neotropical	39	39	0
Palearctic	20	20	0
Unknown	1	1	0

Table 9. Number of host records by host family.

Host Family	Records
Caliscelidae	5
Cicadellidae	131
Delphacidae	2
Eurybrachidae	4

Table 10. Number of host records by host subfamilies.

Subfamily	Hosts No.
Cicadellinae	2
Coelidiinae	1
Deltocephalinae	93
Eurymelinae	2
Iassinae	1
Megophthalminae	19
Neocoelidiinae	1
Typhlocybinae	12
Caliscelinae	5
Delphacinae	2
Platybrachinae	1

Table 11. Number of cicadellid tribes with hosts.

Cicadellid Tribes	Hosts No.
Acostemmini	1
Agalliini	19
Athysanini	3
Bahitini	4
Bonaspeiini	1
Chiasmini	13
Cicadellini	2
Deltocephalini	33
Empoascini	10
Erythroneurini	2
Hecalini	2
Hyalojassini	1
Idiocerini	1
Limotettigini	1
Macropsini	1
Macrostelini	4
Neocoelidiini	1
Opsiini	1
Paralimnini	24
Pendarini	1
Phlepsiini	1
Scaphytopiini	1
Stenometopiini	2
Tinobregmini	1
Undertermined	1

Table 12. Sequenced samples (N=143). Specimens belonging to the same new species are specified in Table 9. UNK-02 location is inferred from the phylogenetic analysis as it formed a clade with high support with a specimen from Arizona, ARI-13. [1] indicates new geographic records; [2] indicates unidentified nymphs; [3] indicates unidentified females; [4] indicates male or sample for which male was available but with atrophied genitalia. Codes with decimals indicate multiple larvae removed from the same host individual.

ID	Locality	Host Family	Subfamily	Tribe	Genera	Species	Larvae ID
ARG-101	Argentina: Chaco	Cicadellidae	Deltocephalinae	Deltocephalini	*Haldorus* (*Haldorellus*)	*furcatus*	Gonatopodinae
ARG-102	Argentina: Chaco	Cicadellidae	Deltocephalinae	Bahitini	*Frequenamia*	*venosula*	Gonatopodinae
ARG-103	Argentina: Corrientes	Cicadellidae	Deltocephalinae	Deltocephalini	*Graminella*	*stelliger expansa*	Gonatopodinae
ARG-104	Argentina: Chaco	Cicadellidae	Deltocephalinae	Athysanini	*Atanus*	*luqueatus equalis*	Gonatopodinae
ARG-16	Argentina: Jujuy	Cicadellidae	Deltocephalinae	Macrostelini	*Balclutha*	*new species*	Gonatopodinae
ARG-34	Argentina: Chaco	Cicadellidae	Deltocephalinae	Deltocephalini	*Haldorus* (*Haldorellus*)	*new species*	Gonatopodinae
ARG-36	Argentina: Chaco	Cicadellidae	Deltocephalinae	Bahitini	*Frequenamia*	*venosula*	Gonatopodinae
ARG-59	Argentina: Misiones	Cicadellidae	Neocoelidiinae	Neocoelidiini	*Coelidiana*	*brasiliensis*	Gonatopodinae
ARG-60	Argentina: Corrientes	Cicadellidae	Cicadellinae	Cicadellini	*Hortensia*	*similis*	Gonatopodinae
ARG-74	Argentina: Jujuy	Cicadellidae	Deltocephalinae	Hecalini	*Spangbergiella*	*felix*	Gonatopodinae
ARG-78	Argentina: Chaco	Caliscelidae	Caliscelinae	Caliscelini	*Plagiopsis*	sp.	Unknown
ARG-79	Argentina: Salta	Cicadellidae	Deltocephalinae	Deltocephalini	*Amplicephalus*	*rotundiceps*	Gonatopodinae
ARG-80	Argentina: Jujuy	Cicadellidae	Deltocephalinae	Bahitini	*Frequenamia*	*venosula*	Gonatopodinae
ARG-81	Argentina: Jujuy	Cicadellidae	Deltocephalinae	Deltocephalini	*Haldorus*	*new species*	Gonatopodinae
ARG-84	Argentina: Buenos Aires	Cicadellidae	Deltocephalinae	Deltocephalini	*Haldorus*	*sexpunctatus*	Gonatopodinae
ARG-85	Argentina: Buenos Aires	Cicadellidae	Deltocephalinae	Deltocephalini	*Haldorus* (*Haldorellus*)	*new species*	Gonatopodinae
ARG-86	Argentina: Buenos Aires	Cicadellidae	Deltocephalinae	Deltocephalini	*Amplicephalus*	*luridus*	Gonatopodinae
ARG-87	Argentina: Buenos Aires	Cicadellidae	Deltocephalinae	Deltocephalini	*Haldorus* (*Haldorellus*)	*new species*	Gonatopodinae
ARG-88	Argentina: Buenos Aires	Cicadellidae	Deltocephalinae	Deltocephalini	*Haldorus*	*sexpunctatus*	Gonatopodinae
ARG-89	Argentina: Jujuy	Cicadellidae	Deltocephalinae	Deltocephalini	*Mendozellus*	*asunctia*	Gonatopodinae
ARG-90	Argentina: Jujuy	Cicadellidae	Deltocephalinae	Deltocephalini	*Mendozellus*	*asunctia*	Gonatopodinae
ARG-91	Argentina: Jujuy	Cicadellidae	Deltocephalinae	Deltocephalini	*NEW GENUS*	*new species*	Gonatopodinae
ARG-92	Argentina: Chaco	Caliscelidae	Caliscelinae	Caliscelini	*Plagiopsis*	sp.	Unknown
ARG-93	Argentina: Corrientes	Cicadellidae	Deltocephalinae	Deltocephalini	*Graminella*	*stelliger expansa*	Gonatopodinae

Table 12 (continued)

ID	Locality	Host Family	Subfamily	Tribe	Genera	Species	Larvae ID
ARG-94	Argentina: Corrientes	Cicadellidae	Deltocephalinae	Deltocephalini	*Graminella*	*stelliger expansa*	Gonatopodinae
ARG-95	Argentina: Corrientes	Cicadellidae	Deltocephalinae	Deltocephalini	*Graminella*	*stelliger expansa*	Gonatopodinae
ARG-98	Argentina: Buenos Aires	Cicadellidae	Deltocephalinae	Deltocephalini	*Haldorus (Haldorellus)*	*new species*	Gonatopodinae
ARG-99	Argentina: Buenos Aires	Cicadellidae	Deltocephalinae	Deltocephalini	*Amplicephalus*	*luridus*	Gonatopodinae
ARI-01	USA: Arizona: Graham Co.	Cicadellidae	Megophthalminae	Agalliini	*Ceratagallia*	*gillettei*	Gonatopodinae
ARI-02	USA: Arizona: Graham Co.	Cicadellidae	Megophthalminae	Agalliini	*Ceratagallia*	*gillettei*	Gonatopodinae
ARI-03	USA: Arizona: Graham Co.	Cicadellidae	Deltocephalinae	Pendarini	*Paraphlepsius*	*micronotatus*	Gonatopodinae
ARI-05	USA: Arizona: Santa Cruz Co.	Cicadellidae	Deltocephalinae	Paralimnini	*Laevicephalus*	sp.	Gonatopodinae
ARI-06	USA: Arizona: Graham Co.	Cicadellidae	Typhlocybinae	Empoascini	*Empoasca*	sp.	Aphelopinae
ARI-08	USA: Arizona: Graham Co.	Cicadellidae	Megophthalminae	Agalliini	*Ceratagallia*	*gillettei*	Gonatopodinae
ARI-13	USA: Arizona: Graham Co.	Cicadellidae	Iassinae	Hyalojassini	*Penestragania*	*robusta*	Gonatopodinae
ARI-14	USA: Arizona: Graham Co.	Cicadellidae	Deltocephalinae	Chiasmini	*Exitianus*	*exitiosus*	Gonatopodinae
ARI-15	USA: Arizona: Graham Co.	Cicadellidae	Typhlocybinae	Empoascini	*Empoasca*	*fabae*	Gonatopodinae
ARI-16	USA: Arizona: Santa Cruz Co.	Cicadellidae	Deltocephalinae	Athysanini	*Cetexa*	*graecula*	Gonatopodinae
ARI-17	USA: Arizona: Graham Co.	Cicadellidae	Deltocephalinae	Scaphytopiini	*Scaphytopius (Cloanthanus)*	*fuscifrons*	Gonatopodinae
ARI-18	USA: Arizona: Graham Co.	Cicadellidae	Megophthalminae	Agalliini	*Ceratagallia*	*gillettei*	Gonatopodinae
ARI-20	USA: Arizona: Graham Co.	Cicadellidae	Megophthalminae	Agalliini	*Ceratagallia*	*gillettei*	Gonatopodinae
ARI-21	USA: Arizona: Graham Co.	Cicadellidae	Megophthalminae	Agalliini	*Ceratagallia*	*gillettei*	Gonatopodinae
ARI-22	USA: Arizona: Graham Co.	Cicadellidae	Megophthalminae	Agalliini	*Ceratagallia*	*gillettei*	Gonatopodinae
ARI-23	USA: Arizona: Graham Co.	Cicadellidae	Megophthalminae	Agalliini	*Ceratagallia*	*gillettei*	Gonatopodinae
ARI-24	USA: Arizona: Graham Co.	Cicadellidae	Typhlocybinae	Empoascini	*Empoasca*	sp.	Aphelopinae
ARI-25	USA: Arizona: Pima Co.	Cicadellidae	Megophthalminae	Agalliini	*Ceratagallia*	*gillettei*	Gonatopodinae
ARI-26	USA: Arizona: Pima Co.	Cicadellidae	Megophthalminae	Agalliini	*Ceratagallia*	*gillettei*	Gonatopodinae
ARI-27	USA: Arizona: Pima Co.	Cicadellidae	Megophthalminae	Agalliini	*Ceratagallia*	*gillettei*	Gonatopodinae

Table 12 (continued)

ID	Locality	Host Family	Subfamily	Tribe	Genera	Species	Larvae ID
ARI-28	USA: Arizona: Pima Co.	Cicadellidae	Megophthalminae	Agalliini	*Ceratagallia*	*gillettei*	Gonatopodinae
ARI-29	USA: Arizona: Pima Co.	Cicadellidae	Megophthalminae	Agalliini	*Ceratagallia*	*gillettei*	Gonatopodinae
ARI-30	USA: Arizona: Graham Co.	Cicadellidae	Megophthalminae	Agalliini	*Ceratagallia*	*gillettei*	Gonatopodinae
ARI-31	USA: Arizona: Graham Co.	Cicadellidae	Megophthalminae	Agalliini	*Ceratagallia*	*gillettei*	Gonatopodinae
ARI-32	USA: Arizona: Graham Co.	Cicadellidae	Megophthalminae	Agalliini	*Ceratagallia*	*gillettei*	Gonatopodinae
ARI-33	USA: Arizona: Graham Co.	Cicadellidae	Megophthalminae	Agalliini	*Ceratagallia*	*gillettei*	Gonatopodinae
ARI-34	USA: Arizona: Graham Co.	Cicadellidae	Megophthalminae	Agalliini	*Ceratagallia*	*gillettei*	Gonatopodinae
AUS-01.1	Australia: Queensland	Eurybrachidae	Platybrachinae	Platybrachini	*Platybrachys*	sp.	Unknown
AUS-01.2	Australia: Queensland	Eurybrachidae	Platybrachinae	Platybrachini	*Platybrachys*	sp.	Unknown
AUS-01.3	Australia: Queensland	Eurybrachidae	Platybrachinae	Platybrachini	*Platybrachys*	sp.	Unknown
AUS-01.4	Australia: Queensland	Eurybrachidae	Platybrachinae	Platybrachini	*Platybrachys*	sp.	Unknown
CAL-01	USA: California: Inyo Co.	Cicadellidae	Deltocephalinae	Chiasmini	*Exitianus*	*exitiosus*	Gonatopodinae
CAL-03	USA: California: Riverside Co.	Cicadellidae	Deltocephalinae	Deltocephalini	*Graminella*	*sonora*	Gonatopodinae
CAL-04	USA: California: Riverside Co.	Cicadellidae	Typhlocybinae	Empoascini	*Empoasca*	sp.	Aphelopinae
CAL-05	USA: California: Riverside Co.	Cicadellidae	Typhlocybinae	Empoascini	*Empoasca*	*fabae*	Aphelopinae
FRA-02	France: Mont. Ventoux	Cicadellidae	Deltocephalinae	Paralimnini	*Jassargus*	*obtusivalvis*	Gonatopodinae
ILI-07	USA: Illinois: Ford Co.	Cicadellidae	Eurymelinae	Idiocerini	*Idiocerus*	*varians*	Gonatopodinae
ILI-09	USA: Illinois: Ford Co.	Cicadellidae	Deltocephalinae	Macrostelini	*Balclutha*	sp.	Gonatopodinae
ILI-10	USA: Illinois: Ford Co.	Cicadellidae	Deltocephalinae	Macrostelini	*Balclutha*	sp.	Gonatopodinae
ILI-11	USA: Illinois: Ford Co.	Cicadellidae	Deltocephalinae	Macrostelini	*Balclutha*	sp.	Gonatopodinae
ILI-12[2]	USA: Illinois: Ford Co.	Cicadellidae	Deltocephalinae	-	-	-	*Anteon* sp. (Anteoninae)
ILI-13[3]	USA: Illinois: Ford Co.	Delphacidae	Delphacinae	Delphacini	-	-	Gonatopodinae

Table 12 (continued)

ID	Locality	Host Family	Subfamily	Tribe	Genera	Species	Larvae ID
ILI-14	USA: Illinois: Vermilion Co.	Cicadellidae	Deltocephalinae	Deltocephalini	*Polyamia*	*caperata*	Gonatopodinae
ILI-15	USA: Illinois: Ford Co.	Cicadellidae	Coelidiinae	Tinobregmini	*Tinobregnus*	*viridescens*	*Anteon* sp. (Anteoninae)
ILI-18	USA: Illinois: Mason Co.	Cicadellidae	Typhlocybinae	Empoascini	*Empoasca*	*fabae*	Aphelopinae
ILI-21	USA: Illinois: Mason Co.	Caliscelidae	Caliscelinae	Peltonotellini	*Bruchomorpha*	*jocosa*	Unknown
ILI-22	USA: Illinois: Mason Co.	Caliscelidae	Caliscelinae	Peltonotellini	*Bruchomorpha*	*abrupta*	Unknown
ILI-24	USA: Illinois: Mason Co.	Cicadellidae	Deltocephalinae	Deltocephalini	*Polyamia*	*caperata*	*Anteon* sp. (Anteoninae)
ILI-25[1]	USA: Illinois: Mason Co.	Dryinidae	Gonatopodinae	-	*Gonatopus*	*elongatus*	Gonatopodinae
ILI-29	USA: Illinois: Mason Co.	Cicadellidae	Deltocephalinae	Paralimnini	*Flexamia*	*sandersi*	Gonatopodinae
ILI-30[1]	USA: Illinois: Mason Co.	Cicadellidae	Deltocephalinae	Stenometopiini	*Stirellus*	*bicolor*	Gonatopodinae
ILI-31	USA: Illinois: Mason Co.	Cicadellidae	Deltocephalinae	Bahitini	*Menosoma*	*cincta*	Gonatopodinae
ILI-32	USA: Illinois: Mason Co.	Cicadellidae	Megophthalminae	Agalliini	*Ceratagallia*	*agricola*	Gonatopodinae
ILI-34	USA: Illinois: Mason Co.	Cicadellidae	Deltocephalinae	Deltocephalini	*Polyamia*	*caperata*	*Anteon* sp. (Anteoninae)
ILI-35	USA: Illinois: Mason Co.	Cicadellidae	Deltocephalinae	Deltocephalini	*Polyamia*	*caperata*	*Anteon* sp. (Anteoninae)
ILI-36	USA: Illinois: Mason Co.	Cicadellidae	Deltocephalinae	Deltocephalini	*Polyamia*	*caperata*	Gonatopodinae
MAD-01	Madagascar	Cicadellidae	Deltocephalinae	Acostemmini	*Acostemma*	sp. nov.	Anteoninae
MEX-01	Mexico: Jalisco	Cicadellidae	Deltocephalinae	Phlepsiini	*Texananus*	sp.	*Anteon* sp. (Anteoninae)
MEX-02	Mexico: Jalisco	Cicadellidae	Deltocephalinae	Deltocephalini	*Amplicephalus*	*trilobatus*	Gonatopodinae
MEX-03[2]	Mexico: Jalisco	Cicadellidae	Deltocephalinae	Paralimnini	-	-	*Anteon* sp. (Anteoninae)
MEX-16	Mexico: Jalisco	Cicadellidae	Deltocephalinae	Deltocephalini	*Graminella*	*comata*	*Anteon* sp. (Anteoninae)
MEX-17	Mexico: Jalisco	Cicadellidae	Deltocephalinae	Chiasmini	*Exitianus*	sp.	*Anteon* sp. (Anteoninae)
MEX-19	Mexico: Jalisco	Cicadellidae	Deltocephalinae	Deltocephalini	*Amplicephalus*	*trilobatus*	Gonatopodinae
MEX-21	Mexico: Jalisco	Cicadellidae	Deltocephalinae	Deltocephalini	*Polyamia*	*satur*	Gonatopodinae

Table 12 (continued)

ID	Locality	Host Family	Subfamily	Tribe	Genera	Species	Larvae ID
MEX-22	Mexico: Jalisco	Cicadellidae	Deltocephalinae	Deltocephalini	*Polyamia*	*satur*	*Anteon* sp. (Anteoninae)
MEX-23	Mexico: Jalisco	Cicadellidae	Deltocephalinae	Deltocephalini	*Polyamia*	*satur*	*Anteon* sp. (Anteoninae)
MEX-24	Mexico: Jalisco	Cicadellidae	Deltocephalinae	Deltocephalini	*Amplicephalus*	*sonorus*	*Anteon* sp. (Anteoninae)
MEX-25	Mexico: Jalisco	Cicadellidae	Deltocephalinae	Deltocephalini	*Amplicephalus*	*sonorus*	*Anteon* sp. (Anteoninae)
MEX-26	Mexico: Jalisco	Cicadellidae	Deltocephalinae	Stenometopiini	*Stirellus*	*bicolor*	Gonatopodinae
MEX-27	Mexico: Cohauila	Cicadellidae	Deltocephalinae	Paralimnini	*Flexamia*	*abreviatta*	*Anteon* sp. (Anteoninae)
MEX-29	Mexico: Cohauila	Cicadellidae	Deltocephalinae	Chiasmini	*Athysanella (Gladionura)*	*wardi*	Gonatopodinae
MEX-30	Mexico: Cohauila	Cicadellidae	Deltocephalinae	Chiasmini	*Athysanella (Gladionura)*	*wardi*	Gonatopodinae
MEX-31	Mexico: Cohauila	Cicadellidae	Deltocephalinae	Chiasmini	*Athysanella (Gladionura)*	*wardi*	Gonatopodinae
MEX-32	Mexico: Cohauila	Cicadellidae	Deltocephalinae	Chiasmini	*Athysanella (Gladionura)*	*wardi*	Gonatopodinae
MEX-33	Mexico: Cohauila	Cicadellidae	Deltocephalinae	Chiasmini	*Athysanella (Gladionura)*	*wardi*	Gonatopodinae
MEX-34	Mexico: Cohauila	Cicadellidae	Deltocephalinae	Paralimnini	*Laevicephalus*	sp.	Gonatopodinae
MEX-35	Mexico: Cohauila	Cicadellidae	Deltocephalinae	Chiasmini	*Athysanella (Gladionura)*	*wardi*	Gonatopodinae
MEX-37	Mexico: Cohauila	Cicadellidae	Deltocephalinae	Chiasmini	*Athysanella (Gladionura)*	*wardi*	Gonatopodinae
MEX-38	Mexico: Cohauila	Cicadellidae	Deltocephalinae	Chiasmini	*Athysanella (Gladionura)*	*wardi*	Gonatopodinae
MEX-39	Mexico: Cohauila	Caliscelidae	Caliscelinae	Peltonotellini	*Aphelonema*	sp.	Gonatopodinae
MEX-40	Mexico: Cohauila	Cicadellidae	Deltocephalinae	Chiasmini	*Athysanella (Gladionura)*	*wardi*	Gonatopodinae
MEX-41	Mexico: Cohauila	Cicadellidae	Deltocephalinae	Chiasmini	*Athysanella (Gladionura)*	*wardi*	Gonatopodinae
MEX-42	Mexico: Durango	Cicadellidae	Deltocephalinae	Deltocephalini	*Graminella*	*barinasensis*	Gonatopodinae
MON-03	Mongolia: Khövsgöl	Cicadellidae	Deltocephalinae	Paralimnini	*Emeljanovianus*	*medius*	Anteoninae
MON-04	Mongolia: Khövsgöl	Cicadellidae	Deltocephalinae	Paralimnini	*Diplocolenus*	*frauenfeldi*	Gonatopodinae
MON-05	Mongolia: Khövsgöl	Cicadellidae	Deltocephalinae	Paralimnini	*Diplocolenus*	*frauenfeldi*	Gonatopodinae
MON-06	Mongolia: Bulgan	Cicadellidae	Deltocephalinae	Paralimnini	*Cosmotettix (Agapelus)*	*aurantiacus*	Gonatopodinae

Table 12 (continued)

ID	Locality	Host Family	Subfamily	Tribe	Genera	Species	Larvae ID
MON-07	Mongolia: Selenge	Cicadellidae	Deltocephalinae	Paralimnini	*Sorhoanus*	*xanthoneurus*	Anteoninae
MON-08	Mongolia: Selenge	Cicadellidae	Deltocephalinae	Paralimnini	*Diplocolenus*	*abdominalis*	Anteoninae
MON-09	Mongolia: Selenge	Cicadellidae	Deltocephalinae	Paralimnini	*Diplocolenus*	*funebris*	Anteoninae
MON-10	Mongolia: Selenge	Cicadellidae	Deltocephalinae	Limotettigini	*related to Limotettix*	-	Gonatopodinae
MON-11	Mongolia: Selenge	Cicadellidae	Deltocephalinae	Paralimnini	*Psammotettix*	*atropidicola*	Anteoninae
MON-13	Mongolia: Selenge	Cicadellidae	Deltocephalinae	Paralimnini	-	-	Gonatopodinae
MON-14	Mongolia: Selenge	Cicadellidae	Deltocephalinae	Paralimnini	*Mogangina (Tungara)*	*chubsugulica*	Anteoninae
MON-15	Mongolia: Selenge	Cicadellidae	Deltocephalinae	Paralimnini	*Mogangina (Tungara)*	*chubsugulica*	Anteoninae
MON-16	Mongolia: Selenge	Cicadellidae	Deltocephalinae	Paralimnini	*Psammotettix*	*striatus*	Gonatopodinae
MON-17	Mongolia: Selenge	Cicadellidae	Deltocephalinae	Paralimnini	*Psammotettix*	*striatus*	Gonatopodinae
MON-18	Mongolia: Selenge	Cicadellidae	Deltocephalinae	Paralimnini	*Diplocolenus*	*funebris*	Gonatopodinae
MON-19	Mongolia: Selenge	Cicadellidae	Deltocephalinae	Paralimnini	*Diplocolenus*	*funebris*	Anteoninae
MON-20	Mongolia: Selenge	Cicadellidae	Deltocephalinae	Paralimnini	*Pantallus*	*alboniger*	Gonatopodinae
MON-21	Mongolia: Selenge	Cicadellidae	Deltocephalinae	Paralimnini	*Pantallus*	*alboniger*	Gonatopodinae
MON-23	Mongolia: Selenge	Cicadellidae	Deltocephalinae	Paralimnini	*Pantallus*	*alboniger*	Gonatopodinae
NEB-01	Nebraska: Lodgepole	Cicadellidae	Deltocephalinae	Hecalini	*Hecalus*	sp.	Gonatopodinae
SAF-11	South Africa: West Cape Prov.	Cicadellidae	Deltocephalinae	Bonaspeiini	*Gcaleka*	sp.	Gonatopodinae
SAF-38	South Africa: West Cape Prov.	Cicadellidae	Typhlocybinae	Empoascini	*Empoasca*	sp.	Aphelopinae
SAF-39	South Africa: West Cape Prov.	Cicadellidae	Typhlocybinae	Empoascini	*Empoasca*	sp.	Aphelopinae
TAI-21	Taiwan: Taichung	Cicadellidae	Typhlocybinae	Empoascini	*Empoasca*	sp.	Aphelopinae
TAI-29[1]	Taiwan: Taichung	Cicadellidae	Deltocephalinae	Opsiini	*Hishimonus*	sp.	*Anteon* sp. (Anteoninae)
TAI-30	Taiwan: Nantou	Delphacidae	Delphacinae	Delphacini	*Sogatella*	*vibix*	Gonatopodinae
THA-01[4]	Thailand: Kanchanaburi Khuean Srinagarindra NP	Cicadellidae	Typhlocybinae	Erythroneurini	-	-	Aphelopinae

Table 12 (continued)

ID	Locality	Host Family	Subfamily	Tribe	Genera	Species	Larvae ID
THA-02[3]	Thailand: Kanchanaburi Khuean Srinagarindra NP	Cicadellidae	Typhlocybinae	Empoascini	-	-	Aphelopinae
THA-10[3]	Thailand: Kanchanaburi Khuean Srinagarindra NP	Cicadellidae	Typhlocybinae	Erythroneurini	-	-	Aphelopinae
THA-12	Thailand: Phetchabun Thung Salaeng Luang NP	Cicadellidae	Cicadellinae	Cicadellini	*Kolla*	*paulula*	Anteoninae
UNK-02	USA: Arizona	Cicadellidae	Deltocephalinae	Athysanini	*Chlorotettix*	*lusorius*	Gonatopodinae
ZAM-01	Zambia: Copperbelt Prov.	Cicadellidae	Eurymelinae	Macropsini	*Pediopsoides*	sp.	*Anteon* sp. (Anteoninae)

Table 13. Samples with unsuccessful sequencing (N=10). [1] indicates new geographic records; [2] indicates unidentified nymphs; [3] indicates unidentified females; [4] indicates male or sample for which male was available but with atrophied genitalia.

ID	Locality	Host Family	Subfamily	Tribe	Genera	Species
ARG-105	Argentina: Jujuy	Cicadellidae	Deltocephalinae	Faltalini	*Clorindaia*	*latiabdoma*
ARG-62	Argentina: Jujuy	Cicadellidae	Deltocephalinae	Deltocephalini	NEW GENUS	new species
AUS-01	Australia: Queensland	Eurybrachidae	Platybrachinae	Platybrachini	*Platybrachys*	sp.
CAL-02	USA: California: Inyo Co.	Cicadellidae	Eurymelinae	Idiocerini	*Idiocerus*	*catalinus*
ECU-01	Ecuador: Orellana	Cicadellidae	Megophthalminae	Agalliini	*Agalliopsis*	*elegans*
ECU-02	Ecuador: Orellana	Cicadellidae	Typhlocybinae	Empoascini	*Empoasca*	*new species*
ILI-06	USA: Illinois: Ford Co.	Cicadellidae	Eurymelinae	Idiocerini	*Idiocerus*	*varians*
ILI-08	USA: Illinois: Ford Co.	Delphacidae	Delphacinae	Delphacini	*Prokelisia*	*crocea*
MEX-43	Mexico: Durango	Cicadellidae	Deltocephalinae	Deltocephalini	*Graminella*	*barinasensis*
MON-02	Mongolia: Khövsgöl	Cicadellidae	Deltocephalinae	Paralimnini	*Diplocolenus*	*altaicus*

Table 14. New host records per country (N=69). In cases where identification reached the genus level, a record was considered new if no dryinid was previously reported to parasitize that genus. Sample name provided for reference to Table 1. [1] Adult record resulting in Northern range expansion.

Sample	Country	Subfamily	Genus	species
ARG-16	Argentina	Deltocephalinae	*Balclutha*	sp. nov.
ARG-34, 98	Argentina	Deltocephalinae	*Haldorus* (*Haldorus*)	sp. nov.
ARG-36, 80, 102	Argentina	Deltocephalinae	*Frequenamia*	*venosula*
ARG-59	Argentina	Neocoelidiinae	*Coelidiana*	*brasiliensis*
ARG-60	Argentina	Cicadellinae	*Hortensia*	*similis*
ARG-62, 91	Argentina	Deltocephalinae	gen. nov.	sp. nov.
ARG-74	Argentina	Deltocephalinae	*Spangbergiella*	*felix*
ARG-78, 92	Argentina	Caliscelinae	*Plagiopsis*	sp.
ARG-79	Argentina	Deltocephalinae	*Amplicephalus*	*rotundiceps*
ARG-81	Argentina	Deltocephalinae	*Haldorus*	sp. nov.
ARG-85, 87	Argentina	Deltocephalinae	*Haldorus* (Haldorus)	sp. nov.
ARG-86, 99	Argentina	Deltocephalinae	*Amplicephalus*	*luridus*
ARG-101	Argentina	Deltocephalinae	*Haldorus* (*Haldorus*)	*furcatus*
ARG-104	Argentina	Deltocephalinae	*Atanus*	*luqueatus* *equalis*
ARI-01-02, 08, 18, 21-23, 25-34	Arizona	Megophthalminae	*Ceratagallia*	*gillettei*
ARI-03	Arizona	Megophthalminae	*Paraphlepsius*	*micronotatus*
ARI-05	Arizona	Deltocephalinae	*Laevicephalus*	sp.
ARI-13	Arizona	Megophthalminae	*Penestragania*	*robusta*
ARI-15	Arizona	Typhlocybinae	*Empoasca*	*fabae*
ARI-16	Arizona	Deltocephalinae	*Cetexa*	*graecula*
ARI-17	Arizona	Deltocephalinae	*Scaphytopius* (*Cloanthanus*)	*fuscifrons*
AUS-01	Australia	Platybrachinae	*Platybrachys*	sp.
CAL-01	California	Eurymelinae	*Idiocerus*	sp.
CAL-02	California	Eurymelinae	*Idiocerus*	*catalinus*
CAL-03	California	Deltocephalinae	*Graminella*	*sonora*
CAL-05	California	Typhlocybinae	*Empoasca*	*fabae*
ECU-01	Ecuador	Megophthalminae	*Agalliopsis*	*elegans*
ECU-02	Ecuador	Typhlocybinae	*Empoasca*	sp. nov.
ILI-06-07	Illinois	Eurymelinae	*Idiocerus*	*varians*
ILI-08	Illinois	Delphacinae	*Prokelisia*	*crocea*
ILI-14, 36	Illinois	Deltocephalinae	*Polyamia*	*caperata*
ILI-15	Illinois	Coelidiinae	*TInobregnus*	*viridescens*
ILI-18	Illinois	Caliscelinae	*Bruchomorpha*	*jocosa*
ILI-21	Illinois	Caliscelinae	*Bruchomorpha*	*abrupta*

Table 14 (continued)

Sample	Country	Subfamily	Genus	species
ILI-22	Illinois	Caliscelinae	*Bruchomorpha*	*jocosa*
ILI-24, 34-35	Illinois	Deltocephalinae	*Polyamia*	*caperata*
ILI-25[1]	Illinois	Gonatopodinae	*Gonatopus*	*elongatus*
ILI-29	Illinois	Deltocephalinae	*Flexamia*	*sandersi*
ILI-30	Illinois	Deltocephalinae	*Stirellus*	*bicolor*
ILI-31	Illinois	Deltocephalinae	*Menosoma*	*cincta*
ILI-32	Illinois	Megophthalminae	*Ceratagallia*	*agricola*
MAD-01	Madagascar	Deltocephalinae	*Acostemma*	sp. nov.
MEX-01	Mexico	Deltocephalinae	*Texananus*	sp.
MEX-02, 19	Mexico	Deltocephalinae	*Amplicephalus*	*trilobatus*
MEX-22-23	Mexico	Deltocephalinae	*Polyamia*	*satur*
MEX-24-25	Mexico	Deltocephalinae	*Amplicephalus*	*sonorus*
MEX-27	Mexico	Deltocephalinae	*Flexamia*	*abbreviata*
MEX-29-33, 35-38, 40-41	Mexico	Deltocephalinae	*Athysanella*	*wardi*
MEX-34	Mexico	Deltocephalinae	*Laevicephalus*	sp.
MEX-39	Mexico	Caliscelinae	*Aphelonema*	sp.
MEX-42-43	Mexico	Deltocephalinae	*Graminella*	*barinasensis*
MON-02	Mongolia	Deltocephalinae	*Diplocolenus*	*altaicus*
MON-03	Mongolia	Deltocephalinae	*Emeljanovianus*	*medius*
MON-06	Mongolia	Deltocephalinae	*Cosmotettix* (*Agapelus*)	*aurantiacus*
MON-07	Mongolia	Deltocephalinae	*Sorhoanus*	*xanthoneurus*
MON-08	Mongolia	Deltocephalinae	*Diplocolenus*	*abdominalis*
MON-09, 19	Mongolia	Deltocephalinae	*Diplocolenus*	*funebris*
MON-10	Mongolia	Deltocephalinae	related to *Limotettix*	sp.
MON-11	Mongolia	Deltocephalinae	*Psammotettix*	*atropidicola*
MON-14	Mongolia	Deltocephalinae	*Mogangina* (*Tungara*)	*chubsugulica*
MON-15	Mongolia	Deltocephalinae	*Mogangina* (*Tungara*)	*chubsugulica*
MON-16-17	Mongolia	Deltocephalinae	*Psammotettix*	*striatus*
MON-18	Mongolia	Deltocephalinae	*Diplocolenus*	*funebris*
MON-20-21, 23	Mongolia	Deltocephalinae	*Pantallus*	*alboniger*
SAF-11	Mongolia	Deltocephalinae	*Gcaleka*	sp.
TAI-29	Taiwan	Deltocephalinae	*Hishimonus*	sp.
TAI-30	Taiwan	Deltocephalinae	*Sogatella*	*vibix*
THA-12	Thailand	Cicadellinae	*Kolla*	*paulula*
UNK-02	?	Deltocephalinae	*Chlorotettix*	*lusorius*
ZAM-01	Zambia	Eurymelinae	*Pediopsoides*	sp.

Table 15. Biogeographic analysis using maximum likelihood. Dryinidae was recovered as having a Neotropical ancestral distribution, 75.91% probability, with a Nearctic ancestry being less favored, 15.50% probability. Number without asterisks (*) indicate equivocal reconstruction. Only most likely reconstruction shown for all clades, except Dryinidae. Values given in percentage (%).

Clade	Indo-Malay	Nearctic	Neotropical	Palearctic
Dryinidae		15.50	75.91*	
Unknown Dryinidae			86.60*	
Anteoninae (*Anteon* + *Lonchodryinus*)			80.58*	
Aphelopinae		87.60*		
Gonatopodinae		86.52*		
Thaumatodryininae + Dryininae + Gonatopodinae		76.37*		
Conganteoninae				55.94
Deinodryinus + MAD-01		52.52		
Deinodryinus		39.52		
Thaumatodryininae		46.95		
Dryininae + Gonatopodinae		61.14		
Dryininae		40.74		

Table 16. Ancestral host maximum likelihood reconstruction. Reconstruction had a marginal probability of 127.71under the Mk1 model and strongly suggested Deltocephalinae leafhoppers as the ancestral host of Dryinidae. Other major clades were not considered due to the incorrect phylogenetic placement of *Deinodryinus*. Asterisks (*) indicates significant results; equivocal reconstructions show no asterisk. Clades with no values indicate unresolved reconstructions. Values given in percentage (%).

Clade	Deltocephalinae	Typhlocybinae	Caliscelinae	Platybrachinae
Dryinidae	96.11*			
Unknown Dryinidae			49.07*	49.07*
Anteoninae (*Anteon* + *Lonchodryinus*) without *Anteon* sp.1	99.99*			
MAD-01 + *Deinodryinus* + Aphelopinae	58.30*	40.88*	-	
Aphelopinae without *Aphelopus* sp.1		99.2*		
Gonatopodinae without *Echthrodelphax* sp.1 and *Gonatopus* sp.1	99.99*			
Dryininae + Gonatopodinae	99.83*			
Anteoninae (*Anteon* + *Lonchodryinus*)				
Deinodryinus + Aphelopinae				
Aphelopinae				
Conganteoninae				
Thaumatodryininae + Dryininae + Gonatopodinae				
Thaumatodryininae				
Dryininae				
Gonatopodinae				

Table 17. Ancestral habitat maximum likelihood reconstruction. Reconstructions had a marginal probability of 91.35 under the Mk1 model. Conganteoninae, Thaumatodryininae and Dryininae had no distribution data for the whole clade, likely affecting these results. Other major clades were not considered due to the incorrect phylogenetic placement of *Deinodryinus*. Asterisks (*) indicates significant results; equivocal reconstructions show no asterisk. Clades with no values indicate unresolved reconstructions. Values given in percentage (%).

Clade	Forest	Grass-shrub-scrub
Dryinidae	55.84	44.16
Unknown Dryinidae	90.14*	9.86
Anteoninae (*Anteon* + *Lonchodryinus*) without *Anteon* sp.1	62.55	37.44
Deinodryinus + MAD-01 + Aphelopinae	28.02	71.98
Aphelopinae without *Aphelopus* sp.1	21.63	78.36
Gonatopodinae without *Echthrodelphax* sp.1 and *Gonatopus* sp.1	53.12	46.88
Conganteoninae		
Deinodryinus + Aphelopinae		
Aphelopinae		
Thaumatodryininae		
Dryininae		
Gonatopodinae		
Dryininae + Gonatopodinae		
Thaumatodryininae + Dryininae + Gonatopodinae		

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APPENDIX A. NEXUS TREEFILE RESULTING FROM 28S ML PHYLOGENETIC ANALYSIS.

begin trees;
tree tree_1 = [&R]
(Cleptes_seoulensis:41.0,((Chrysis_cembricola:1.0,Chrysis_principalis:1.0):39.0,('Sclerogibba_sp.':39.0,((((('AUS01.1|Desudaboides|sp. nov.|Piocerini|Poiocerinae':1.0,'AUS01.2|Desudaboides|sp. nov.|Piocerini|Poiocerinae':1.0):1.0,('AUS01.3|Desudaboides|sp. nov.|Piocerini|Poiocerinae':1.0,'AUS01.4|Desudaboides|sp. nov.|Piocerini|Poiocerinae':1.0):1.0):1.0,(('ARG92|Genus|sp.|Peltonotellini|Caliscelinae':1.0,'ARG78|Genus|sp.|Peltonotellini|Caliscelinae':1.0):1.0,('ILI21|Bruchomorpha|jocosa|Peltonotellini|Caliscelinae':1.0,'ILI22|Bruchomorpha|abrupta|Peltonotellini|Caliscelinae':1.0):1.0):1.0):35.0,((Anteon_sp1:17.0,((((Anteon_sp2:1.0,'ZAM01|Pediopsoides|sp.|Macropsini|Eurymelinae':1.0):1.0,('TAI29|Genus|sp.|Tribe|Deltocephalinae':1.0,'ILI12|Genus|sp.|Tribe|Typhlocybinae':1.0):1.0):7.0,('MEX27|Flexamia|abreviatta|Paralimnini|Deltocephalinae':8.0,(('MEX17|Genus|sp.|Tribe|Deltocephalinae':4.0,('MEX23|Polyamia|satur|Deltocephalini|Deltocephalinae':3.0,('ILI35|Polyamia|caperata|Deltocephalini|Deltocephalinae':2.0,('MEX03|Genus|sp.|Tribe|Deltocephalinae':1.0,'MEX24|Amplicephalus|sonorus|Deltocephalini|Deltocephalinae':1.0):1.0):1.0):1.0):3.0,('MEX16|Graminella|comata|Deltocephalini|Deltocephalinae':6.0,('MEX25|Amplicephalus|sonorus|Deltocephalini|Deltocephalinae':5.0,((Anteon_sp5:2.0,(Anteon_sp3:1.0,Anteon_sp4:1.0):1.0):2.0,('MEX01|Genus|sp.|Tribe|Deltocephalinae':3.0,('MEX22|Polyamia|satur|Deltocephalini|Deltocephalinae':2.0,('ILI24|Polyamia|caperata|Deltocephalini|Deltocephalinae':1.0,'ILI34|Polyamia|caperata|Deltocephalini|Deltocephalinae':1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):7.0,((Anteon_sp7:1.0,Anteon_wushense:1.0):14.0,(Anteon_sp8:14.0,(Lonchodryinus_sp1:13.0,(Lonchodryinus_subapterus:12.0,('MON03|Emeljanovianus|medius|Paralmnini|Deltocephalinae':11.0,(('MON08|Diplocolenus|abdominalis|Paralimnini|Deltocephalinae':5.0,('MON19|Diplocolenus|funebris|Paralimnini|Deltocephalinae':4.0,('MON07|Sorhoanus|xanthoneurus|Paraliminini|Deltocephalinae':3.0,('MON11|Psammotettix|atropidicola|Paralimnini|Deltocephalinae':2.0,('MON09|Diplocolenus|funebris|Paralimnini|Deltocephalinae':1.0,'MON14|Mogangina|chubsugulica|Paralimnini|Deltocephalinae':1.0):1.0):1.0):1.0):1.0):5.0,((Anteon_sp9:1.0,'THA12|Kolla|paulula|Cicadellini|Cicadellinae':1.0):8.0,(Anteon_sp6:8.0,((Anteon_sp10:1.0,Anteon_sp11:1.0):6.0,(Anteon_sp12:6.0,(Anteon_sp13:5.0,((Anteon_sp16:2.0,('MON15|Mogangina|chubsugulica|Paralimnini|Deltocephalinae':1.0,Anteon_sp15:1.0):1.0):2.0,(Anteon_sp14:3.0,(Anteon_sp17:2.0,(Anteon_sp18:1.0,'ILI15|Tinobregnus|viridescens|Tinobregmini|Coelidiinae':1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):20.0,(Fiorianteon_junonium:36.0,(('MAD01|Acostemma|sp. nov.|Acostemini|Deltocephalinae':13.0,(Deinodryinus_sp3:12.0,((Deinodryinus_incaicus:2.0,(Deinodryinus_sp2:1.0,Deinodryinus_sp1:1.0):1.0):9.0,(Deinodryinus_sp4:10.0,((Deinodryinus_sp5:2.0,(Deinodryinus_finnamorei:1.0,Deinodryinus_sp6:1.0):1.0):7.0,(Aphelopus_sp1:8.0,('THA01|Genus|sp.|Erythroneurini|Typhlocybinae':7.0,('TAI21|Genus|sp.|Empoascini|Typhlocybinae':6.0,(('THA10|Genus|sp.|Erythroneurini|Typhlocybinae':4.0,('ILI18|Empoasca|fabae|Empoascini|Typhlocybinae':3.0,('ARI24|Empoasca|sp.|Empoascini|Typhlocybinae':2.0,('CAL04|Empoasca|sp.|Empoascini|Typhlocybinae':1.0,'CAL05|Empoasca|fabae|Empoascini|Typhlocybinae':1.0):1.0):1.0):1.0):1.0,(('SAF38|Genus|sp.|Empoascini|Typhlocybinae':1.0,'SAF39|Genus|sp.|Empoascini|Typhlocybinae':1.0):3.0,('THA02|Genus|sp.|Empoascini|Typhlocybinae':3.0,(Aphelopus_sp3:2.0,('ARI06|Empoasca|sp.|Empoascini|Typhlocybinae':1.0,Aphelopus_sp2:1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):22.0,((Thaumatodryinus_macilentus:1.0,Thaumatodryinus_merinus:1.0):33.0,(((Dryinus_sp8:2.0,(Dryinus_sp9:1.0,Dryinus_sp10:1.0):1.0):8.0,((Dryinus_sp11:8.0,(Dryinus_sp12:7.0,(Dryinus_sp14:6.0,(Dryinus_sp16:5.0,('Dryinus_sp18.2':4.0,(Dryinus_stantoni:3.0,(Dryinus_sp17:2.0,(Dryinus_parvulus:1.0,Dryinus_sp18:1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0,(Dryinus_spangleri:6.0,((Dryinus_sp5:2.0,(Dryinus_striatus:1.0,'Dryinus_striatus.2':1.0):1.0):3.0,((Dryinus_sp4:1.0,Dryinus_sp7:1.0):3.0,((Dryinus_bicolor:1.0,Dryinus_bolivianus:1.0):2.0,(Dryinus_sp1:2.0,(Dryinus_sp2:1.0,Dryinus_sp3:1.0):1.0):1.0):1.0):1.0):1.0):3.0):1.0):23.0,(Echthrodelphax_sp1:32.0,(Gonatopus_sp1:31.0,('ILI31|Menosoma|cincta|Bahitini|Deltocephalinae':30.0,((('MON23|Pantallus|alboniger|Paralimnini|Deltocephalinae':1.0,'MON18|Diplocolenus|funebris|Paralimnini|Deltocephalinae':1.0):6.0,(('MON17|Psammotettix|striatus|Paralimnini|Deltocephalinae':2.0,('MON10|Norva|sp.|Opsiini|Deltocephalinae':1.0,'MON21|Pantallus|alboniger|Paralimnini|Deltocephalinae':1.0):1.0):4.0,('MON20|Pantallus|alboniger|Paralimnini|Deltocephalinae':5.0,('MON13|Genus|sp.|Tribe|Subf':4.0,('FRA02|Jassargus|obtusivalvis|Paralimnini|Deltocephalinae':3.0,('MON04|Diplocolenus|fraunfeldi|Paralimnini|Deltocephalinae':2.0,('MON16|Psammottetix|striatus|Paralimnini|Deltocephalinae':1.0,'MON05|Diplocolenus|fraunfeldi|Paralimnini|Deltocephalinae':1.0):1.0):1.0):1.0):1.0):1.0):1.0):22.0,('ILI13|Genus|sp.|Delphacini|Delphacinae':28.0,('

Appendix A (continued)

MEX21|Polyamia|satur|Deltocephalini|Deltocephalinae':27.0,(('ARI26|Ceratagallia|gillettei|Agalliini|Megophthalminae':15.0,(('ARI22|Ceratagallia|gillettei|Agalliini|Megophthalmiane':2.0,('ARI01|Ceratagallia|gillettei|Agalliini|Megophthalminae':1.0,'ARI18|Ceratagallia|gillettei|Agalliini|Megophthalminae':1.0):1.0):12.0,('ARI02|Ceratagallia|gillettei|Agalliini|Megophthalminae':13.0,('ARI28|Ceratagallia|gillettei|Agalliini|Megophthalminae':12.0,('ARI30|Ceratagallia|gillettei|Agalliini|Megophthalminae':11.0,('ARI29|Ceratagallia|gillettei|Agalliini|Megophthalminae':10.0,('ARI21|Ceratagallia|gillettei|Agalliini|Megophthalminae':9.0,('ARI31|Ceratagallia|gillettei|Agalliini|Megophthalminae':8.0,('ARI23|Ceratagallia|gillettei|Agalliini|Megophthalminae':7.0,('ARI34|Ceratagallia|gillettei|Agalliini|Megophthalminae':6.0,('ARI25|Ceratagallia|gillettei|Agalliini|Megophthalminae':5.0,('ARI08|Ceratagallia|gillettei|Agalliini|Megophthalminae':4.0,('ARI27|Ceratagallia|gillettei|Agalliini|Megophthalminae':3.0,('ARI32|Ceratagallia|gillettei|Agalliini|Megophthalminae':2.0,('ILI32|Ceratagalliae|agricola|Agalliini|Megophthalminae':1.0,'ARI20|Ceratagallia|gillettei|Agalliini|Megophthalminae':1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):11.0,('MEX42|Graminella|barinasensis|Deltocephalini|Deltocephalinae':25.0,('ARG104|Atanus|luqueatus|equalis|Athysanini|Deltocephalinae':24.0,(('NEB01|Hecalus|sp.|Hecalini|Deltocephalinae':1.0,Gonatopus_sp2:1.0):22.0,((Gonatopus_sp4:1.0,'ILI29|Flexamia|sandersi|Paralimnini|Deltocephalinae':1.0):21.0,('CAL03|Graminella|sonora|Deltocephalini|Deltocephalinae':21.0,('ARG59|Coelidiana|brasiliensis|Neocoelidiini|Neocoelidiinae':20.0,(('ARG79|Amplicephalus|rotundiceps|Deltocephalini|Deltocephalinae':5.0,('ARG86|Amplicephalus|luridus|Deltocephalni|Deltocephalinae':4.0,('ARG34|Haldorus|sp.
nov.|Deltocephalini|Deltocephalinae':3.0,('ARG85|Haldorus|sp.
nov.|Deltocephalini|Deltocephalinae':2.0,('ARG81|Haldorus|sp.
nov.|Deltocephalini|Deltocephalinae':1.0,'ARG99|Amplicephalus|luridus|Deltocephalini|Deltocephalinae':1.0):1.0):1.0):1.0):14.0,(('UNK02|Chlorotettix|lusorius|Athysanini|Deltocephalinae':1.0,'ARI13|Penestragania|robusta|Hyalojassini|Iassinae':1.0):17.0,(('ARI05|Genus|sp.|Tribe|Deltocephalinae':3.0,('ILI09|Genus|sp.|Tribe|Deltocephalinae':2.0,('ILI11|Genus|sp.|Tribe|Deltocephalinae':1.0,'ILI10|Genus|sp.|Tribe|Deltocephalinae':1.0):1.0):1.0):14.0,(('ARG88|Haldorus|sexpunctatus|Deltocephalini|Deltocephalinae':1.0,'ARG84|Haldorus|sexpunctatus|Deltocephalini|Deltocephalinae':1.0):15.0,('ARG103|Graminella|stelliger|expansa|Deltocephalini|Deltocephalinae':15.0,('ARG94|Graminella|stelliger|expansa|Deltocephalini|Deltocephalinae||reversed|':14.0,('ARG95|Graminella|stelliger|expansa|Deltocephalini|Deltocephalinae':13.0,(('ILI07|Idiocerus|varians|Idiocerini|Eurymelinae':2.0,('ARG60|Hortensia|similis|Cicadellini|Cicadellinae':1.0,'ARG93|Graminella|stelliger|expansa|Deltocephalini|Deltocephalinae':1.0):1.0):10.0,(('MEX39|Aphelonema|sp.|Peltonotellini|Caliscelinae':6.0,(Echthrodelphax_sp2:5.0,(('ARI16|Chlorotettix|sp.|Athysanini|Deltocepahalinae':2.0,('MEX02|Amplicephalus|trilobatus|Deltocephalini|Deltocephalinae':1.0,'MEX19|Amplicephalus|trilobatus|Deltocephalini|Deltocephalinae
':1.0):1.0):2.0,(('TAI30|Sogatella|vibix|Delphacini|Delphacinae':1.0,Haplogonatopus_oratorius:1.0):2.0,(Eucamptonyx_sp:2.0,('ARI14|Exitianus|exitosus|Chiasmini|Deltocephalinae':1.0,'CAL01|Idiocerus|sp.|Idiocerini|Eurymelinae':1.0):1.0):1.0):1.0):1.0):1.0):5.0,((Gonatopus_sp3:8.0,('ARG74|Spangbergiella|felix|Hecalini|Deltocephalinae':7.0,(('ARG87|Haldorus|sp. nov.|Deltocephalini|Deltocephalinae':5.0,('ARG98|Haldorus|sp.
nov.|Deltocephalini|Deltocephalinae':4.0,('ARG101|Haldorus|furcatus|Deltocephalini|Deltocephalinae':3.0,('ARG89|Mendozellus|asunctia|Deltocephalini|Deltocephalinae':2.0,('ARG91|Genus nov.|sp.
nov.|Deltocephalini|Deltocephalinae':1.0,'ARG90|Mendozellus|asunctia|Deltocephalini|Deltocephalinae':1.0):1.0):1.0):1.0):1.0):1.0,(('MEX26|Stirellus|bicolor|Stenometopiini|Deltocephalinae':1.0,'ARI03|Paraphlepsius|micronotatus|Pendarini|Deltocephalinae':1.0):3.0,(('MEX40|Athysanella|wardi|Chiasmini|Deltocephalinae':1.0,'MEX41|Athysanella|wardi|Chiasmini|Deltocephalinae':1.0):2.0,('MEX29|Athysanella|wardi|Chiasmini|Deltocephalinae':2.0,('MEX35|Athysanella|wardi|Chiasmini|Deltocephalinae':1.0,'ILI25|Dryinidae|Gonatopus|elongatus||Gonatopodinae':1.0):1.0):1.0):1.0):2.0):1.0):1.0):2.0,(((('SAF11|Genus|sp.|Tribe|Deltocephalinae':1.0,'ARI33|Ceratagallia|gillettei|Agalliini|Megophthalminae':1.0):4.0,('ARG16|Balclutha|sp.
nov.|Macrostelini|Deltocephalinae':4.0,((Epigonatopus_sp2:1.0,Epigonatopus_sp1:1.0):2.0,('ILI14|Polyamia|caperata|Deltocephalini|Deltocephalinae':2.0,(Gonatopus_cyphonotus:1.0,'ILI36|Polyamia|caperata|Deltocephalini|Deltocephalinae':1.0):1.0):1.0):1.0):1.0):4.0,(('ARI17|Scaphytopius|fuscifrons|Scaphytopiini|Deltocephalinae':1.0,'ARI15|Empoasca|fabae|Empoascini|Typhlocybinae':1.0):7.0,((('MON06|Cosmotettix|aurantiacus|Paralimnini|Deltocephalinae':1.0,'ILI30|Genus|sp.|Tribe|Deltocephalinae':1.0):2.0,('ARG36|Frequenamia|venosula|Bahitini|Deltocephalinae':2.0,('ARG80|Frequenamia|venosula|Bahitini|Deltocephalinae':1.0,'ARG102|Frequenamia|venosula|Bahitini|Deltocephalinae':1.0):1.0):1.0):4.0,(Gonatopus_peculiaris:6.0,(('MEX34|Genus|sp.|Tribe|Deltocephalinae':1.

Appendix A (continued)

0,'MEX30|Athysanella|wardi|Chiasmini|Deltocephalinae':1.0):4.0,('MEX31|Athysanella|wardi|Chiasmini|Deltocephalinae':4.0,('MEX38|Athysanella|wardi|Chiasmini|Deltocephalinae':3.0,('MEX32|Athysanella|wardi|Chiasmini|Deltocephalinae':2.0,('MEX37|Athysanella|wardi|Chiasmini|Deltocephalinae':1.0,'MEX33|Athysanella|wardi|Chiasmini|Deltocephalinae':1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0):1.0);
end;

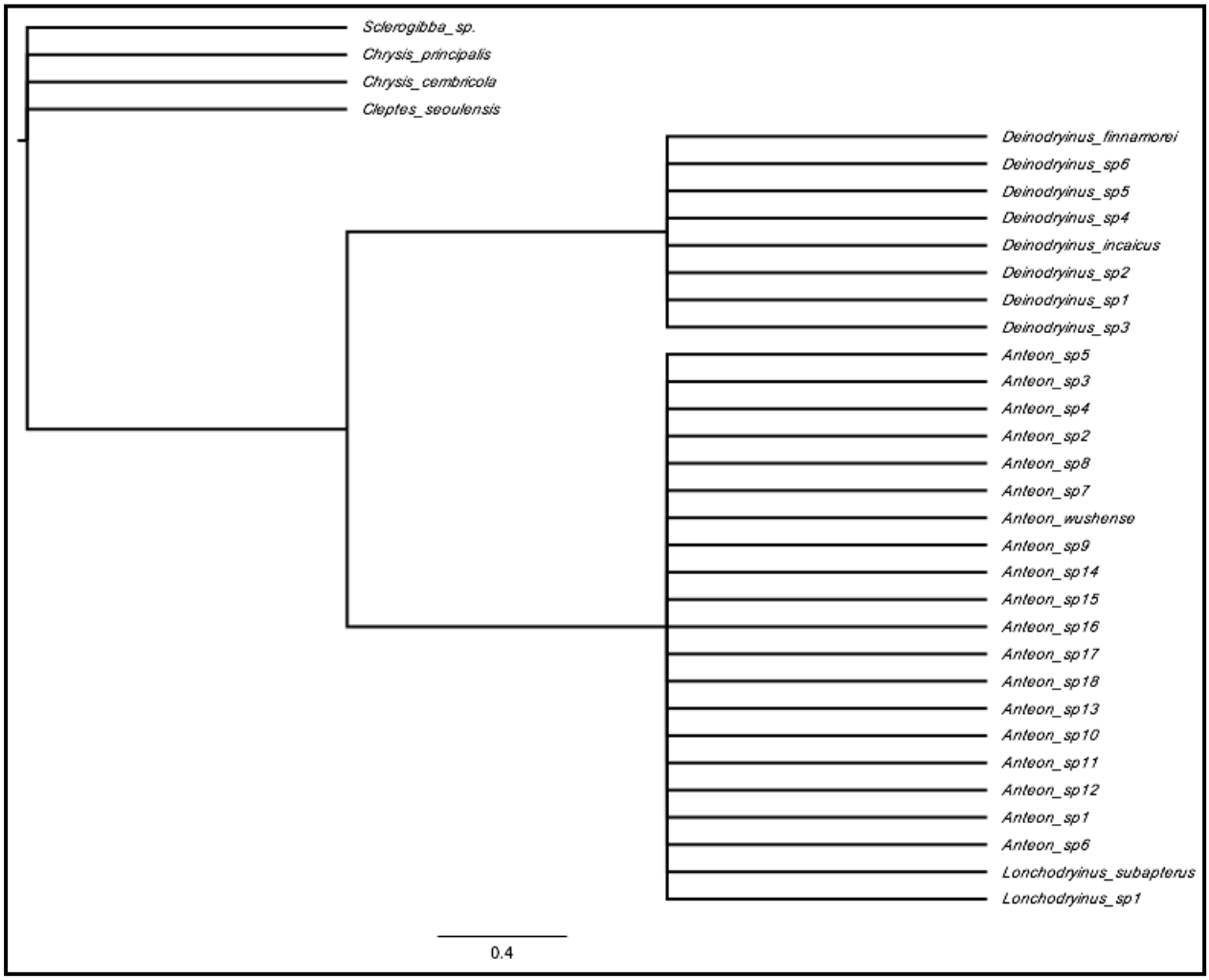
APPENDIX B. ANTEONINAE CONSTRAINT TREE FOR CONSTRAINT ML ANALYSIS IN NEWICK FORMAT.

Here, Anteoninae is forced to be a monophyletic clade composed of the genera *Anteon*, *Lonchodryinus* and *Deinodryinus*, as previously found in a four-genes phylogeny (Tribull 2015).

(((Deinodryinus_finnamorei,Deinodryinus_sp6,Deinodryinus_sp5,Deinodryinus_sp4,Deinodryinus_incaicus,Deinodryinus_sp2,Deinodryinus_sp1,Deinodryinus_sp3),(Anteon_sp5,Anteon_sp3,Anteon_sp4,Anteon_sp2,Anteon_sp8,Anteon_sp7,Anteon_wushense,Anteon_sp9,Anteon_sp14,Anteon_sp15,Anteon_sp16,Anteon_sp17,Anteon_sp18,Anteon_sp13,Anteon_sp10,Anteon_sp11,Anteon_sp12,Anteon_sp1,Anteon_sp6,Lonchodryinus_subapterus,Lonchodryinus_sp1)),Sclerogibba_sp.,Chrysis_principalis,Chrysis_cembricola,Cleptes_seoulensis);

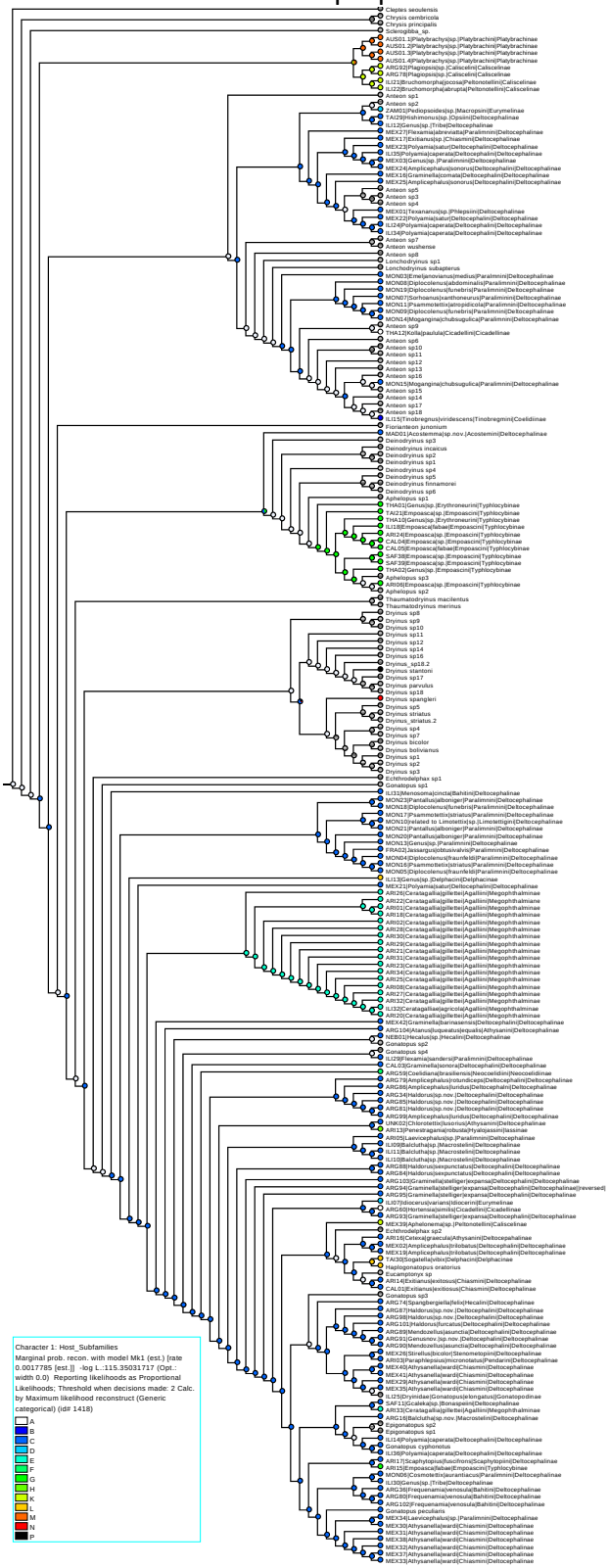
APPENDIX C. ANTEONINAE CONSTRAINT TREE FOR CONSTRAINT ML ANALYSIS IN TREE FORMAT.

Details as in Appendix B.



APPENDIX D. ANCESTRAL HOST RECONSTRUCTION USING ML UNDER THE MK1 MODEL.

Pie charts indicate proportional likelihood for each state.



APPENDIX E. ANCESTRAL ENVIRONMENT RECONSTRUCTION USING ML UNDER THE MK1 MODEL.

Pie charts indicate proportional likelihood for each state.