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Phylogenetic relationships among the subfamilies of Dryinidae (Hymenoptera, Chrysidoidea) as reconstructed by molecular sequencing

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Abstract

Previously, the only published phylogenetic analysis of Dryinidae was a morphological analysis of just 32 characters. Herein, I present the first analysis of molecular sequence data examining the relationships among several of the major subfamilies of Dryinidae. A total of 77 specimens of Dryinidae from seven subfamilies, two specimens of *Chrysis* (Chrysididae), one specimen of *Cleptes* (Chrysididae), and one specimen of *Sclerogibba* (Sclerogibbidae) were examined utilizing molecular sequence data from nuclear 18S and 28S genes and mitochondrial Cytochrome Oxidase Subunit I (COI) and Cytochrome b (Cytb) genes. Dryininae were rendered nonmonophyletic due to the placement of *Thaumatodryinus*, which was sister to the remainder of Dryininae and Gonatopodinae. To establish monophyly of Dryininae, Thaumatodryininae were resurrected for *Thaumatodryinus*.

Keywords

Pincer Wasps, Molecular Systematics, Phylogeny, Phylogenetics

Introduction

Dryinidae are the third largest family within Chrysidoidea, containing 15 subfamilies, 50 genera, and over 1700 species found worldwide (Olmi 1994b, Olmi and Virla 2014, Olmi et al. 2014). These wasps are parasitoids and predators of Auchenorrhyncha and

have huge potential as agricultural biocontrol agents, particularly for rice, fruit, and sugarcane pests (Sahragard et al. 1991, Olmi 1989, Mora-Kepfer and Espinoza 2009, Virla et al. 2011). In one species, *Gonatopus flavifemur* Esaki & Hashimoto, 1932, a single female was recorded as having attacked 466 planthoppers (as both food and hosts) over its 19-day adult life (Chua and Dyck 1982, Sahragard et al. 1991). Most dryinid species are host generalists that attack a wide variety of Auchenorrhyncha, often with host species belonging to different genera or even different families (Guglielmino and Olmi 1997, Guglielmino et al. 2013).

With only one or two world experts exclusively studying Dryinidae at any one time, the family has an interesting, but sparse, taxonomic history. Kieffer (1914) wrote the first world monograph of Dryinidae, with the first revisionary taxonomy for the family coming from Richards (1939, 1953). Outside of small agricultural studies and taxonomic descriptions, there was little focus on Dryinidae until the publication of Olmi (1984), a 1913-page world monograph that revised much of the taxonomy and provided keys throughout the family. Since then, there has been a growth in known dryinid diversity and host-records and the production of several large regional monographs (Olmi 1994a, b, Olmi 2005, Olmi 2007, Xu et al. 2013, Olmi and Virla 2014).

Currently, the fifteen subfamilies consist of four fossil subfamilies: Burmadryininae Olmi et al., 2014, Palaeoanteoninae Olmi & Bechly, 2001, Ponomarenkoinae Olmi 2010, and Protodryininae Olmi & Guglielmino, 2012 – and eleven extant subfamilies: Anteoninae Perkins, 1912, Aphelopinae Perkins, 1912, Apoaphelopinae Olmi, 2007, Apodryininae Olmi, 1984, Bocchinae Richards, 1939, Conganteoninae Olmi, 1984, Dryininae Haliday, 1833, Erwiniinae Olmi & Guglielmino, 2010, Gonatopodinae Kieffer, 1906, Plesiodryininae Olmi, 1987, and Transdryininae Olmi, 1984. The five largest subfamilies, Anteoninae, Gonatopodinae, Dryininae, Bocchinae, and Aphelopinae are found worldwide and comprise over ninety percent of the known diversity of Dryinidae (Olmi and Virla 2014, Xu et al. 2013). Conganteoninae contains about 15 species found in the Palearctic, Afrotropical, and Oriental regions, Plesiodryininae are known from a single species in the Nearctic region, Erwiniinae are known from a single species in the Neotropical region, Apoaphelopinae are known from two species in South Africa and Mozambique, Apodryininae are known from 13 species (with a Gondwanan distribution) and Transdryininae are known from two species from Australia (Olmi 1984, Olmi and Guglielmino 2010, Xu et al. 2013, Olmi and Virla 2014).

Over half of the described species of Dryinidae are found within three genera– *Anteon* Jurine, 1807, *Dryinus* Latreille, 1804, and *Gonatopus* Ljungh, 1810. A multiplicity of genera were synonymized within these three (refer to Olmi and Virla 2014 and Xu et al. 2013 for a complete list), but only within *Gonatopus* and *Dryinus* were the synonymized genera delimited amongst species groups. Olmi (1993) first synonymized *Chelothelius* Reinhard, 1863, *Mesodryinus* Kieffer, 1906, *Perodryinus* Perkins, 1907, *Tridryinus* Kieffer, 1913, *Bocchoides* Benoit, 1954, and *Alphadryinus* Olmi, 1984 within *Dryinus* and *Dicondylus* Haliday, 1830, *Pseudogonatopus* Perkins, 1905, *Agonatopoides* Perkins, 1907, *Apterodryinus* Perkins, 1907, *Donisthorpina* Richards, 1939, *Plectrogonatopoides* Ponomarenko, 1975, and *Acrodontochelys* Currado, 1976

within *Gonatopus* based on the lack of genus-level synapomorphies in the males of these synonymized genera. *Trichogonatopus* Kieffer, 1909 was also synonymized with *Gonatopus* upon the discovery of male specimens in Virla et al. (2010). Olmi (1993), Xu et al. (2013), and Olmi and Virla (2014), provided morphological keys to describe four species groups within *Dryinus* and 12 in *Gonatopus*.

Olmi (1993) also synonymized Thaumatodryininae Perkins, 1905 as a genus within Dryininae, *Thaumatodryinus* Perkins, 1905, along with moving a Gonatopodinae genus, *Pseudodryinus*, Olmi 1989, to Dryininae on the basis of mandibular character similarity in males. Currently, males of both *Pseudodryinus* and *Thaumatodryinus* are distinguishable from the other genera in Dryininae by having quadridentate mandibles, with *Thaumatodryinus* males presenting mandibular teeth that usually progress larger from anterior to posterior, whereas in *Pseudodryinus*, the four teeth of the mandible are irregularly sized. Females of *Thaumatodryinus* are easily distinguished from other Dryininae by the presence of long hairs on flagellomeres 3 – 8 (Mita 2009, Xu et al. 2013).

There is very little published on the phylogenetic relationships of the subfamilies within Dryinidae. Olmi (1994a) stated "we cannot discuss species affinities, because evolution has followed completely different paths in males and females, and female affinities are completely different from male affinities", and did not attempt to combine morphological data from both sexes to reconstruct a phylogeny. Olmi (1994a) presented a tree, but only included female specimens from four subfamilies found within Denmark and Fennoscandia and did not make clear how characters were coded and analyzed. In Carpenter (1999), a cladogram was reconstructed from 32 characters based on the taxonomic keys and descriptions of Massimo Olmi from both sexes. Given the growth in known dryinid diversity since then, neither study reflects the current subfamily classification and only addressed a small number of morphological features, although both placed Aphelopinae as the basal subfamily of Dryinidae and placed Gonatopodinae and Dryininae as sister groups (as in Olmi 1994a) or as closely associated in a polytomy that also contained Transdryininae and (Apodryininae + Plesiodryininae) (Carpenter 1999). There are no published molecular phylogenies, but DNA has been used to link the highly modified females of *Gonatopus javanus* Perkins, 1912 to males, which are similar looking throughout the genus, and to explore intraspecific genetic variation (Mita and Matsumoto 2012, Mita et al. 2013). Herein, I present the first analysis of molecular sequence data examining the relationships among several of the major subfamilies.

Methods

Materials

Phylogenetic relationships were inferred from 77 specimens of Dryinidae with one specimen of *Sclerogibba* Riggio & De Stefani-Perez, 1888 (Chrysidoidea: Sclerogibbidae), two species of *Chrysis* Linnaeus, 1761 (Chrysidoidea: Chrysididae), and *Cleptes seoulensis* Tsuneki, 1959 (Chrysidoidea: Chrysididae) as outgroup taxa. The majority

of specimens came from two sources: Instituto Nacional de Pesquisas da Amazônia (INPA) and Canadian National Collections (CNC). Materials from the CNC were sorted from bulk alcohol materials from a variety of institutions and collectors, as detailed in Suppl. material 1. Additionally, several specimens were provided courtesy of Massimo Olmi, Toshiharu Mita and Pierre Tripotin. Specimens were stored in 95 percent ethanol and refrigerated prior to extraction. As these materials were acquired from unsorted bulk Malaise, yellow pan trap, and sweep net samples, they have not been accessioned in collections. Materials will be returned to their original institutions following the completion of this work and subsequent description of new species.

Laboratory protocols

Genomic DNA was isolated using a QIAGEN DNeasy Tissue Kit following the manufacturer's protocols, with the exception of using non-destructive lysing techniques (Paquin and Vink 2009). This allowed for specimens to be pinned and identified after extraction protocols. PCR amplification was accomplished using General Electric PuReTaq Ready-To-Go beads with the following primers: the 18S region was amplified using 18SF2 (5'-CTA CCA CAT CCA AGG AAG GCA G-3') and 18SR2 (5'-AGA GTC TCG TTC GTT ATC GGA-3') (Rokas et al. 2002), 28S D2-D3 was amplified using For28Vesp (5' AGA GAG AGT TCA AGA GTA CGT G-3') and Rev28SVesp (5'-GGA ACC AGC TAC TAG ATG G-3') (Hines et al. 2007). Cytochrome Oxidase I (COI) was amplified for the Folmer/barcode region using LCO (5'- GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Vrijenhoek 1994). The Cytochrome b (Cytb) region was amplified using CB1 (5'-TAT GTA CTA CCA TGA GGA CAA ATA TC-3') and CB2 (5'-ATT ACA CCT CCT AAT TTA TTA GGA AT-3') (Simon et al. 1994). Thermocycler protocols are detailed in Suppl. material 2, with assistance from Jongok Lim. Sequencing was performed at the American Museum of Natural History (AMNH) in the Sackler Institute for Comparative Genomics on an ABI 3730.

Analyses

Sequences were assembled and edited in Geneious 5.4 (Kearse et al. 2012). Mitochondrial genes COI and Cytb were checked for stop codons and numts and aligned using the translation alignment algorithm within Geneious. 18S and 28S were aligned using MAAFT, using the E-INS-I algorithm as implemented in Geneious. This algorithm was chosen for its accuracy in difficult alignments (Morrison 2009) and its recent use in the Hymenoptera Tree of Life project, which provided sequences for outgroup taxon *Chrysis cembricola* Krombein, 1958 (Klopfstein et al. 2013) The concatenated matrix was assembled in SeqMatrix (Vaidya et al. 2011), resulting in a final matrix of 6594 characters, with 13 percent missing data.

Phylogenetic analyses were performed using parsimony, Bayesian and maximum likelihood approaches. For parsimony, TNT (Goloboff et al. 2008) was used with the new technology search algorithms with the following parameters modified from default: 200 ratchet iterations, upweighting percentage 8, downweighting 4; 50 cycles of drift; minimum length hit 25 times with gaps treated as missing data. Jackknife resampling (Farris et al. 1996) support values were calculated using GC-values from a symmetric resampling of 1000 replicates. Separate analyses were performed using equal weighting and implied weighting as implemented by the setK script in TNT (courtesy of J. Salvador Arias).

PartitionFinder (Lanfear et al. 2012) was used to select models of molecular evolution for the RAxML (Stamatakis 2014) and MrBayes (Ronquist et al. 2012) analyses for each ribosomal gene, and each codon for COI and Cytb. For the models available for implementation in RAxML, each partition was returned as GTR+I+G. Using RAxML 8.1.11 XSEDE on the Cipres server, 20 independent analyses were performed with different starting seed values and 1000 rapid bootstrapping (BS) replicates, choosing the tree with the best known likelihood (BKL) score amongst those independent searches (method adapted from Munro et al. 2011). Additionally, Garli 2.1 (Bazinet et al. 2014) on www.molecularevolution.org was utilized to see if the same topology was returned as the best tree, with 1000 bootstrap replicates.

For Bayesian analyses, Mr Bayes 3.2.3 (Ronquist et al. 2012) XSEDE was utilized with the following partitions: K80+I+G for 18S and GTR+I+G for 28S, HKY+I+G for the 1st positions in COI and Cytb, and GTR+I+G for the 2nd and 3rd positions in COI and Cytb.

In MrBayes, default parameters were used, with the exception of allowing enough time for 15,000,000 generations.

Trees were visualized in Figtree v.1.3.1 (Rambaut 2007)

Results

The topologies of the equal weighting and implied weighting analyses in TNT (parsimony) were the same, with the equal weighting analysis recovering nine trees with a best score of 8562 steps (CI 0.287 RI 0.641) and the implied weighting (K = 20.527) analysis recovering nine trees with a best score of 200. The best RAxML tree from 20 separate analyses had a final optimization likelihood of -44251.166938 (Fig. 2), and had the same topology as the tree produced by Garli, and the MrBayes analysis produced an average standard deviation of split frequencies (ASDSF) of 0.010179, with 25 percent of samples discarded as burn-in (Fig. 3).

Results were largely congruent for parsimony, likelihood, and Bayesian approaches in terms of higher-level topology (Figs 1–3), while species-level topologies were more variable. Apodryininae (as represented by *Madecadryinus politus* Olmi, 2007) were the sister taxon to all other Dryinidae in every analysis. The greatest difference among analyses were among Aphelopinae, Bocchinae and

Conganteoninae. In all three trees, Aphelopinae and Conganteoninae were recovered as monophyletic, but since Bocchinae were represented by one species, its monophyly could not be tested. In the Bayesian analysis, Bocchinae were the sister group to the remainder of Dryinidae excluding Apodryininae, with Aphelopinae and Conganteoninae as sister groups. In the parsimony and likelihood analyses, Bocchinae were the sister group to Conganteoninae, with Aphelopinae sister to (Conganteoninae + Bocchinae). The remaining subfamily topologies were the same in all three analyses – Anteoninae, Aphelopinae, and Gonatopodinae were monophyletic, with Anteoninae as the sister subfamily to ((*Thaumatodryinus* + (Dryininae *partim* + Gonatopodinae). Dryininae were paraphyletic due to the placement of *Thaumatodryinus merinus* Olmi, 2004 and *Thaumatodryinus macilentus* De Santis & Vidal Sarmiento, 1974, which were sister to a monophyletic Gonatopodinae and the remainder of Dryininae.

Many of the genera tested were found to be nonmonophyletic. Within Anteoninae, *Lonchodryinus* Kieffer, 1905 was the only genus found as monophyletic, as was *Epigonatopus* Perkins, 1905 in Gonatopodinae. *Dryinus* and *Thaumatodryinus* were the only genera from Dryininae tested, although all four of the *Dryinus* 'species groups' defined by Olmi (1993), were examined. Species groups were only defined for females, so undescribed male dryinid specimens could not be assessed. However, *Dryinus* Group 1 was found nonmonophyletic due to the placement of *Dryinus striatus* Fenton, 1927, although *Dryinus* Group 2 and *Dryinus* Group 4 were monophyletic. *Dryinus* Group 3 could not be assessed due to the sampling of a single specimen. *Gonatopus* was not monophyletic, nor were any of its species groups.

Discussion

The validity of Thaumatodryininae

Olmi (1993) synonymized Thaumatodryininae with Dryininae, placing *Thaumatodryinus* close to the Dryininae genus *Pseudodryinus.* Olmi (1989) had originally attributed *Pseudodryinus* to Gonatopodinae on the basis of lacking a spur (1, 0, 2 tibial formula), but later examination of *Pseudodryinus* specimens by Olmi revealed a tibial formula of 1, 1, 2, allowing for the genus to be moved to Dryininae. At that time, previously unknown males of *Pseudodryinus* were discovered, and were shown to have quadridentate mandibles, as opposed to the tridentate mandibles found in all other male Dryininae (Olmi 1993). Olmi proposed that these males belonged to Thaumatodryininae, and then further noted that it would be unfeasible to have the females of *Pseudodryinus* within Dryininae and the males of *Pseudodryinus* within Thaumatodryininae. To preserve *Pseudodryinus* as a valid genus, *Thaumatodryinus* (the only genus within Thaumatodryininae) was synonymized within Dryininae.

In the molecular analyses presented here, the two different species of *Thaumatodryinus* were monophyletic and sister to Gonatopodinae + Dryininae. Molecular

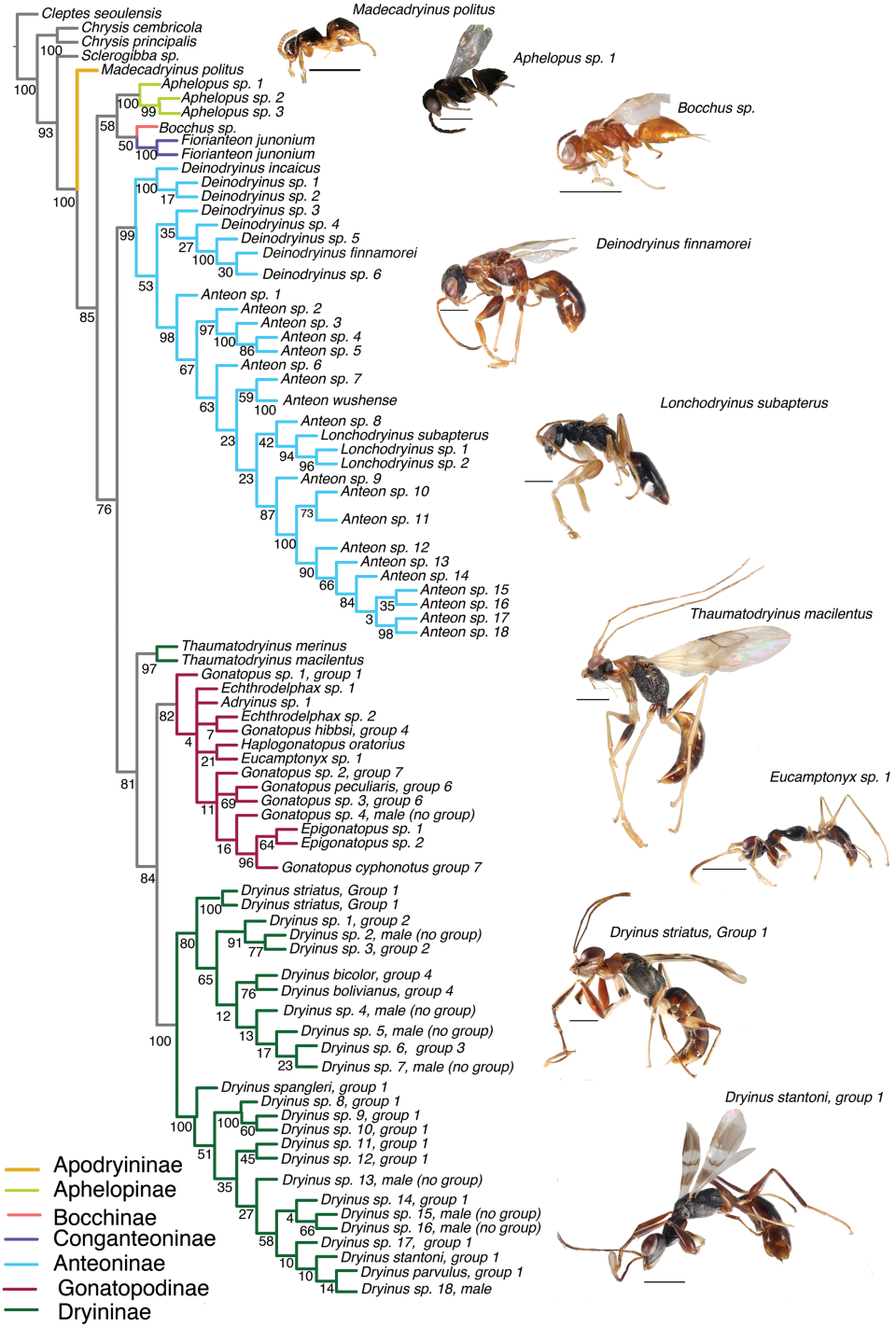

Figure 1. Parsimony support tree. Jackknife support for nodes given in GC-values (frequency differences) from 1000 replicates. CI 0.287 RI 0.641. Scale bar in all images is 1.0 mm.

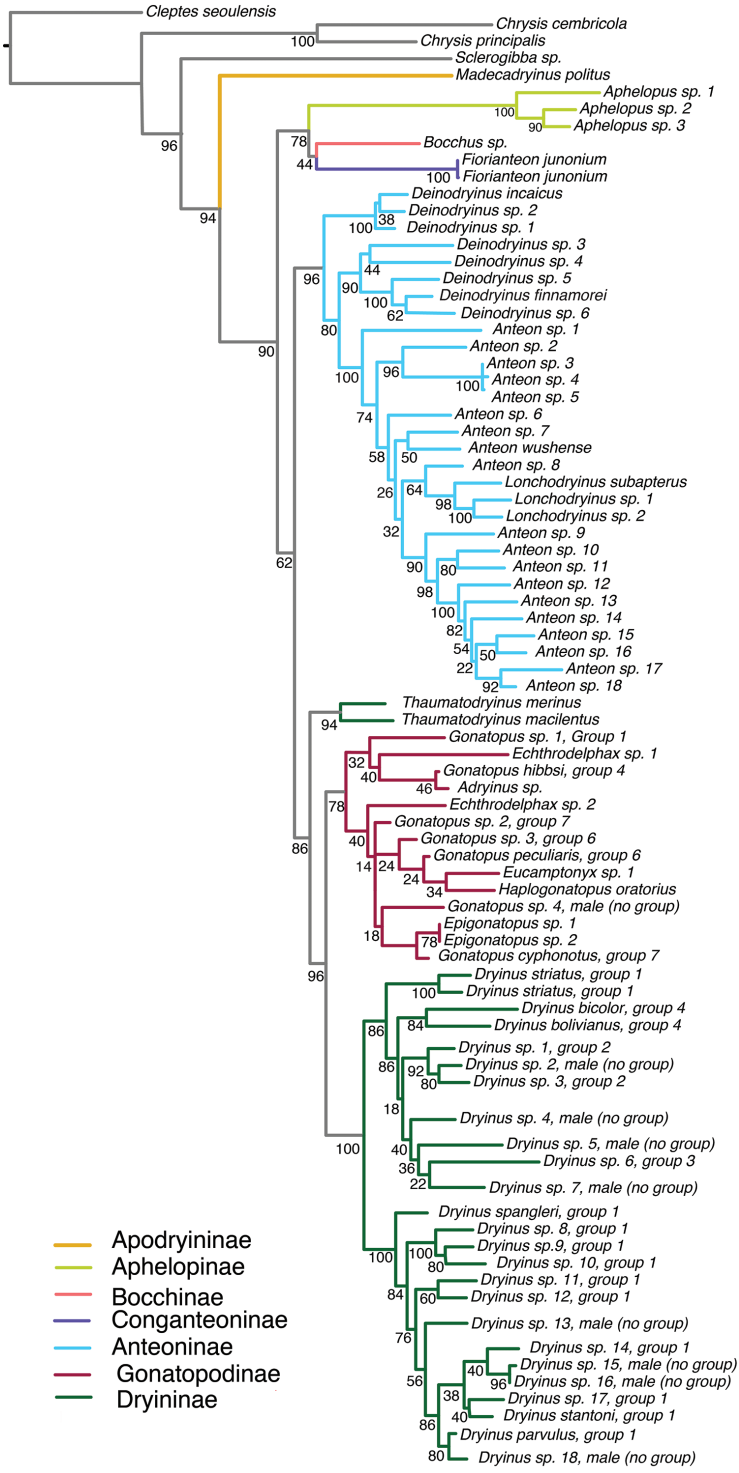

Figure 2. Likelihood support tree. Rapid Bootstrap support values shown at nodes.

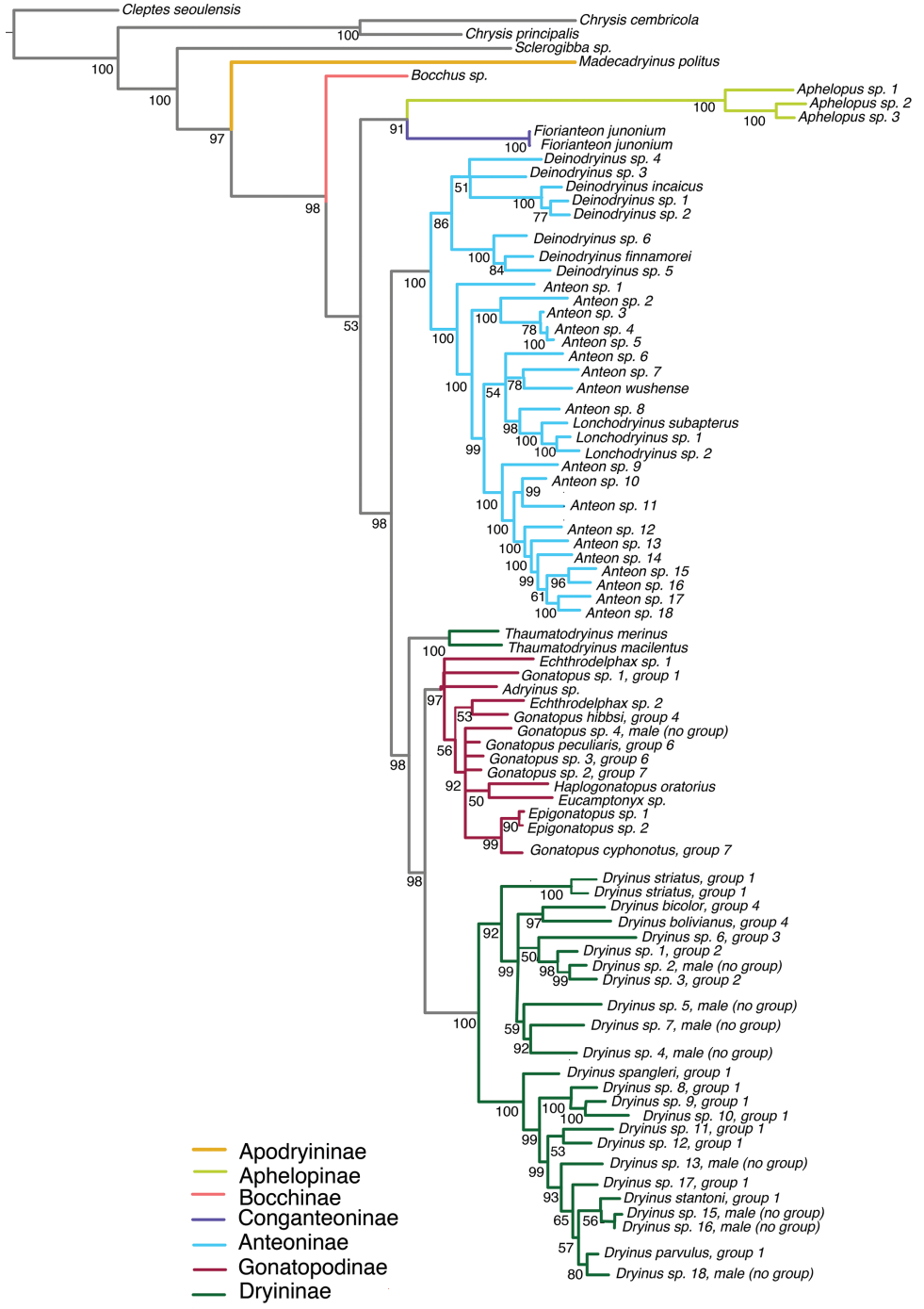

Figure 3. Bayesian support tree. Support probabilities shown at nodes as a percent.

data from *Thaumatodryinus macilentus* were taken from a female specimen, while molecular data from *Thaumatodryinus merinus* come from a male. Unfortunately, neither male nor female specimens of *Pseudodryinus* with viable DNA were available to test their placement within Dryininae or Thaumatodryininae. To establish the monophyly of Dryininae, and retain Gonatopodinae as a separate subfamily, I resurrect Thaumatodryininae, containing the genus *Thaumatodryinus*. The defining synapomorphy of Thaumatodryininae is the presence of long hairs on flagellomeres 3 – 8 in females (Xu et al. 2013).

Evolution of the chela

The tree produced by Olmi only treated Aphelopinae, Anteoninae, Dryininae, and Gonatopodinae from Fennoscandia and Denmark (Olmi 1994a), and similarly found Dryininae and Gonatopodinae as sister groups (*Thaumatodryinus* was not included in the cladogram). Olmi (1994a) placed Anteoninae as sister to (Dryininae + Gonatopodinae), which was found in this study. Carpenter (1999) also found Anteoninae as the sister group to the clade that contained Dryininae and Gonatopodinae. This study diverges from these past two trees in the basal lineage of Dryinidae. In both Olmi (1994a) and Carpenter (1999), Aphelopinae were considered the basal lineage of Dryinidae on the basis of the lack of the characteristic pincer-like chela. Here, Apodryininae were found as the basal lineage of Dryinidae and while not all subfamilies of Dryinidae were considered, this suggests that the loss of the chela is a derived trait of Aphelopinae. Erwiniinae (known only from the type species) are also achelate, but were not included in this study.

Sampling of genera and species groups of Dryinus and Gonatopus

Several of the smaller subfamilies were not represented in this study because of their scarcity – Apoaphelopinae are known from two species, Erwiniinae from one species, Plesiodryininae from one species, and Transdryininae from two species.

Sampling of the genera of the subfamilies was also incomplete. Within Dryininae, only *Dryinus* was treated, although all four of the species groups were included. *Megadryinus* Richards, 1953 (known from three species), *Gonadryinus* Olmi, 1991 (known from one species), and *Pseudodryinus* (known from ten species) were absent. Given the shared characteristic of having quadridentate mandibles in males, *Thaumatodryinus* and *Pseudodryinus* might be related, but without a specimen from which viable DNA could be sequenced, the placement of *Pseudodryinus* could not be assessed.

Within Gonatopodinae, only five of the twelve species groups of *Gonatopus* were assessed. *Epigonatopus* Perkins, 1905, which is only known from Australia, was found

monophyletic, and *Echthrodelphax* Perkins, 1903 was nonmonophyletic. All other genera assessed (*Adryinus* Olmi, 1984, *Haplogonatopus* Perkins, 1905, and *Eucamptonyx* Perkins, 1907) were only represented by a single specimen. DNA-viable specimens from *Pentagonatopus* Olmi, 1984 (known from three species), *Pareucamptonyx* Olmi, 1991 (known from two species) *Esagonatopus* Olmi, 1984, (known from six species), *Gynochelys* Brues, 1906 (known from two species), and *Neodryinus* Perkins, 1905 (known from 49 species) were unavailable.

Within Anteoninae, three out of four extant genera were included, with *Metanteon* Olmi, 1984 (known only from the type species) not included. Conganteoninae were only represented by one genus, *Fiorianteon* Olmi, 1984, and did not include the other genus, *Conganteon* Benoit, 1951. Bocchinae were only represented by *Bocchus* Ashmead, 1893, and did not include *Mirodryinus* Ponomarenko, 1972 and *Mystrophorus* Förster, 1856. Aphelopinae were only represented by *Aphelopus* Dalman, 1823, and did not include *Crovettia* Olmi, 1984. Apodryininae were only represented by *Madecadryinus* Olmi, 2007, and did not include the six other genera.

Conclusion

In all analyses, *Thaumatodryinus* was well-supported and Thaumatodryininae were resurrected here, bringing the total subfamilies of Dryinidae to 16.

The validity of species groups within *Dryinus* and *Gonatopus* remains questionable. Some species groups, like *Dryinus* Group 4, which was originally a separate genus, *Perodryinus*, were easily recovered as monophyletic while *Dryinus* Group 1, which contains several synonymized genera, was not recovered as monophyletic. This may be because the larger species groups share synonymized genera – for example, *Dryinus* species groups 1, 2, and 3 all contain synonymized species from *Mesodryinus*. Shared synonymized genera are found within the *Gonatopus* species groups as well.

In continuing molecular studies, specimens from each of the species groups of *Gonatopus* and *Dryinus* should be included, as well as all of the genera of the subfamilies, where sampling permits. In particular interest would be to find morphological synapomorphies at the generic level for male Dryinidae.

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Supplementary material 1

Specimen information and gene coverage

Authors: C. M. Tribull

Data type: specimens data

Explanation note: Locality, museum collection, collector, and sex listed for each specimen in study. Genbank accession numbers provided for sequences sourced from genbank. Success of sequencing for each specimen is indicated by a filled green box.

Supplementary material 2

Primer protocols

Authors: C. M. Tribull

Data type: primer data

Explanation note: Primer names and thermocycler conditions for each gene. Provided courtesy of Jongok Lim.