MOLECULAR AND MORPHOMETRIC PHYLOGENETICS OF DRYINIDAE AND BETHYLIDAE (HYMENOPTERA: CHRYSIDOIDEA)

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By

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ABSTRACT

Aculeata (Hymenoptera) is largely known for its bees, ants, and social wasps, from which most people would immediately recognize honey bees and paper wasps. However, sister to the clade of Apoidea and Vespoidea is the much smaller, and infinitely more enigmatic Chrysidoidea, which contains seven extant families that are relatively understudied. Unlike the other superfamilies, Chrysidoidea is exclusively parasitic – although this behavior ranges from ectoparasitoidism to endoparasitoidism to cleptoparasitism (targeting solitary wasps and bees), as seen in Chrysididae (the cuckoo wasps).

Dryinidae, which contains about 1700 species worldwide in 16 subfamilies, are parasitoids of Auchenorrhyncha (leafhoppers, planthoppers, and their allies) and are known to attack major rice and fruit crop pests. In Chapters II and III, the relationships within the family were investigated at the subfamily level. In Chapter II, a phylogeny reconstructed from 18S, 28S, Cytochrome Oxidase I (COI) and Cytochrome b (CytB) resulted in the resurrection of *Thaumatodryinus* to Thaumatodryininae to preserve the monophyly of Dryininae. Chapter III examined the utility of landmark analysis in parsimony using the methods implemented by Catalano et al. (2010) and subsequent publications. The trees constructed from the landmark analyses were incongruent with the

combined molecular and morphological phylogeny, but landmark analyses could be utilized effectively to reconstruct species-level phylogenies for *Dryinus* and *Gonatopus*, both of which were found as nonmonophyletic in Chapters II and III.

Chapters IV and V focused on *Epyris*, the largest genus within the subfamily Epyrinae, and Bethylidae as a whole. *Epyris* has long been suspected of being a taxonomic wastebasket, but the molecular phylogeny reconstructed from 16S, 18S, 28S, COI, and CytB is the first phylogeny to sample the worldwide breadth of its species diversity. *Epyris* was shown to be nonmonophyletic, although the type specimen, *Epyris niger* Westwood, was recovered in a clade with a distinct synapomorphy of large, nearly touching, scutellar pits. In Chapter V, five new species from *Epyris sensu stricto* were described from Western Australia and Queensland, and a key was provided to the known female *Epyris* of Australia.

For anyone that I would consider as family,
regardless of blood,
and all of my teachers

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And finally, to my family, who have always been supportive, and only a little concerned, as I flung myself to the far corners of the world in search of very tiny wasps.

But wasps, like most other creatures, are pretty complicated.

-Howard Ensign Evans, in *Wasp Farm*

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CHAPTER 1
GENERAL INTRODUCTION

Background I: An Overview of Phylogenetic Research in Chrysidoidea

When the word 'wasp' comes to mind, most people think of the social wasps – large, often brightly colored, occasionally aggressive, 'pests' that are known for making an unwanted appearance at outdoor parties or under the eaves of our homes. However, the vast majority of Hymenoptera are small, solitary parasitoids that have evolved to make hosts out of living caterpillars, beetle larvae, and other hapless insects (Quicke 1997; O'Neill 2001). Parasitism of wood-boring insects is the ground-plan behavior of the Apocrita - the "true" wasps, defined by the "wasp waist" formed by the incorporation of the first abdominal segment into the thorax, and it contains several mega-diverse superfamilies of parasitoid wasps (Goulet and Huber 1993). Within Apocrita lies the infraorder Aculeata, which contains the ants, bees, and stinging wasps, in which the defining trait is the development of the primitive ovipositor into a sting that is utilized to deliver venom in both defensive and offensive maneuvers. This development allowed for the evolution of the social wasps, bees, and ants, and subsequently, many of our cultural and social connections with insects (Grimaldi and Engel 2005).

While social Hymenoptera gain the most attention in Aculeata, the primitive reproductive behavior is still based in parasitoidism, as seen throughout the extant superfamily Chrysidoidea (Figure 1.1). This superfamily is composed of seven extant families and a single extinct family, Plumalexiidae, which shares characteristics with Plumariidae, is known from males in amber, and might instead be the sister family to Chrysidoidea. (Grimaldi and Engel 2005; Brothers 2011). Compared to the two other

superfamilies of Aculeata, Apoidea and Vespoidea, Chrysidoidea has the smallest portion of species and is excluded from the other two superfamilies by an equal number of antennomeres in both sexes and an exposed seventh metasomal tergum that is evenly sclerotized in females.

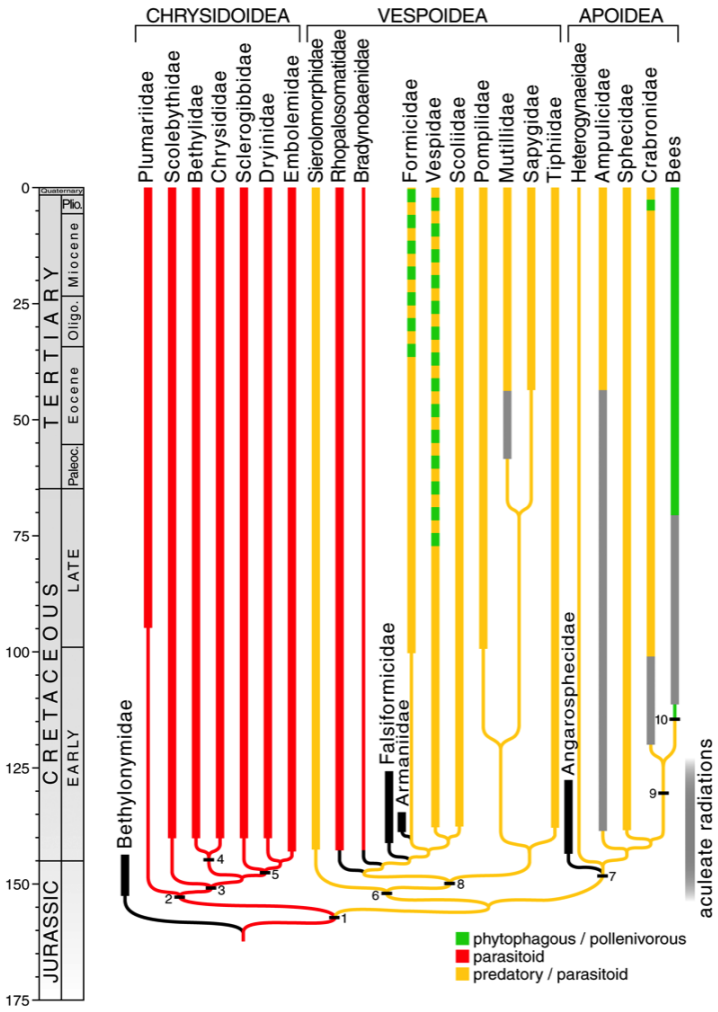

Figure 1.1 from Grimaldi and Engel (2005). Relationships of families of Aculeata.

Relationships amongst the families of Chrysidoidea have been analyzed several times in the past few decades, most notably in Brothers (1999, 2011) Brothers and Carpenter (1993), Carpenter (1986, 1999), and Ronquist (1999), each using solely morphological characters. Molecular phylogenetic studies to elucidate the relationships amongst Hymenoptera exist, but Chrysidoidea, as a relatively small and understudied superfamily, is

never represented by all seven families (Heraty et al. 2011; Sharkey et al. 2012; Klopfstein et al. 2013; Payne et al. 2013). For example, Sharkey et al. (2012), which examined the relationships of the superfamilies of Hymenoptera, only represented Chrysidoidea with a single bethylid. Heraty et al. (2011) sampled four of the families (Chrysididae, Bethylidae, Sclerogibbidae, and Scolebythidae) and continuously found the superfamily to be nonmonophyletic, despite the obvious morphological synapomorphies known to define the superfamily. Thus far, there has been only one attempt to combine the morphological and molecular data for Chrysidoidea, in which the superfamily was recovered as monophyletic with Plumariidae as the outgroup to all extant Chrysidoidea (Carpenter et al. *In preparation*).

THE EXTANT FAMILIES OF CHRYSIDOIDEA

PLUMARIIDAE

Plumariidae is the most basal family of the extant Chrysidoidea, with seven modern genera that are found strictly in arid habitats of southern Africa and South America, suggesting that the lineage arose in Gondwana in the Mid-Late Jurassic (Grimaldi and Engel 2005). Of the seven genera, four are monotypic and the most speciose genus, *Plumarius* Philip, contains just sixteen described species. Like most families of Chrysidoidea, males and females are highly sexually dimorphic with wingless females and winged males (Evans 1966; Carpenter 1999; Diez et al. 2012). Beyond this sexual dimorphism, nothing is known of their biology. The only putative plumariid fossils were recently moved to a new extinct family, Plumalexiidae

Figure 1.1 from Grimaldi and Engel (2005). Relationships of families of Aculeata

(Brothers 2011)

SCOLEBYTHIDAE

The next family to arise after Plumariidae, Scolebythidae, is known from South and Central America, South Africa, Madagascar, Australia, Fiji, Thailand, and China (Engel and Grimaldi 2007; Azevedo et al. 2011). Like Plumariidae, little is known of the biology except for records of ectoparasitoidism on wood-boring beetles (Grimaldi and Engel 2005). Oddly, there are more fossil species of Scolebythidae than there are living species, which are found in amber fossils from the Early Cretaceous to the Early Miocene across the Northern Hemisphere (Grimaldi and Engel 2005; Engel and Grimaldi 2007).

CHRYSIDIDAE

With 3,000 species found worldwide, Chrysididae is the largest family within Chrysidoidea and has the greatest diversity of hosts and parasitic behaviors, ranging across ectoparasitism on Lepidoptera, Hymenoptera, and the eggs of Phasmatodea (Kimsey and Bohart 1990). Additionally, this is the only family within Chrysidoidea to exhibit cleptoparasitism, with records of chrysidids attacking the nests of solitary wasps and bees (Rosenheim 1987).

Chrysididae, along with Bethylidae, are both known from Early Cretaceous fossil records (Grimaldi and Engel 2005). With vivid, iridescent bodies that stand in sharp contrast to the dull browns and blacks that dominate the rest of Chrysidoidea, Chrysididae has attracted the attention of many entomologists, and was most comprehensively examined and taxonomically revised in Kimsey and Bohart (1990).

BETHYLIDAE

Bethylidae, which is found as the sister group to Chrysididae in all morphological analyses, contains about 2,700 species worldwide, and parasitize Coleoptera and Lepidoptera (Evans 1964; Gordh and Móczár 1990). Bethylids are natural predators of *Hypothenemus hampei* (the coffee berry borer) and *Amyelois transitella* (navel orange worm) (Abraham et al. 1990; Legner and Gordh 1992). Evans (1964, 1969) covered the family extensively, revising much of the original taxonomic work by Kieffer (1905, 1907, 1914), but there were no true phylogenetic studies of the family until Sorg (1988) and Carr et al. (2010). Within the past ten years, there have been multiple examinations of the subfamily level taxonomy and subsequent revisions. Of the greatest relevance to this dissertation is the work of Alencar and Azevedo (2013), in which the largest subfamily Epyrinae *sensu* Evans was divided into two separate subfamilies, Epyrinae *sensu* Alencar and Azevedo and Scleroderminae *sensu* Alencar and Azevedo.

SCLEROGIBBIDAE

Sclerogibbidae is a worldwide family, composed of 22 species of ectoparasitoids of Embioptera (the webspinners), with little known of their biology (Lim et al. 2013). They are considered the sister-group to (Embolemidae + Dryinidae) (Grimaldi and Engel 2005; Carpenter et al. *In preparation*). The fossil record consists of two specimens, from Early Cretaceous Lebanese amber and Miocene Dominican amber (Grimaldi and Engel 2005).

EMBOLEMIDAE

Embolemidae is the sister family to the Dryinidae, and little is known of the biology outside of obligate parasitoidism of planthopper (Auchenorrhyncha) nymphs (Olmi et al. 2014a). The family is composed of two genera – *Embolemus* Westwood and

Ampulicomorpha Ashmead, and has a worldwide distribution (Olmi 1996; Olmi et al. 2014a; Olmi et al. 2014b)

DRYINIDAE

Dryinidae is the third largest family within Chrysidoidea and contains about 1700 species worldwide (Olmi 1984; Olmi 1989; Olmi and Virla 2014; Xu et al. 2013). Dryinids are parasitoids and predators of Auchenorrhyncha, and naturally attack several major rice, fruit, and sugarcane pests (Sahragard et al. 1991; Olmi 1989; Mora-Kepfer and Espinoza 2009, Virla et al. 2010). Interestingly, dryinids blur the line between ectoparasitoidism, which is present in all other families of Chrysidoidea, and endoparasitoidism, with female dryinids depositing eggs inside hosts. The wasp larvae develop internally through the first 2-3 instar molts, before partially migrating externally, protecting themselves by forming a thylacium out of the exuviae of previous molts (Grimaldi and Engel, 2005). Currently, there are eleven extant subfamilies and four fossil subfamilies (Grimaldi and Engel 2005; Xu et al. 2013). Males and females are extremely sexually dimorphic, leading to an abundance of species that are described from one sex and a complicated history of systematic revision, explained in detail in the section below and in chapter II.

BACKGROUND II: A BRIEF OVERVIEW OF PHYLOGENETIC RESEARCH WITHIN DRYINIDAE

Dryinidae was first placed as a genus within Proctotrupoidea in Latreille (1805) and was not raised to family status until Haliday (1833). The most recent classification comes from Olmi and Virla (2014) and Xu et al. (2013). Despite a recent abundance of

taxonomic revisions, mostly from Massimo Olmi, there has been little phylogenetic research in Dryinidae.

This may be because of a supposed difficulty in coding characters in a family that exhibits huge sexual dimorphism. In "The Dryinidae and Embolemidae (Hymenoptera: Chrysidoidea) of Fennescandia and Denmark", Massimo Olmi writes "1) We can discuss female affinities, because females show clear and different evolutionary levels; 2) we can discuss male affinities, but with great difficulty since males are very uniform and their differential morphological characteristics are usually very slight; 3) 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" (Olmi 1994, p. 31 – 32). Carpenter (1999) pointed out that this statement was a confusion of data sources (from male and female morphological data sets) with true phylogenetic relationships. Carpenter (1999) featured the first subfamily-level morphological phylogeny from 32 characters that were analyzed in NONA, but the tree is now outdated in terms of subfamily classification. Until Tribull (2015, *in press*, presented in chapter II), Carpenter (1999) remained the sole published phylogeny of Dryinidae at any taxonomic level.

Despite a lack of phylogenetic testing, taxonomic revisions have continued at the subfamily and genus level since Carpenter (1999). Of the ten subfamilies initially examined, Laberitinae and Transdryininae have been synonymized with Anteoninae and Dryininae, respectively. Several new subfamilies have been erected and the largest genera within Dryinidae, *Dryinus* and *Gonatopus*, have been attributed species groups

from numerous synonymized genera, as elaborated upon in the introduction to chapter two.

BACKGROUND III: INTRODUCTION TO SYSTEMATIC WORK IN BETHYLIDAE

The term Bethylidae was first used in Haliday (1839), was changed to a superfamily Bethyloidea in Förster (1856), and then changed back to Bethylidae in Ashmead (1902). Independently, Cameron (1883) placed Bethylinae as a subfamily in Proctotrupidae, which Dalle Torre and De (1898) would further split into two subfamilies Bethylinae and Pristocerinae. Ashmead (1902) and Brown (1906) placed Bethylidae within Vespoidea, with Bethylidae containing two subfamilies Bethylinae and Dryinidae.

Jean-Jacques Kieffer expanded the known diversity of Bethylidae in several publications, eventually summarized in Kieffer (1908) and Kieffer (1914) with a total of 660 species in 102 genera and five tribes – Pristocerini, Sclerodermini, Epyrini, Mesitiini, and Bethylini, all within Bethylinae. At that point, Bethylidae contained Anteoninae, Embolemininae, Bethylinae, and Sclerogibbinae.

Berland (1928) re-established Bethyloidea, raising Kieffer's Bethylinae to Bethylidae, with each of the five tribes elevated to Pristocerinae, Scleroderminae, Epyrinae, Mesitiinae, and Bethylinae. This classification remained until Evans (1964), when he collapsed Scleroderminae sensu Berland into Epyrinae, and created three tribes – Sclerodermini, Cephalonomiini, and Epyrini. Galodoxinae was described by Nagy (1974), but synonymized as a genus, *Galodoxa*, within Sclerodermini in Lanes and Azevedo (2008). Afgoioginae (Argaman 1988) was synonymized within Pristocerinae in Terayama (2003).

In the past decade, most of the revisions have been at the genus level and largely focused on synonymizing the genera described by Kieffer (1914). The latest higher-level taxonomic revision came as a result of Alencar and Azevedo (2013). Using over 200 morphological characters, the authors found that Epyrinae *sensu* Evans was not monophyletic and Sclerodermini (in which Cephalonomiini had been synonymized in Lanes and Azevedo [2008]) was not sister to Epyrini. Sclerodermini and Epyrini were raised to subfamily status as Scleroderminae and Epyrinae.

While Epyrinae was now well supported as a subfamily, the relationships amongst the genera were largely unknown. This is certainly the case for *Epyris*, which contains numerous genera synonymized by Evans (1964, 1969) and Azevedo and Alencar (2010a, 2010b). There have been no detailed morphological or molecular analyses at the genus level, although Alencar and Azevedo (2013) did find that the two species of *Epyris* included in their morphological analysis were not monophyletic. Carr et al. (2010) found similar results, but their sample size was also small (4 specimens of *Epyris* in the single 16S and 28S gene trees and 2 specimens in the combined tree).

BACKGROUND IV: GEOMETRIC MORPHOMETRICS AND ITS APPLICATIONS IN ENTOMOLOGY AND CLADISTIC PHYLOGENETICS.

The analysis of shape data, or geometric morphometrics, has been utilized in entomology to delimit species groups, study geographical variation, caste delimitation, and even responses to pesticides (Daly 1985; Bethke et al. 1991; Hartfelder and Engels 1992; Perrard et al. 2012). Geometric morphometric data, however, have been little applied to phylogenetic research. There has been a division between geometric morphometrics and systematics because morphometric shapes were seen as incompatible

with phylogenetic research, stemming from a belief that any incorporation of morphometric data would be phenetic (MacLeod 2002; Adams et al. 2013).

However, recent studies have reopened the idea of combining shape data and phylogenetics. Catalano et al. (2010), Goloboff and Catalano (2011), and Catalano and Goloboff (2012) explored the use of the landmark data as phylogenetic information that can be utilized by the parsimony program TNT (Goloboff et al. 2008). This approach treats the landmark configurations (where a configuration is a shape made up of landmarks) as single continuous characters in TNT (Farris, 1970; Goloboff et al. 2006). The method implemented finds the value for ancestral landmark points that minimizes the distances between ancestor and descendant landmark points. Before using the script in TNT, all landmarks should be aligned to remove information resulting from size and orientation, within programs like IMP, MorphoJ, or the Morphometrics package in R. In Catalano and Goloboff (2012), a new dynamic alignment method was designed so that landmark alignment can be accomplished in coordination with the initial optimizations in TNT.

Unfortunately, there have been relatively few implementations of GM as cladistic data, possibly due to the heavy computational costs (Catalano et al. 2010) or the time required to take photos of specimens, identify homologous landmarks, and digitally add landmarks to all of the specimens. Currently, there are just three records of using the methods in the literature – an analysis of shell shape in *Conus* (Cruz et al. 2012), an analysis of the quadrate in nonavian theropods (Hendrickx *in press*), and an analysis of wing venation in Vespinae (Perrard et al. *in press*). In Catalano et al. (2014), it was found that using a small number of landmark configurations (1 – 2) in a study of Musteloidea

resulted in trees that were highly incongruent from the morphological and molecular data trees. Similarity between the trees improved as more landmark configurations were added to the GM analysis.

EXPLORATIONS OF THIS DISSERTATION, MATERIAL SOURCES, AND FUNDING

This dissertation is divided by taxonomic subject – Chapters II and III focus on the subfamily level systematics of Dryinidae, and chapters IV and V investigates the systematics of Epyrinae, with a focus on the largest genus, *Epyris*.

Chapter II is a molecular analysis of the subfamilies of Dryinidae, which concludes with the re-elevation of *Thaumatodryinus* to the subfamily Thaumatodryininae. Chapter III is an exploration of geometric morphometrics in cladistic analysis in combination with traditional morphological and molecular techniques. Unlike Chapter II, Chapter III utilizes a different dataset of Dryinidae that are female-only, to avoid issues of variability in the morphometric analysis.

Chapter IV is a molecular phylogeny of Epyrinae, with a focus on the largest genus within Bethylidae, *Epyris*, which has long been suspected of being a taxonomic wastebasket. *Epyris* is shown to be non-monophyletic, although the clade that contains the type, *Epyris niger* Westwood, is well supported and features a distinct morphological synapomorphy in the shape of the scutellar pits. Chapter V describes five new species of *Epyris* from this clade, specimens of which were loaned to the author following a field and museum collection trip to Australia in summer 2014.

The samples used in this dissertation come from several domestic and international field trips taken by the author in the past four years. Domestic trips included California, Oregon, Washington, and Florida (Summer 2012), and the Southwestern

Research station in Portal Arizona (Summer 2013). International trips were to the Canadian National Collections (Summer 2013), the Universidade Federal do Espírito Santo (Winter 2014), the Queensland Museum, the Australian National Insect Collection (Summer 2014), the British Museum of Natural History, and the Muséum National D'Histore Naturelle (Winter 2015). Major funding was provided by the Richard Gilder Graduate School at the American Museum of Natural History, the National Science Foundation Graduate Research Fellowship Program, and a National Science Foundation Doctoral Dissertation Improvement Grant.

REFERENCES

Abraham, Y. J., D. Moore, and G. Godwin. 1990. "Rearing and aspects of biology of *Cephalonomia stephanoderis* and *Prorops nasuta* (Hymenoptera: Bethylidae) parasitoids of the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae)." *Bulletin of Entomological Research* 80:121-128.

Adams, D.C., F. J. Rohlf, and D.E. Slice. 2013. "A field comes of age: geometric morphometrics in the 21st century." *Hystrix, the Italian Journal of Mammalogy* 24: 7-14.

Argaman, Q. 1988. "A new subfamily of Bethylidae allied to Pristocerinae (Hymenoptera)". *Bolletino della Società Entomologica Italiana* 120: 139-152.

Alencar, I.D.C.C., and C.O. Azevedo. 2013. "Reclassification Epyrini (Hymenoptera: Bethylidae): a tribal approach with commentary on their genera." *Systematic Entomology* 38: 45-80.

Ashmead, W.H. 1902. "Classification of the fossorial, predaceous and parasitic wasps, or the superfamily Vespoidea. No.9. Family XXXII." *Canadian Entomology* 34: 268-273.

Azevedo, C.O., and I.D.C.C. Alencar. 2010a. "Rediscovery of *Melanepyris* (Hymenoptera: Bethylidae): a new synonym of *Epyris*." *Zoologica (Curitiba*) 27: 403-407.

Azevedo, C.O., and I.D.C.C. Alencar. 2010b. "Rediscovery of the Afrotropical genus *Trissepyris* Kieffer (Hymenoptera: Bethylidae), a new synonym of *Epyris* Westwood". *Journal of Afrotropical Zoology* 6: 3-7.

Azevedo, C.O., Z. Xu, and R.A. Beaver. 2011. "A new species of *Pristapenesia* Brues (Hymenoptera, Scolebythidae) from Asia." *Zootaxa* 2750: 60-64.

Berland, L. 1928. Bethylidae. In Faune de France 19 – Hyménoptères Vespiformes II. Office Central de Fannistique, Paul Lechevalier, Paris: 96-137.

Bethke, J.A., T.D. Paine, and G.S. Nuessly.1991. "Comparative biology, morphometrics, and development of two populations of *Bemisia tabaci* (Homoptera: Aleyrodidae) on cotton and poinsettia." *Annals of the Entomological Society of America* 84: 407-411.

Brothers, D.J. 1999. "Phylogeny and evolution of wasps, ants and bees (Hymenoptera, Chrysidoidea, Vespoidea and Apoidea)." *Zoologica Scripta* 28: 233-250.

Brothers, D.J. 2011. "A new Late Cretaceous family of Hymenoptera, and phylogeny of the Plumariidae and Chrysidoidea (Aculeata)." *ZooKeys* 130 (2011): 515-542.

Brothers, D.J., and J.M. Carpenter. 1993. “Phylogeny of Aculeata: Chrysidoidea and Vespoidea (Hymenoptera).” *Journal of Hymenoptera Research* 2: 227-304.

Brown, R.E. 1906. "A catalogue of Philippine Hymenoptera, with description of new species." *Philippine Journal of Science* 1: 683-695.

Cameron, P. 1883. "IX. Descriptions of new genera and species of Hymenoptera." *Transactions of the Royal Entomological Society of London* 31: 187-197.

Carpenter, J.M. 1986. "Cladistics of the Chrysidoidea (Hymenoptera)." *Journal of the New York Entomological Society* 94: 303-330.

Carpenter, J.M. 1999. "What do we know about chrysidoid (Hymenoptera) relationships?" *Zoologica Scripta* 28: 215-231.

Carpenter, J.M., D. Agosti, C.O. Azevedo, C.M. Brabant, D.J. Brothers, J. Dubois, L.S. Kimsey, V. Lohrmann, M. Ohl, L. Packer, S. Schulmeister. *In preparation*. Phylogeny of the Aculeata: Results of the Hymatol Taxonomic Working Group

Carr, M.J., P.W. Young, and P.J. Mayhew. 2010. "Phylogeny of bethylid wasps (Hymenoptera: Bethylidae) inferred from 28S and 16S rRNA genes." *Insect Systematics & Evolution* 41: 55-73.

Catalano, S.A., and P.A. Goloboff. 2012. "Simultaneously mapping and superimposing landmark configurations with parsimony as optimality criterion." *Systematic biology* 61:392-400.

Catalano, S.A., P.A. Goloboff, and N.P. Giannini. 2010. "Phylogenetic morphometrics (I): the use of landmark data in a phylogenetic framework." *Cladistics* 26: 539-549.

Catalano, S.A., M.D. Ercoli, and F.J. Prevosti. 2014. "The More, the Better: The Use of Multiple Landmark Configurations to Solve the Phylogenetic Relationships in Musteloids." *Systematic Biology*: syu107.

Cruz, R.A.L., M.A. Josefa, R. Pante, and J.F. Rohlf. 2012. “Geometric morphometric analysis of shall shape variation in *Conus* (Gastropoda: Conidae).” *Zoological Journal of the Linnean Society* 165: 296-310. \

Dalla T. and C.G. De. 1898. Bethylinae, Pristocerinae. In Catalogus Hymenopterorum hucusque descriptorum Systematicus et synonymicus. Chalcididae et Proctotrupidae, 5: 1-598.

Daly, H.V. 1985. "Insect morphometrics." *Annual Review of Entomology* 30: 415-438.

Diez, Patricia A., P. Fidalgo, and A. Roig-Alsina. 2013. "Revision of the *ocellatus* species-group of the genus *Plumarius* Philippi 1873 (Hymenoptera, Chrysidoidea, Plumariidae), with description of three new species from Argentina." *Zootaxa* 3721: 85-91.

Engel, M.S., and D.A. Grimaldi. 2007. "Cretaceous Scolebythidae and phylogeny of the family (Hymenoptera: Chrysidoidea)." *American Museum Novitates*: 1-16.

Evans, H.E. 1964. "A synopsis of the american Bethylidae (Hymenoptera, Aculeata)." *Bulletin of the Museum of Comparative Zoology* 132: 1-222.

Evans, H.E. 1966 "Discovery of the female *Plumarius* (Hymenoptera, Plumariidae)." *Psyche: A Journal of Entomology* 73: 229-237.

Evans, H.E. 1969. "A revision of the genus *Epyris* in the Americas (Hymenoptera: Bethylidae)." *Transactions of the American Entomological Society* 95: 181-352.

Farris, J.S. 1970. "Methods for computing Wagner trees." *Systematic Biology* 19: 83-92.

Förster, A. 1856. "Hymenopterologische Studien II: Chalacidiae und Proctotrupii." *Aachen. Ernst ter Meer* 2: 1-152. Bethylidae)." *Transactions of the American Entomological Society* 95: 181-352.

Goloboff, P.A., C.I. Mattoni, and A.S. Quinteros. 2006. "Continuous characters analyzed as such." *Cladistics* 22: 589-601.

Goloboff, P.A., J.S. Farris, and Kevin C. Nixon. 2008. "TNT, a free program for phylogenetic analysis." *Cladistics* 24: 774-786.

Goloboff, P.A., and S.A. Catalano. 2011. "Phylogenetic morphometrics (II): algorithms for landmark optimization." *Cladistics* 27: 42-51.

Gordh, G., and L. Móczár. 1990. "A catalog of the World Bethylidae (Hymenoptera, Aculeata)." *Memoirs of the American Entomological Institute* 46: 1–364.

Goulet, H., and J.T. Huber. 1993. *Hymenoptera of the World: an Identification Guide to Families*. Research Branch, Agriculture Canada.

Grimaldi, D., and M.S. Engel. 2005. *Evolution of the Insects*. Cambridge University Press.

Haliday, A.H. 1833. "An essay on the classification of the Parasitic Hymenoptera of Britain, which correspond with the *Ichneumones minuti* of Linnaeus." *The Entomological Magazine* 1: 259-273.

Haliday, A.H. 1839. Hymenoptera Britannica, Part 1. Oxyura. Addendum to volume 1, Hymenopterorum synopsis ad methodum fallenil utplurimum accomodata. Hippolytus Bailliere, London.

Hartfelder, K., and W. Engels. 1992. "Allometric and multivariate analysis of sex and caste polymorphism in the neotropical stingless bee, *Scaptotrigona postica*." *Insectes Sociaux* 39: 251-266.

Hendrickx, C. 2015. "The nonavian theropod quadrate II: systematic usefulness, major trends and cladistic and phylogenetic morphometrics analyses." *In preparation*. DOI 10.7287/peerj.preprints.380v2

Heraty, J., F. Ronquist, J.M. Carpenter, D. Hawks, S. Schulmeister, A.P. Dowling, D. Murray et al. 2011. "Evolution of the hymenopteran megaradiation." *Molecular Phylogenetics and Evolution* 60: 73-88.

Kieffer, J-J. 1905. "Description de nouveaux Proctotrypides exotiques." *Annales de la Société scientifique de Bruxelles* 29: 95–142.

Kieffer, J-J. 1907. Dryinidae. In P. Wytsman, Genera Insectorum, Fasc. 54: 1-33

Kieffer, J-J. 1908. Bethylidae. In P. Wytsman, Genera Insectorum, Fasc. 76: 1-50.

Kieffer, J-J. 1914. Bethylidae. Das Tierreich 41: 1–205.

Kimsey, L.S., and R.M. Bohart. 1990. *The chrysidid wasps of the world*. Oxford University Press.

Klopfstein, S., L.Vilhelmsen, J.M. Heraty, M. Sharkey, and F. Ronquist. 2013. "The hymenopteran tree of life: evidence from protein-coding genes and objectively aligned ribosomal data." *PLOS one* 8, no. 8: e69344.

Lanes, G.O., and C.O. Azevedo. 2008. "Phylogeny and Taxonomy of Sclerodermini (Hymenoptera, Bethylidae, Epyrinae)." *Insect Systematics & Evolution* 39: 55-86.

Latreille, P.A. 1805. Histoire naturelle generale et particulière des crustacés et des insectes, 13. F. Dufart, Paris. 1-432.

Legner, F.E., and G. Gordh. 1992. "Lower Navel Orange worm (Lepidoptera: Phycitidae) Population Densities Following Establishment *of Goniozus legneri* (Hymenoptera: Bethylidae) in California." *Journal of Economic Entomology* 85: 2153-2160.

Lim, J., M. Olmi, I. Kim, and S. Lee. 2013. "First record of Sclerogibbidae (Hymenoptera: Chrysidoidea), ectoparasitoids of Embiidina (Insecta: Neoptera) from Cambodia." *Korean Society of Applied Entomology*: 273.

Mora-Kepfer, F., and A. M. Espinoza. 2009. "Parasitism and predation of the planthopper *Tagosodes orizicolus* (Homoptera: Delphacidae) by a dryinid parasitoid in Costa Rica." *Revista de biologia tropical* 57: 203-211.

MacLeod, N. 2002. "Phylogenetic signals in morphometric data." *Morphology, shape and phylogeny*: 100-138.

Nagy, C. "A new bethylid subfamily allied to Protopristocerinae (Hymenoptera Bethylidae)." *Bollettino della Societá Entomologica Italiana* 106 (1974): 126-129.

Olmi, M. 1984. "A revision of the Dryinidae (Hymenoptera)." *Memoirs of the American Entomological Institute* 37: 1-1913.

Olmi, M. 1989. "Supplement to the revision of the world Dryinidae (Hymenoptera, Chrysidoidea)." *Frustula Entomologica, Nuova Serie* 26: 15-19.

Olmi, M. 1994. *The Dryinidae and Embolemidae (Hymenoptera: Chrysidoidea) of Fennoscandia and Denmark*. Vol. 30. Brill.

Olmi, M. 1996. "A revision of the world Embolemidae (Hymenoptera Chrysidoidea)." *Frustula entomologica* 17: 85-146.

Olmi, M., A.P. Rasnitsyn, D.J. Brothers, and A. Guglielmino 2014a. "The first fossil Embolemidae (Hymenoptera: Chrysidoidea) from Burmese amber (Myanmar) and Orapa Kimberlitic deposits (Botswana) and their phylogenetic significance." *Journal of Systematic Paleontology* 12: 623-625.

Olmi, M., T. Mita, and, A. Guglielmino. 2014b. "Revision of the Embolemidae of Japan (Hymenoptera: Chrysidoidea), with description of a new genus and two new species". *Zootaxa* 3739: 423-440.

Olmi, M., and E.G. Virla. 2014. "Dryinidae of the Neotropical region (Hymenoptera: Chrysidoidea)." *Zootaxa* 3792: 1-534.

O'Neill, K. M. 2001. *Solitary wasps: behavior and natural history*. Cornell University Press.

Payne, A., P.M. Barden, W.C. Wheeler, and J.M. Carpenter. 2013. "Direct optimization, sensitivity analysis, and the evolution of the hymenopteran superfamilies." *American Museum Novitates* 3789: 1-20.

Perrard, A., C. Villemant, J. M. Carpenter, and M. Baylac. 2012. "Differences in caste dimorphism among three hornet species (Hymenoptera: Vespidae): forewing size, shape and allometry." *Journal of Evolutionary Biology* 25: 1389-1398.

Perrard, A., F. Lopez-Osorio, and J.M. Carpenter. 2014. "Phylogeny, landmark optimization, and the use of wing venation to study the evolution of social wasps (Hymenoptera: Vespidae: Vespinae) *In press for Cladistics.*

Quicke, D.L.J. 1997. *Parasitic wasps*. Chapman & Hall Ltd.

Ronquist, F. 1999. "Phylogeny of the Hymenoptera (Insecta): the state of the art." *Zoologica Scripta* 28: 3-11.

Rosenheim, J.A. 1987. "Host location and exploitation by the cleptoparasitic wasp *Argochrysis armilla*: the role of learning (Hymenoptera: Chrysididae)." *Behavioral Ecology and Sociobiology* 21: 401-406.

Sahragard, A., M.A. Jervis, and N.A.C. Kidd. 1991 "Influence of host availability on rates of oviposition and host- feeding, and on longevity in *Dicondylus indianus* Olmi (Hym., Dryinidae), a parasitoid of the Rice Brown Planthopper, *Nilaparvata lugens* Stål (Hem., Delphacidae)." *Journal of Applied Entomology* 112: 153-162.

Sharkey, M.J., J.M. Carpenter, L. Vilhelmsen, J. Heraty, J. Liljeblad, A.P.G. Dowling, S. Schulmeister et al. 2012. "Phylogenetic relationships among superfamilies of Hymenoptera." *Cladistics* 28: 80-112.

Sorg, M. 1988. "Zur Phylogenie und Systematik der Bethylidae (Insecta: Hymenoptera: Chrysidoidea)." *Sonderveröffentlichungen des Geologishcen Instiuts der Universität zu Köln* 63:1-146.

Terayama, M. 2003. "Phylogenetic systematics of the family Bethylidae (Insecta: Hymenoptera) Part I. Higher classification." *The Academic Reports of the Faculty of Engineering of Tokyo Polytechnic University* 26:1-15.

Tribull, C. 2015. "Phylogenetic relationships of the subfamilies of Dryinidae as reconstructed by molecular sequencing." *In press for Journal of Hymenoptera Research.*

Virla, E.G., M.S. Espinoza, and M. Olmi. 2010. "A contribution to the knowledge of Dryinidae of Argentina: descriptions of the hitherto unknown males of *Gonatopus argentinus* Olmi and *Trichogonatopus richardsi* Olmi (Hymenoptera: Chrysidoidea)". *Frustula Entomologica, Nuova Serie* 31: 125-134.

Xu, Z., M. Olmi, and J. He. 2013. "Dryinidae of the Oriental region (Hymenoptera: Chrysidoidea)." *Zootaxa* 3614: 001-460.

CHAPTER II

PHYLOGENETIC RELATIONSHIPS AMONG THE SUBFAMILIES OF DRYINIDAE (HYMENOPTERA: CHRYSIDOIDEA) AS RECONSTRUCTED BY MOLECULAR SEQUENCING

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 (leaf hoppers, plant hoppers, and their allies) and thus 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 and 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, 1994b; 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 and Bechly, 2001, Ponomarenkoinae Olmi 2010, and Protodryininae Olmi and 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 and Guglielmino, 2010, Gonatopodinae Kieffer, 1906, Plesiodryininae Olmi, 1987, and Transdryininae Olmi, 1984. The five most speciose 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 contain 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, 1953, 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. Virla et al. (2010) also synonymized *Trichogonatopus* Kieffer, 1909 with *Gonatopus* upon the discovery of male specimens. 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 all of the taxa of 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 Appendix A. 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 Appendix B, 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. The 18S and 28S loci 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, of which 1,101 were parsimony informative, 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 (Farris et al.

1996) support values were reported as frequency differences (GC) from 1000 replicates on the strict consensus topology. 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 assess if the same topology was returned as the best tree, with 1000 bootstrap replicates.

For Bayesian analyses, MrBayes 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 1^{st} positions in COI and CytB, and GTR+I+G for the 2^{nd} and 3^{rd} 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 most parsimonious trees with a best score of 200 (Figure 2.1). Average jackknife support values were 52.8. The best RAxML tree from 20 separate analyses had a final optimization likelihood of -44251.166938 (Figure 2.2), 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 (Figure 2.3).

Results were largely congruent for parsimony, likelihood, and Bayesian approaches in terms of higher-level topology, 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 only 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 *T. macilentus* De

Santis and 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, *Lonchondryinus* 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

A. 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 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 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).

B. 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 also 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 its determination of 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.

C. 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 also was 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 utility 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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REFERENCES

Bazinet, A.L., D.J. Zwickl, and M. P. Cummings. 2014. "A gateway for phylogenetic analysis powered by grid computing featuring GARLI 2.0." *Systematic Biology* 63: 812-818.

Carpenter, J.M. 1999 "What do we know about chrysidoid (Hymenoptera) relationships?" *Zoologica Scripta* 28: 215–231.

Chua, T. H., and V. A. Dyck. 1982. "Assessment of *Pseudogonatopus flavifemur* E. & H. (Dryinidae: Hymenoptera) as a biocontrol agent of the rice brown planthopper." In *Proceedings of the International Conference in Tropics:* 253-265.

Farris, J.S., V.A. Albert, M. Källersjö, D. Lipscomb, and A. G. Kluge. 1996. "Parsimony jackknifing outperforms neighbor- joining." *Cladistics* 12: 99-124.

Goloboff, P.A., J.S. Farris, and K.C. Nixon. 2008. "TNT, a free program for phylogenetic analysis." *Cladistics* 24: 774-786.

Guglielmino, A., and M. Olmi. 1997 "A host-parasite catalog of world Dryinidae (Hymenoptera: Chrysidoidea)." *Contributions on Entomology, International* 2: 165–298.

Guglielmino, A., M. Olmi, and C. Bückle. 2013. "An updated host-parasite catalogue of world Dryinidae (Hymenoptera: Chrysidoidea)." *Zootaxa* 3740: 1-113.

Hines, H. M., J.H. Hunt, T. K. O'Connor, J.J. Gillespie, and S.A. Cameron. 2007. "Multigene phylogeny reveals eusociality evolved twice in vespid wasps." *Proceedings of the National Academy of Sciences* 104: 3295-3299.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton et al. 2012. "Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data." *Bioinformatics* 28: 1647-1649.

Kieffer J-J. 1914. Bethylidae. Das Tierreich, 41. R. Friedlander und Sohn. (Berlin): 1–595.

Klopfstein, S., L. Vilhelmsen, J.M. Heraty, M. Sharkey, and F. Ronquist. 2013. "The hymenopteran tree of life: evidence from protein-coding genes and objectively aligned ribosomal data." *PLOS ONE* 8: e69344.

Lanfear, R., B. Calcott, S.Y.W Ho, and S. Guindon. 2012. "PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses." *Molecular Biology and Evolution* 29: 1695-1701.

Mita, T. 2009. "A taxonomic study of the Dryininae (Hymenoptera: Dryinidae) of Japan, with description of a new species of *Pseudodryinus*." *Zootaxa* 2168: 45-56.

Mita, T., and Y. Matsumoto. 2012. "First description of the male of *Gonatopus javanus* (RCL Perkins) determined by mitochondrial COI sequence (Hymenoptera: Dryinidae)." *Entomological Science* 15: 214-218.

Mita, T., S. Sanada-Morimura, M. Matsumura, and Y. Matsumoto. 2013. "Genetic variation of two apterous wasps *Haplogonatopus apicalis* and *H. oratorius* (Hymenoptera: Dryinidae) in East Asia." *Applied Entomology and Zoology* 48: 119-124.

Mora-Kepfer, F., and A. M. Espinoza. 2009. "Parasitism and predation of the planthopper *Tagosodes orizicolus* (Homoptera: Delphacidae) by a dryinid parasitoid in Costa Rica." *Revista de biologia tropical* 57: 2003-2011.

Morrison, D. A. 2009. "Why would phylogeneticists ignore computerized sequence alignment?" *Systematic Biology* 58: 150-158.

Munro, J. B., J.M. Heraty, R.A. Burks, D. Hawks, J. Mottern, A. Cruaud, J. Rasplus, and P. Jansta. 2011. "A molecular phylogeny of the Chalcidoidea (Hymenoptera)." *PLOS ONE* 6: e27023.

Olmi, M. 1984. “A revision of the Dryinidae (Hymenoptera).” *Memoirs of the American Entomological Institute* 37: 1–1913.

Olmi, M. 1989. “Supplement to the revision of the world Dryinidae (Hymenoptera, Chrysidoidea).” *Frustula Entomologica (Nuova Serie*) 12: 109–395.

Olmi, M. 1993 “A new generic classification for Thaumatodryininae, Dryininae, and Gonatopodinae, with descriptions of new species (Hymenoptera Dryinidae).” *Bollettino di Zoologia agraria e di Bachicoltura, Ser: II* 25: 57–89.

Olmi, M. 1994a. “The Dryinidae and Embolemidae (Hymenoptera: Chrysidoidea) of Fennoscandia and Denmark.” *Fauna Entomologica Scandinavia* 30: 1–100.

Olmi, M. 1994b. “Taxonomic studies on the Dryinidae of Mozambique (Hymenoptera: Chrysidoidea).” *Oriental Insects* 28: 67–80.

Olmi, M. 2005. “A new fossil dryinid in amber from the Dominican Republic: *Harpactosphecion scheveni* n. sp. (Hymenoptera Dryinidae).” *Frustula Entomologica (Nuova Serie)* 26: 15–19.

Olmi, M. 2007. “New species of Afrotropical Dryinidae (Hymenoptera: Chrysidoidea), with description of a new genus and a new subfamily.” *African Invertebrates 48*: 199–232.

Olmi, M, and A. Guglielmino. 2010. “Description of Erwiniinae, new subfamily of Dryinidae from Ecuador (Hymenoptera: Chrysidoidea).” *Zootaxa* 2605: 56–62.

Olmi, M., Z. Xu, and A. Guglielmino. 2014. “Descriptions of new fossil taxa of Dryinidae (Hymenoptera: Chrysidoidea) from Burmese amber (Myanmar).” *Acta Entomologica Musei Nationalis* Pragae. 54: 703–714.

Olmi M., and E.G. Virla. 2014. “Dryinidae of the Neotropical Region (Hymenoptera: Chrysidoidea).” *Zootaxa* 3792: 1–534.

Paquin, P., and C.J. Vink. 2009. "Testing compatibility between molecular and morphological techniques for arthropod systematics: a minimally destructive DNA extraction method that preserves morphological integrity, and the effect of lactic acid on DNA quality." *Journal of Insect Conservation* 13: 453-457.

Rambaut A. (2007) FigTree, a graphical viewer of phylogenetic trees. http://tree.bio.ed.ac.uk/software/figtree.

Richards, O.W. 1939. “The British Bethylidae (Hymenoptera).” *The Transactions of the Royal Entomological Society of London* 89: 185–344.

Richards, O.W. 1953. “The classification of the Dryinidae (Hymenoptera), with descriptions of new species.” *Transactions of the Royal Entomological Society of London* 104: 51–70.

Rokas, A., J.A.A Nylander, F. Ronquist, and G.N. Stone. 2002. "A maximum-likelihood analysis of eight phylogenetic markers in gallwasps (Hymenoptera: Cynipidae): implications for insect phylogenetic studies." *Molecular Phylogenetics and Evolution* 22: 206-219.

Ronquist, F., M. Teslenko, P. van der Mark, D. L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M.A. Suchard, and J.P. Huelsenbeck. 2012. "MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space." *Systematic Biology* 61: 539-542.

Sahragard A., M.A. Jervis, N.A.C. Kidd. 1991. “Influence of host availability on rates of oviposition and host- feeding, and on longevity in *Dicondylus indianus* Olmi (Hym., Dryinidae), a parasitoid of the Rice Brown Planthopper, *Nilaparvata lugens* Stål (Hemiptera: Delphacidae)”. *Journal of Applied Entomology* 112: 153–162.

Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994. "Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers." *Annals of the Entomological Society of America* 87: 651-701.

Stamatakis, A. 2014. "RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies." *Bioinformatics* 30: 1312-1313.

Vaidya, G., D.J. Lohman, and R. Meier. 2011. "SequenceMatrix: concatenation software for the fast assembly of multi- gene datasets with character set and codon information." *Cladistics* 27: 171-180.

Virla E.G., M.S. Espinosa, M. Olmi. 2010. "A contribution to the knowledge of Dryinidae of Argentina: descriptions of the hitherto unknown males of *Gonatopus argentinus* Olmi and *Trichogonatopus richardsi* Olmi (Hymenoptera: Chrysidoidea)." *Frustula Entomologica (Nuova Serie)* 31: 124–134.

Virla E.G, M.S. Espinosa, and G. Moya-Raygoza. 2011. "First host record for *Anteon pilicorne* (Ogloblin) (Hymenoptera: Dryinidae), a parasitoid of Cicadellidae, including the corn leafhopper (Hemiptera: Cicadellidae)." *Neotropical Entomology* 40: 285–287.

Vrijenhoek, R. 1994 "DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates." *Molecular Marine Biology and Biotechnology* 3: 294-299.

Xu, Z., M.Olmi, and J. He. 2013. "Dryinidae of the Oriental region (Hymenoptera: Chrysidoidea)." *Zootaxa*: 001-460.

Figure 2.1 Strict consensus of nine most Parsimonious trees. Jackknife support for nodes given in GC-values (frequency differences) from 1000 replicates. Average support value was 52.8. CI 0.287 RI 0.641. Scale bar in all images is 1.0 mm

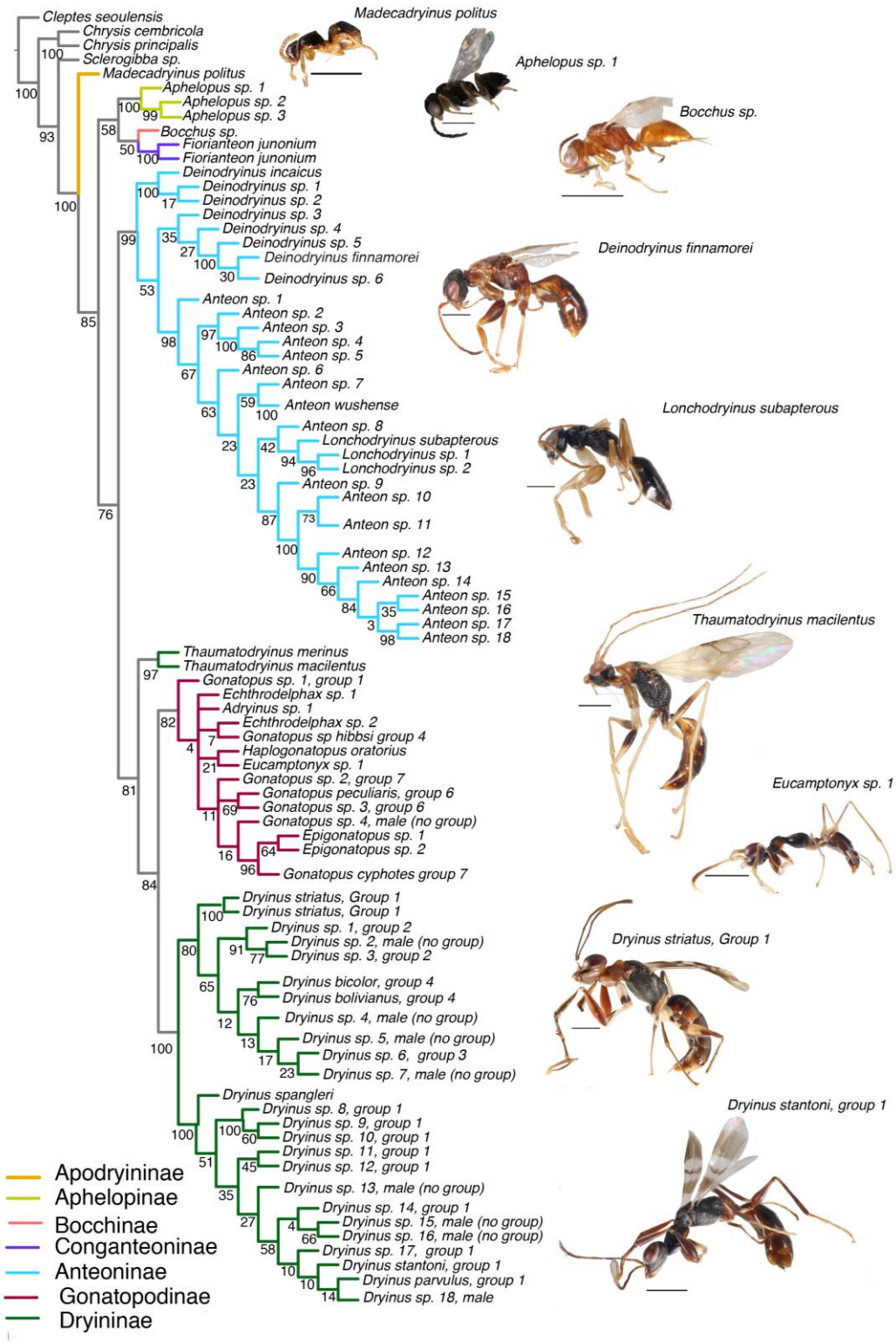

Cleptes seoulensis
Chrysis cembricola
Chrysis principalis
Sclerogibba sp.
Madecadryinus politus
Aphelopus sp. 1
Aphelopus sp. 2
Aphelopus sp. 3
Bocchus sp.
Fiorianteon junonium
Fiorianteon junonium
Deinodryinus incaicus
Deinodryinus sp. 1
Deinodryinus sp. 2
Deinodryinus sp. 3
Deinodryinus sp. 4
Deinodryinus sp. 5
Deinodryinus finnamorei
Deinodryinus sp. 6
Anteon sp. 1
Anteon sp. 2
Anteon sp. 3
Anteon sp. 4
Anteon sp. 5
Anteon sp. 6
Anteon sp. 7
Anteon wushense
Anteon sp. 8
Lonchodryinus subapterous
Lonchodryinus sp. 1
Lonchodryinus sp. 2
Anteon sp. 9
Anteon sp. 10
Anteon sp. 11
Anteon sp. 12
Anteon sp. 13
Anteon sp. 14
Anteon sp. 15
Anteon sp. 16
Anteon sp. 17
Anteon sp. 18
Thaumatodryinus merinus
Thaumatodryinus macilentus
Gonatopus sp. 1, group 1
Echthrodelphax sp. 1
Adryinus sp. 1
Echthrodelphax sp. 2
Gonatopus sp hibbsi group 4
Haplogonatopus oratorius
Eucamptonyx sp. 1
Gonatopus sp. 2, group 7
Gonatopus peculiaris, group 6
Gonatopus sp. 3, group 6
Gonatopus sp. 4, male (no group)
Epigonatopus sp. 1
Epigonatopus sp. 2
Gonatopus cyphotes group 7
Dryinus striatus, Group 1
Dryinus striatus, Group 1
Dryinus sp. 1, group 2
Dryinus sp. 2, male (no group)
Dryinus sp. 3, group 2
Dryinus bicolor, group 4
Dryinus bolivianus, group 4
Dryinus sp. 4, male (no group)
Dryinus sp. 5, male (no group)
Dryinus sp. 6, group 3
Dryinus sp. 7, male (no group)
Dryinus spangleri
Dryinus sp. 8, group 1
Dryinus sp. 9, group 1
Dryinus sp. 10, group 1
Dryinus sp. 11, group 1
Dryinus sp. 12, group 1
Dryinus sp. 13, male (no group)
Dryinus sp. 14, group 1
Dryinus sp. 15, male (no group)
Dryinus sp. 16, male (no group)
Dryinus sp. 17, group 1
Dryinus stantoni, group 1
Dryinus parvulus, group 1
Dryinus sp. 18, male
Apodryininae
Aphelopinae
Bocchinae
Conganteoninae
Anteoninae
Gonatopodinae
Dryininae
Madecadryinus politus
Aphelopus sp. 1
Bocchus sp.
Deinodryinus finnamorei
Lonchodryinus subapterous
Thaumatodryinus macilentus
Eucamptonyx sp. 1
Dryinus striatus, Group 1
Dryinus stantoni, group 1

Figure 2.2 Likelihood support tree. Rapid Bootstrap support values shown at nodes.

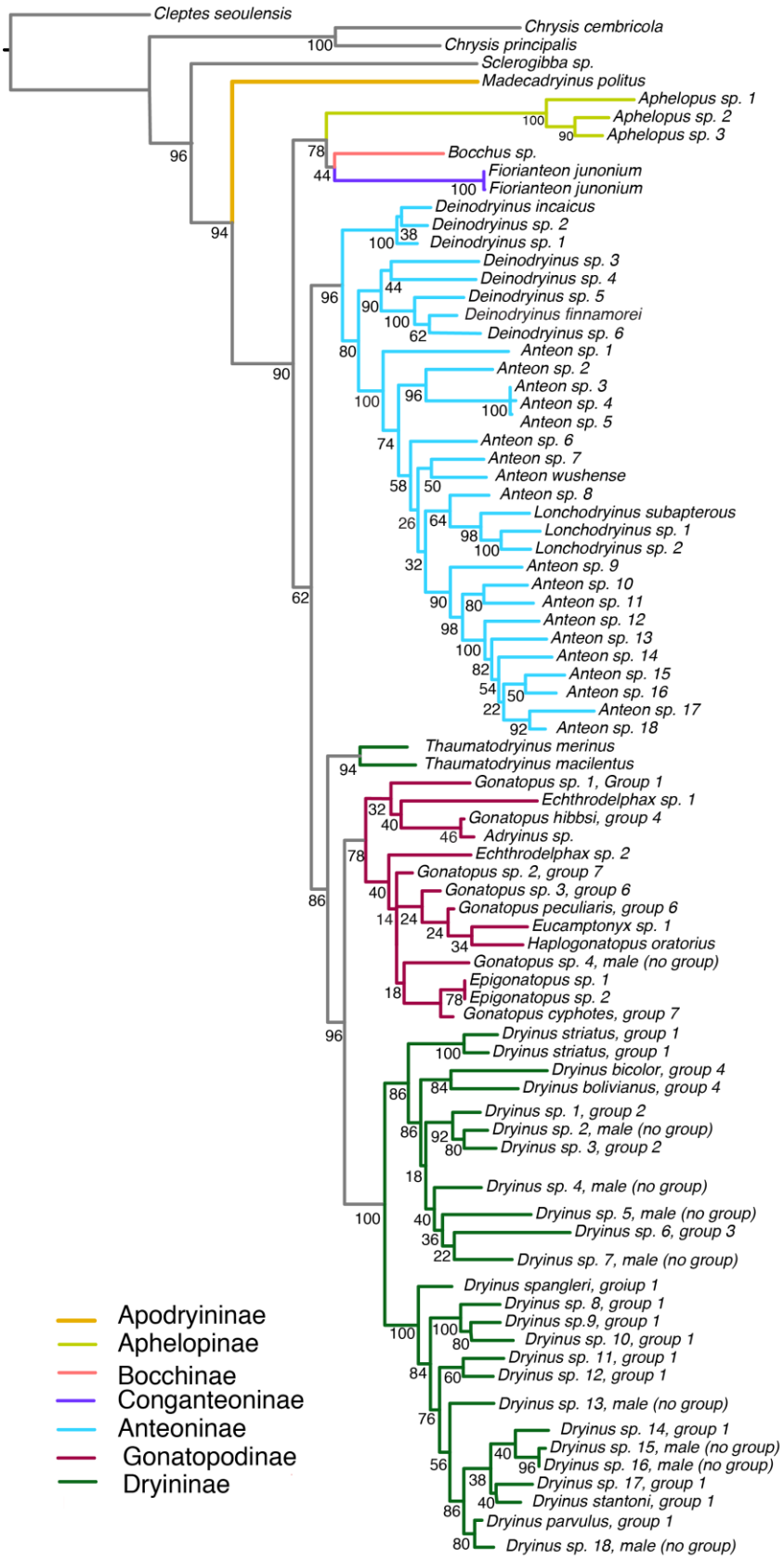
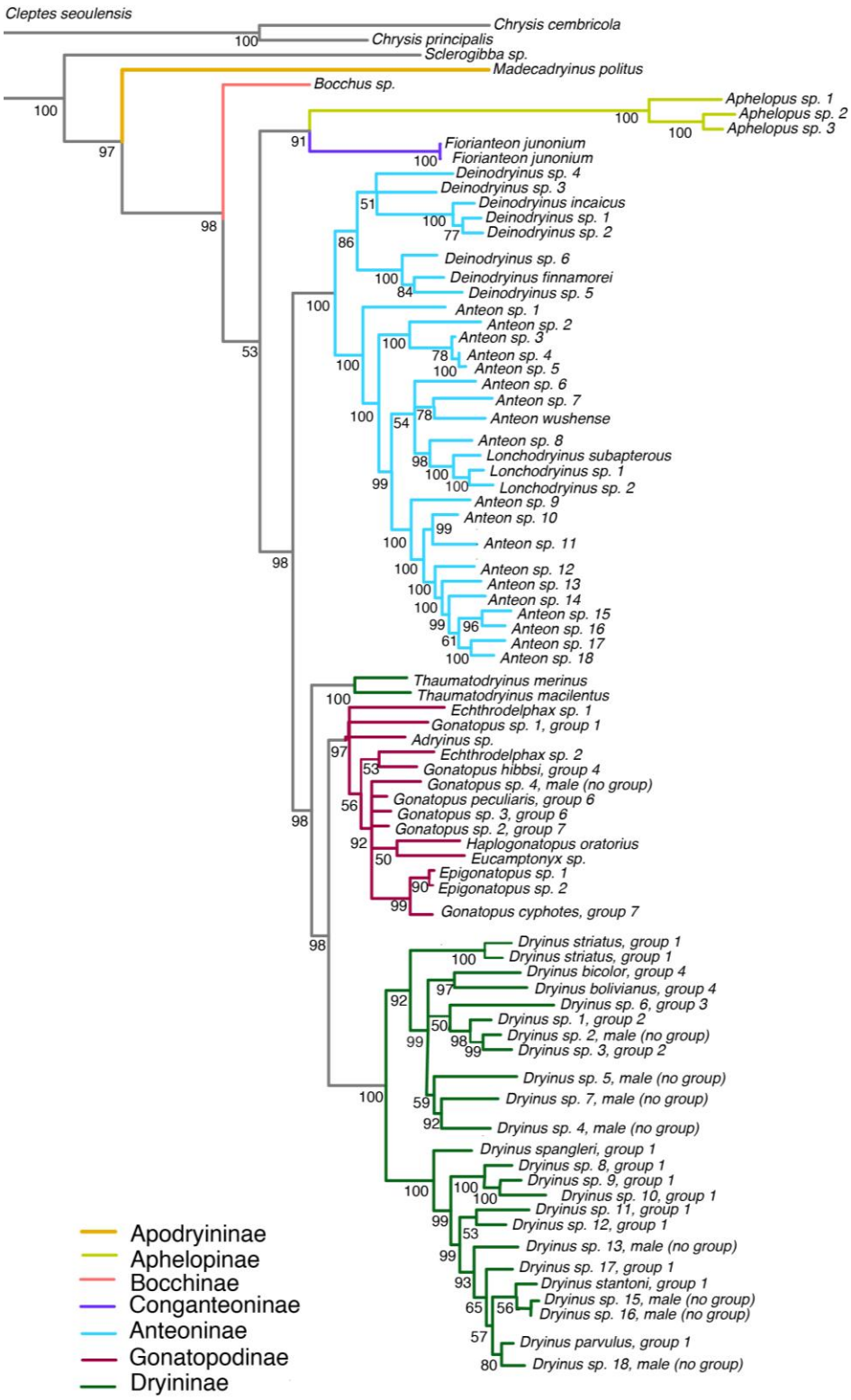

Cleptes seoulensis
Chrysis cembricola
Chrysis principalis
Sclerogibba sp.
Madecadryinus politus
Aphelopus sp. 1
Aphelopus sp. 2
Aphelopus sp. 3
Bocchus sp.
Fiorianteon junonium
Fiorianteon junonium
Deinodryinus incaicus
Deinodryinus sp. 2
Deinodryinus sp. 1
Deinodryinus sp. 3
Deinodryinus sp. 4
Deinodryinus sp. 5
Deinodryinus finnamorei
Deinodryinus sp. 6
Anteon sp. 1
Anteon sp. 2
Anteon sp. 3
Anteon sp. 4
Anteon sp. 5
Anteon sp. 6
Anteon sp. 7
Anteon wushense
Anteon sp. 8
Lonchodryinus subapterous
Lonchodryinus sp. 1
Lonchodryinus sp. 2
Anteon sp. 9
Anteon sp. 10
Anteon sp. 11
Anteon sp. 12
Anteon sp. 13
Anteon sp. 14
Anteon sp. 15
Anteon sp. 16
Anteon sp. 17
Anteon sp. 18
Thaumatodryinus merinus
Thaumatodryinus macilentus
Gonatopus sp. 1, Group 1
Echthrodelphax sp. 1
Gonatopus hibbsi, group 4
Adryinus sp.
Echthrodelphax sp. 2
Gonatopus sp. 2, group 7
Gonatopus sp. 3, group 6
Gonatopus peculiaris, group 6
Eucamptonyx sp. 1
Haplogonatopus oratorius
Gonatopus sp. 4, male (no group)
Epigonatopus sp. 1
Epigonatopus sp. 2
Gonatopus cyphotes, group 7
Dryinus striatus, group 1
Dryinus striatus, group 1
Dryinus bicolor, group 4
Dryinus bolivianus, group 4
Dryinus sp. 1, group 2
Dryinus sp. 2, male (no group)
Dryinus sp. 3, group 2
Dryinus sp. 4, male (no group)
Dryinus sp. 5, male (no group)
Dryinus sp. 6, group 3
Dryinus sp. 7, male (no group)
Dryinus spangleri, groiup 1
Dryinus sp. 8, group 1
Dryinus sp.9, group 1
Dryinus sp. 10, group 1
Dryinus sp. 11, group 1
Dryinus sp. 12, group 1
Dryinus sp. 13, male (no group)
Dryinus sp. 14, group 1
Dryinus sp. 15, male (no group)
Dryinus sp. 16, male (no group)
Dryinus sp. 17, group 1
Dryinus stantoni, group 1
Dryinus parvulus, group 1
Dryinus sp. 18, male (no group)
Apodryininae
Aphelopinae
Bocchinae
Conganteoninae
Anteoninae
Gonatopodinae
Dryininae

Figure 2.3. Bayesian support tree. Support probabilities shown at nodes as a percent.


Cleptes seoulensis
Chrysis cembricola
Chrysis principalis
Sclerogibba sp.
Madecadryinus politus
Bocchus sp.
Aphelopus sp. 1
Aphelopus sp. 2
Aphelopus sp. 3
Fiorianteon junonium
Fiorianteon junonium
Deinodryinus sp. 4
Deinodryinus sp. 3
Deinodryinus incaicus
Deinodryinus sp. 1
Deinodryinus sp. 2
Deinodryinus sp. 6
Deinodryinus finnamorei
Deinodryinus sp. 5
Anteon sp. 1
Anteon sp. 2
Anteon sp. 3
Anteon sp. 4
Anteon sp. 5
Anteon sp. 6
Anteon sp. 7
Anteon wushense
Anteon sp. 8
Lonchodryinus subapterous
Lonchodryinus sp. 1
Lonchodryinus sp. 2
Anteon sp. 9
Anteon sp. 10
Anteon sp. 11
Anteon sp. 12
Anteon sp. 13
Anteon sp. 14
Anteon sp. 15
Anteon sp. 16
Anteon sp. 17
Anteon sp. 18
Thaumatodryinus merinus
Thaumatodryinus macilentus
Echthrodelphax sp. 1
Gonatopus sp. 1, group 1
Adryinus sp.
Echthrodelphax sp. 2
Gonatopus hibbsi, group 4
Gonatopus sp. 4, male (no group)
Gonatopus peculiaris, group 6
Gonatopus sp. 3, group 6
Gonatopus sp. 2, group 7
Haplogonatopus oratorius
Eucamptonyx sp.
Epigonatopus sp. 1
Epigonatopus sp. 2
Gonatopus cyphotes, group 7
Dryinus striatus, group 1
Dryinus striatus, group 1
Dryinus bicolor, group 4
Dryinus bolivianus, group 4
Dryinus sp. 6, group 3
Dryinus sp. 1, group 2
Dryinus sp. 2, male (no group)
Dryinus sp. 3, group 2
Dryinus sp. 5, male (no group)
Dryinus sp. 7, male (no group)
Dryinus sp. 4, male (no group)
Dryinus spangleri, group 1
Dryinus sp. 8, group 1
Dryinus sp. 9, group 1
Dryinus sp. 10, group 1
Dryinus sp. 11, group 1
Dryinus sp. 12, group 1
Dryinus sp. 13, male (no group)
Dryinus sp. 17, group 1
Dryinus stantoni, group 1
Dryinus sp. 15, male (no group)
Dryinus sp. 16, male (no group)
Dryinus parvulus, group 1
Dryinus sp. 18, male (no group)
Apodryininae
Aphelopinae
Bocchinae
Conganteoninae
Anteoninae
Gonatopodinae
Dryininae

CHAPTER III: MORPHOMETRICS, MORPHOLOGY, AND MOLECULES IN DRYINIDAE (HYMENOPTERA: CHRYSIDOIDEA) SYSTEMATICS – HOW DO THE TREES COMPARE?

INTRODUCTION

Morphometric data have been utilized to study insects for at least the past fifty years, typically to differentiate between species groups and study geographical variation (Stower et al. 1960; Medler 1962; Kim et al. 1967). Recently, however, attention has turned to the use of geometric morphometrics (GM) in cladistic analyses. Originally, this concept was strongly opposed because of an erroneous connection between phenetics and landmark analysis as a result of much of the groundbreaking analytical work of morphometrics being within the sphere of pheneticists (MacLeod 2002) or an inability to recognize the use of continuous characters in cladistics (Adams et al. 2013). However, morphometric data can be considered as continuous characters, and cladistics can accommodate the use of such characters, as explained in Catalano et al. (2010). Their method involved using Farris optimization for continuous characters for each landmark in a shape (referred to as a 'landmark configuration') to find the value for ancestral points that minimized the sum of the distances between ancestor/descendant points (Farris, 1970; Goloboff et al. 2006). A follow-up paper, Goloboff and Catalano (2011), explained the algorithm for finding the optimal landmark positions in which a grid was used to assign costs to every optimal and suboptimal point in a 2-D or 3-D space in a similar manner as used for step-matrix characters (Goloboff, 1998) and then calculating the geometric median for each point. Goloboff and Catalano (2011) realized that the computational time required for this operation was extremely prohibitive and found that

partially randomizing the grid size and using smaller nested grids centered at the best position for each node helped to find more precise estimations in a shorter amount of time. Finally, in Catalano and Goloboff (2012), a method for aligning landmarks using the parsimony criterion (dynamic alignment) as opposed to *a priori* alignment using Procrustes methods (Rohlf and Slice 1990) or resistant-fit theta rho analysis (RFTRA) (Siegel and Benson 1982; Benson et al. 1982) was implemented as part of the landmark package in TNT (Goloboff et al. 2008). This method still required that configurations be scaled to centroid size, as implemented in several morphometrics programs (Sheets 2003; Klingenberg 2011).

As the first implementation of landmark configuration analysis under parsimony was fairly recent (Catalano et al. 2010), the method has been utilized only a handful of times in the literature – an analysis of shell shape in *Conus* (Cruz et al. 2012), of nonavian theropod quadrates (Hendrickx, *in press*), of Musteloidea skeletons (Catalano et al. 2014), and of the utility of wing venation in Vespinae (Perrard, *in press*). Catalano et al. (2014) provided a method for resampling landmark data in TNT, along with a response to criticisms from studies that found morphometric phylogenies that were incongruent with the phylogenies produced by traditional data, although none of these studies implemented landmark analyses through parsimony (Panchetti et al. 2008; Scalica and Panchetti 2011). While they used different morphometric methods, the authors of those critiques had analyzed just a single shape. Catalano et al. (2014) found that the inclusion of just one configuration, which was analyzed as a single, continuous character under a Catalano et al. (2010) implementation, led to a tree that was incongruent with traditional data. Including multiple configurations, and thus multiple characters, increased

the similarity between morphometric phylogenies and molecular and/or morphological phylogenies.

Herein, I present an analysis of Dryinidae using landmark configuration data, traditional morphology, and molecular data. This is an extension of the previous chapter, but utilizes a different set of specimens. To deal with issues of high variation resulting from the extreme sexual dimorphism of Dryinidae disrupting landmark analysis (elaborated upon in the discussion), the dataset is female-only. Additionally, the methods of Klingenberg and Gidaszewski (2010) were implemented to examine for phylogenetic signal within the shape data. For a complete review of the current systematic research within Dryinidae, please refer to the introduction of the previous chapter.

MATERIALS AND METHODS

Fifty specimens of Dryinidae, one specimen of Bethylidae, and one specimen of Embolemidae were utilized in this study. The specimens of Dryinidae utilized were slightly different than in the previous chapter, with all specimens being female and the addition of specimens that were not sequenced in the previous chapter (please refer to Appendix C). The exclusion of males was to avoid issues of high variance due to sexual dimorphism in the morphometric analysis, which can result in a deficient superimposition. Fifteen specimens of Dryininae, 10 specimens of Gonatopodinae, 18 specimens of Anteoninae, 2 specimens of Aphelopinae, 2 specimens of Conganteoninae, 1 specimen of Thaumatodryininae, 1 specimen of Bocchinae, and 1 specimen of Apodryininae formed the sample of Dryinidae.

A. Molecular methods

Molecular sequencing and alignment methods were the same as in the previous

chapter. In summary: 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') (Vrijenhoek1994). 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). Sequencing was performed at the American Museum of Natural History (AMNH) in the Sackler Institute for Comparative Genomics on an ABI 3730.

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. The 18S and 28S loci were aligned using MAAFT, using the E-INS-I algorithm as implemented in Geneious (Morrison 2009). The concatenated matrix was assembled in SeqMatrix (Vaidya et al. 2010), and when combined with the morphological and landmark data below, resulted in a final matrix of 3163 characters, 1187 of which were parsimony informative, with 14% missing data.

Phylogenetic analyses were performed using TNT (Goloboff et al. 2008) 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. Support values are symmetric resampling reported as frequency differences of 1000 replicates (Goloboff et al. 2003), with an average value of 69.7.

B. Morphological Methods

A morphological matrix was provided courtesy of Toshiharu Mita. Male morphological characters were removed, and additional characters added, to result in a final matrix of 50 characters (see Appendix II). These characters were carefully selected to avoid any overlap between the morphometric characters and the morphological characters. Characters were coded after DNA had been extracted from specimens, which were then pinned and analyzed with a Nikon SMZ1500 dissecting microscope. The process of whole body DNA extraction did lighten and clear some surface structures, but did not affect coding characters from the morphological matrix or photographing landmark configurations.

C. Morphometric Characters

Five landmark configurations (shapes) were examined: The head (dorsal view), the claw of the chela (lateral), the fifth tarsal segment of the chela (lateral), and two shapes from the lateral view of the thorax – the pronotum and the remainder of the thorax through the propodeum (hereby referred to as the mesosoma for simplicity, please refer to Figure 3.1 for landmark placement guides). The latter two shapes were separated because of the strong articulation and movement between the pronotum and the remainder of the thorax. For the head, mesosoma, and pronotum, images were acquired using a Microptics system, and for the chela claw and fifth tarsal segment, images were acquired after slide-mounting chela and using a compound microscope with live photography. These shapes

were chosen because of the striking variation seen amongst the subfamilies and the inability to easily characterize this shape variation in discrete morphological character codes. In most Gonatopodinae, for example, the metanotum is elongate and conspicuously humped, giving them an almost ant-like appearance (Olmi 1994). However, there is variation in the shape of the hump in its severity, how posteriorly it is located, and the proportion of the mesosoma that it comprises. None of these details are easily captured in morphological codes. As another example, in Anteoninae, the chela claw is a relatively simple, scythe-like shape, whereas most Dryininae and Gonatopodinae chela claws are elongate and typically possess an apical tooth. Yet the angle of the curve of the claw, even in the simple ones found throughout Anteoninae, is extremely variable (Figure 3.1).

Landmarks were placed using TpsDig2 software (Rohlf 2001). Both landmarks and sliding semilandmarks were placed for the fifth tarsal segment, chela, pronotum, and mesosoma, while only landmarks were placed for the head. Landmarks were utilized where homologous points could be identified in every specimen, while semilandmarks were used to measure shapes that could not be easily defined by homologous points- such as the curve of the chela. Semilandmarks were placed using the curve drawing function in TpsDIG2, so that equal numbers of semilandmarks were equally spaced in every curve. Figure 3.1 provides a schematic for how landmarks were placed.

Once landmarks were digitized, they were further configured in IMP8 (Sheets 2014) in which semilandmarks were slid to minimize the bending energy along their X-axis using the subprogram SemiLand. All configurations were superimposed using a generalized Procrustes analysis (Rohlf and Slice 1990). Total shape variations were

examined using Principal Component Analyses (PCA) in PCAGen (Sheets 2014) by projecting the coordinates into the linear tangent space (Rohlf 1999). This was done for two reasons – a) to check for any mistakes in landmark placement and ordering and b) to see if taxonomic groups clustered together and if there were any outliers driving variation within subfamilies.

D. Phylogenetic signal of landmark data

To test for phylogenetic signal of the different landmark configurations, the methods described in Klingenberg and Gidaszewski (2010), were utilized. First, the phylogenetic tree (from the molecular and morphological matrix) was mapped within the tangent shape space by computing the ancestral states that minimized the squared parsimony length (Maddison 1991). The squared length of the tree within the tangent space was then used to test the phylogenetic signal. With a strong phylogenetic signal in the landmark data, closely related species would be near each other in morphometric space. As a result, the average amount of shape change along the branches of the tree should be small. If the phylogenetic signal were low, the tree would have a much higher cost to accommodate the greater shape changes along the branches of the phylogeny. The null hypothesis (no phylogenetic signal in the landmark data) could then be tested by a permutation procedure in which shapes were swapped along the tips of the phylogenetic tree, as explained in Siddall (2000). If there was no phylogenetic signal, than the cost of the morphometric changes will not change significantly with the permutations of the tips of the tree. If there was a strong phylogenetic signal, than swapping the shapes would result in a longer tree than the original tree provided.

To implement this test, A. Perrard provided an R script from Perrard et al. (2014) that read the newick format tree generated by the morphological and molecular dataset, computed the ancestral state reconstruction in squared parsimony, plotted the phylomorphospace on the principal component axes, and calculated the tree lengths of the random permutations and the phylogenetic signal with a significance cut off of 0.05.

E. Phylogenetic analysis of landmark data

TPS coordinates were converted to a TNT-readable format using the R-script Caronte (available at https://github.com/atorresgalvis/Caronte--2D-) and each configuration was run separately using static alignment with the command *run land_searches.run 0 0* (no re-alignment, and at the fastest/least exhaustive settings) to assess the congruency between each configuration and the morphological and molecular tree. The five combined configurations were then analyzed together on a cluster of seven 4-core CPUs, with assistance from Santiago Catalano. The search parameters were 32 Random Addition Sequences (Wagner trees) followed by TBR (tree bisection reconnection algorithm) with a 7x7 grid of cells, and one level of nested Sankoff (*cell 7 nes 1 1*). Resampling was also performed on Santiago Catalano's cluster in a similar manner to symmetric resampling (Goloboff et al. 2003), with 120 pseudo-replicates and a single run of RAS + TBR for each replicate, with values reported as frequency differences. Resampling was performed for two different datasets – the individual landmarks alone and the entire shape configurations.

RESULTS

A. Molecular + traditional morphological characters

The subfamily-level topology of the molecular and morphological tree was essentially the same as in the previous chapter –Apodryininae (represented by *Madecadryinus politus*) were the sister group to all other subfamilies of Dryinidae; Bocchinae were weakly placed as the sister to (Aphelopinae + Conganteoninae); Anteoninae were sister to (Thaumatodryininae + (Gonatopodinae + Dryininae)); Thaumatodryininae were well-supported and was sister to (Gonatopodinae + Dryininae) (Figure 3.2) with a frequency difference resampling score of 79.

B. Principal Component Analyses

Principal Component Analyses for each of the morphometric configurations revealed that taxa from subfamilies rarely clustered together in distinctive, well-spaced groups. Examining the specimens, there were anatomical outliers in each of the configuration sets that resulted in large morphological variation. Within Dryininae, for example, two specimens of *Dryinus* had extremely truncated chela and fifth tarsal segments – the *Dryinus* species group four, originally known as *Perodryinus* Perkins 1907 and synonymized with *Dryinus* in Olmi (1993). Despite belonging to the same genus, the variation throughout *Dryinus* was some of the most extreme due to several morphologically distinct species groups. Similarly, within Gonatopodinae, there was huge variation in the shape of the mesosoma – especially between the more ant-like *Gonatopus* and the more Dryininae-like *Echthrodelphax*. The tightest clusters of shapes were most apparent in the chela and fifth tarsal segment for Gonatopodinae (Figures 3.3 and 3.4).

C. Phylogenetic Signal

Using the methods of Klingenberg and Gidaszewski (2010), the mesosoma, chela, fifth tarsal segment, and head configurations were shown to have significant phylogenetic signal ($p < .05$), meaning that the null hypothesis of a complete lack of phylogenetic signal could be rejected. The pronotum was reported to not have any significant phylogenetic signal. Figure 3.5 shows that while these configurations contained significant phylogenetic signal, the influence on the phylogeny was incomplete, as many lineages mapped into the first and second principal components were overlapping, poorly separated, and with nodes that were extremely divergent – this is most obvious in the single, elongate branches in the chela, fifth tarsal segment, and pronotum visualizations.

D. Morphometric analyses

1. Single configurations

No single landmark configuration analyzed as cladistic data was able to recover the topology from the molecular and morphological tree (Figures 3.6, 3.7, and 3.8)

2. All landmarks together

The five landmark configurations analyzed together produced a phylogeny that was also incongruent with the molecular and morphological phylogeny. None of the subfamilies were monophyletic, and support values were generally low, although higher when resampling was performed for individual landmarks as opposed to whole configurations. (Figure 3.9)

E. Combined analysis

Unsurprisingly, the addition of five more characters did not change the subfamily-level topology of the combined morphometric, molecular, and morphological analysis when compared to the molecular and morphological tree alone. Support values were

lower than in the molecular and morphological tree, most likely due to the incongruence between datasets. The only support value that was increased by the inclusion of landmark data in the analysis was the node that places *Madecadryinus politus* (Apodryininae) as the sister to all other dryinids (Figure 3.2).

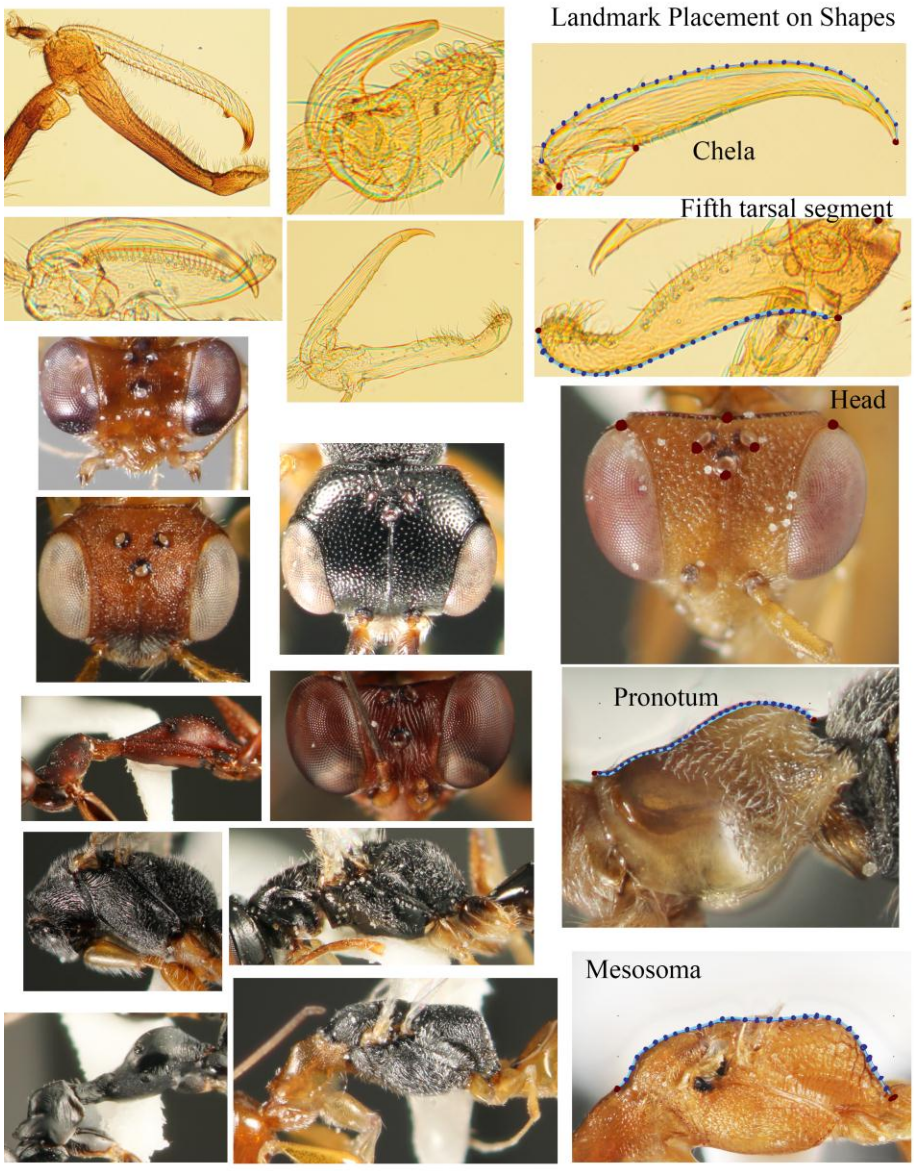


Figure 3.1 Schematic of landmark placement in the chela claw, fifth tarsal segment, head, pronotum, and mesosoma (right) and examples of diversity of Dryinidae on left for these structures. Images not to scale.

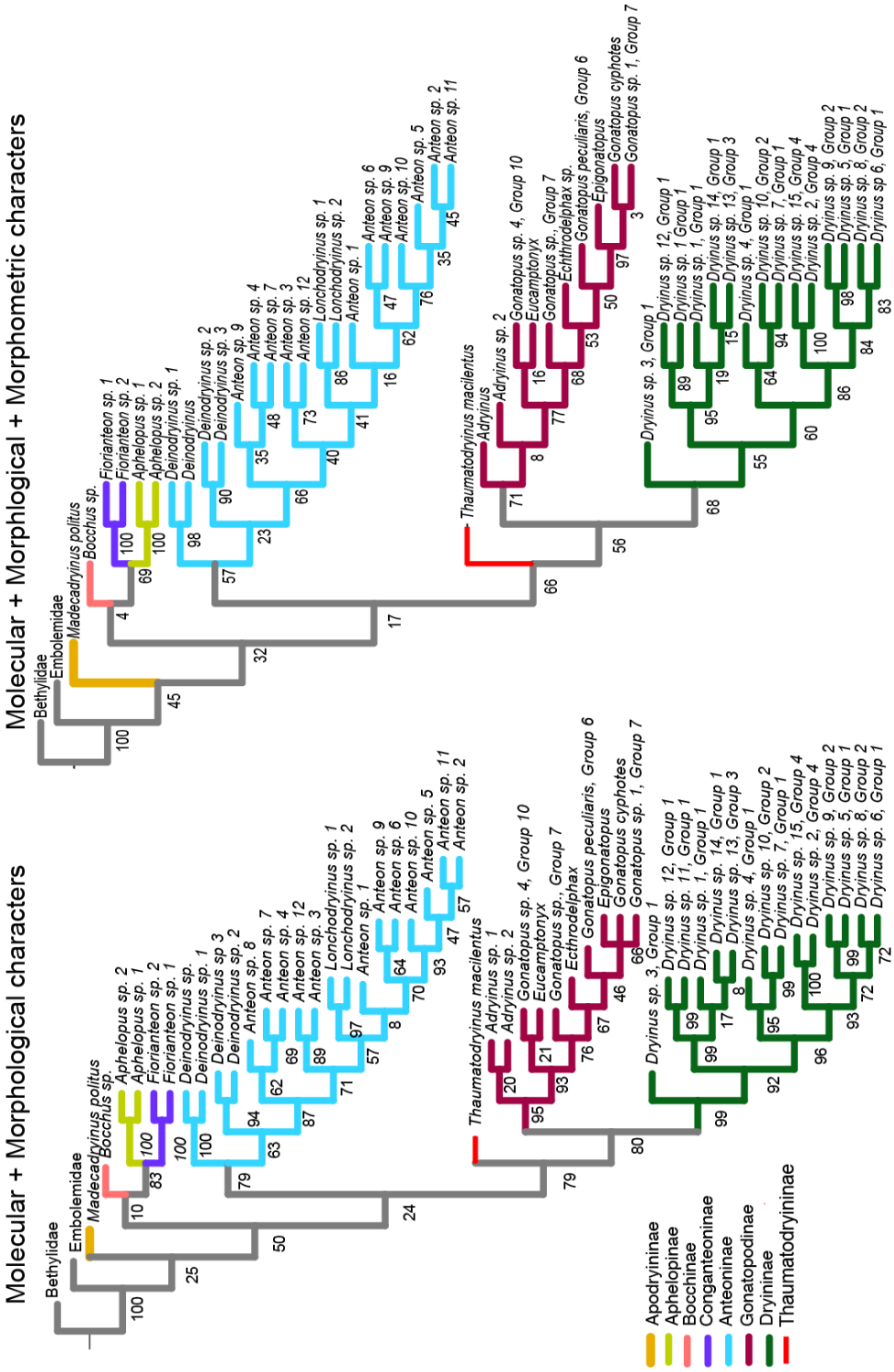


Figure 3.2 Comparison of phylogenetic trees from molecular and morphological data (left) and molecular, morphological, and morphometric data (right). Support values are symmetric resampling reported as frequency differences.

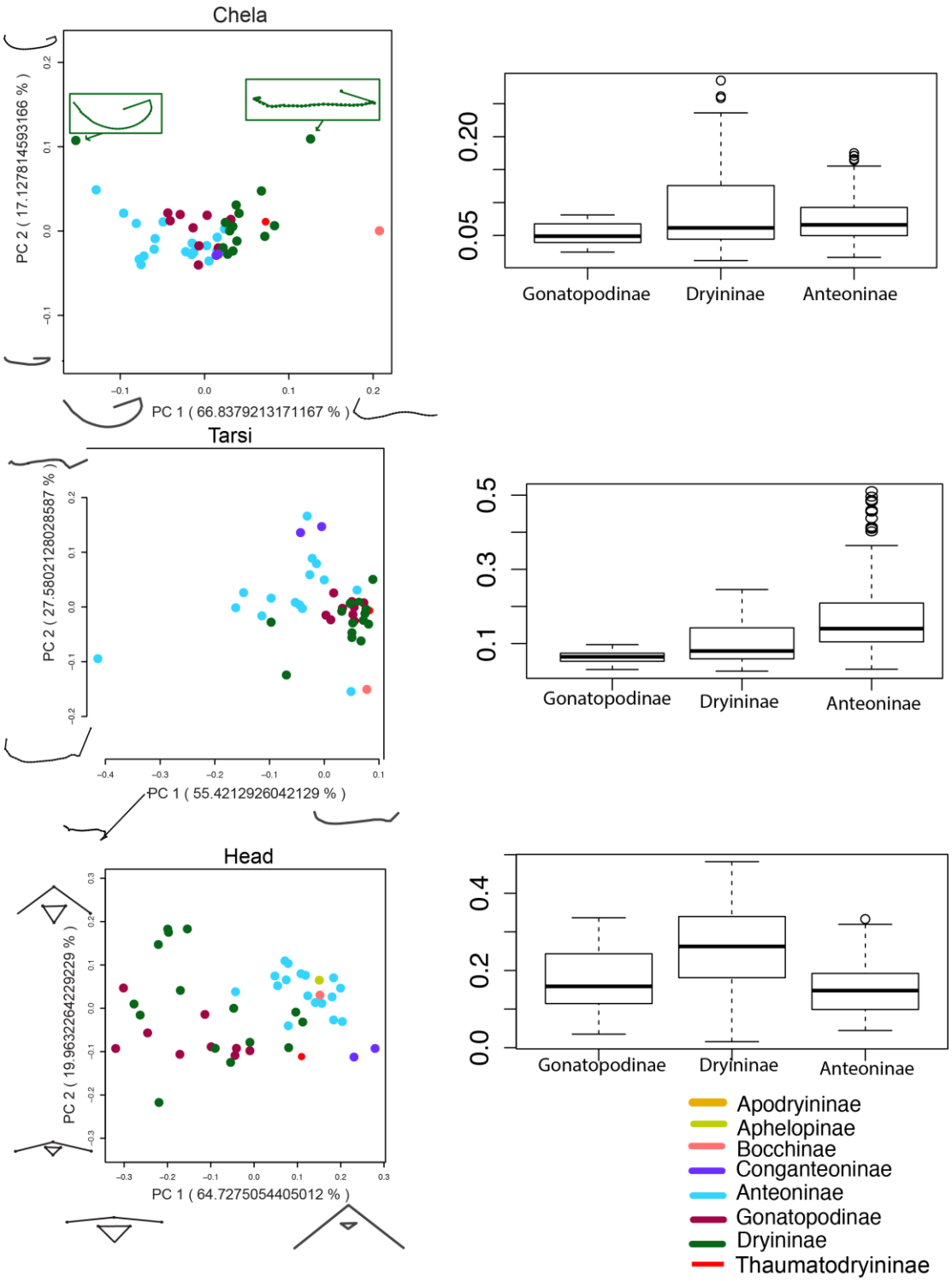


Figure 3.3. PCA plots with extremes of shape variation at the axes and plots of shape distribution for the three largest subfamilies. Extremes of shape difference of the chela claw in Dryininae shown in boxes

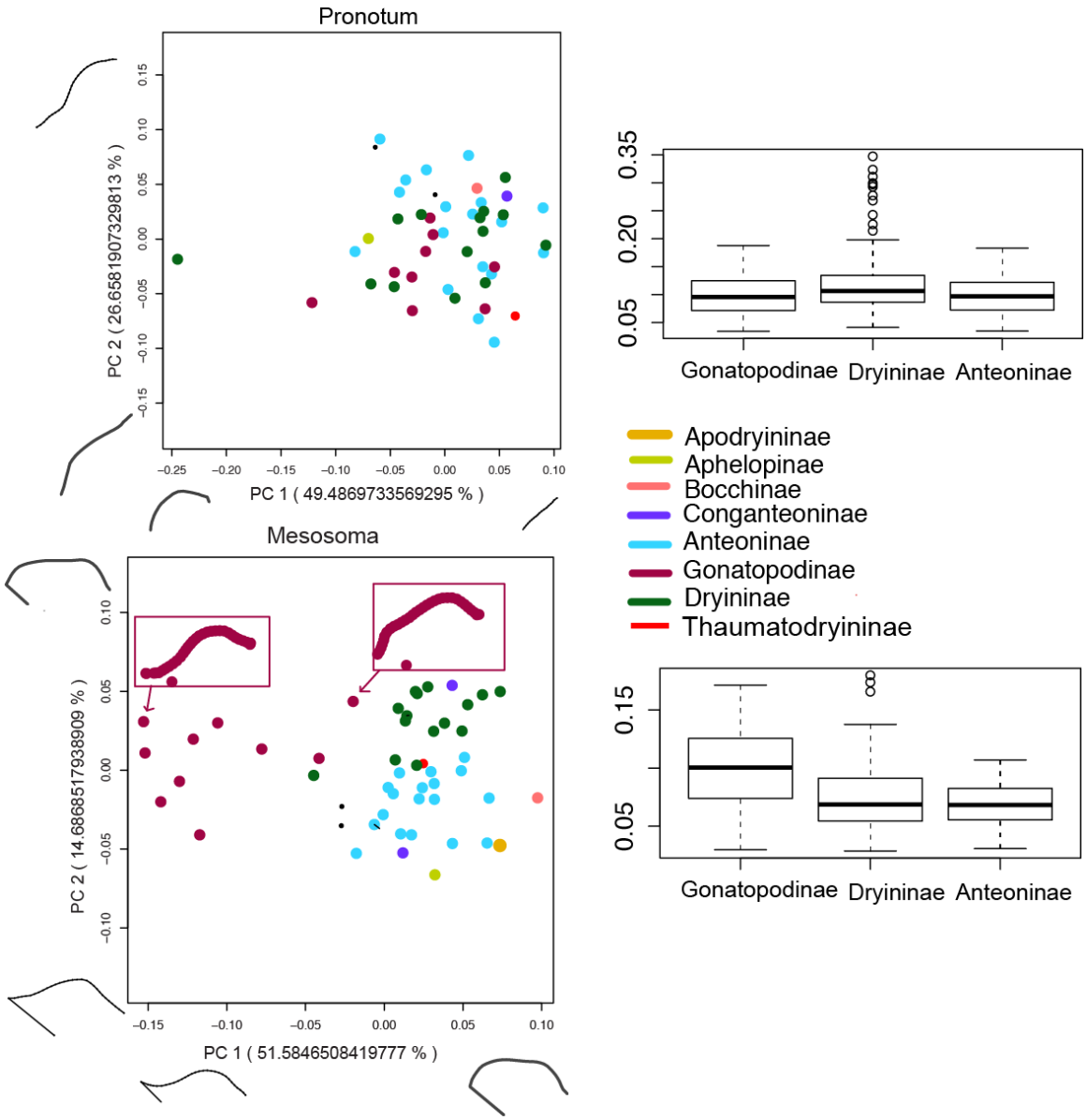


Figure 3.4 PCA plots with extremes of shape variation at the axes and plots of shape distribution for the three largest subfamilies. Extremes of shape difference of the mesosoma in Gonatopodinae shown in boxes

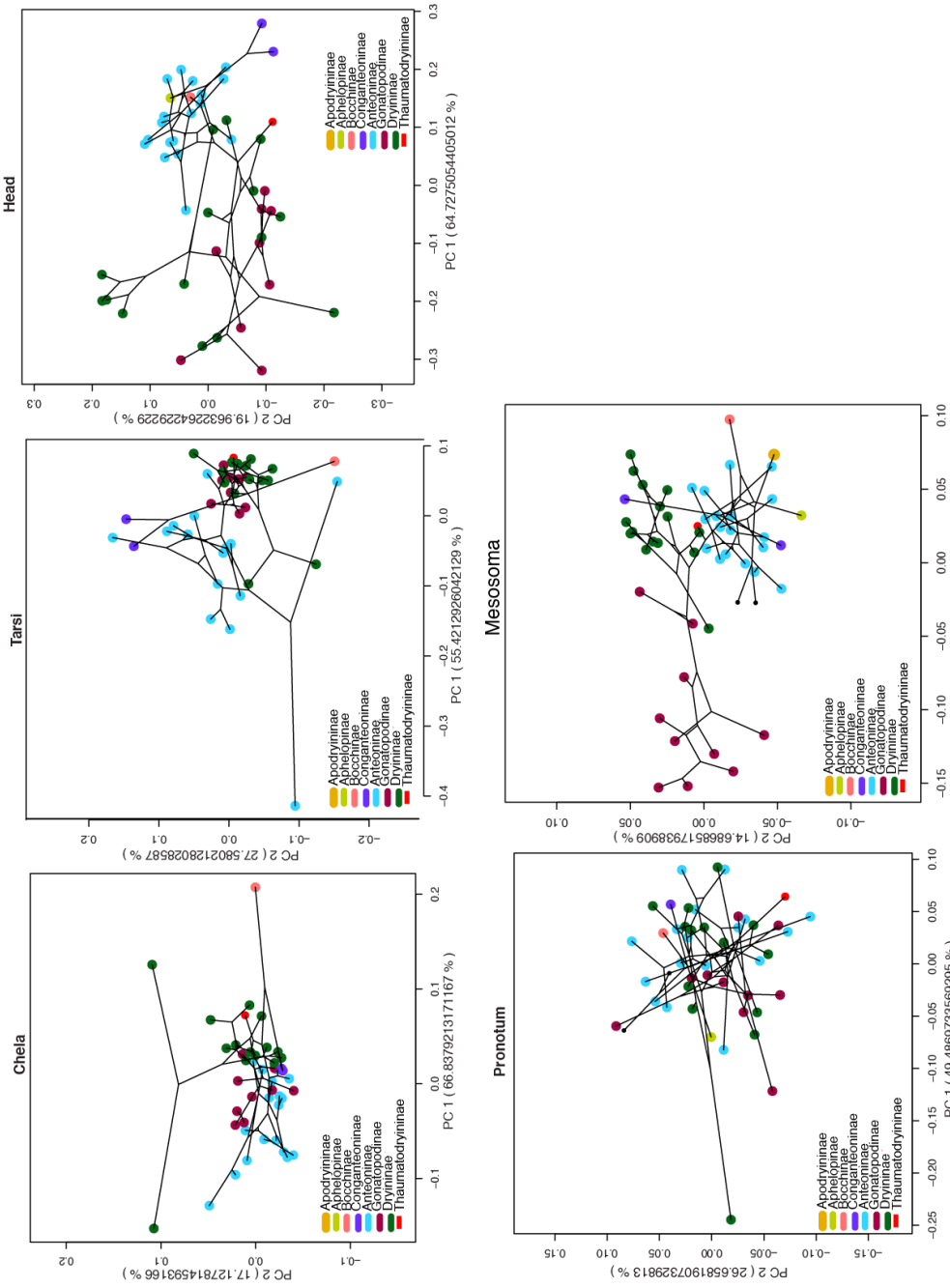


Figure 3.5. Projection of phylogeny (from morphological + molecular tree) onto the tangent shape space in the first and second principal components.

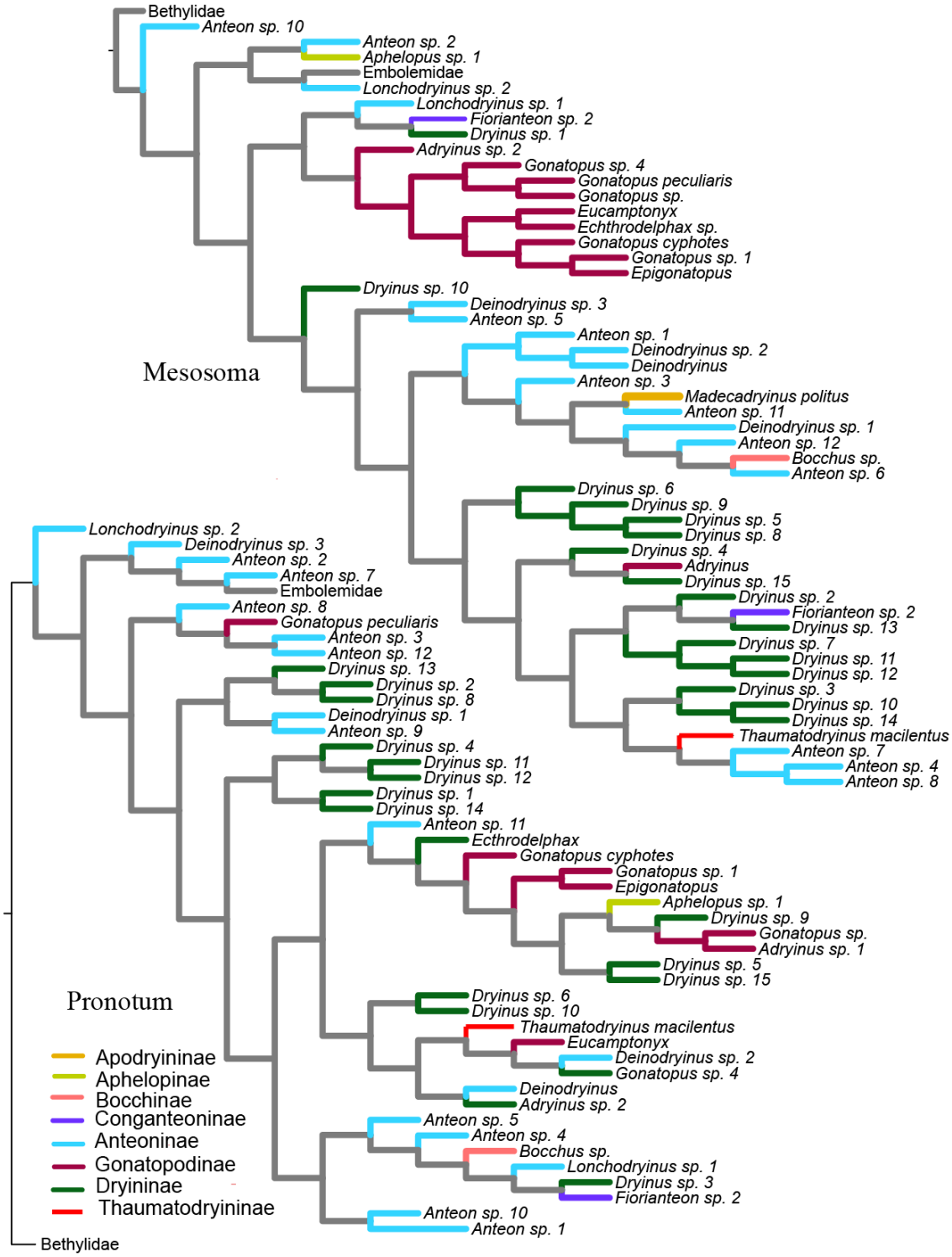


Figure 3.6 Trees produced by analyzing single shapes in TNT using the methods of Catalano et al. (2010). Top (mesosoma) and bottom (pronotum).

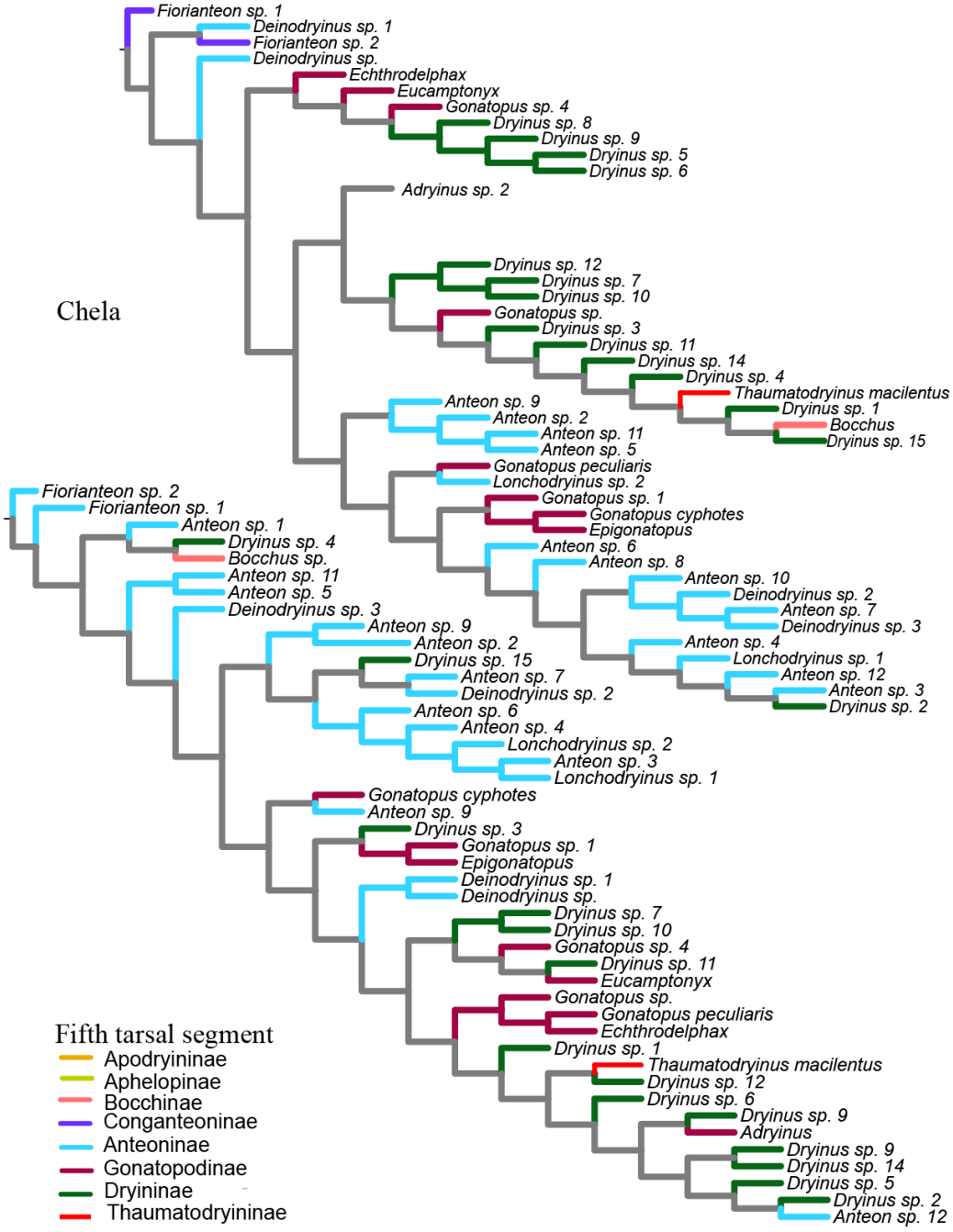


Figure 3.7. Trees produced by analyzing single shapes in TNT using the methods of Catalano et al. (2010). Top (chela claw) and bottom (fifth tarsal segment).

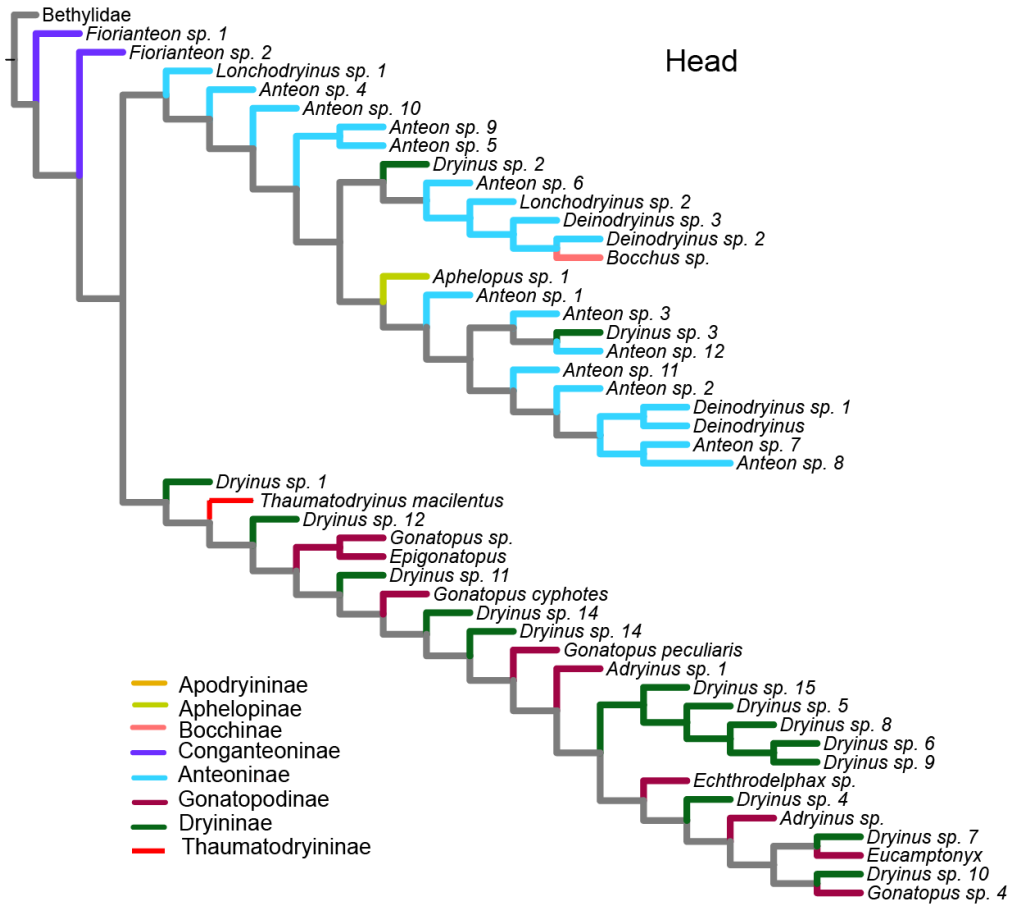


Figure 3.8. Tree produced by analyzing single shape (head) in TNT using the methods of Catalano et al. (2010).

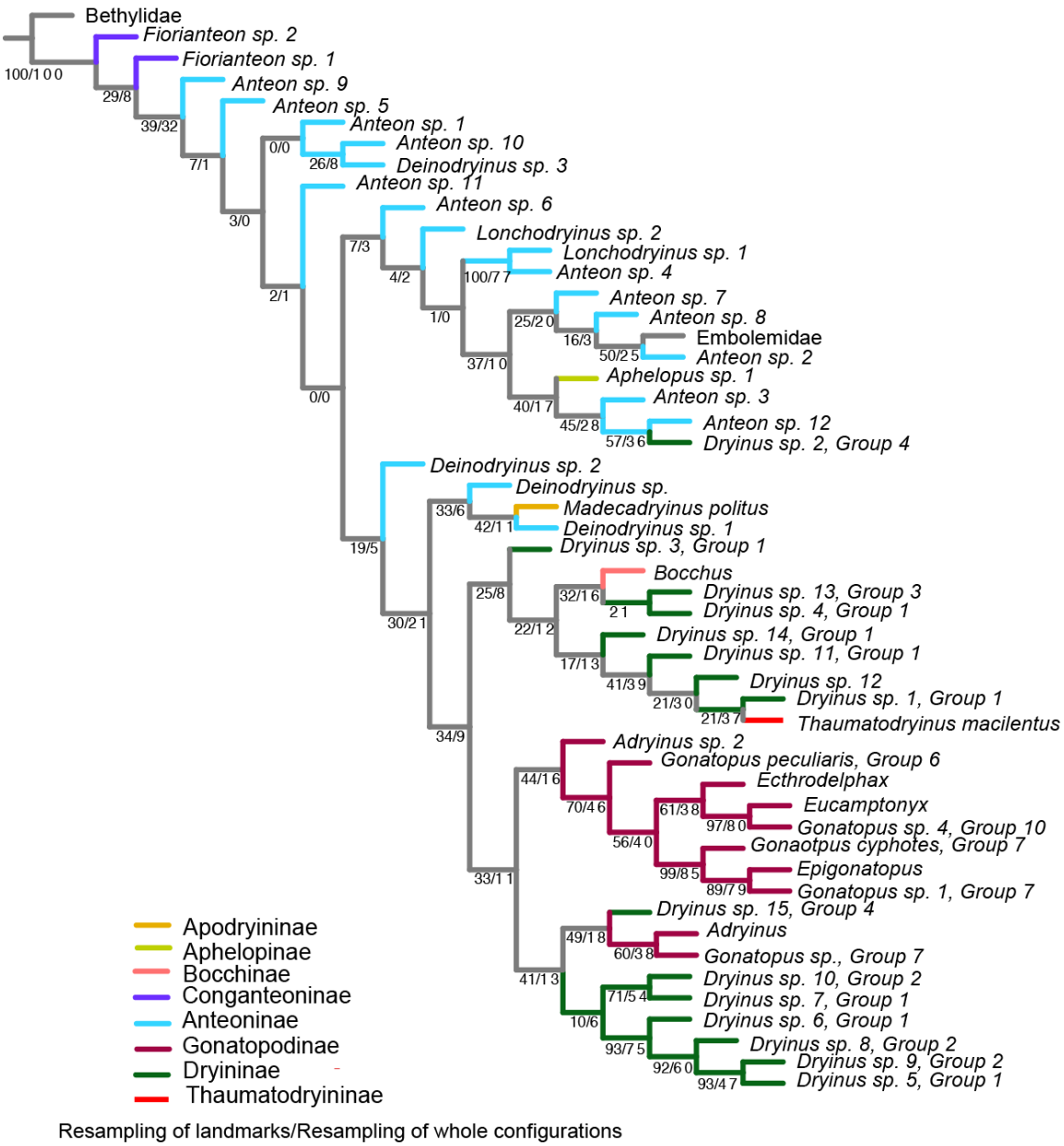


Figure 3.9. Trees produced by analyzing all five shapes in TNT using the methods of Catalano et al. (2010) and Catalano et al. (2014) for resampling of both individual landmarks and whole configurations (landmarks analyzed together).

DISCUSSION

To answer the question posed in the title of this chapter, the tree generated by morphometric data alone was incongruent to the tree produced by the molecular and morphological data. In the morphometric tree, both subfamilies and genera were nonmonophyletic. Discussions of why landmark analysis produced an incongruent tree are worth considering, particularly as an exploration for a new methodology in which systematists and morphometricians are still trying to place its use.

First, the PCA visualizations revealed that variance within subfamilies for each configuration tended to be high (see boxplots in Figures 3.3 and 3.4), and that specimens from subfamilies generally did not cluster together in distinct groups. In cases like *Dryinus* and *Gonatopus*, much of the variation within these genera came from the morphologically diverse species groups. In this study, *Dryinus* is represented by all four of the species groups, which as discussed in the last chapter, contain numerous synonymized genera. *Gonatopus* was represented by three species groups, and Gonatopodinae in whole was represented by the addition of *Echthrodelphax, Adryinus, Eucamptonyx*, and *Epigonatopus*. *Echthrodelphax* and *Adryinus* lack the ant-like, wingless shape of *Gonatopus*, *Eucamptonyx*, and *Epigonatopus*, and this resulted in high variation in the pronotum and metanotum.

The presence of a phylogenetic signal in the landmark configurations does not mean that the tree produced will be congruent with the phylogeny generated by the combination of discrete molecular and morphological character data. Using a test data set of nine species of *Drosophila* and landmarks from wing venation, Klingenberg and Gidaszewski (2010) had a similar result of significant phylogenetic signal but low

congruence with the phylogenetic trees from traditional data sources. Klingenberg and Gidaszewski (2010) assumed a Brownian model of evolution, which makes the assumption that there were no selective pressures on the shapes that were being analyzed. The same assumption was applied to the five shapes analyzed here. Certainly with the two shapes of the chela (the claw and the fifth tarsal segment), evolution could be driven by host-parasite co-evolution as chela are modified to grasp different types of hosts (Olmi 1994). Klingenberg and Gidaszewski (2010) used a different method to construct trees from their shapes (a variant of building Euclidean Steiner trees that minimized the sum of squared distances of shape change), but even while using landmark analysis under parsimony implementation, a significant phylogenetic signal in the configurations did not translate into congruence with the phylogeny generated from molecular and morphological data.

Finally, there is the question presented by Catalano et al. (2014) – how many configurations are necessary to successfully reconstruct the phylogeny? In Catalano et al. (2014), a method was developed to quantify the congruence between an accepted phylogeny and the phylogeny reconstructed by one or more landmark configurations using a score based on the number of SPR moves needed to convert the morphometric tree into the accepted phylogeny, divided by (# taxa – 2). The results indicated that single landmark configurations were insufficient and that congruence increased with more landmark configurations.

Santiago Catalano performed this analysis on the morphometric dataset here and found that the level of congruence trended towards increasing with more landmark configurations, even though the five-configuration tree was still incongruent with the

phylogenetic tree. As found in 8 other experimental morphometric datasets (Catalano 2015, *in preparation*, presented at the Willi Hennig Society Conference 2015), there was a positive correlation between the number of morphometric landmark configurations and the congruence to the tree produced by traditional types of data in this study of dryinid morphometrics. Unfortunately, Catalano did not provide explanation of *which* landmark configurations shared the most congruence with the tree produced from the molecular and morphological dataset.

CONCLUSION

The inclusion of geometric morphometrics as phylogenetic data that can be used in a cladistic analysis is a fairly new approach, and the pros, cons, and applicability of the technique is still being assessed in the literature. This specific study utilizing five morphometric shapes in comparison to a molecular and morphological tree resulted in trees that lacked congruence, but the reasons why bring more data to an on-going conversation about morphometrics and phylogenetics. For example, the congruence of the morphometric tree seems to improve with the inclusion of more morphometric characters, but this brings up the question of "How many configurations are necessary to reconstruct the phylogeny?"

A tree reconstructed from five traditional morphological characters would probably not be congruent compared to a tree reconstructed from 3000 molecular characters, so it is unsurprising that 5 landmark configurations were also incongruent. However, collecting morphometric landmarks for phylogenetic analyses is much more labor intensive than collecting traditional morphological characters. Unless working from an already published set of photos or CT scans, authors must obtain two-dimensional

photos or three-dimensional scans of each structure they plan to analyze. Ideally, multiple specimens should represent each species. Morphometrics is costly in terms of labor hours, and, if institutions charge for CT scanning time, perhaps financially prohibitive as well. However, not every physical feature should be analyzed by geometric morphometrics and perhaps it should only be considered for characters that are variable in terms of shapes that are not easily categorized by morphological coding. In this study, for example, landmarks were included from the ocellar triangle, which has often been represented in species-level diagnostic keys of Dryinidae as a proportion of measurements. Using shape variation instead of measurements could more effectively capture the changes in the shape of the ocellar triangle throughout Dryinidae.

Levels of high variation resulting in an insufficient superimposition were probably detrimental to this analysis at the subfamily level, but GM in cladistics could work at a smaller taxonomic range. In the previous chapter, the species groups of *Dryinus* were mostly nonmonophyletic, and *Gonatopus* was nonmonophyletic as well. Working at the species-level, geometric morphometrics could be utilized to reconstruct a phylogeny examining the relationships of the species groups within *Dryinus* and *Gonatopus*. This technique could be particularly useful for cases where specimens are too old to have DNA extracted and traditional morphological codes fail to delimit species. In Xu et al. (2013) and Olmi and Virla (2014), most of the characters for species level keys were based on color, relative lengths of various structures, and comparisons between species which were awkward to translate into morphological codes. Landmark analysis could successfully provide data in cases where variation is small, but morphological codes fail to capture differences at the species-level.

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REFERENCES

Adams, D.C., F. J. Rohlf, and D. E. Slice. 2013. "A field comes of age: geometric morphometrics in the 21st century." *Hystrix, the Italian Journal of Mammalogy* 24: 7-14.

Benson, R.H., R.E. Chapman, and A.F. Siegel. 1982. "On the measurement of morphology and its change." *Paleobiology*: 328-339.

Catalano, S.A., P.A. Goloboff, and N.P. Giannini. 2010. "Phylogenetic morphometrics (I): the use of landmark data in a phylogenetic framework." *Cladistics* 26: 539-549.

Catalano, S.A., and P.A. Goloboff. 2012. "Simultaneously mapping and superimposing landmark configurations with parsimony as optimality criterion." *Systematic Biology* 61: 392-400.

Catalano, S.A., M.D. Ercoli, and F.J. Prevosti. 2014. The more, the better: The use of multiple landmark configurations to solve phylogenetic relationships in musteloids. *Systematic Biology*: syu107

Cruz, R., Allan L., M. Josefa, R. Pante, and F. J. Rohlf. 2012. "Geometric morphometric analysis of shell shape variation in Conus (Gastropoda: Conidae)." *Zoological Journal of the Linnean Society* 165: 296-310.

Farris, J.S. 1970. "Methods for computing Wagner trees." *Systematic Biology* 19: 83-92.

Goloboff, P.A. 1998. "Tree searches under Sankoff parsimony." *Cladistics* 14: 229-237.

Goloboff, P. A., Mattoni, C. I., and Quinteros, A. S. 2006. "Continuous characters analyzed as such." *Cladistics* 22: 589-601.

Goloboff, P.A., J.S. Farris, and K.C. Nixon. 2008. "TNT, a free program for phylogenetic analysis." *Cladistics* 24: 774-786.

Goloboff, P.A., J.S. Farris, M. Källersjö, B. Oxelman, and C.A. Szumik. 2003. "Improvements to resampling measures of group support." *Cladistics* 19: 324-332.

Goloboff, P.A., and S.A. Catalano. 2011. "Phylogenetic morphometrics (II): algorithms for landmark optimization." *Cladistics* 27: 42-51.

Hendrickx, C. 2015. "The nonavian theropod quadrate II: systematic usefulness, major trends and cladistic and phylogenetic morphometrics analyses." *In preparation*. DOI 10.7287/peerj.preprints.380v2

Hines, H. M., J.H. Hunt, T.K. O'Connor, J.J. Gillespie, and S.A. Cameron. 2007. "Multigene phylogeny reveals eusociality evolved twice in vespid wasps." *Proceedings of the National Academy of Sciences* 104: 3295-3299.

Kearse, M., R. Moir, A.Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton et al. 2012. "Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data." *Bioinformatics* 28: 1647-1649.

Kim, K. C., H. C. Chiang, and B.W. Brown. 1967. "Morphometric differences among four biotypes of *Ostrinia nubilalis* (Lepidoptera: Pyralidae)." *Annals of the Entomological Society of America* 60: 796-801.

Klingenberg, C. P., and N.A. Gidaszewski. 2010. "Testing and quantifying phylogenetic signals and homoplasy in morphometric data." *Systematic Biology* 59: 245-261.

Maddison, W.P. 1991. "Squared-change parsimony reconstructions of ancestral states for continuous-valued characters on a phylogenetic tree." *Systematic Biology* 40: 304-314.

MacLeod, N. "Phylogenetic signals in morphometric data." *Morphology, Shape and Phylogeny* (2002): 100-138.

Medler, J.T. "Morphometric studies on bumble bees." *Annals of the Entomological Society of America* 55: 212-218.

Morrison, D.A. "Why would phylogeneticists ignore computerized sequence alignment?" 2009. *Systematic Biology* 58: 150-158.

Olmi, Massimo. 1993. "A new generic classification for Thaumatodryininae, Dryininae and Gonatopodinae, with descriptions of new species (Hymenoptera Dryinidae)." *Bollettino di Zoologia agraria e di Bachicoltura, Ser. II, 25,* 57-89.

Olmi, M. 1994. "The Dryinidae and Embolemidae (Hymenoptera: Chrysidoidea) of Fennoscandia and Denmark." *Fauna Entomologica Scandinavica* 30: 1–100.

Olmi, M., and E.G. Virla. 2014. "Dryinidae of the Neotropical region (Hymenoptera: Chrysidoidea)." *Zootaxa* 3792: 1-534.

Panchetti, F., M. Scalici, G. Maria Carpaneto, and G. Gibertini. 2008. "Shape and size variations in the cranium of elephant-shrews: a morphometric contribution to a phylogenetic debate." *Zoomorphology* 127: 69-82.

Perrard, A., F. Lopez-Osorio, and J.M. Carpenter. 2014. "Phylogeny, landmark optimization, and the use of wing venation to study the evolution of social wasps (Hymenoptera: Vespidae: Vespinae) *In press for Cladistics.*

Rokas, A., J.A.A. Nylander, F. Ronquist, and G.N. Stone. 2002. "A maximum-likelihood analysis of eight phylogenetic markers in gallwasps (Hymenoptera: Cynipidae): implications for insect phylogenetic studies." *Molecular Phylogenetics and Evolution* 22: 206-219.

Rohlf, F.J. 1999. "Shape statistics: Procrustes superimpositions and tangent spaces." *Journal of Classification* 16: 197-223.

Rohlf, F.J. 2001. TPSDig2: a program for landmark development and analysis. *See http://life.bio.sunysb.edu/morph*.

Rohlf, F.J., and D. Slice. 1990. "Extensions of the Procrustes method for the optimal superimposition of landmarks." *Systematic Biology* 39: 40-59.

Siddall, M.E. 2000. "Computer-Intensive Randomizations in Systematics." *Cladistics* 17: S34-S52.

Siegel, A.F., and R.H. Benson. 1982. "A robust comparison of biological shapes." *Biometrics*: 341-350.

Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994. "Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers." *Annals of the Entomological Society of America* 87: 651-701.

Scalici, M., and F. Panchetti. 2011. "Morphological cranial diversity contributes to phylogeny in soft-furred sengis (Afrotheria, Macroscelidea)." *Zoology* 114: 85-94.

Sheets, H.D. 2014. IMP-integrated morphometrics package – Version 8.

Stower, W. J., D. E. Davies, and I. B. Jones. "Morphometric studies of the desert locust, *Schistocerca gregaria* (Forsk.)." 1960. *The Journal of Animal Ecology*: 309-339.

Vaidya, G., D.J. Lohman, and R. Meier. 2011. "SequenceMatrix: concatenation software for the fast assembly of multi- gene datasets with character set and codon information." *Cladistics* 27: 171-180.

Vrijenhoek, R. 1994 "DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates." *Molecular marine biology and biotechnology* 3: 294-299.

Xu, Z., M. Olmi, and J. He. 2013. "Dryinidae of the Oriental region (Hymenoptera: Chrysidoidea)." *Zootaxa* 3614: 001-460.

CHAPTER IV
A MOLECULAR PHYLOGENY OF EPYRINAE (HYMENOPTERA: BETHYLIDAE) WITH A FOCUS ON THE PROBLEMATIC GENUS *EPYRIS* WESTWOOD

INTRODUCTION

Bethylidae, also known as the flat wasps (Azevedo 2014a), are the second largest family within Chrysidoidea, and the sister group to Chrysididae (Brothers and Carpenter 1993; Ronquist et al. 1999; Carr et al. 2010; Azevedo 2014a). Containing nearly 2700 species in five extant subfamilies, they are parasitoids of Coleoptera and Lepidoptera. While not as commonly utilized as Ichneumonidae or Braconidae as agricultural biocontrols, bethylids are known to naturally attack *Hypothenemus hampei* (the coffee berry borer) and *Amyelois transitella* (navel orange worm) (Abraham et al. 1990; Legner and Gordh 1992). As one of the commonly collected Chrysidoidea families, Bethylidae have been included in a number of recent Hymenoptera molecular phylogenies (Heraty et al. 2011; Klopfstein et al. 2013), but there has been only one molecular phylogenetic study of the relationships within the family itself (Carr et al. 2010).

Epyrinae, one of the larger subfamilies of Bethylidae, have been subjected to particular systematic scrutiny over the past few decades. In its original form, Epyrinae were composed of two tribes, Epyrini and Sclerodermini, as proposed by Kieffer (1914). Both tribes were raised to subfamily level by Berland (1928), but were collapsed back to tribal status by Evans (1964), with the addition of a third tribe, Cephalonomiini. Evans proposed that Sclerodermini and Cephalonomiini were related to Epyrini by a series of "intermediate genera" (e.g., *Laelius* Ashmead, *Plastanoxus* Ashmead, *Nesepyris*

Bridwell) and while Evans proposed a hypothesis of the relationships of the subfamilies, he did not perform phylogenetic analyses to test these relationships (Evans 1964).

Evans diagnosed Epyrini by having 13 antennal segments, laterally placed eyes, a median projecting lobe on the clypeus, well-defined notauli, and a pronotal disk bordered dorsally by carinae with the posterolateral angles foveolate. Unfortunately, these characters are highly variable within Epyrini, and are present in *other* subfamilies of Bethylidae (Sorg 1988; Carpenter 1999; Terayama 2003; Alencar and Azevedo 2013). Phylogenetic examination of Epyrinae and its tribes began with Lanes and Azevedo (2008), in which Sclerodermini were established as monophyletic only when they contained Cephalonomiini, which were then merged under Sclerodermini.

Carr et al. (2010) provided the first molecular phylogeny utilizing mitochondrial 16S and nuclear 28S genes, and found that Epyrini and Sclerodermini each were monophyletic, but Epyrinae as a whole were not monophyletic. Sclerodermini (*sensu* Lanes and Azevedo 2008) were sister to Mesitiinae, although a small sampling number prevented the authors from proposing a taxonomic revision (Carr et. al. 2010) Finally, in Alencar and Azevedo (2013), Epyrini and Sclerodermini (*sensu* Lanes and Azevedo 2008) were subjected to a thorough morphological analysis in which characters available only through dissection were examined. Sampling throughout Bethylidae, they found that Epyrinae (*sensu* Evans 1964) were not monophyletic, with all recovered topologies (Bethylinae + Sclerodermini + ((Mesitiinae + Pristocerinae) + Epyrini))). For the first time, synapomorphies were ascribed to Epyrini: posterior sulcus of pronotal disc punctate, epicoxal sulcus of propleuron circular, anterior margin of petiolar root convex with median narrow emargination, petiole not segmented, with root and body fused,

aedeagus short, and apex of aedeagus not reaching cuspis maximum height. Epyrini and Sclerodermini were re-elevated to subfamily status as Epyrinae and Scleroderminae, respectively.

While Epyrinae were well defined as a subfamily, several of its 16 extant genera were taxonomically disputable. Krombein (1996) had attempted to examine *Neodisepyris* Kurian and could not find any specimens, or the vast majority of the types described in Kurian (1952, 1954, 1955) despite the published deposit records. Alencar and Azevedo (2013) were unable to locate the Baker Collection in the Philippines in which the single species of *Leptepyris* Kieffer and the two species of *Xenepyris* Kieffer were deposited. Many of the genera in Epyrinae Kieffer described, such as *Melanepyris* Kieffer, *Pristepyris* Kieffer, *Trissepyris* Kieffer, and *Neurepyris* Kieffer have been synonymized with other genera, (Alencar and Azevedo 2009; Azevedo and Alencar 2009; Azevedo and Alencar 2010a; Azevedo and Alencar 2010b; Alencar and Azevedo 2011), but the written descriptions alone were insufficient to re-analyze *Leptepyris* and *Xenepyris*.

Waichert and Azevedo (2009) analyzed *Rhabdepyris* through a morphological phylogeny and found that the genus was not monophyletic, and that the species it contained could probably be transferred to *Laelius*, *Anisepyris* Kieffer, and *Chlorepyris* Kieffer. They expanded the descriptions of these genera to accommodate the *Rhabdepyris* species that were analyzed, but as all of the species of *Rhabdepyris* could not be examined, it was not discarded as a genus. The type species of *Rhabdepyris*, *Rhabdepyris myrmecophilus*, remained the type of *Rhabdepyris*, which still contains forty species. Barbosa and Azevedo (2011) synonymized *Allepyris* Kieffer, *Paralaelius* Kieffer, and

Prolaelius Kieffer with *Laelius* based on the results of a similar morphological phylogeny.

The most speciose genus within Bethylidae, with 275 species described worldwide, *Epyris* Westwood is likely a taxonomic wastebasket as it cannot be defined by unique autapomorphies (Carpenter 1999; Alencar and Azevedo 2013). In Alencar and Azevedo (2013) it was found as paraphyletic, but was only represented by three species, and Carr et al. (2010) only featured two specimens in their combined analysis of 28S and 16S genes (four specimens of *Epyris* were utilized in the individual gene trees and were paraphyletic in both cases).

Epyris has numerous generic synonyms - *Dolus* Motschulsky, *Muellerella* Saussure, *Parepyris* Kieffer, *Psilepyris* Kieffer, *Artiepyris* Kieffer, *Trissepyris* Kieffer, and *Melanepyris* Kieffer (Evans 1964; Evans 1969; Krombein 1987). Evans (1964) synonymized *Muellerella*, *Parepyris*, and *Psilepyris* and Evans (1969) synonymized *Artiepyris* within the *dodecatomus* species group of *Epyris*. Evans (1969) split the Nearctic and Neotropical *Epyris* into nine species groups, although admitted that some groups were "distinctive, easily recognized, and undoubtedly natural taxa (e.g. the *montivagus and depressigaster groups*)" while other species groups were decidedly arbitrary, extremely broad, and connected by "intermediary species", such as in the *tricostatus* and *rufipes* groups. Evans (1969) provided a tree to how these species groups were related and derived from *Rhabdepyris*, but did not provide any phylogenetic analyses. He later removed one of the species groups, *montivagus* and placed them in a separate genus, *Bakeriella* (Evans 1979).

Outside of Howard Evans' work, several genera have been synonymized within *Epyris*, such as *Melanepyris* Kieffer and *Trissepyris* Kieffer (Azevedo and Alencar 2010a; Azevedo and Alencar 2010b). *Calyozina*, a genus that was diagnosed by males possessing ramose antennae, was revised by Krombein (1992) so that species that contained males with a reduced third antennal flagellomere were now part of the *staphylinoides* group of *Epyris*. The trait of the reduced third antennal flagellomere is shared with the *dodecatomus* species group.

The combination of a lack of synapomorphies, numerous synonymized genera, and species groups that are likely arbitrary means that the largest genus within Bethylidae is questionable and likely a taxonomic wastebasket. To examine *Epyris* and the other large genera within Epyrinae, a molecular phylogeny is presented below using nuclear 18S, 28S, and mitochondrial 16S, Cytochrome Oxidase Subunit I, and Cytochrome B genetic markers. Unlike other studies that have included *Epyris* in their molecular and morphological phylogenies, this is the first study that attempted to represent the vast diversity of *Epyris* by focusing on the sampling from the various species groups and numerous geographical regions.

MATERIALS AND METHODS

Materials

Phylogenetic relationships were inferred from 176 specimens of Bethylidae with three specimens of Chrysididae (two from Chrysididae and one from Cleptinae), which are the sister group to Bethylidae (Grimaldi and Engel 2005), as outgroup taxa. Specimens were provided from collection visits to the Universidade Federal do Espírito Santo (UFES), the Canadian National Collections (CNC), the British Museum of Natural

History (BNHM), the Muséum National D'Histore Naturelle (MNHM), the Queensland Museum (QLD), and the Australian National Insect Collection (ANIC) where bethylid samples from a variety of worldwide collecting efforts were accessioned. These materials, which were sorted from a variety of Malaise trap, yellow pan trap, and sweep netting catches, aimed to represent the worldwide distribution of Epyrinae, with 25 countries and a large focus on areas of known diversity. As these specimens came from bulk alcohol and by-catch collections, they have not been identified or accessioned in collections in most cases. After this study, and the identification of new species, they will be deposited in their home collections.

Ninety-two specimens of *Epyris* were utilized, along with 23 specimens of *Holepyris*, nine *Laelius*, 20 *Chlorepyris*, nine *Anisepyris*, four *Bakeriella*, and three *Trachepyris*. While Waichert and Azevedo (2009) showed that *Rhabdepyris* was non-monophyletic, and suggested that many species could be moved to *Chlorepyris* and *Laelius*, the authors did not officially revise the taxonomy. For that reason, in the study presented here, *Rhabdepyris* species that could be transferred according to their recommendations as *Laelius sensu* Waichert and Azevedo and *Chlorepryis sensu* Waichert and Azevedo are listed as *Laelius/Rhabdepyris* and *Chlorepyris/Rhabdepyris.* There were three specimens of *Laelius sensu* Ashmead, with another six *Laelius sensu* Waichert and Azevedo. One genus of Epyrinae, *Trachepyris*, has synapomorphies that are only found within females (with males appearing as identical to those of *Epyris*), so that only females were identifiable and three specimens were included (Krombein 1987). Potential males of *Trachepyris* are elaborated upon in the discussion. Attempts were made to extract DNA from *Disepyris* Kieffer, *Formosiepyris* Terayama and *Aspidepyris*

Evans, but these specimens were too old for successful extraction. In addition, specimens of *Isobrachium* Ashmead, *Leptepyris*, *Xenepyris*, and *Neodisepyris* were not examined. *Calyozina sensu* Krombein 1987 was not included, although specimens of the *staphylinoides* species group of *Epyris*, which was originally part of *Calyozina* sensu Enderlein, 1912, were analyzed. To test the subfamily-level findings of Carr et al. (2010) and Alencar and Azevedo (2013), two Bethylinae, five Pristocerinae, four Mesitiinae, and five Scleroderminae were also included.

Laboratory protocols

Specimens were stored in 95 percent ethanol and refrigerated prior to extraction. 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 16S region was amplified using 16SF1 (5'-CAC CTG TTT ATC AAA AAC AT-3') and 16SR1 (5'-CGT CGA TTT GAA CTC AAA TC-3') (Dowton and Austin 1994), 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), the 28S D2-D3 region 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) and 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 Appendix B, 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 8 (Kearse et al. 2012). Protein coding genes (COI and Cytb) were checked for stop codons and numts and aligned using the translation alignment algorithm within Geneious. 16S, 18S, and 28S genes were aligned using MAAFT, using the E-INS-I algorithm as implemented in Geneious 8. This algorithm was chosen for its accuracy in difficult alignments (Morrison 2009). The concatenated matrix was assembled in SeqMatrix (Vaidya et al. 2010), resulting in a final matrix of 7584 characters, 1,727 of which were parsimony informative sites, with 13 % missing data.

Phylogenetic analyses were performed using parsimony, maximum likelihood, and Bayesian approaches. For parsimony, TNT (Goloboff et al. 2008) was used with the default new technology search algorithms with the following parameters modified: 200 ratchet iterations, upweighting percentage 8, downweighting 4; 50 cycles of drift; minimum length hit 25 times with gaps treated as missing data. Resampling (Goloboff et al. 2003) 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 (courtesy of Salvador Arias) in TNT, with a reported k value of 39.189454.

PartitionFinder (Lanfear et al. 2012) was used to select models of molecular evolution for Garli (Stamatakis 2014) and MrBayes (Ronquist et al. 2012) analyses for each ribosomal gene and each codon for COI and Cytb. Each partition was returned as GTR+I+G. Likelihood analyses were performed using the molecularevolution.org server version of Garli, which analyzes datasets to determine the number of search replicates needed to find the tree topology with the best likelihood score within a 95 percent probability (Bazinet et al. 2014). One thousand Bootstrap replicates were also performed on the molecularevolution.org server and the python script Sumtrees (Sukumaran and Holder, 2008) was used to summarize the results on the best likelihood score tree. For Bayesian analyses, Mr Bayes 3.2.3 (Ronquist et al. 2012) on the XSEDE platform on Cipres with the default parameters, allowing for 20, 000, 000 generations, was utilized.

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

RESULTS

Analytical Output

In TNT (parsimony), 42,992,005,590 rearrangements were examined and ten trees were retained with a best score of 23060 for the unweighted search with a consistency index of 0.206 and a retention index of 0.570. The tree generated by the symmetric resampling analysis is presented in Figure 4.1, with an average support value of 54.5. For the weighted search, 47,937,592,033 rearrangements were examined with five trees retained and a best score of 304.13225. The strict consensus for the weighted and unweighted trees was the same. The average of the support values were

After 28 replicates, the Garli (likelihood) analysis had a best likelihood score of -93083.105577 (Figure 4.2) and the MrBayes analysis (Bayesian) had an average

standard deviation of 0.029261for the split frequencies at the end of 20,000,000 generations (Figure 4.3). In all three analyses, branches were not collapsed below a set support value cut-off of zero.

Subfamily Topology

Epyrinae *sensu* Alencar and Azevedo were recovered as monophyletic with strong support values (73 in the parsimony analysis, 87 in the likelihood analysis, and 100 in the Bayesian analysis), as were Scleroderminae *sensu* Alencar and Azevedo, Pristocerinae, Bethylinae, and Mesitiinae. Subfamily topologies in all analyses were (Bethylinae + (Pristocerinae + (Mesitiinae + Scleroderminae) + Epyrinae))).

Relationships within Epyrinae

Two major divisions of the genera within Epyrinae were recovered in every analysis: *Anisepyris*, *Laelius*, *Chlorepyris*, and *Rhabdepyris* formed a clade, and *Trachepyris*, *Epyris*, *Bakeriella*, and *Holepyris* formed another. Resampling for these two divisions were high, with support values of 99 in the parsimony analysis, 100 in the likelihood analysis, and 100 in the Bayesian analysis. Shared throughout each analysis was the recovery of *Anisepyris* and *Holepyris* as monophyletic and a non-monophyletic *Epyris*.

In the parsimony analysis, *Chlorepyris sensu* Waichert and Azevedo was polyphyletic, as was *Laelius sensu* Waichert and Azevedo. However, *Laelius sensu* Ashmead was monophyletic. The monophyletic *Anisepyris* was the sister genus to *Chlorepyris in partim*, which was entirely from Australia with the exception of a single taxon from Peru. In the likelihood analysis, *Anisepyris* was the sister group to a clade that contained a monophyletic *Laelius sensu* Waichert and Azevedo and a nonmonophyletic

Chlorepyris sensu Waichert and Azevedo. In the Bayesian analysis, both *Laelius sensu* Waichert and Azevedo and *Anisepyris* were monophyletic, together with a nonmonophyletic *Chlorepyri*s forming a polytomy.

In all analyses, the remainder of Epyrinae were a nonmonophyletic *Epyris* and monophyletic *Holepyris*, *Bakeriella*, and *Trachepyris*. The Bayesian and likelihood topologies of the clades recovered were the same, excluding the presence of a polytomy at the basal node that connected *Trachepyris*, *Bakeriella*, *Holepyris*, and the various groups of *Epyris* that were sister to those genera in the Bayesian analysis. In both trees, the *Epyris staphylinoides* species group (which was considered as *Calyozina* prior to Krombein 1992) was the sister group to *Epyris*, *Trachepyris*, *Bakeriella*, and *Holepyris*.

In the parsimony tree, the clades of the nonmonophyletic *Epyris* (including the *staphylinoides* group), *Trachepyris*, *Bakeriella*, and *Holepyris* were presented as an undifferentiated polytomy in which (*Bakeriella* + the *Epyris staphylinoides* group) formed a clade. Besides presenting as a polytomy, the placement of *Bakeriella* and the *staphylinoides* group were the key difference between the parsimony and nearly identical likelihood and Bayesian trees.

DISCUSSION

The phylogenies presented here supported the revision Alencar and Azevedo (2013) made in separating Sclerodermini and Epyrini into separate subfamilies, and clearly showed that *Epyris* is not monophyletic and thus is an unnatural genus. As seen in

Figure 4.1. Unweighted parsimony support tree. CI = 0.206, RI = 0.570. Symmetric resampling support for nodes reported as GC-values (frequency differences) from 1000 replicates. Print-size expansion in Appendix F.

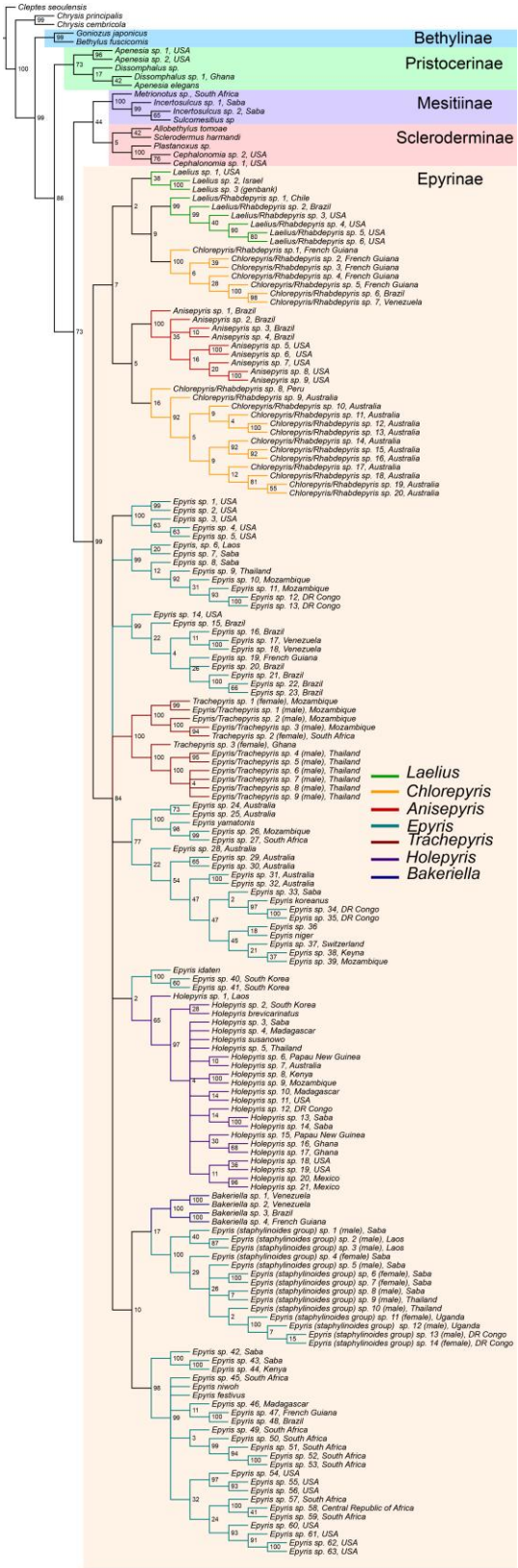

Cleptes seoulensis
Chrysis principalis
Chrysis cembricola
Goniozus japonicus
Bethylus fuscicornis
Bethylinae
Apenesia sp. 1, USA
Apenesia sp. 2, USA
Dissomphalus sp.
Dissomphalus sp. 1, Ghana
Apenesia elegans
Pristocerinae
Metrionotus sp., South Africa
Incertosulcus sp. 1, Saba
Incertosulcus sp. 2, Saba
Sulcomesitius sp
Mesitiinae
Allobethylus tomoae
Scleroderma harmandi
Plastanoxus sp.
Cephalonomia sp. 2, USA
Cephalonomia sp. 1, USA
Scleroderminae
Laelius sp. 1, USA
Laelius sp. 2, Israel
Laelius sp. 3 (genbank)
Laelius/Rhabdepyris sp. 1, Chile
Laelius/Rhabdepyris sp. 2, Brazil
Laelius/Rhabdepyris sp. 3, USA
Laelius/Rhabdepyris sp. 4, USA
Laelius/Rhabdepyris sp. 5, USA
Laelius/Rhabdepyris sp. 6, USA
Chlorepyris/Rhabdepyris sp.1, French Guiana
Chlorepyris/Rhabdepyris sp. 2, French Guiana
Chlorepyris/Rhabdepyris sp. 3, French Guiana
Chlorepyris/Rhabdepyris sp. 4, French Guiana
Chlorepyris/Rhabdepyris sp. 5, French Guiana
Chlorepyris/Rhabdepyris sp. 6, Brazil
Chlorepyris/Rhabdepyris sp. 7, Venezuela
Anisepyris sp. 1, Brazil
Anisepyris sp. 2, Brazil
Anisepyris sp. 3, Brazil
Anisepyris sp. 4, Brazil
Anisepyris sp. 5, USA
Anisepyris sp. 6, USA
Anisepyris sp. 7, USA
Anisepyris sp. 8, USA
Anisepyris sp. 9, USA
Chlorepyris/Rhabdepyris sp. 8, Peru
Chlorepyris/Rhabdepyris sp. 9, Australia
Chlorepyris/Rhabdepyris sp. 10, Australia
Chlorepyris/Rhabdepyris sp. 11, Australia
Chlorepyris/Rhabdepyris sp. 12, Australia
Chlorepyris/Rhabdepyris sp. 13, Australia
Chlorepyris/Rhabdepyris sp. 14, Australia
Chlorepyris/Rhabdepyris sp. 15, Australia
Chlorepyris/Rhabdepyris sp. 16, Australia
Chlorepyris/Rhabdepyris sp. 17, Australia
Chlorepyris/Rhabdepyris sp. 18, Australia
Chlorepyris/Rhabdepyris sp. 19, Australia
Chlorepyris/Rhabdepyris sp. 20, Australia
Epyris sp. 1, USA
Epyris sp. 2, USA
Epyris sp. 3, USA
Epyris sp. 4, USA
Epyris sp. 5, USA
Epyris, sp. 6, Laos
Epyris sp. 7, Saba
Epyris sp. 8, Saba
Epyris sp. 9, Thailand
Epyris sp. 10, Mozambique
Epyris sp. 11, Mozambique
Epyris sp. 12, DR Congo
Epyris sp. 13, DR Congo
Epyris sp. 14, USA
Epyris sp. 15, Brazil
Epyris sp. 16, Brazil
Epyris sp. 17, Venezuela
Epyris sp. 18, Venezuela
Epyris sp. 19, French Guiana
Epyris sp. 20, Brazil
Epyris sp. 21, Brazil
Epyris sp. 22, Brazil
Epyris sp. 23, Brazil
Trachepyris sp. 1 (female), Mozambique
Epyris/Trachepyris sp. 1 (male), Mozambique
Epyris/Trachepyris sp. 2 (male), Mozambique
Epyris/Trachepyris sp. 3 (male), Mozambique
Trachepyris sp. 2 (female), South Africa
Trachepyris sp. 3 (female), Ghana
Epyris/Trachepyris sp. 4 (male), Thailand
Epyris/Trachepyris sp. 5 (male), Thailand
Epyris/Trachepyris sp. 6 (male), Thailand
Epyris/Trachepyris sp. 7 (male), Thailand
Epyris/Trachepyris sp. 8 (male), Thailand
Epyris/Trachepyris sp. 9 (male), Thailand
Epyris sp. 24, Australia
Epyris sp. 25, Australia
Epyris yamatonis
Epyris sp. 26, Mozambique
Epyris sp. 27, South Africa
Epyris sp. 28, Australia
Epyris sp. 29, Australia
Epyris sp. 30, Australia
Epyris sp. 31, Australia
Epyris sp. 32, Australia
Epyris sp. 33, Saba
Epyris koreanus
Epyris sp. 34, DR Congo
Epyris sp. 35, DR Congo
Epyris sp. 36
Epyris niger
Epyris sp. 37, Switzerland
Epyris sp. 38, Keyna
Epyris sp. 39, Mozambique
Epyris idaten
Epyris sp. 40, South Korea
Epyris sp. 41, South Korea
Holepyris sp. 1, Laos
Holepyris sp. 2, South Korea
Holepyris brevicarinatus
Holepyris sp. 3, Saba
Holepyris sp. 4, Madagascar
Holepyris susanowo
Holepyris sp. 5, Thailand
Holepyris sp. 6, Papau New Guinea
Holepyris sp. 7, Australia
Holepyris sp. 8, Kenya
Holepyris sp. 9, Mozambique
Holepyris sp. 10, Madagascar
Holepyris sp. 11, USA
Holepyris sp. 12, DR Congo
Holepyris sp. 13, Saba
Holepyris sp. 14, Saba
Holepyris sp. 15, Papau New Guinea
Holepyris sp. 16, Ghana
Holepyris sp. 17, Ghana
Holepyris sp. 18, USA
Holepyris sp. 19, USA
Holepyris sp. 20, Mexico
Holepyris sp. 21, Mexico
Bakeriella sp. 1, Venezuela
Bakeriella sp. 2, Venezuela
Bakeriella sp. 3, Brazil
Bakeriella sp. 4, French Guiana
Epyris (staphylinoides group) sp. 1 (male), Saba
Epyris (staphylinoides group) sp. 2 (male), Laos
Epyris (staphylinoides group) sp. 3 (male), Laos
Epyris (staphylinoides group) sp. 4 (female) Saba
Epyris (staphylinoides group) sp. 5 (male), Saba
Epyris (staphylinoides group) sp. 6 (female), Saba
Epyris (staphylinoides group) sp. 7 (female), Saba
Epyris (staphylinoides group) sp. 8 (male), Saba
Epyris (staphylinoides group) sp. 9 (male), Thailand
Epyris (staphylinoides group) sp. 10 (male), Thailand
Epyris (staphylinoides group) sp. 11 (female), Uganda
Epyris (staphylinoides group) sp. 12 (male), Ugandia
Epyris (staphylinoides group) sp. 13 (male), DR Congo
Epyris (staphylinoides group) sp. 14 (female), DR Congo
Epyris sp. 42, Saba
Epyris sp. 43, Saba
Epyris sp. 44, Kenya
Epyris sp. 45, South Africa
Epyris nwoh
Epyris festivus
Epyris sp. 46, Madagascar
Epyris sp. 47, French Guiana
Epyris sp. 48, Brazil
Epyris sp. 49, South Africa
Epyris sp. 50, South Africa
Epyris sp. 51, South Africa
Epyris sp. 52, South Africa
Epyris sp. 53, South Africa
Epyris sp. 54, USA
Epyris sp. 55, USA
Epyris sp. 56, USA
Epyris sp. 57, South Africa
Epyris sp. 58, Central Republic of Africa
Epyris sp. 59, South Africa
Epyris sp. 60, USA
Epyris sp. 61, USA
Epyris sp. 62, USA
Epyris sp. 63, USA
Epyrinae
Laelius
Chlorepyris
Anisepyris
Epyris
Trachepyris
Holepyris
Bakeriella

Figure 4.2. Likelihood tree. Rapid Bootstrap support values shown at nodes. Print size expansion in Appendix F.

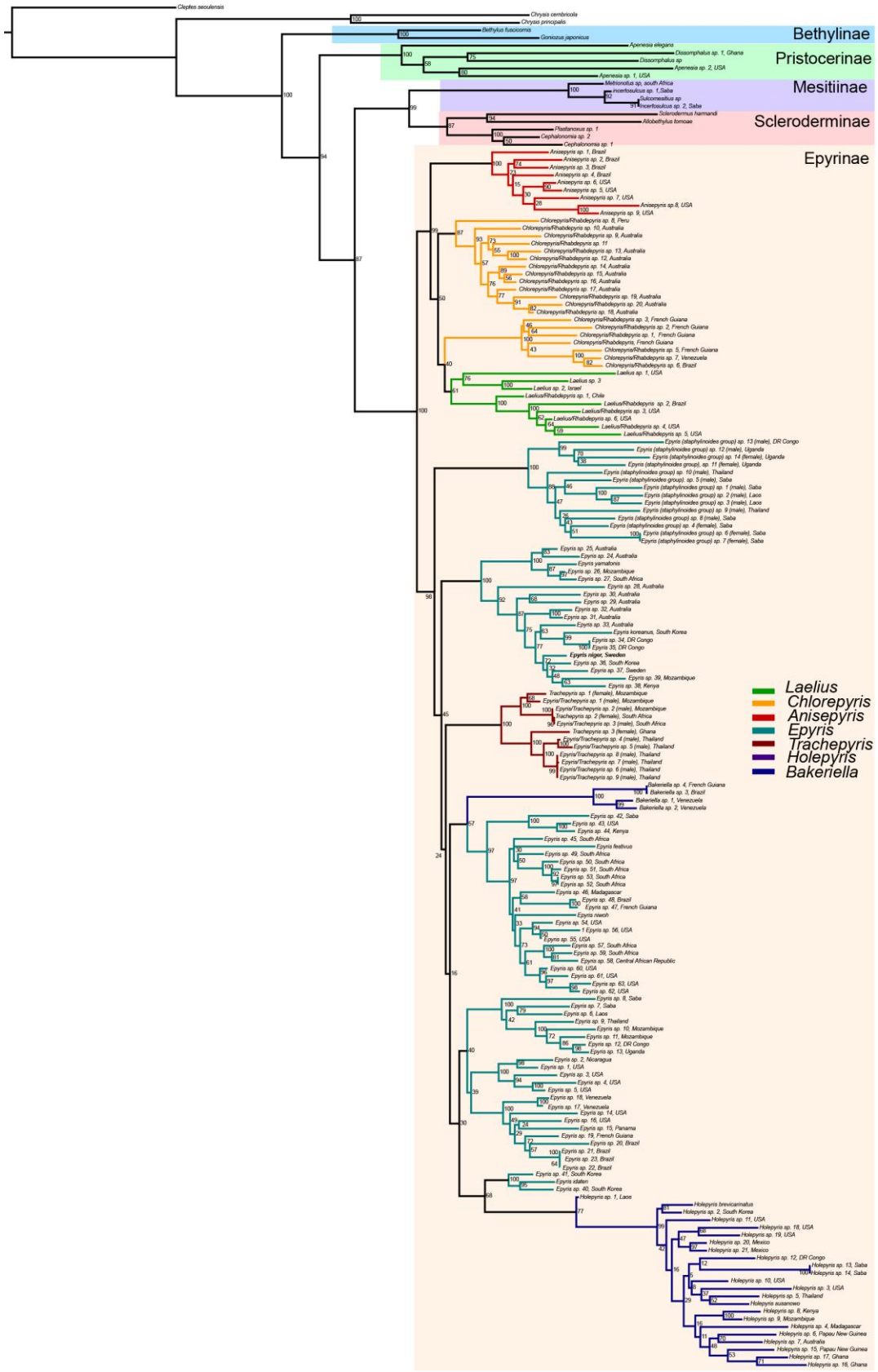
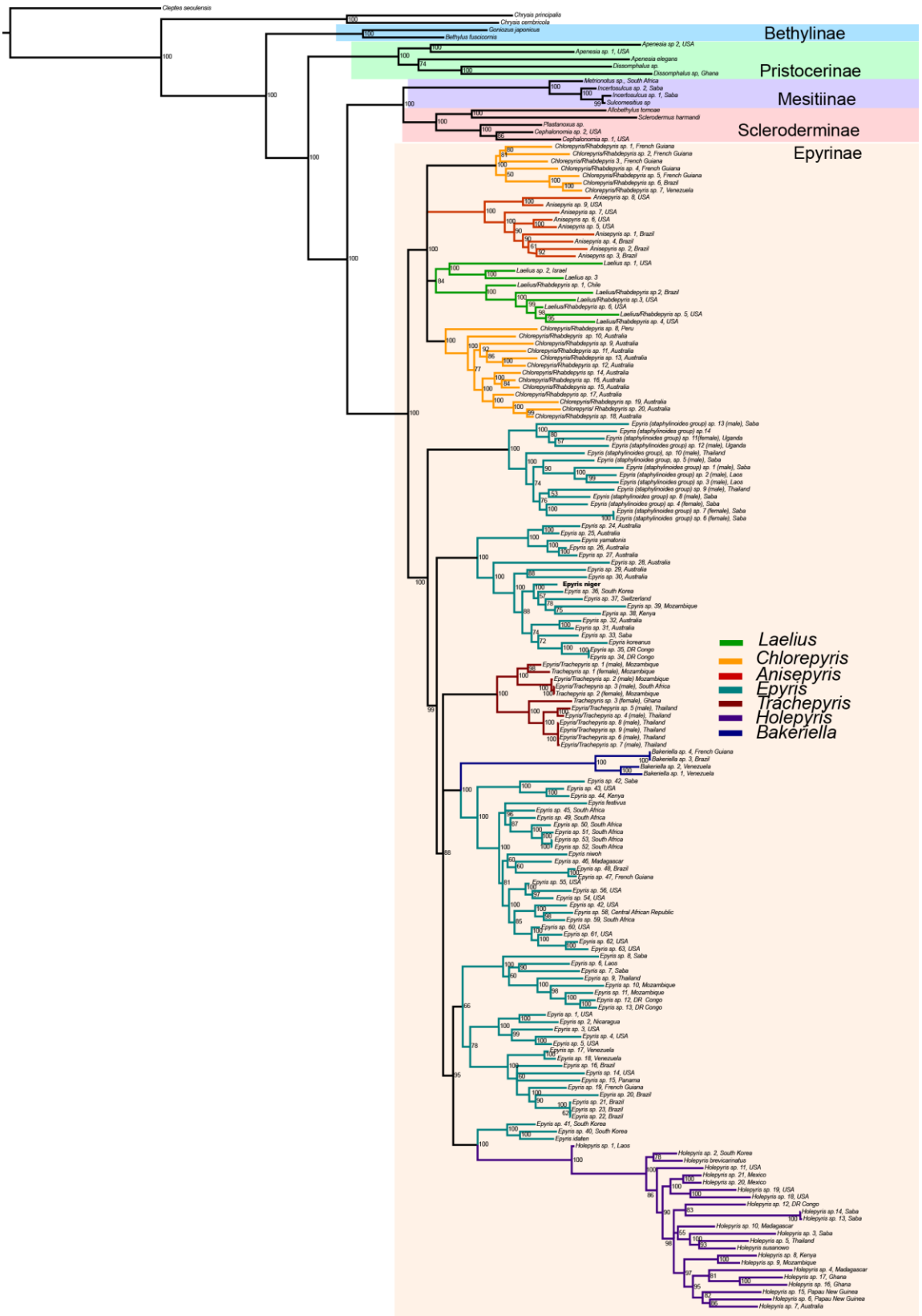

Cleptes seculensis
Chrysis cembricola
Chrysis principalis
Bethylus fuscicornis
Goniozus japonicus
Bethylinae
Apenesia elegans
Dissomphalus sp. 1, Ghana
Dissomphalus sp
Apenesia sp. 2, USA
Pristocerinae
Apenesia sp. 1, USA
Meiriomolus sp. south Africa
Incertosulcus sp. 1,Saba
Sulcomesitius sp
Incertosulcus sp. 2, Saba
Mesitiinae
Scleroderma harmandi
Afobethylus tomoae
Plastanoxus sp. 1
Cephalonomia sp. 2
Cephalonomia sp. 1
Sclerodermine
Epyrinae
Anisepyris sp. 1, Brazil
Anisepyris sp. 2, Brazil
Anisepyris sp. 3, Brazil
Anisepyris sp. 4, Brazil
Anisepyris sp. 6, USA
Anisepyris sp. 5, USA
Anisepyris sp. 7, USA
Anisepyris sp.8, USA
Anisepyris sp. 9, USA
Chlorepyris/Rhabdepyris sp. 8, Peru
Chlorepyris/Rhabdepyris sp. 10, Australia
Chlorepyris/Rhabdepyris sp. 9, Australia
Chlorepyris/Rhabdepyris sp. 11
Chlorepyris/Rhabdepyris sp. 13, Australia
Chlorepyris/Rhabdepyris sp. 12, Australia
Chlorepyris/Rhabdepyris sp. 14, Australia
Chlorepyris/Rhabdepyris sp. 15, Australia
Chlorepyris/Rhabdepyris sp. 16, Australia
Chlorepyris/Rhabdepyris sp. 17, Australia
Chlorepyris/Rhabdepyris sp. 19, Australia
Chlorepyris/Rhabdepyris sp. 20, Australia
Chlorepyris/Rhabdepyris sp. 18, Australia
Chlorepyris/Rhabdepyris sp. 3, French Guiana
Chlorepyris/Rhabdepyris sp. 2, French Guiana
Chlorepyris/Rhabdepyris sp. 1, French Guiana
Chlorepyris/Rhabdepyris, French Guiana
Chlorepyris/Rhabdepyris sp. 5, French Guiana
Chlorepyris/Rhabdepyris sp. 7, Venezuela
Chlorepyris/Rhabdepyris sp. 6, Brazil
Laelius sp. 1, USA
Laelius sp. 3
Laelius sp. 2, Israel
Laelius/Rhabdepyris sp. 1, Chile
Laelius/Rhabdepyris sp. 2, Brazil
Laelius/Rhabdepyris sp. 3, USA
Laelius/Rhabdepyris sp. 6, USA
Laelius/Rhabdepyris sp. 4, USA
Laelius/Rhabdepyris sp. 5, USA
Epyris (staphylinoides group) sp. 13 (male), DR Congo
Epyris (staphylinoides group) sp. 12 (male), Uganda
Epyris (staphylinoides group) sp. 14 (female), Uganda
Epyris (staphylinoides group), sp. 11 (female), Uganda
Epyris (staphylinoides group) sp. 10 (male), Thailand
Epyris (staphylinoides group), sp. 5 (male), Saba
Epyris (staphylinoides group) sp. 1 (male), Saba
Epyris (staphylinoides group) sp. 2 (male), Laos
Epyris (staphylinoides group) sp. 3 (male), Laos
Epyris (staphylinoides group) sp. 9 (male), Thailand
Epyris (staphylinoides group) sp. 8 (male), Saba
Epyris (staphylinoides group) sp. 4 (female), Saba
Epyris (staphylinoides group) sp. 6 (female), Saba
Epyris (staphylinoides group) sp. 7 (female), Saba
Epyris sp. 25, Australia
Epyris sp. 24, Australia
Epyris yamatonis
Epyris sp. 26, Mozambique
Epyris sp. 27, South Africa
Epyris sp. 28, Australia
Epyris sp. 30, Australia
Epyris sp. 29, Australia
Epyris sp. 32, Australia
Epyris sp. 31, Australia
Epyris sp. 33, Australia
Epyris koreanus, South Korea
Epyris sp. 34, DR Congo
Epyris 35, DR Congo
Epyris nitger, Sweden
Epyris sp. 36, South Korea
Epyris sp. 37, Sweden
Epyris sp. 39, Mozambique
Epyris sp. 38, Kenya
Trachepyris sp. 1 (female), Mozambique
Epyris/Trachepyris sp. 1 (male), Mozambique
Epyris/Trachepyris sp. 2 (male), Mozambique
Trachepyris sp. 2 (female), South Africa
Epyris/Trachepyris sp. 3 (male), South Africa
Trachepyris sp. 3 (female), Ghana
Epyris/Trachepyris sp. 4 (male), Thailand
Epyris/Trachepyris sp. 5 (male), Thailand
Epyris/Trachepyris sp. 8 (male), Thailand
Epyris/Trachepyris sp. 7 (male), Thailand
Epyris/Trachepyris sp. 6 (male), Thailand
Epyris/Trachepyris sp. 9 (male), Thailand
Bakeriella sp. 4, French Guiana
Bakeriella sp. 3, Brazil
Bakeriella sp. 1, Venezuela
Bakeriella sp. 2, Venezuela
Laelius
Chlorepyris
Anisepyris
Epyris
Trachepyris
Holepyris
Bakeriella
Epyris sp. 42, Saba
Epyris sp. 43, USA
Epyris sp. 44, Kenya
Epyris sp. 45, South Africa
Epyris festivus
Epyris sp. 49, South Africa
Epyris sp. 50, South Africa
Epyris sp. 51, South Africa
Epyris sp. 53, South Africa
Epyris sp. 52, South Africa
Epyris sp. 46, Madagascar
Epyris sp. 48, Brazil
Epyris sp. 47, French Guiana
Epyris niwoh
Epyris sp. 54, USA
Epyris sp. 56, USA
Epyris sp. 55, USA
Epyris sp. 57, South Africa
Epyris sp. 59, South Africa
Epyris sp. 58, Central African Republic
Epyris sp. 60, USA
Epyris sp. 61, USA
Epyris sp. 63, USA
Epyris sp. 62, USA
Epyris sp. 8, Saba
Epyris sp. 7, Saba
Epyris sp. 6, Laos
Epyris sp. 9, Thailand
Epyris sp. 10, Mozambique
Epyris sp. 11, Mozambique
Epyris sp. 12, DR Congo
Epyris sp. 13, Uganda
Epyris sp. 2, Nicaragua
Epyris sp. 1, USA
Epyris sp. 3, USA
Epyris sp. 4, USA
Epyris sp. 5, USA
Epyris sp. 18, Venezuela
Epyris sp. 17, Venezuela
Epyris sp. 14, USA
Epyris sp. 16, USA
Epyris sp. 15, Panama
Epyris sp. 19, French Guiana
Epyris sp. 26, Brazil
Epyris sp. 21, Brazil
Epyris sp. 23, Brazil
Epyris sp. 22, Brazil
Epyris sp. 41, South Korea
Epyris xilalen
Epyris sp. 40, South Korea
Holepyris sp. 1, Laos
Holepyris brevicarinatus
Holepyris sp. 2, South Korea
Holepyris sp. 11, USA
Holepyris sp. 18, USA
Holepyris sp. 19, USA
Holepyris sp. 20, Mexico
Holepyris sp. 21, Mexico
Holepyris sp. 12, DR Congo
Holepyris sp. 13, Saba
Holepyris sp. 14, Saba
Holepyris sp. 10, USA
Holepyris sp. 3, USA
Holepyris sp. 5, Thailand
Holepyris susanowo
Holepyris sp. 8, Kenya
Holepyris sp. 9, Mozambique
Holepyris sp. 4, Madagascar
Holepyris sp. 6, Papau New Guinea
Holepyris sp. 7, Australia
Holepyris sp. 15, Papau New Guinea
Holepyris sp. 17, Ghana
Holepyris sp. 16, Ghana

Figure 4.3. Bayesian Tree. Support probabilities shown at nodes as a percent. Print size expansion in Appendix F.


Bethylinae
Pristocerinae
Mesitiinae
Scleroderminae
Epyrinae
Laelius
Chlorepyris
Anisepyris
Epyris
Trachepyris
Holepyris
Bakeriella

previous studies, *Epyris* had a close association with *Holepyris, Bakeriella*, and *Trachepyris* (Alencar and Azevedo 2013)

Less well supported was the suggestion of removing *Rhabdepyris* to *Laelius sensu* Waichert and Azevedo, *Chlorepyris sensu* Waichert and Azevedo, and *Anisepyris sensu* Waichert and Azevedo. As proposed by Waichert and Azevedo (2009), *Rhabdepyris sensu* Kieffer was not monophyletic, and the genus is likely artificial, but the results of this tree do not fully support the revisions made in that article. *Laelius sensu* Waichert and Azevedo 2009 was monophyletic in the likelihood and Bayesian analyses, but not the parsimony analysis. In all cases, *Chlorepyris* sensu Waichert and Azevedo 2009 was nonmonophyletic, although the same two clades that constitute *Chlorepyris* were recovered in each analysis. With further morphological study, one of these clades could represent *Rhabdepyris* – including *Rhabdepyris myrmecophilus*, the type species, would be necessary in further analyses.

Figure 4.4 Example of a male from the *Epyris staphylinoides* species group. Scale bar = 0.5mm

While *Epyris* is clearly nonmonophyletic, there were clades within *Epyris* that were recovered in each analysis with high support. The most recognizable of these was the *staphylinoides* group, which contains males that have the

third flagellomere reduced to an inconspicuous ring on otherwise visually striking ramose antennae (Figure 4.4). These *Epyris* were a part of *Calyozina sensu* Enderlein until Krombein (1992) synonymized them with *Epyris*. Unfortunately, specimens from *Calyozina sensu* Krombein were unavailable for analysis.

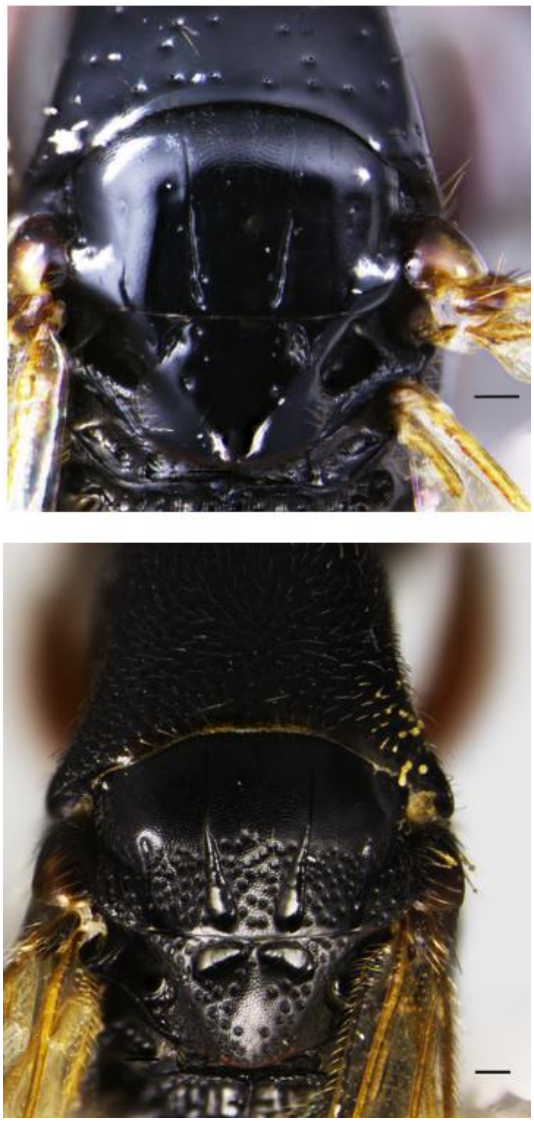


Figure 4.5. Example of variation in scutellar pit size. Top: *Epyris* with a more typical representation of well-spaced scutellar pits. Bottom: *Epyris with* large, nearly touching pits that are found in *Epyris niger* and all specimens of *Epyris* in that clade. Scale bar = 0.1 mm

The type species of *Epyris*, *Epyris niger* Westwood, was recovered in a well-supported clade in every analysis that contained both male and female specimens with large scutellar pits, as opposed to the small, well-spaced scutellar pits that are found throughout the rest of *Epyris* (Figure 4.5). This synapomorphy could take two forms- two large, oval-shaped pits that were obviously separated, or a groove that at first glace seemed uninterrupted, but contained ridges on the interior that divided it into separate pits. These specimens were found in specimens from the Palearctic, Ethiopian, and Oriental regions. Large scutellar pits are similarly found in *Bakeriella*, which is

only known from Neotropical and Nearctic regions, but this clade of *Epyris* differs in the sculpturing of the propodeal disc by lacking sinuous paramedian carinae (Azevedo 2014b).

Other clades of *Epyris* were shared between the parsimony, likelihood, and Bayesian analyses, but finding morphological synapomorphies to define these clades was difficult. With specimens from Neotropical and Nearctic regions, Evans (1969) was used to key specimens to species group and this study found that the two largest species groups, *tricostatus* and *rufipes*, were nonmonophyletic.

CONCLUSION

This study points to a need to taxonomically reassess *Epyris*, which is the largest genus in both Epyrinae and Bethylidae as a whole. With numerous genus synonyms and questionable species group delimitations, it cannot be defined by autapomorphies. However, the type species, *Epyris niger*, was recovered in a well-supported clade defined by the presence of large, nearly-touching scutellar pits. In the future revision of *Epyris*, this clade would be retained as *Epyris sensu stricto*.

Another necessary step is the discovery of synapomorphies that can aid in identifying both males and females of the genera and species groups within Epyrinae. This need was particularly evident after several '*Epyris'* males fell into the clade that contains *Trachepyris*, a genus that is only readily identifiable by unique characters that are only found in females.

Redefining *Epyris* could be accomplished by collapsing all the genera that fell into the clade that contained *Epyris* within *Epyris*. Two of the genera that were found within that clade – *Trachepyris* and *Bakeriella*, are small, and are extremely similar to

Epyris. Trachepyris males, for instance, are indistinguishable from *Epyris*, and both *Trachepyris* and *Bakeriella* possess separated scutellar pits, although *Bakeriella* often (but not always) possess vertical carinae on the pronotum. However, this becomes unfeasible considering *Holepyris*, which also fell into the clade but is large, containing at least 120 species found worldwide with a synapomorphy of a clypeus with large lateral lobes, and is routinely recovered as monophyletic (Alencar and Azevedo 2013).

Instead of trying to collapse other genera within *Epyris* (adding to an already large taxonomic wastebasket) or reclassifying the genus based on the results of this study, more phylogenetic data is probably needed before *Epyris* could be revised. Creating a thorough morphological matrix as used to examine the previous tribal classification of Epyrinae in Alencar and Azevedo (2013) could provide greater resolution while potentially resulting in the discovery of synapomorphies for new genera, should *Epyris* be split. Alternatively, and perhaps additionally, the amplification of nuclear protein-coding genes could resolve many of the ambiguities in the trees presented here. Although difficult to amplify, nuclear protein-coding genes have been utilized in a variety of Hymenoptera molecular phylogenies (Klopfstein et al. 2013; Danforth et al. 2013) and could aid future molecular analyses of Epyrinae and *Epyris*.

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REFERENCES

Abraham, Y. J., D. Moore, and G. Godwin. 1990. "Rearing and aspects of biology of *Cephalonomia stephanoderis* and *Prorops nasuta* (Hymenoptera: Bethylidae) parasitoids of the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae)." *Bulletin of Entomological Research* 80: 121-128.

Alencar, I.D.C.C., and C.O. Azevedo. 2009. "Rediscovery of *Planepyris* Kieffer: a new synomym of *Trachepyris* Kieffer (Hymenoptera, Bethylidae)." *Doriana* 8: 1-12.

Alencar, I.D.C.C., and C.O. Azevedo. 2011. "Review of *Neurepyris* Kieffer, 1905 (Hymenoptera, Bethylidae), a new synonym of *Pristocera* Klug, 1808." *European Journal of Taxonomy* 4: 1–12.

Alencar, I.D.C.C., and C.O. Azevedo. 2013. "Reclassification of Epyrini (Hymenoptera: Bethylidae): a tribal approach with commentary on their genera." *Systematic Entomology*: 38, 45-80.

Azevedo, C.O. 2014a. "Flat wasp, a common name for the bethylids." *Hamuli* 5: 7.

Azevedo, C.O. 2014b. "Synopsis of *Bakeriella* Kieffer, 1910 (Hymenoptera: Bethylidae)." *Zootaxa* 3878: 501–535

Azevedo, C.O., and I.D.C.C. Alencar. 2010a. "Rediscovery of *Melanepyris* (Hymenoptera: Bethylidae): a new synonym of Epyris." *Zoologia (Curitiba)* 27: 403–407.

Azevedo, C.O., and I.D.C.C. Alencar. 2010b. "Rediscovery of the Afrotropical genus *Trissepyris* Kieffer (Hymenoptera: Bethylidae), a new synonym of *Epyris* Westwood." *Journal of Afrotropical Zoology* 6: 3–7.

Bazinet, A.L., D.J. Zwickl, and M.P. Cummings. 2014."A gateway for phylogenetic analysis powered by grid computing featuring GARLI 2.0." *Systematic Biology* 63: 812-818.

Barbosa, D.N., and C.O. Azevedo. 2011. "Phylogeny and reclassification of *Laelius* (Hymenoptera: Bethylidae) and description of four new species." *Insect Systematics & Evolution* 42: 237-275.

Berland, L. 1928. "Hyménoptères Vespiformes II." *Faune de France* 19: 1–215.

Brother, D.J., and Carpenter, J.M. 1993. "Phylogeny of Aculeata: Chrysidoidea and Vespoidea (Hymenoptera)." *Journal of Hymenoptera Research* 2: 227–304.

Carpenter, J.M. 1999. “What do we know about chrysidoid (Hymenoptera) relationships?” *Zoologica Scripta* 28: 215–231.

Carr, M, J. Peter W. Young, and P.J. Mayhew. 2010. "Phylogeny of bethylid wasps (Hymenoptera: Bethylidae) inferred from 28S and 16S rRNA genes." *Insect Systematics & Evolution* 41: 55-73.

Danforth, B.N., S.Cardinal, C. Praz, E.A.B. Almeida, and D. Michez. 2013. “The impact of molecular data on our understanding of bee phylogeny and evolution.” *The Annual Review of Entomology* 58: 57-58.

Dowton, M., and A.D. Austin. 1994. "Molecular phylogeny of the insect order Hymenoptera: Apocritan relationships." *Proceedings of the National Academy of Sciences* 91: 9911-9915.

Evans, H.E. 1964. “A synopsis of the American Bethylidae (Hymenoptera, Aculeata).” *Bulletin of the Museum of Comparative Zoology* 132: 1–222.

Evans, H.E. 1969. “A revision of the genus *Epyris* in the Americas (Hymenoptera: Bethylidae).” *Transactions of the American Entomological Society*: 181–352.

Evans, H.E. 1979. “A reconsideration of the genus *Bakeriella* (Hymenoptera: Bethylidae).” New York Entomological Society 87: 256–266.

Goloboff, P.A., James S. Farris, and Kevin C. Nixon. 2008. "TNT, a free program for phylogenetic analysis." *Cladistics* 24: 774-786.

Goloboff, P.A., James S. Farris, Mari Källersjö, Bengt Oxelman, and Claudia A. Szumik. 2003. "Improvements to resampling measures of group support." *Cladistics* 19: 324-332.

Grimaldi, D., and M.S. Engel. 2005. *Evolution of the Insects*. Cambridge University Press.

Heraty, J., F. Ronquist, J.M. Carpenter, D. Hawks, S. Schulmeister, A.P. Dowling, D. Murray et al. 2011. "Evolution of the hymenopteran megaradiation." *Molecular Phylogenetics and Evolution* 60: 73-88.

Hines, H.M., J.H. Hunt, T.K. O'Connor, J.J. Gillespie, and S.A. Cameron. 2007. "Multigene phylogeny reveals eusociality evolved twice in vespid wasps." *Proceedings of the National Academy of Sciences* 104: 3295-3299.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton et al. "Geneious Basic: an integrated and extendable desktop software platform for

the organization and analysis of sequence data." 2012. *Bioinformatics* 28: 1647-1649.

Kieffer, J.J. 1914. “Bethylidae.” *Das Tierreich* 41: 1–205.

Klopfstein, S., L. Vilhelmsen, J.M. Heraty, M. Sharkey, and F. Ronquist. 2013 "The hymenopteran tree of life: evidence from protein-coding genes and objectively aligned ribosomal data." *PLOS ONE:* 8: e69344.

Krombein, K.V. 1987. "Biosystematic studies of Ceylonese wasps. XVIII. The species of *Trachepyris* Kieffer (Hymenoptera: Bethylidae: Epyrinae)." *The Pan-Pacific Entomologist (USA)* (1987).

Krombein, K.V. 1992. “Systematics of the genera of Epyrinae with ramose male antennae (Hymenoptera: Bethylidae).” *Proceedings of the Entomological Society of Washington* 94: 345–360.

Krombein, K.V. 1996. “Biosystematic Studies of Ceylonese Wasps, XXI: A Revision of the Bethylinae and Epyrinae (Cephalonomiini and Sclerodermini) (Hymenoptera: Bethylidae).” *Smithsonian Contributions to Zoology* 579: 1– 28.

Kurian, C. 1952. "Descriptions of four new and record of one known Bethyloidea (Parasitic Hymenoptera) from India." *Agra University Journal of Research* 1: 63-72.

Kurian, C.1954. “Descriptions of Five New and Records and Redescriptions of Two Known Bethyloidea (Parasitic Hymenoptera) from India.” *Agra University Journal of Research* 3: 417–440.

Kurian, C. 1955. “Bethyloidea (Hymenoptera) from India.” *Agra University Journal of Research* 4: 67–156.

Lanes, G.O., and C.O. Azevedo. 2008. "Phylogeny and Taxonomy of Sclerodermini (Hymenoptera, Bethylidae, Epyrinae)." *Insect Systematics & Evolution* 39: 55-86.

Lanfear, R., B. Calcott, S.Y.W Ho, and S. Guindon. 2012. “PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses." *Molecular Biology and Evolution* 29: 1695-1701.

Legner, F.E., and Gordon G. 1992. "Lower Navel Orange worm (Lepidoptera: Phycitidae) Population Densities Following Establishment of *Goniozus legneri* (Hymenoptera: Bethylidae) in California." *Journal of Economic Entomology* 85: 2153-2160.

Morrison, D.A. 2009. "Why would phylogeneticists ignore computerized sequence alignment?" *Systematic Biology* 58: 150-158.

Paquin, P., and C.J. Vink. 2009. "Testing compatibility between molecular and morphological techniques for arthropod systematics: a minimally destructive DNA extraction method that preserves morphological integrity, and the effect of lactic acid on DNA quality." *Journal of Insect Conservation* 13: 453-457.

Rambaut, A. 2007. FigTree, a graphical viewer of phylogenetic trees. *See http://tree.bio.ed.ac.uk/software/figtree*.

Rokas, A., J.A.A. Nylander, F. Ronquist, and G.N. Stone. 2002. "A maximum-likelihood analysis of eight phylogenetic markers in gallwasps (Hymenoptera: Cynipidae): implications for insect phylogenetic studies." *Molecular Phylogenetics and Evolution* 22: 206-219.

Ronquist, F., A.P. Rasnitsyn, A. Roy, K. Eriksson, and M. Lindgren. 1999. "Phylogeny of the Hymenoptera: a cladistic reanalysis of Rasnitsyn's (1988) data." *Zoologica Scripta* 28: 13-50.

Ronquist, F., M. Teslenko, P.van der Mark, D. L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M.A. Suchard, and J.P. Huelsenbeck. 2012. "MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space." *Systematic Biology* 61: 539-542.

Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994. "Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers." *Annals of the entomological Society of America* 87: 651-701.

Sorg, M. 1988. "Zur Phylogenie und Systematik der Bethylidae (Insecta: Hymenoptera: Chrysidoidea)." *Sonderveröffentlichungen des Geologishcen Instituts der Universität zu Köln* 63: 1–146.

Stamatakis, A. 2014. "RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies." *Bioinformatics* 30: 1312-1313.

Sukumaran, J., and M. T. Holder. 2008. "SumTrees: summarization of split support on phylogenetic trees." *Part of the DendroPy Phylogenetic Computation Library Version* 2, no. 3.

Terayama, M. 2003. "Phylogenetic systematics of the family Bethylidae (Insecta: Hymenoptera) Part I. Higher classification." *The Academic Reports of the Faculty of Engineering of Tokyo Polytechnic University* 26: 1-15.

Vaidya, G., D.J. Lohman, and R. Meier. 2011. "SequenceMatrix: concatenation software for the fast assembly of multi- gene datasets with character set and codon information." *Cladistics* 27: 171-180.

Vrijenhoek, R. 1994. "DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates." *Molecular Marine Biology and biotechnology* 3: 294-299.

Waichert, C., and C.O. Azevedo. 2009. “Phylogenetic analysis of Rhabdepyris (Hymenoptera: Bethylidae) and redefinition of generic limits based on morphological characters.” *Zootaxa* 2284: 1–29.

CHAPTER V

NEW SPECIES OF *EPYRIS SENSU STRICTO* WESTWOOD (BETHYLIDAE: EPYRINAE) FROM WESTERN AUSTRALIA AND QUEENSLAND

INTRODUCTION

In the previous chapter, a molecular phylogeny of Epyrinae was presented in which *Epyris* was shown to be a non-monophyletic genus with multiple, strongly supported clades spread throughout a polytomy (in the case of the parsimony analysis) that contained a monophyletic *Holepyris*, *Trachepyris*, and *Bakeriella*. One of these clades contained the type species of *Epyris*, *Epyris niger* Westwood, and all specimens in this clade shared a synapomorphy of having large, sub-rectangular scutellar pits that were nearly touching. This was in contrast to the small, usually circular, but always well-separated scutellar pits that are found through the rest of *Epyris*.

Outside of synonymizing other genera within *Epyris*, there has been no revisionary work in the genus since Howard Evans's delimitation of species groups of Neotropical and Nearctic *Epyris* in Evans (1964, 1969). The genus is understudied compared to the other genera of Bethylidae, and all of the recent morphological and molecular studies have shown it as nonmonophyletic (Carr et al. 2010; Alencar and Azevedo 2013). Given the lack of keys and descriptions for *Epyris* outside of the major works by Kieffer (1914) and Evans (1964, 1969), many of the specimens in the previous chapter were undescribed. However, instead of adding to an already large taxonomic wastebasket, only specimens from *Epyris sensu stricto* are described here.

Seven out of the 19 specimens in the *Epyris sensu stricto* clade came from Australia, where relatively little is known of bethylid diversity. There are 50 species of Bethylidae described from Australia, with 90 percent of them endemic and five non-endemic species that are cosmopolitan, and were likely introduced with invasive grain pests (Berry 1998; Gordh 1990; Azevedo 2005; Azevedo 2006). Most of the Epyrinae fall within *Rhabdepyris*, from which 18 species are known. According to Azevedo (2006), there are three species of *Epyris* from Australia, but only two species are listed in Gordh and Móczár (1990) and the CSIRO Atlas of Living Australia Website: *Epyris lutescens* Kieffer and *Epyris fulvimanus* Kieffer. Possibly, Azevedo (2006) erroneously included *Rhabdepyris platycephalus* Westwood, which was originally described as *Epyris* in Westwood (1874) and moved to *Rhabdepyris* in Kieffer (1907).

Epyris fulvimanus is known only from Queensland, and *Epyris lutescens* is known only known from Victoria – both states on the eastern side of Australia. Almost all Bethylidae known from Australia come from the eastern states of Queensland, Victoria, and New South Wales (Azevedo 2006), and almost no species are known from Western Australia. Sampling studies in Western Australia would suggest they are rare – in a sampling study of the invertebrates of Barrow Island (off the Northwest coast of Western Australia), 15 morphotypes of Bethylidae were identified from 1,873 total morphotypes of insects (Callan et al. 2011), and 11 species were identified amongst 895 species in a sampling survey of Western Australia forests (Majer et al., 2000). Three of the species described below came from Western Australia and the other two species came from Queensland.

MATERIALS AND METHODS

Material was provide by the following institutions:

CNC	The Canadian National Collections, Ottawa, Canada
CSIRO	The Commonwealth Scientific and Industrial Research Organization
QM	The Queensland Museum, Brisbane, Australia
ANIC	The Australian National Insect Collection, Canberra, Australia

Materials accessed at the CNC were from a trip to sift through Bethylidae in ethanol from a variety of collection trips by various entomologists that had been deposited at the CNC for safekeeping and examination. Two species described here, *Epyris loisae* Tribull and *Epyris herschae* Tribull, come from a series of malaise trap catches from M.E. Irwin and F.D. Parker during a CSIRO/ANIC expedition to survey insects in April and May 2003.

The following three species, *Epyris carpenteri* Tribull, *Epyris azevedoi* Tribull, and *Epyris fulgeocauda* Tribull, were obtained during a museum collection trip to the Queensland Museum and the Australian National Insect Collections. *Epyris carpenteri* was collected in Western Australia and accessioned to the Australian National Insect Collection. The two species from Queensland, *Epyris azevedoi* and *Epyris fulgeocauda* Tribull, were accessioned at the Australian National Collections and Queensland Museum, respectively. The specimens from Western Australia were stored in ninety-five percent ethanol and refrigerated prior to being utilized in the molecular phylogeny in the previous chapter. The material from Queensland was not included in the molecular phylogeny, but as these specimens also share the distinct scutellar pits, they are described below.

Images were taken using a Nikon SMZ 1500 with a Nikon Digital Sight DS – U3 camera system with NIS-Elements for stacking images and taking measurements. For mandibles, drawings were made from photos on a Wacom Cintiq tablet in Photoshop CC.

The following abbreviations in the descriptions were adopted from Azevedo (2001) and Evans (1964, 1969) TL, total length; LH, length of head, excluding mandibles; WH, maximum width of head; WF, minimum width of frons; HE, eye height in lateral view; WOT, maximum width of ocellar triangle; POL, width between posterior ocelli; AOL, width between posterior ocellus and anterior ocellus; OOL, width between posterior ocellus and compound eye; LFW, maximum length of forewing; LP, length of pronotum excluding anterior collar; WP, maximum width of pronotum.

All species described below are female, and given the sexual dimorphism in Bethylidae, can only be compared to *Epyris fulvimanus*. *Epyris lutescens* is only known from the male holotype and is not discussed in the diagnosis. The descriptions conclude with a key to the known species of *Epyris sensu stricto* from Australia.

Genus *Epyris* Westwood

Epyris Westwood 1832: 129. Type-species *Epyris niger* Westwood 1832, by monotypy.

Calyoza Westwood *in* Hope 1837: 56, fig.11. Type species *Calyoza staphylinoides* Hope 1837, by monotypy. Synonymized by Krombein, 1992: 346.

Dulos Motschulsky 1863: 27. Type-species *Dolus politus* Motschulsky 1863, by subsequent designation of Krombein 1987: 357. Synonymized by Krombein, 1987: 357.

Muellerella Saussure 1892: pl. 25, fig. 20. Type-species *Muellerella amabilis* Saussure 1892, by monotypy. Synonymized by Evans 1964: 104.

Homoglenus Kieffer 1904: 388. Type-species *Homoglenus punctatus* Kieffer 1904, by monotypy. Synonymized by Terayama, 2003: 16.

Trissepyris Kieffer 1905: 109. Type-species *Trissepyris ruficeps* Kieffer 1905, by monotypy. Synonymized by Azevedo & Alencar 2010b: 3.

Paracalyoza Cameron 1909: 377. Type-species *Paracalyoza hirtipennis* Cameron 1909, by monotypy. Synonymized by Krombein 1992: 346.

Parepyris Kieffer 1913: 108. Type-species *Parepyris interruptus* Kieffer 1913, by subsequent designation of Kieffer 1914: 410. Synonymized by Evans 1964: 104.

Psilepyris Kieffer 1913: 108. Type-species *Epyris indivisus* Kieffer 1913, by subsequent designation of Kieffer 1914: 401. Synonymized by Evans 1964: 104.

Artiepyris Kieffer 1913: 108. Type-species *Epyris dodecatomus* Kieffer 1913, by monotypy. Synonymized by Evans, 1969: 181.

Melanepyris Kieffer 1913: 108. Type-species *Epyris imicola* Kieffer 1913, by monotypy. Synonymized by Azevedo & Alencar 2010a: 403.

Pseudocalyoza Turner 1915: 298. Type-species *Pseudocalyoza subramosa* Turner 1915, by monotypy. Synonymized by Krombein 1992: 346.

Calyozella Enderlein 1920: 24. Type-species *Calyozella flavipennis* Enderlein 1920, by original designation. Synonymized by Krombein, 1992: 346.

Epyris sensu stricto is defined by having the notable characteristic of the type species, *Epyris niger Westwood*, 1832 – large, nearly colliding scutellar pits on the dorsal surface of the scutellum. In other previously-named species of *Epyris*, these pits are small and often well separated.

1. *Epyris loisae* Tribull, sp. nov.

Type material. *Holotype*. Female. Deposited in the Australian National Insect Collection. Collection data - Australia: Western Australia - 30 km ESE Three Rivers Station; malaise in dry bed of Gascoyne River; 24.IV - 7.V. 2003; collected by ME Irwin and FD Parker; 504 m; 25°13.6'S, 118°56.9'E (GPS). *Paratypes*: 1 Female, same locality as holotype.

Diagnosis *Epyris loisae* differs most obviously from *Epyris fulvimanus* in the sculpturing of the propodeum – the lateral sides of *E. fulvimanus* are smooth, as opposed to the strongly reticulated lateral sides and deeply foveolate posterolateral corners of the propodeal disc in *Epyris loisae*. Additionally, in *E. fulvimanus,* only the tibia are castaneous, and the mesoscutum in *E. loisae* is much longer, 1.5x the length of the scutellum as opposed to being equal length in *E. fulvimanus.*

Description. FEMALE (holotype). Body length when flattened, 8.5 mm; LFW: 3.72 mm.

Color. Body, black; mandible, palpi, tip of clypeus, antennae castaneous. Metasomal segments black with castaneous borders. Legs castaneous, except trochanters dark castaneous. (Figure 5.1)

Head. Hairy, and strongly punctate– punctures deep, close together, but irregularly spaced on a shining surface. Mandibles with long hairs, three teeth progressing larger posteriorly to anteriorly – apical tooth largest and sharpened, middle tooth smaller, but still sharpened, upper tooth round. Clypeus flattened apically, emarginate, with sharp median ridge and long hairs. Eyes small, LH 2.6 x HE, with short, erect hairs. Antennal flagellomeres (starting with the scape) in the following proportions

– 4: 1.4: 1.5: 1.3: 1.2: 1.2: 1.2: 1.2: 1.1: 1.1: 1.0: 1.1: 1.8. LH 1.0 x WH; HE 1.0 x OOL; WF 2.28 x WOT; POL 1.2 x AOL.

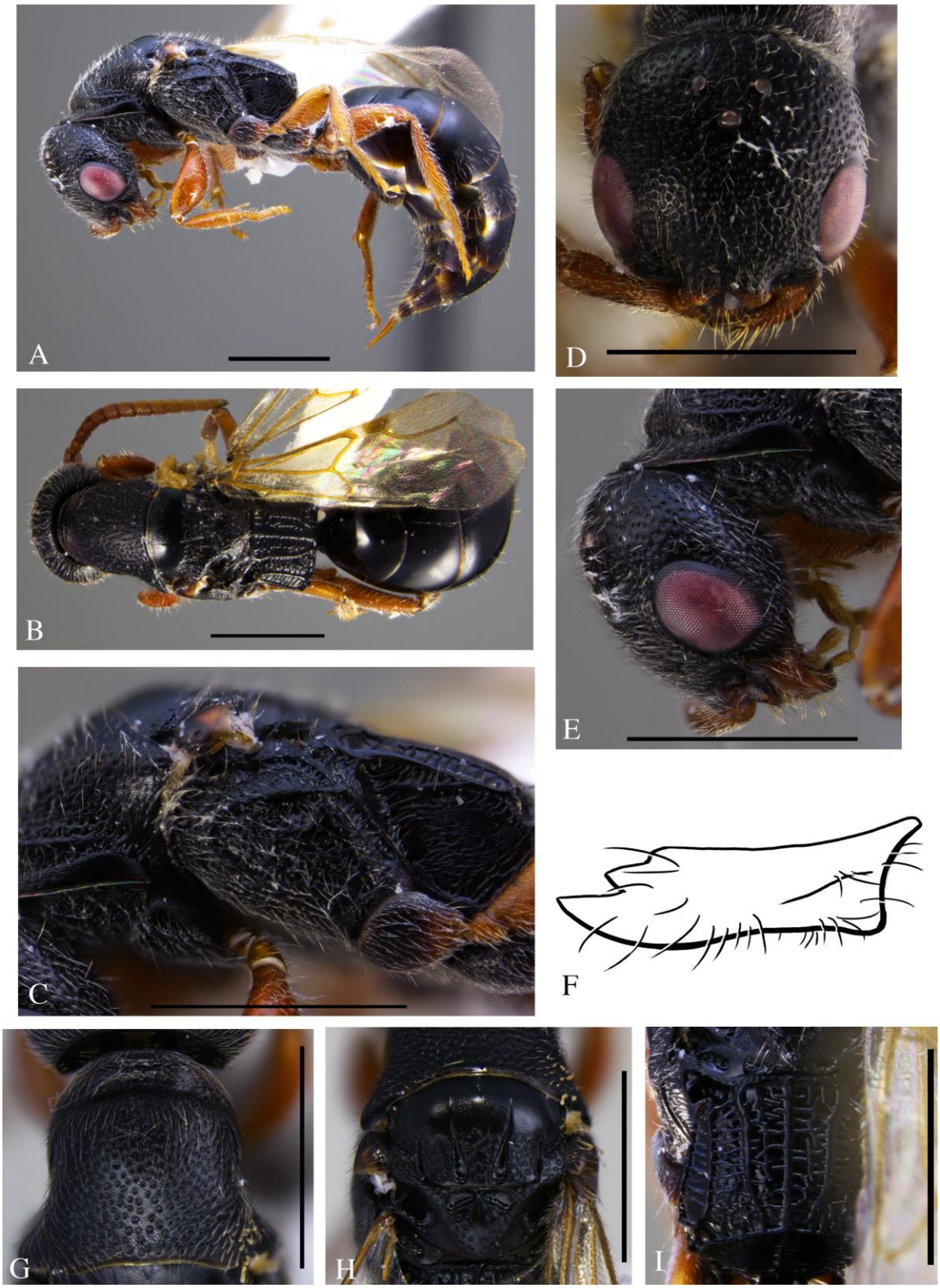
Mesosoma. Pronotal disc 0.6 x as long as wide, sculptured in the same manner as the head. Mesoscutum coriaceous, 1.5 x the length of the scutellum with punctures only on the posterior half. Notauli are narrow anteriorly and divergent, broadening posteriorly and converging, separated by 1.1 x their posterior width. Scutellum coriaceous with punctures and hairs on the lateral edges. Scutellar pits large, subrectangular, separated by 0.3 x their width and slanting posteriorly laterally. Propodeal disc 0.67 x as long as wide with three carinae reaching the posterior margin; submedian carinae present, as well as strong sublateral carinae. Posterolateral corners foveolate. Entirety of propodeum strongly reticulated on shining surface. Forewing hyaline with veins light castaneous and stigma dark castaneous. Transverse-median vein strongly arched, convex posteriorly, but not meeting anal vein.

Metasoma. Petiolate and smooth. Terga 3 – 7 with sparse punctures and sparse, pale hairs.

Etymology. The species name is in memoriam to Lois Tribull, the author's mother.

Distribution. Holotype and paratype taken in the same malaise trap

Figure 5.1: *Epyris loisae sp. nov.* A. Lateral habitus, B. Dorsal habitus, C. Lateral detail of mesoscutum, D. Head, E. Lateral view of head, F. Drawing of mandible, G. Pronotum, H. Scutellum, and I. Propodeum. Scale bar = 1.0 mm.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1. *Epyris herschae* Tribull, sp. nov.

Type material. *Holotype*. Female. Deposited in the Australian National Collections/CSIRO. Collection data - Australia: Western Australia - W Cobra Station; malaise across pool in wide rocky wash: 26.IV/ 10.V.2003; ME Irwin, FD Parker; 360 m; 24°10.2'S, 116°23.0'E (GPS)

Paratypes: 1 Female, same locality as holotype.

Diagnosis. Unlike other species of *Epyris* described from Australia, *Epyris herschae* is iridescent blue on the head, pronotum, mesoscutum, and scutellum. Its mandibles are also unique – under high magnification, it is possible to see that the posterior tooth of the mandible is cleft.

Description. FEMALE (holotype). Body length when flattened 3.44 mm; LFW: 2.23 mm.

Color. Head, pronotum, mesoscutum, and scutellum iridescent blue under light; Antennal scape and clypeus dark castaneous, mandibles dark castaneous at base, light castaneous at teeth, palpi and remainder of antennae castanseous. Metasomal segments castaneous, with first and last segments light castaneous. Coxa, trochanter, and femur castaneous, tibia dark castaneous towards femur and light castaneous towards tarsi, tarsi light castaneous. (Figure 5.2)

Head. Lightly coriaceous, but shining and iridescent, with many pale hairs. Punctures small and evenly spaced about 3x their diameter apart. Mandibles with long hairs, three teeth progressing larger anteriorly to posteriorly with the posterior tooth possessing a distinctive cleft, which divides it into two small points. Clypeus with rounded median lobe and sparse hairs. Eyes with short, erect hairs, LH 2.4 x HE.

Antennal flagellomeres (starting with the scape) in the following proportions – 4.5: 1.8: 1.3: 1.3: 1.3: 1.1: 1.1: 1.1: 1.0: 1.1: 1.1: 1.3: 2.1. LH 1.1 x WH; HE 1.0 x OOL; WF 2.6 x WOT; POL 1.1 x AOL.

Mesosoma. Pronotal disc 0.6 x as long as wide, sculptured in the same manner as the head. Mesoscutum coriaceous, 1.2X the length of the scutellum, with punctures as on the head and pronotum on the posterior half. Notauli are narrow anteriorly and divergent, broadening widely posteriorly and converging, separated by 5 x their posterior width. Scutellum coriaceous with sparse pale hairs. Scutellar pits large, subrectangular, separated by 0.1 x their width, and very slightly slanting laterally posteriorly. Within each scutellar pit is a single raised division that does not meet the surface of the scutellum, so that the scutellar pits do not appear fully divided. Propodeal disc 0.72 x as long as wide with three carinae reaching the posterior margin; submedian carinae present, as well as longitudinal carinae. Posterolateral corners foveolate. Entirety of propodeum strongly reticulated on shining surface. Forewing hyaline with veins castaneous and pterostigma somewhat darker castaneous. Transverse-median vein arching, convex posteriorly, and meeting anal vein. Metacarpus absent

Metasoma. Petiolate and smooth. Terga 3 – 7 with sparse punctures and sparse, pale hairs.

Etymology. The species is named for Rebecca Hersch, who was instrumental in guiding the author (and many other graduate students) through learning molecular techniques at the Sackler Institute for Comparative Genomics at the American Museum of Natural History.

Distribution. Holotype and paratype taken from the same malaise trap locality listed above.

1. *Epyris carpenteri* Tribull, sp. nov.

Type material. *Holotype*. Female. Deposited in the Australian National Collections/CSIRO. Collection data - Australia: Western Australia – 21 km N Albany; Malaise Trap; Hillbrook Nature Reserve; 11-14 October 1999; Collected by M. Court, S. Cunningham. 34 51.493'S 117 48.525E.

Diagnosis *Epyris carpenteri* differs from *E. fulvimanus* in the sculpturing of the propodeum: *E. carpenteri* has lateral carinae that do not reach the posterior margin of the propodeum, and do not converge as much towards the posterior margin, appearing straighter than in *E. fulvimanus*. The posterolateral corners of the propodeum in *E. carpenteri* are more rounded than as in *E. fulvimanus*. In addition, the notauli are much thinner at their posterior ends, and more divergent anteriorly than in *E. fulvimanus*.

Description. FEMALE (holotype). Body length when flattened; 4.15 mm LFW: 2.26 mm.

Color. Body, Black; Scape and clypeus dark castaneous, mandibles dark castaneous at base, light castaneous at teeth, palpi and remainder of antennae castanseous, torulus light castaneous. Metasoma castaneous, with distal end light castaneous. Coxa, trochanter, and femur castaneous, tibia dark castaneous towards femur and light castaneous towards tarsi, tarsi light castaneous. (Figure 5.3)

Head. Shining black. Punctures small and evenly spaced about 3 x their diameter apart. Mandibles with long hairs, bidentate, with upper tooth smaller than the apical tooth. Clypeus with median lobe flattened apically and raised median ridge. Eyes

with short, erect hairs, LH 2.5 x HE. Antennal flagellomeres (starting with the scape) in the following proportions – 3.75: 1.25: 1.0: 1.25: 1.1: 1.0: 1.0: 1.1: 1.1: 1.1: 1.25: 1.25: 1.75. LH 1.1 x WH; HE 0.70 x OOL; WF 2.2 x WOT; POL 1.4 x AOL.

Mesosoma. Pronotal disc 0.63 x as long as wide, sculptured in the same manner as the head. Mesoscutum coriaceous, 1.0 x the length of the scutellum, with punctures as on the head and pronotum on the posterior half. Notauli are narrow anteriorly and divergent, broadening widely posteriorly and convergent, separated by 4.0 x their posterior width. Scutellum coriaceous with sparse pale hairs and punctures along the lateral edges. Scutellar pits large, subrectangular, separated by 0.1 x their width. Propodeal disc 0.70 x as long as wide with three carinae, of which only the median carina reaches the posterior margin with the lateral carinae 0.75 x as long as the median carina; submedian carinae present, as well as strong sublateral carinae. Entirety of propodeum strongly reticulated on shining surface. Wings hyaline, veins and pterostigma light castaneous. Transverse-median vein gently arched posteriorly and meeting anal vein.

Metasoma. Petiolate and smooth. Terga 3 – 7 with sparse punctures and sparse, pale hairs.

Etymology. The species is named for the chair of the author's dissertation committee, James M. Carpenter, in recognition of the four years of support and guidance through the dissertation process.

Distribution. Holotype and paratype taken from the same malaise trap locality listed above.

Figure 5.2: *Epyris herschae sp. nov.* A. Lateral habitus, B. Dorsal habitus, C. Lateral detail of mesoscutum, D. Head, E. Lateral view of head, F. Drawing of mandible, G. Pronotum, H. Scutellum, and I. Propodeum. Scale bar = 1.0 mm.

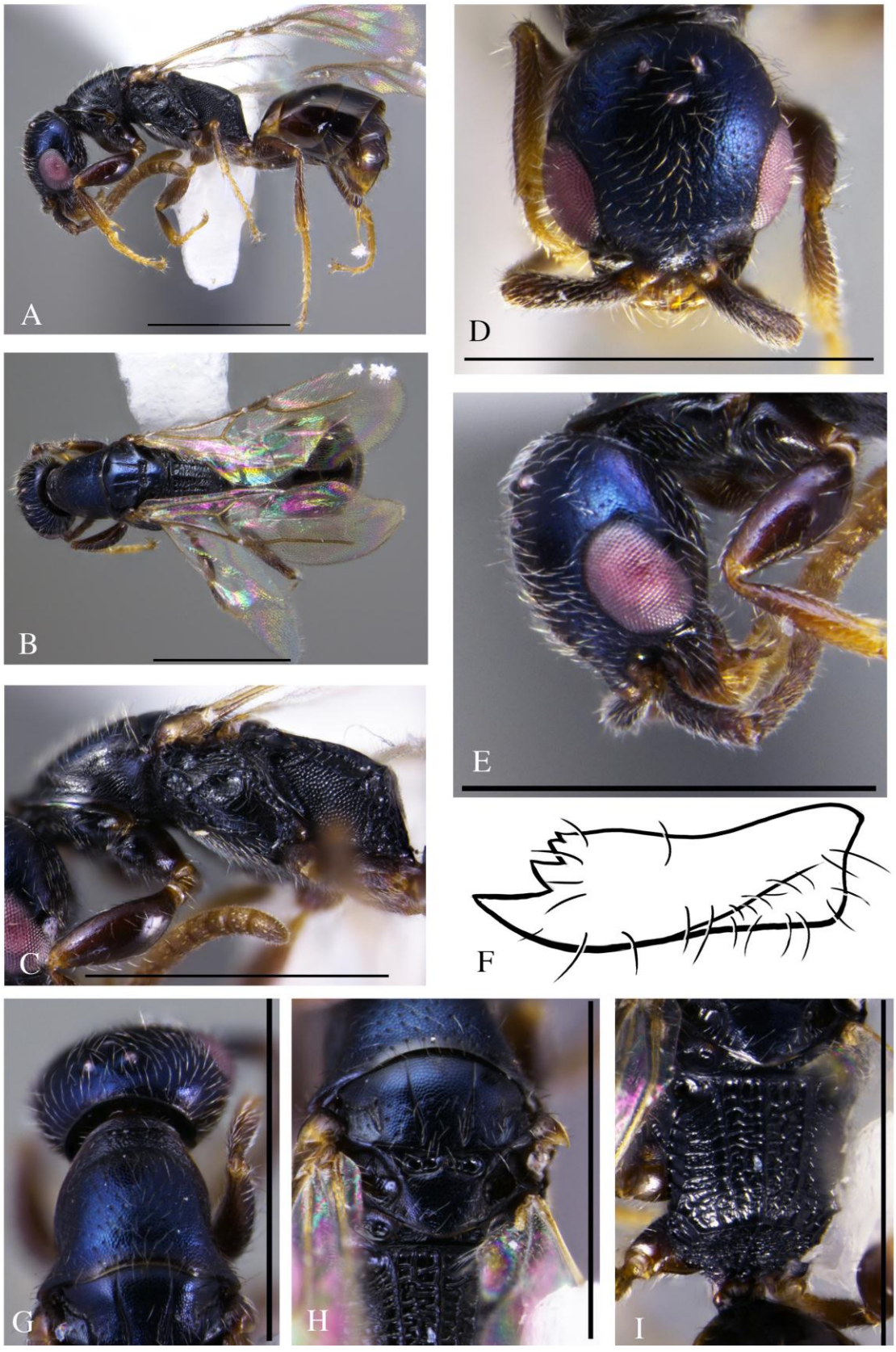
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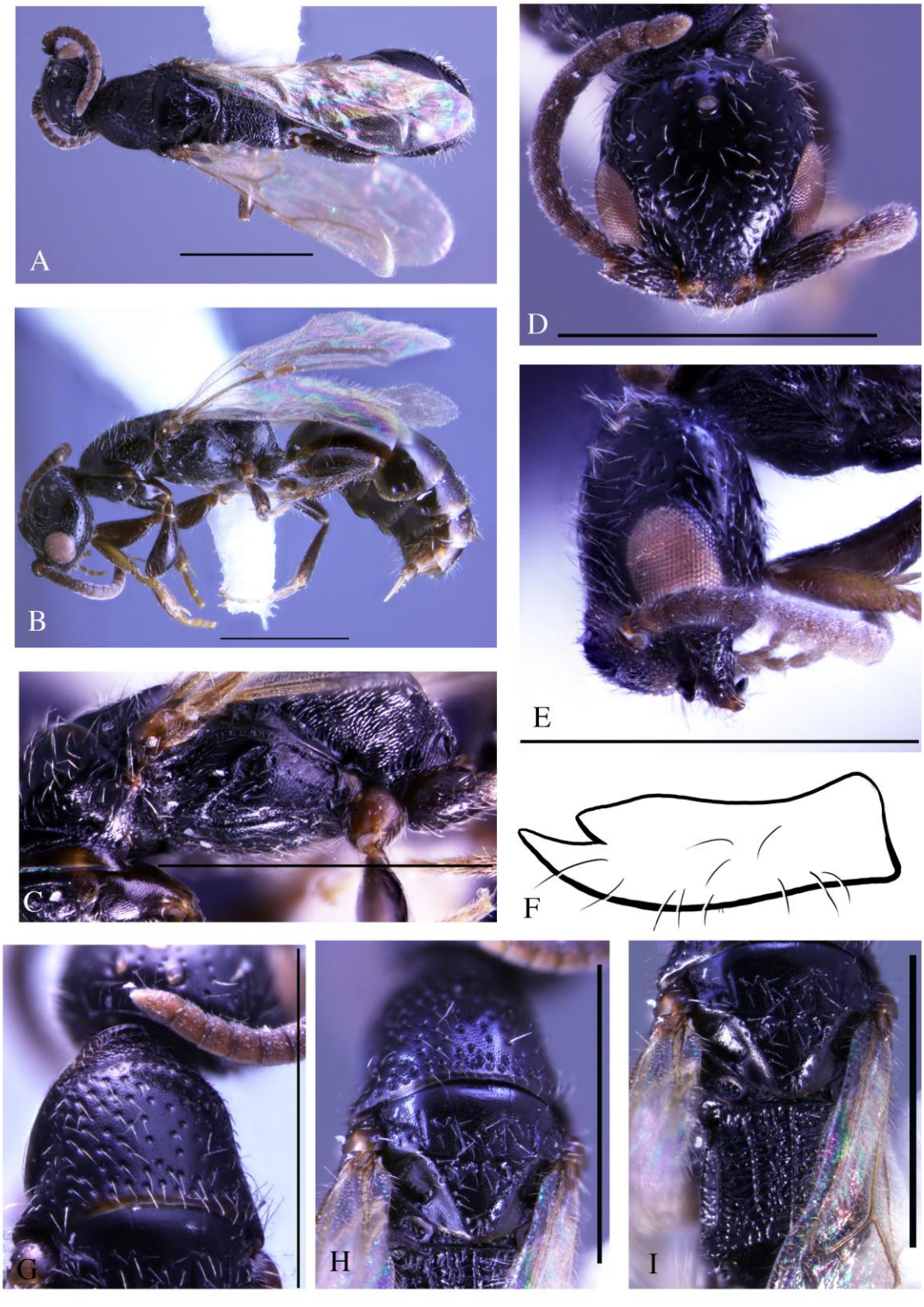
Figure 5.3: *Epyris carpenteri sp. nov*. A. Lateral habitus, B. Dorsal habitus, C. Lateral detail of mesoscutum, D. Head, E. Lateral view of head, F. Drawing of mandible, G. Pronotum, H. Scutellum, and I. Propodeum. Scale bar = 1.0 mm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The following two species were not included in the previous molecular chapter, but are *Epyris* with enlarged scutellar pits, and can be placed within *Epyris sensu stricto*. These species were from Queensland, from which only one species was previously known, *E. fulvimanus.*

1. *Epyris azevedoi* Tribull, sp. nov.

Type material. *Holotype*. Female. Deposited in the Australian National Collections/CSIRO. Collection data - Australia: Queensland. 17.37S 145.34E 100m; BS3 Massey Crk QLD; 6 Mar- 5 Apr 1995; P. Zborowski. Flight Intercept Trap James Cook University (East).

Diagnosis. *Epyris azevedoi* differs from *Epyris fulvimanus* in the densely punctate surface of the face and pronotum. While both species have punctures, those of *E. azevedoi* are very close together and occasionally colliding as opposed to the separation of 1.0 x their diameter as in *E. fulvimanus.* The mesoscutum is longer in *E. azevedoi*, approximately 1.3 x as long as the scutellum, as opposed to being the same length in *E. fulvimanus*. The propodeum in *E. azevedoi* is reticulated on the lateral margins, as opposed to the smooth lateral margins of *E. fulvimanus*.

Description. FEMALE (holotype). Body length when flattened 8.46 mm; LFW: 4.00 mm.

Color. Body, Black; Scape and clypeus dark castaneous, mandibles dark castaneous at base, light castaneous at teeth, palpi and remainder of antennae castanseous, torulus somewhat lighter. Metasoma castaneous, with distal end light castaneous. Coxa, trochanter, femur castaneous, and tarsi dark castaneous with tarsi progressively lighter castaneous. (Figure 5.4)

Head. Deeply punctate on coriaceous (where it is visible between the punctures) surface. Punctures large, deep, and spaced so closely together that they occasionally share a side. Mandibles, hairy, with four teeth, the largest apical one sharpened, as is the smaller one above. Between the second and fourth sharpened tooth is an intermediary rudimentary tooth, which is rounded. Clypeus with median lobe flattened apically and with a small emargination and raised median ridge. Eyes with erect hairs (longer than on other species), LH 2.4 x HE, LH 1.0 x WH, HE 1.2 x OOL, WF 2.1 x WOT; POL 1.2 x AOL. Antennal flagellomeres (starting with the scape) in the following proportions – 2.5: 1.4: 1.0: 1.2: 1.4: 1.25: 1.2: 1.3: 1.2: 1.1: 1.2: 1.0: 1.6.

Mesosoma. Pronotal disc 0.56 x as long as wide, sculptured in the same manner as the head. Mesoscutum coriaceous, 1.3 x the length of the scutellum, with punctures as on the head and pronotum on the posterior half. Notauli are narrow for half their length and slightly divergent anteriorly, broadening widely posteriorly and convergent, separated by 3.3 x their posterior width. Scutellum coriaceous with sparse pale hairs large pits. Scutellar pits large, oval-shaped, separated by 0.3 x their length and slanted posteriorly laterally. Propodeal disc 0.73 x as long as wide with three carinae, submedian carinae present, as well as strong sublateral carinae. Posterolateral corners foveolate. Entirety of propodeum strongly reticulated on shining surface. Wings subhyaline and yellow-hued with castaneous veins and pterostigma. Transverse-median vein strongly convex posteriorly and meeting anal vein.

Metasoma. Petiolate and smooth. Terga 3 – 7 with sparse, pale hairs.

Etymology. The species is named for Celso Azevedo, the current expert in Bethylidae, who has been instrumental in helping the author obtain species and visit collections in Brazil.

Distribution. Only known from holotype.

1. *Epyris fulgeocauda* Tribull, sp. nov.

Type material. *Holotype*. Female. Deposited in the Queensland Museum. Collection data - Australia: Queensland: SEQ 25°27'S 150°08'E. Boggom 12/1. (Nathan, G.) via Taroom. 13Nov96-1997; Cook and Monteith; Baited Flight Intercept #43. QM Registration # TM33399.

Diagnosis. *Epyris fulgeocauda* differs from *Epyris fulvimanus,* and the rest of the species described above, in having the entirety of its metasoma bright castaneous. In addition, unlike *E. fulvimanus,* the punctures on the surface of the face and pronotum are small, extremely shallow, and almost nonexistent. Unlike all other specimens of *Epyris* in Australia, the lateral sides of the propodeal disc are strongly transversely striate.

Description. FEMALE (holotype). Body length when flattened 3.65 mm; LFW: 1.80 mm.

Color. Head and mesosoma, black; Scape, castaneous, with remainder of antennae a somewhat lighter castaneous; Clypeus, dark castaneous at base, lightening at apex; mandibles, black at base, lightening to castaneous apically; palpi, castaneous; legs, light castaneous throughout. (Figure 5.5)

Head. Strongly coriaceous, although somewhat shining, and hairy throughout with pits very small, hidden amongst the coriaceous surface. Mandibles tridentate and

slender, with teeth progressing smaller to larger posteriorly to anteriorly, with numerous hairs on the anterior margin. Clypeus with median lobe pointed and with a very small raised median ridge. Eyes with numerous short, erect, pale hairs, LH 2.6 x HE, LH 1.1 x WH, HE .89 x OOL, WF 3.1 x WOT, POL 0.8 x AOL. Antennal flagellomeres (starting with the scape) din the following proportions – 3.6: 1.7: 1.2: 1.0: 1.3: 1.2: 1.2: 1.5: 1.2: 1.2: 1.3: 1.0: 2.2.

Mesosoma. Pronotal disc 0.57 x as long as wide, sculptured in the same manner as the head. Mesoscutum coriaceous, 1.2 x the length of the scutellum. Notauli are narrow for 2/3 their length, slightly divergent anteriorly, separated by 5.5 x their posterior width. Scutellum coriaceous with sparse pale hairs large pits. Scutellar pits large, subrectangular, but with a convex curve to the posterior and anterior margins. Pits are separated by 0.1 x their length, almost touching. Propodeal disc 0.92 x as long as wide with three carinae and lateral surface strongly transversely reticulate. Posterolateral corners foveolate. Entirety of propodeum strongly reticulated on shining surface. Wings hyaline with castaneous veins and dark castaneous pterostigma. Transverse-median vein gently arching and meeting anal vein.

Metasoma. Petiolate and smooth. Terga 4 – 7 with sparse, pale hairs.

Etymology. The species is named for the conspicuously bright metasoma – “fulgeo” is Latin for flash, and “cauda” is Latin for tail.

Distribution. Only known from holotype.

Figure 5.4: *Epyris azevedoi sp. nov*. A. Lateral habitus, B. Dorsal habitus, C. Lateral detail of mesoscutum, D. Head, E. Lateral view of head, F. Drawing of mandible, G. Pronotum, H. Scutellum, and I. Propodeum. Scale bar = 1.0 mm

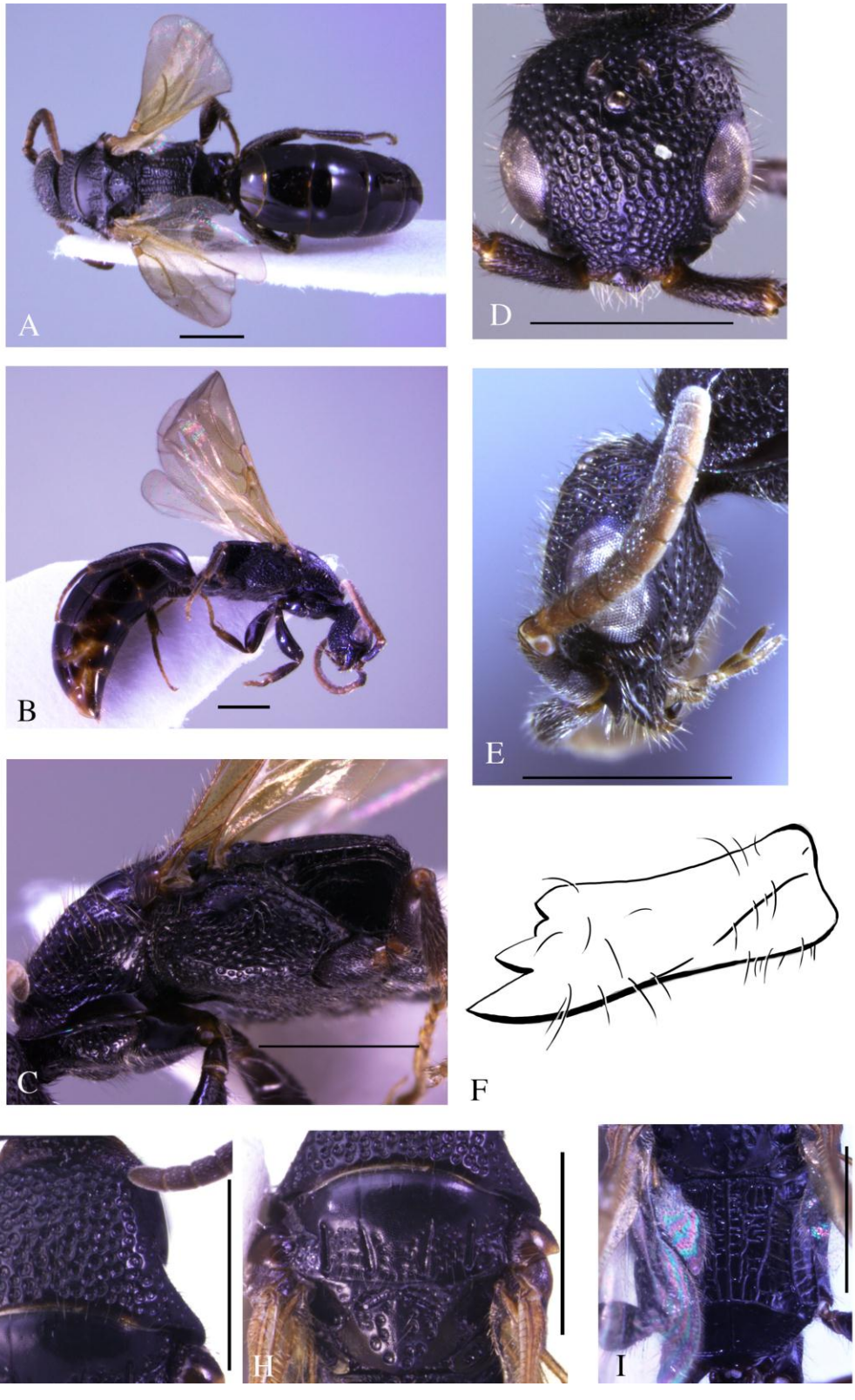
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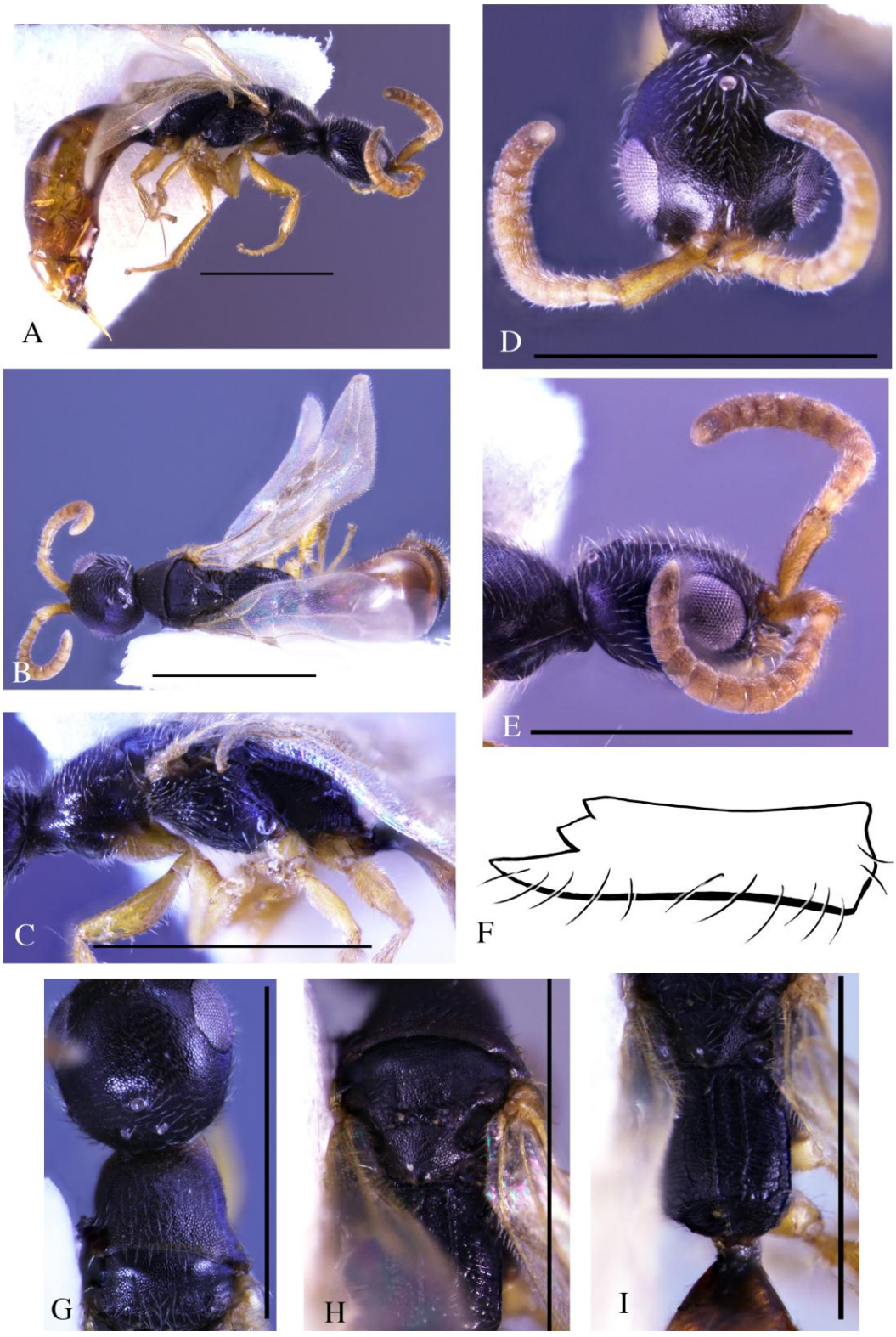
Figure 5.5. *Epyris fulgeocauda sp. nov.* A. Lateral habitus, B. Dorsal habitus, C. Lateral detail of mesoscutum, D. Head, E. Lateral view of head, F. Drawing of mandible, G. Pronotum, H. Scutellum, and I. Propodeum. Scale bar = 1.0 mm.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KEY TO THE KNOWN AUSTRALIAN SPECIES OF *EPYRIS*

FEMALES

1. Metasoma bright castaneous throughout……..…*Epyris fulgeocauda*

- Metasoma at least partially black or dark castaneous …….. 2

2. Mandibles bidentate ……*Epyris carpenteri*

- Mandibles with more than two teeth…… 3

3. Mandibles with a rounded, intermediate rudimentary tooth between the first two, regularly sharpened teeth…….*Epyris azevedoi*

- Mandibles without an intermediate rudimentary tooth, all teeth progressing in size posteriorly to anteriorly……4

4. First tooth on mandible with distinct cleft, divided into two sharpened points, body with iridescent blue color……..……..*Epyris herschae*

- Mandibles with teeth simple……..5

5. Lateral sides of propodeal disc smooth, not reticulate…….*Epyris fulvimanus*

6. Lateral sides of propodeal disc with reticulations on shining surface… *Epyris loisae*

REFERENCES

Azevedo, C.O. 2001. "Systematics of the Neotropical *Dissomphalus* Ashmead (Hymenoptera, Bethylidae) of the *bicavatus* group". *Revista Brasileira de Entomologia* 45: 173-205.

Azevedo, C.O. 2005. "A new species of *Allobethylus* (Hymenoptera: Bethylidae) from Australia, with a key to world species." *Zootaxa* 1074: 25-30.

Azevedo, C.O. 2006. "Insecta, Hymenoptera, Bethylidae: range extension and filling gaps in Australia*." Check List*: *The Journal of Biodiversity Data* 2: 42-44.

Alencar, I.D.C.C., and C.O. Azevedo. 2013. "Reclassification of Epyrini (Hymenoptera: Bethylidae): a tribal approach with commentary on their genera." *Systematic Entomology* 38: 45- 80.

Berry, J.A. 1998. "The bethyline species (Hymenoptera: Bethylidae: Bethylinae) imported into New Zealand for biological control of pest leafrollers." *New Zealand Journal of Zoology* 25: 329-333.

Callan, S.K., J.D. Majer, K. Edwards, and D. Moro 2011. "Documenting the terrestrial invertebrate fauna of Barrow Island, Western Australia." *Australian Journal of Entomology* 50: 323-343.

Carr, M.J., P.W. Young, and P.J. Mayhew. 2010. "Phylogeny of bethylid wasps (Hymenoptera: Bethylidae) inferred from 28S and 16S rRNA genes." *Insect Systematics & Evolution* 41: 55-73.

Evans, H.E. 1964. "A synopsis of the American Bethylidae (Hymenoptera, Aculeata)." *Bulletin of the Museum of Comparative Zoology* 132: 1-222.

Evans, H.E. 1969. "A revision of the genus *Epyris* in the Americas (Hymenoptera: Bethylidae)." *Transactions of the American Entomological Society*: 181-352.

Gordh, G. 1998. "A new species of *Sierola* parasitic on moth larvae in Western Australia (Hymenoptera: Bethylidae)." *Proceedings of the Hawaiian Entomological Society* 33: 83-88.

Gordh, G. 1990. "*Apenesia evansi sp. n.* (Hymenoptera, Bethylidae) from Australia, with comments on phoretic copulation in bethylids." *Journal of Australian Entomological Society* 29: 167-170.

Gordh, G., and L. Móczár. 1990. "A catalog of the World Bethylidae (Hymenoptera, Aculeata)." *Memoirs of the American Entomological Institute* 46: 1-364.

Kieffer, J-J. 1914. “Bethylidae.” *Das Tierreich* **41**: 1-595.

Westwood, J. O. 1874. Thesaurus Entomologicus Oxoniensis. Oxford, Clarendon Press, 205 p., 40 pls.

Majer, J. D., H.F. Recher, and S. Ganesh. 2000. “Diversity patterns of eucalypt canopy arthropods in eastern and western Australia.” *Ecological Entomology* 25: 295-306.

CHAPTER VI: GENERAL CONCLUSIONS

GENERAL OVERVIEW: CHRYSIDOIDEA SYSTEMATICS AND WHAT THIS DISSERTATION ADDS

Much of Chrysidoidea has been overlooked by hymenopterists over the years, largely because of the scarcity of some of the smaller families, like Sclerogibbidae, Scolebythidae, Embolemidae, and Plumariidae. Chrysididae and Bethylidae are arguably the best studied, and with a sudden increase in regional monographs over the past decade, Dryinidae easily follows (Grimaldi and Engel 2005, Xu et al. 2013; Olmi and Virla 2014). Phylogenetic examinations of Chrysidoidea are scant (Carpenter 1986; Brothers and Carpenter 1993; Carpenter 1999) and publications on bethylid and dryinid phylogenetics are limited.

Only within the past three decades have hymenopterists attempted to untangle the classification of Bethylidae and their work has resulted in a number of taxonomic revisions (Sorg 1988; Krombein 1992; Lanes and Azevedo 2008; Alencar and Azevedo 2009; Waichert and Azevedo 2009; Azevedo and Alencar 2010a; Azevedo and Alencar 2010b; Alencar and Azevedo 2011; Azevedo 2014). Of the handful of phylogenetic studies, only one of them has utilized molecular techniques (Carr et al. 2010). Of the most significance to this dissertation was the work of Alencar and Azevedo (2013), in which Epyrini *sensu* Evans and Sclerodermini *sensu* Azevedo and Lanes were elevated to subfamily status as Epyrinae *sensu* Alencar and Azevedo and Scleroderminae *sensu* Alencar and Azevedo. Unfortunately, while Epyrinae and Scleroderminae were well supported as subfamilies, several of the genera within Epyrinae were nonmonophyletic. The largest genus of Epyrinae (and all of Bethylidae), *Epyris* Westwood, 1832, has been nonmonophyletic in every study that it has

been sampled in, and has been considered a taxonomic wastebasket (Sorg 1988; Carpenter 1999; Alencar and Azevedo 2013).

Within Dryinidae, there have been no published phylogenetic studies of the family besides Carpenter (1999), but there have been numerous taxonomic revisions (Olmi 1984; Olmi 1993; Virla et al. 2010). Many of these revisions were re-examinations of dryinids from earlier in the century and are probably valid as attempts to tidy up taxonomic divisions that were written in the 1800s and early 1900s (Latreille 1805; Haliday 1833; Haliday 1839; Kieffer 1914). However, quite a few of these recent revisions have been synonymies of multiple genera with *Dryinus*, *Gonatopus*, and *Anteon*, resulting in the vast majority of Dryinidae now being placed within these three genera (Olmi 1993; Xu et al. 2013; Olmi and Virla 2014).

In summary, this dissertation sought to address some of the phylogenetic questions in Bethylidae and Dryinidae. For Dryinidae, the family was treated at the subfamily level using molecular sequencing in Chapter II, and as a vehicle to examine the use of geometric morphometrics as phylogenetic data in Chapter III. For Bethylidae, the focus was on *Epyris*, and its relationship to the other genera of Epyrinae in Chapters IV and V.

A BRIEF REVIEW OF THE RESULTS OF EACH RESEARCH CHAPTER

CHAPTER II

Dryinidae, also known as the "Pincer Wasps," contains over 1700 species and 16 subfamilies and are found worldwide (Olmi and Virla 2014; Xu et al. 2014; Tribull *In press*). They are parasitoids and predators of Auchenorrhyncha and attack several economically important agricultural pests (Olmi 1989; Sahragard et al. 1991). However,

little is known of the systematics of these wasps, and the only phylogenetic study was based on a now-outdated taxonomic classification (Carpenter 1999).

This chapter examined the relationships of the largest subfamilies (Anteoninae, Dryininae, Gonatopodinae, and Aphelopinae) and several smaller ones (Apodryininae, Bocchinae, and Conganteoninae) by reconstructing a phylogeny from nuclear 18S and 28S genes and mitochondrial Cytochrome Oxidase Subunit I (COI) and Cytochrome b (Cytb) genes. The phylogenies were slightly different among the parsimony, likelihood, and Bayesian analyses, mostly in regard to the placement of Bocchinae - in the parsimony and likelihood analyses, Bocchinae were the sister subfamily to Conganteoninae, with Aphelopinae sister to (Conganteoninae + Bocchinae). In the Bayesian analysis, Bocchinae were the sister group to the remainder of Dryinidae excluding Apodryininae with Aphelopinae and Conganteoninae as sister to each other.

The remaining subfamily topology in all three analyses was the same – Anteoninae, Aphelopinae, and Gonatopodinae were monophyletic, with Anteoninae as sister to ((*Thaumatodryinus* + (Dryininae *in partim* + Gonatopodinae).

Thaumatodryinus Perkins, 1905 had previously been classified as its own family, Thaumatodryininae Perkins, 1905, and had been moved to Dryininae as a genus in Olmi (1993). In chapter II, it was recovered outside of Dryininae in all analyses with strong support, and to preserve the monophyly of Dryininae, *Thaumatodryinus* was resurrected to subfamily status as Thaumatodryininae. Other results included the loss of the chela in Aphelopinae as a derived characteristic, since Apodryininae do possess a chela and were found as the sister subfamily to all remaining dryinids included in this study. Additionally, the four species groups of *Dryinus* and the species groups of *Gonatopus* that were included

were never monophyletic throughout the analyses, suggesting that the synonymies of numerous genera were problematic to the current taxonomic utility of *Gonatopus* and *Dryinus.*

CHAPTER III

Chapter III was another analysis of the subfamily classification of Dryinidae, but examined the incorporation of shape data as phylogenetic information. Landmark data were collected from five landmark configurations (shapes composed of landmarks) – two from the chela (the claw and fifth tarsal segment), a dorsal view of the head, the pronotum (lateral), and the mesosoma (lateral). Principal Component Analyses (PCA) revealed that there was high variation throughout the subfamilies for most of the shapes analyzed. A test for phylogenetic signal using the methods of Klingenberg and Gidaszewski (2010) and Perrard et al. (2014) revealed that there was phylogenetic signal in all shapes excepting the pronotum. However, when using the methods of Catalano et al. (2010) and Goloboff and Catalano (2011), the phylogeny reconstructed by any single landmark configuration under parsimony, or all five landmark configurations together, was incongruent with the tree produced by the morphological and molecular data. Combining landmarks, morphology, and morphometrics resulted in the same topology, but with lower support values throughout most of the tree. As found in Catalano et al. (2014), analyzing more landmark configurations together resulted in greater congruence with the traditional phylogenetic tree, although all five configurations together were still incongruent.

A significant phylogenetic signal did not translate into congruence with the molecular and morphological data. In Klingenberg and Gidaszewski (2010), their method for detecting phylogenetic signal assumed a Brownian model of evolution for the shape

analysis. Clearly, this assumption did not hold for the analysis of shapes for Dryinidae, possibly because of strong selection pressures from host-parasite co-evolution in shapes like the fifth tarsal segment and chela claw, which are modified to grasp hosts (Olmi 1994).

High amounts of variation resulted in a deficient superimposition when sampling across all of Dryinidae, and limiting this analysis to species-level within a genus or genus-level within a single subfamily could alleviate this problem. This application could be particularly useful for examining the species groups of *Dryinus* and *Gonatopus*, which were difficult to sample in the molecular phylogeny, but are present in large numbers in the Hymenoptera collection at the American Museum of Natural History.

CHAPTER IV

A phylogeny of Epyrinae, with a focus on the largest genus, *Epyris*, was reconstructed from molecular data utilizing mitochondrial 16S, COI, and Cytb, and nuclear 18S and 28S genes. *Anisepyris*, *Rhabdepyris*, *Holepyris*, *Trachepyris*, *Bakeriella*, *Chlorepyris* and *Laelius* were analyzed in addition to *Epyris*, representing most of the extant genera of Epyrinae.

Epyrinae *sensu* Alencar and Azevedo were recovered as monophyletic in the parsimony, likelihood, and Bayesian analyses, but results within Epyrinae were more variable. Common to all three analyses was a split in Epyrinae in which one clade contained *Anisepyris*, *Laelius*, *Chlorepyris*, and *Rhabdepyris* and the other contained *Trachepyris*, *Epyris*, *Bakeriella*, and *Holepyris*. Additionally, in all analyses, *Anisepyris* and *Holepyris* were monophyletic, and *Epyris* was nonmonophyletic.

Within the non-monophyletic *Epyris*, several clades were recovered in the three analyses. The first of these was the *Epyris staphylinoides* group, which was easily

recognizable by males having a third antennal flagellomere reduced to an inconspicuous ring and the remainder of the antennae as strikingly ramose. Up until Krombein (1992), this group had been considered part of *Calyozina.* The second recognizable clade of *Epyris sensu stricto* contained the type species of *Epyris, Epyris niger* Westwood, 1832, and all specimens in this clade (whether male or female, and located throughout the world) possessed the synapomorphy of large scutellar pits that were barely separated. This stands in contrast to the remainder of *Epyris* in which the scutellar pits are variable in size, but always well separated. Finally, *Trachepyris* was well supported as a genus, but like the *Epyris staphylinoides* group, it is only recognizable by synapomorphies found within one sex, in which males are indistinguishable from *Epyris,* but females have unique mandibular and antennal characteristics. There were other clades that were routinely found within *Epyris,* but finding morphological synapomorphies to define them was difficult. Species groups within *Epyris* have been defined before, notably in Evans (1964, 1969), but only applicable to Nearctic and Neotropical *Epyris.* Keying out *Epyris* from these regions, some of Evan's larger species groups, *tricostatus* and *rufipes,* were nonmonophyletic and placed with specimens from Thailand and South Africa.

CHAPTER V

While many of the specimens of *Epyris* in the previous chapter were undescribed, creating species descriptions for a genus that was shown to be a taxonomic wastebasket would only be adding to the eventual task of reclassifying *Epyris*. For this reason, I chose to only describe new species that fell within the clade that contained the type species, *Epyris niger* or *Epyris sensu stricto*.

While almost all of the species of *Epyris* within that clade were listed as *Epyris sp.*, a lack of published keys made it difficult to determine if those specimens had already been described. In the cases where keys were published, most of the work was done before the advent of easily accessible photography and standardized entomological terms (Kieffer 1910, 1913; Benoit 1952). To diagnose new species from the numerous *Epyris* described from South Africa, the Central African Republic, the Democratic Republic of Congo, Kenya, and Mozambique, it would be necessary to examine the types.

However, several of the specimens that fell into *Epyris sensu stricto* came from Australia, from which only two species of *Epyris* are known – *Epyris fulvimanus* Kieffer, 1907 and *Epyris lutescens* Kieffer, 1905. Additionally, both of these species are known from Queensland and New South Whales, with no record of *Epyris* described from Western Australia (Azevedo 2006).

In this chapter, five species of *Epyris* from Australia were described. Three were collected in Western Australia, and two were collected in Queensland, and were received as loans following visits to the Queensland Museum and Australian National Insect Collections in summer 2014. They were diagnosed by comparisons to the description and photos of the holotype of *Epyris fulvimanus* (provided by Celso Azevedo). *Epyris lutescens* is only known as a male holotype, and as all new species were described from females, comparisons could not be made to *Epyris lutescens. Epyris loisae, E. herschae,* and *E. carpenteri* were included in the molecular sequencing of the previous chapter and are known from Western Australia. *Epyris azevedoi* and *Epyris fulgeocauda*, which are both known from Queensland, were loaned as pinned specimens so were not included in the molecular sequencing. However,

they do possess the large scutellar pits of the *Epyris sensu stricto* and warranted inclusion in the descriptions. A key to the known female *Epyris* of Australia was also included.

FUTURE DIRECTIONS

A. Dryinidae

Analyzing specimens of dryinids that represent the smaller, more cryptic subfamilies (such as Apoaphelopinae, Erwiniinae, Transdryininae, and Plesiodryininae) is instrumental to answering questions about how Dryinidae evolved and how structures like the chela and wings were gained and lost in the various subfamilies. Plesiodryininae and Transdryininae both possess chela, but the one female type of Erwiniinae, *Erwinius prognatus*, lacks chela and the two species of Apoaphelopinae are only known from males. These subfamilies are exceedingly rare (monotypic or known by just a couple of species) and could not be sequenced for this dissertation. However, incorporating these specimens in future studies would be essential to examining the placement of these smaller subfamilies and any eventual studies on taxonomy, host-parasite evolution, or biogeography.

B. Epyris (Bethylidae: Epyrinae)

Now that *Epyris* has been convincingly shown to be nonmonophyletic, hymenopterists can begin the work of reclassifying *Epyris* and redefining the remainder of the genus. A revision of *Epyris* would contain *Epyris niger* (the type) and the other specimens that have large scutellar pits. Reclassifying the rest of *Epyris* requires a thorough morphological examination of the types and a consideration of previously suggested species groups from Evans (1964, 1969) and Krombein (1987, 1991). Additionally, the phylogeny presented in this dissertation lacked specimens from some of the smaller genera in Epyrinae like *Formosiepyris* and *Disepyris*, which have been proposed as closely related to *Holepyris*

(Alencar and Azevedo 2013). The inclusion of these specimens, and the sequencing of more genes (particularly nuclear protein-coding genes) could help to resolve the polytomy that was present in the parsimony tree and the low support values of the Bayesian and likelihood trees in chapter IV.

C. *Morphometric methodology*

Landmark analysis could be particularly well suited to reconstructing phylogenies of pinned materials that are unsuitable for molecular sequencing. For example, the American Museum of Natural History has the largest collection of Dryinidae in the United States, and one of the largest in the world, but the material is unsuitable for genetic work. This material could be ideal for reconstructing the phylogeny of the species groups within *Dryinus* and *Gonatopus*, which were both found as nonmonophyletic in the molecular sequencing of chapter II. Additionally, most of the pinned material is already prepped for morphometric work with chela mounted on flexible slides on the specimen pins.

Landmark analysis could fill in a data gap where sequencing and traditional morphological coding fails. Morphological matrices can provide data where museum specimens are too old for sequencing, but finding characters to separate species can be quite difficult. This is particularly true of Dryinidae, in which keys to the species are based off of relative measurements of ocelli, or color patterns (Xu et al. 2013; Olmi and Virla 2014). Landmark analysis could subsequently add to morphological phylogenies by allowing for the examination of shapes that would be difficult to code, even as continuous characters using length and width measurements.

REFERENCES

Alencar, I.D.C.C., and C.O. Azevedo. 2009. "Rediscovery of *Planepyris* Kieffer, a new synonym of *Trachepyris* Kieffer." *Doriana* 8: 1-12.

Alencar, I.D.C.C., and C.O. Azevedo. 2011. Review of *Neurepyris* Kieffer, 1905 (Hymenoptera, Bethylidae), a new synonym of *Pristocera* Klug, 1808. *European Journal of Taxonomy* 4: 1-12.

Alencar, I.D.C.C., and C.O. Azevedo. 2013. "Reclassification Epyrini (Hymenoptera: Bethylidae): a tribal approach with commentary on their genera." *Systematic Entomology* 38: 45-80.

Azevedo, C.O. 2006. "Insecta, Hymenoptera, Bethylidae: range extension and filling gaps in Australia." *Checklist* 2: 42-44.

Azevedo, C.O. 2014. "Synopsis of *Bakeriella* Kieffer, 1910 (Hymenoptera: Bethylidae)." *Zootaxa* 3878: 501-535.

Azevedo, C.O., and I.D.C.C. Alencar. 2010a. "Rediscovery of *Melanepyris* (Hymenoptera: Bethylidae): a new synonym of *Epyris*." *Zoologica (Curitiba*) 27: 403-407.

Azevedo, C.O., and I.D.C.C. Alencar. 2010b. "Rediscovery of the Afrotropical genus *Trissepyris* Kieffer (Hymenoptera: Bethylidae), a new synonym of *Epyris* Westwood". *Journal of Afrotropical Zoology* 6: 3-7.

Benoit, P.L.G. 1952. Bethylidae (Hym.) nord africains nouveaux ou peu connus du Museo Civico di Storia Naturale di Genova. *Doriana, Genoa Mus. Civ. di Storia Nat. "G. Doria." Annali* 1:1-7.

Brothers, D.J., and J.M. Carpenter. 1993. "Phylogeny of Aculeata: Chrysidoidea and Vespoidea (Hymenoptera)." *Journal of Hymenoptera Research* 2: 227-304.

Carpenter, J.M. 1986. "Cladistics of the Chrysidoidea (Hymenoptera)." *Journal of the New York Entomological Society*: 303-330.

Carpenter, J.M. 1999. "What do we know about chrysidoid (Hymenoptera) relationships?" *Zoologica Scripta* 28: 215-231.

Carr, M.J., P.W. Young, and P.J. Mayhew. 2010. "Phylogeny of bethylid wasps (Hymenoptera: Bethylidae) inferred from 28S and 16S rRNA genes." *Insect Systematics & Evolution* 41: 55-73.

Catalano, S.A., P.A. Goloboff, and N.P. Giannini. 2010. "Phylogenetic morphometrics (I): the use of landmark data in a phylogenetic framework." *Cladistics* 26: 539-549.

Catalano, S.A., M.D. Ercoli, and F.J. Prevosti. 2014. "The More, the Better: The Use of Multiple Landmark Configurations to Solve the Phylogenetic Relationships in Musteloids." *Systematic Biology* doi: 10.1093/sysbio/syu107.

Evans, H.E. 1964. "A synopsis of the American Bethylidae (Hymenoptera, Aculeata)." *Bulletin of the Museum of Comparative Zoology* 132: 1-222.

Evans, H.E. 1969. "A revision of the genus *Epyris* in the Americas (Hymenoptera: Bethylidae)." *Transactions of the American Entomological Society* 95: 181-352.

Goloboff, P.A., and S.A. Catalano. 2011. "Phylogenetic morphometrics (II): algorithms for landmark optimization." *Cladistics* 27: 42-51.

Grimaldi, D., and M.S. Engel. 2005. *Evolution of the Insects*. Cambridge University Press.

Haliday, A.H. 1833. "An essay on the classification of the Parasitic Hymenoptera of Britain, which correspond with the *Ichneumones minuti* of Linnaeus." *The Entomological Magazine* 1: 259-273.

Haliday, A.H. 1839. Hymenoptera Britannica, Part 1. Oxyura. Addendum to volume 1, Hymenopterorum synopsis ad methodum fallenil utplurimum accomodata. Hippolytus Bailliere, London.

Kieffer, J-J. 1910. "Description de nouveaux Bethylides (Hymen.)" *Annales de la Société entomologique de France* 79: 136-140.

Kieffer, J-J. 1914. Bethylidae. Das Tierreich 41: 1–205.

Klingenberg, C.P., and N.A. Gidaszewski. 2010 "Testing and quantifying phylogenetic signals and homoplasy in morphometric data." *Systematic Biology* 59: 245-261.

Krombein, K.V. 1987. "Biosystematic studies of Ceylonese wasps. XVIII. The species of Trachepyris Kieffer (Hymenoptera: Bethylidae: Epyrinae)." *The Pan-Pacific Entomologist (USA)*:

Krombein, K.V. 1992. "Systematics of the genera of Epyrinae with ramose male antennae (Hymenoptera: Bethylidae)." *Proceedings of the Entomological Society of Washington* 94: 345-360.

Lanes, G.O., and C.O. Azevedo. 2008. "Phylogeny and Taxonomy of Sclerodermini (Hymenoptera, Bethylidae, Epyrinae)." *Insect Systematics & Evolution* 39: 55-86.

Latreille, P.A. 1805. Histoire naturelle generale et particulière des crustacés et des insectes, 13. F. Dufart, Paris, 432 pp

Olmi, M. 1984. "A revision of the Dryinidae (Hymenoptera)." *Memoirs of the American*

Entomological Institute 37: 1-1913.

Olmi, M. 1989. “Supplement to the revision of the world Dryinidae (Hymenoptera, Chrysidoidea).” *Frustula Entomologica, Nuova Serie* 26: 15-19.

Olmi, M. 1993. “A new generic classification for Thaumatodryininae, Dryininae, and Gonatopodinae, with descriptions of new species (Hymenoptera, Dryinidae).” *Bollettino di Zoologia agrarian e di Bachicoltura* 25: 57-89.

Olmi, M., and A. Guglielmino. 2010. "Description of Erwiniinae, new subfamily of Dryinidae from Ecuador (Hymenoptera: Chrysidoidea)." *Zootaxa* 2605: 56-62.

Olmi, Massimo, and Eduardo G. Virla. 2014. "Dryinidae of the Neotropical region (Hymenoptera: Chrysidoidea)." *Zootaxa* 3792: 1-534.

Olmi, M., A. Guglielmino, S.A. Natha, and AA Nota Alfredo. 2012. "A catalogue of Dryinidae of Mozambique, with description of a new species (Hymenoptera: Chrysidoidea)." *African Invertebrates* 53: 715-731.

Perrard, A., M. Baylac, J. M. Carpenter, and C. Villemant. 2014 "Evolution of wing shape in hornets: why is the wing venation efficient for species identification?" *Journal of Evolutionary Biology* 27: 2665-2675.

Sahragard, A., M.A. Jervis, and N.A.C. Kidd. 1991 "Influence of host availability on rates of oviposition and host- feeding, and on longevity in *Dicondylus indianus* Olmi (Hym., Dryinidae), a parasitoid of the Rice Brown Planthopper, *Nilaparvata lugens* Stål (Hem., Delphacidae)." *Journal of Applied Entomology* 112: 153-162.

Sorg, M. 1988. “Zur Phylogenie und Systematik der Bethylidae (Insecta: Hymenoptera: Chrysidoidea).” *Sonderveröffentlichungen des Geologishcen Instiuts der Universität zu Köln* 63:1-146.

Virla, E.G., M.S. Espinoza, and M. Olmi. 2010. “A contribution to the knowledge of Dryinidae of Argentina: descriptions of the hitherto unknown males of *Gonatopus argentinus* Olmi and *Trichogonatopus richardsi* Olmi (Hymenoptera: Chrysidoidea)”. *Frustula Entomologica, Nuova Serie* 31: 125-134.

Waichert, C., and C.O. Azevedo. 2009. "Phylogenetic analysis of *Rhabdepyris* (Hymenoptera: Bethylidae) and redefinition of generic limits based on morphological characters." *Zootaxa* 2284: 1-29.

Xu, Z., M. Olmi, and J. He. 2013. "Dryinidae of the Oriental region (Hymenoptera: Chrysidoidea)." *Zootaxa* 3614: 001-460.

APPENDIX A
SPECIMEN INFORMATION FOR CHAPTER II

Terminal name	Locality	Accessioned	Collector	Sex
Adryinus sp.	Brazil	INPA	K Schoening	Female
Anteon sp. 1	Belize	CNC	A Desjardins	Female
Anteon sp. 2	Kenya	CNC	S Kimani	Female
Anteon sp. 3	Curacao	CNC	L Masner	Male
Anteon sp. 4	Curacao	CNC	L Masner	Female
Anteon sp. 5	Curacao	CNC	L Masner	Female
Anteon sp. 6	Australia	CNC	ME Irwin, Fl	Male
Anteon sp. 7	Democratic I	CNC	BD Gill	Female
Anteon sp. 8	South Korea	CNC	N/A	Male
Anteon sp. 9	Thailand	CNC	M Sharkey	Female
Anteon sp. 10	Thailand	CNC	M Sharkey	Female
Anteon sp. 11	South Korea	CNC	N/A	Female
Anteon sp. 12	South Korea	CNC	N/A	Female
Anteon sp. 13	Canada	CNC	RE & LJT L	Female
Anteon sp. 14	Canada	CNC	Goulet, Bouc	Female
Anteon sp. 15	Germany	CNC	N/A	Female
Anteon sp. 16	Canada	CNC	RE & LJT L	Female
Anteon sp. 17	USA	CNC	T Pucci	Female
Anteon sp. 18	USA	CNC	W Godwin	Female
Anteon wushense	South Korea	CNC	N/A	Female
Aphelopus sp. 1	Canada	CNC	RE & LJT L	Female
Aphelopus sp. 2	South Korea	CNC	N/a	Female
Aphelopus sp. 3	South Korea	CNC	N/A	Female
Bocchus sp. 1	Australia	CNC	ME Irwin, Fl	Femlae
Deinodryinus incaicus	French Guia	CNC	A Desjardins	Female
Deinodryinus finnamorei	Uruguay	CNC	N/A	Female
Deinodryinus sp. 1	French Guia	CNC	A Desjardins	Female
Deinodryinus sp. 2	French Guia	CNC	A Desjardins	Male
Deinodryinus sp. 3	South Africa	CNC	L Masner	Male

Terminal name	Locality	Accessioned	Collector	Sex
Adryinus sp.	Brazil	INPA	K Schoening	Female
Anteon sp. 1	Belize	CNC	A Desjardins	Female
Anteon sp. 2	Kenya	CNC	S Kimani	Female
Anteon sp. 3	Curacao	CNC	L Masner	Male
Anteon sp. 4	Curacao	CNC	L Masner	Female
Anteon sp. 5	Curacao	CNC	L Masner	Female
Anteon sp. 6	Australia	CNC	ME Irwin, Fl	Male
Anteon sp. 7	Democratic I	CNC	BD Gill	Female
Anteon sp. 8	South Korea	CNC	N/A	Male
Anteon sp. 9	Thailand	CNC	M Sharkey	Female
Anteon sp. 10	Thailand	CNC	M Sharkey	Female
Anteon sp. 11	South Korea	CNC	N/A	Female
Anteon sp. 12	South Korea	CNC	N/A	Female
Anteon sp. 13	Canada	CNC	RE & LJT L	Female
Anteon sp. 14	Canada	CNC	Goulet, Bouc	Female
Anteon sp. 15	Germany	CNC	N/A	Female
Anteon sp. 16	Canada	CNC	RE & LJT L	Female
Anteon sp. 17	USA	CNC	T Pucci	Female
Anteon sp. 18	USA	CNC	W Godwin	Female
Anteon wushense	South Korea	CNC	N/A	Female
Aphelopus sp. 1	Canada	CNC	RE & LJT L	Female
Aphelopus sp. 2	South Korea	CNC	N/a	Female
Aphelopus sp. 3	South Korea	CNC	N/A	Female
Bocchus sp. 1	Australia	CNC	ME Irwin, Fl	Femlae
Deinodryinus incaicus	French Guia	CNC	A Desjardins	Female
Deinodryinus finnamorei	Uruguay	CNC	N/A	Female
Deinodryinus sp. 1	French Guia	CNC	A Desjardins	Female
Deinodryinus sp. 2	French Guia	CNC	A Desjardins	Male
Deinodryinus sp. 3	South Africa	CNC	L Masner	Male

Table 2.1: Specimen information and sequencing completion (in green)

Terminal name	Locality	Accessioned	Collector	Sex
Adryinus sp.	Brazil	INPA	K Schoeninger	Female
Anteon sp. 1	Belize	CNC	A Desjardins	Female
Anteon sp. 2	Kenya	CNC	S Kimani	Female
Anteon sp. 3	Curacao	CNC	L Masner	Male
Anteon sp. 4	Curacao	CNC	L Masner	Female
Anteon sp. 5	Curacao	CNC	L Masner	Female
Anteon sp. 6	Australia	CNC	ME Irwin, FD Parker	Male
Anteon sp. 7	Democratic Repu	CNC	BD Gill	Female
Anteon sp. 8	South Korea	CNC	N/A	Male
Anteon sp. 9	Thailand	CNC	M Sharkey	Female
Anteon sp. 10	Thailand	CNC	M Sharkey	Female
Anteon sp. 11	South Korea	CNC	N/A	Female
Anteon sp. 12	South Korea	CNC	N/A	Female
Anteon sp. 13	Canada	CNC	RE & LJT Leech	Female
Anteon sp. 14	Canada	CNC	Goulet, Boudreault, Badiss	Female
Anteon sp. 15	Germany	CNC	N/A	Female
Anteon sp. 16	Canada	CNC	RE & LJT Leech	Female
Anteon sp. 17	USA	CNC	T Pucci	Female
Anteon sp. 18	USA	CNC	W Godwin	Female
Anteon wushense	South Korea	CNC	N/A	Female
Aphelopus sp. 1	Canada	CNC	RE & LJT Leech	Female
Aphelopus sp. 2	South Korea	CNC	N/a	Female
Aphelopus sp. 3	South Korea	CNC	N/A	Female
Bocchus sp. 1	Australia	CNC	ME Irwin, FD Parker	Femlae
Deinodryinus incaicus	French Guiana	CNC	A Desjardins	Female
Deinodryinus finnamorei	Uruguay	CNC	N/A	Female
Deinodryinus sp. 1	French Guiana	CNC	A Desjardins	Female
Deinodryinus sp. 2	French Guiana	CNC	A Desjardins	Male
Deinodryinus sp. 3	South Africa	CNC	L Masner	Male

Table 2.1 continued

Terminal name	Locality	Accessioned	Collector	Sex
Deinodryinus sp. 3	South Africa	CNC	L Masner	Male
Deinodryinus sp. 4	USA	CNC	L Masner	Female
Deinodryinus sp. 5	Brazil	INPA	K Schoeninger	Female
Deinodryinus sp. 6	Brazil	INPA	K Schoeninger	Female
Dryinus bicolor	Belize	CNC	L Masner	Female
Dryinus bolivianus	Brazil	INPA	K Schoeninger	Female
Dryinus parvulus	Thailand	M Olmi	M Olmi	Female
Dryinus sp. 1	Brazil	INPA	K Schoeninger	Female
Dryinus sp. 2	French Guiana	CNC	A Desjardins	Male
Dryinus sp. 3	Brazil	INPA	K Schoeninger	Femlae
Dryinus sp. 4	French Guiana	CNC	A Desjardins	Male
Dryinus sp. 5	French Guiana	CNC	A Desjardins	Male
Dryinus sp. 6	Israel	CNC	ME Irwin	Female
Dryinus sp. 7	French Guiana	CNC	A Desjardins	Male
Dryinus sp. 8	Australia	CNC	C Burwell	Female
Dryinus sp. 9	Australia	CNC	C Burwell	Female
Dryinus sp. 10	Australia	CNC	C Burwell	Female
Dryinus sp. 11	Thailand	CNC	M Sharkey	Female
Dryinus sp. 12	Vietnam	AMNH	JM Carpenter, D Grimaldi	Femlae
Dryinus sp. 18	Israel	CNC	ME Irwin	Male
Dryinus sp. 14	Papua New Guinea	CNC	L Leblanc, M Kalamen	Female
Dryinus sp. 16	Thailand	M Olmi	M Olmi	Male
Dryinus sp. 17	Thailand	CNC	M Sharkey	Female
Dryinus sp. 18	South Korea	CNC	N/A	Male
Dryinus spangleri	Madagascar	M Olmi	M Olmi	Female
Dryinus stantoni	Thailand	CNC	M Sharkey	Female
Dryinus striatus	Bolivia	CNC	L Masner	Female
Dryinus striatus	Brazil	CNC	L Masner	Female
Echthrodelphax sp. 1	USA	CNC	W Godwin	Female
Echthrodelphax sp. 2	Ghana	CNC	R Longair, A Tungbari	Female
Epigonatopus sp. 1	Australia	N/A	C Tribull	Female
Epigonatopus sp. 2	Australia	N/A	C Tribull	Female

Table 2.1: continued

Terminal name	Locality	Accessioned	Collector	Sex	18	28	COI	CytB
Eucamptonyx sp.	Brazil	INPA	K Schoeninger	Female				
Fiorianteon junonium	Japan	CNC	K Yamagishi	Female				
Fiorianteon junonium	Japan	CNC	K Yamagishi	Female				
Gonatopus cyphotes	USA	CNC	Goulet & Boudreault	Female				
Gonatopus hibbsi	Ecuador	CNC	P Hibbs	Female				
Gonatopus peculiaris	Canada	CNC	Goulet, Boudreault, Badiss	Female				
Gonatopus sp. 1	Brazil	INPA	K Schoeninger	Femlae				
Gonatopus sp. 2	Belize	CNC	L Masner	Female				
Gonatopus sp. 3	USA	N/A	Carly Tribull	Female				
Gonatopus sp. 4	French Guiana	CNC	A Desjardins	Male				
Haplogonatopus oratorius	GENBANK	N/A	N/A	N/A	JX413910.1	JX413953.1		AB723612.1
Lonchodryinus subapterous	France	N/A	Pierre Tripotin	Female				
Lonchodryinus sp. 1	USA	CNC	Goulet & Boudreault	Female				
Lonchodryinus sp. 2	USA	CNC	Goulet & Boudreault	Female				
Madecadryinus politus	Madagascar	CAS	BL Fisher	Female				
Thaumatodryinus merinus	Madagascar	N/A	Massimo Olmi	Male				
Thaumatodryinus macilentus	Brazil	INPA	K Schoeninger	Female				
Outgroups								
Chrysis cembricola	GENBANK	N/A	N/A	N/A	GQ410611.1	GQ374718.1	GQ374633.1	
Chrysis principalis	GENBANK	N/A	N/A	N/A	JX413894.1	JX413938.1		JX413981.1
Cleptes seoulensis	GENBANK	N/A	N/A	N/A	JX413896.1	JX413940.1		JX413983.1
Sclerogibba sp.	GENBANK	N/A	N/A	N/A	JX413877.1	JX413923.1		JX413966.1

APPENDIX B
PRIMER PROTOCOLS FOR CHAPTERS II AND IV

Primer Protocols

CytB

Primer names:	CB1/CB2
Heated Lid	110 °C
Initial Temp.	95°C for 5 minutes
Start Cycle	40 cycles
Denaturation	95°C for 1 minute
Annealing	42°C for 1 minute
Extension	72°C for 1 minute
End Cycle	
Final Extension	72°C for 10 minutes

COI

Primer names:	HCO2198/LCO1490
Heated Lid	110°C
Initial Temp.	95°C for 5 minutes
Start Cycle	35 cycles
Denaturation	95°C for 1 minute
Annealing	40°C for 1 minute
Extension	72°C for 1.5 minutes
End Cycle	
Final Extension	72°C for 10 minutes

18S

Primer names:	18SF2/18SR2
Heated Lid	110°C
Initial Temp.	95°C for 5 minutes
Start Cycle	34 cycles
Denaturation	95°C for 30 seconds
Annealing	56°C for 40 seconds
Extension	72°C for 40 seconds
End Cycle	
Final Extension	72°C for 10 minutes

28S

Primer names:	For28SVesp/Rev28SVesp
Heated Lid:	110°C
Initial Temp.	94°C for 5 minutes
Start Cycle	35 cycles
Denaturation	94°C for 1 minute
Annealing	50°C for 1 minute
Extension	72°C for 1 minute
End Cycle	
Final Extension	72°C for 5 minutes

16S

Primer names:	16Saf/16Sar
Heated Lid:	110°C
Initial Temp.	94°C for 5 minutes
Start Cycle	34 cycles
Denaturation	94°C for 1 minute
Annealing	45°C for 1 minute
Extension	72°C for 1 minute
End Cycle	
Final Extension	72°C for 5 minutes

APPENDIX C
SPECIMEN INFORMATION FOR CHAPTER III

Table 3.1 Specimens and presence (green) in various datasets

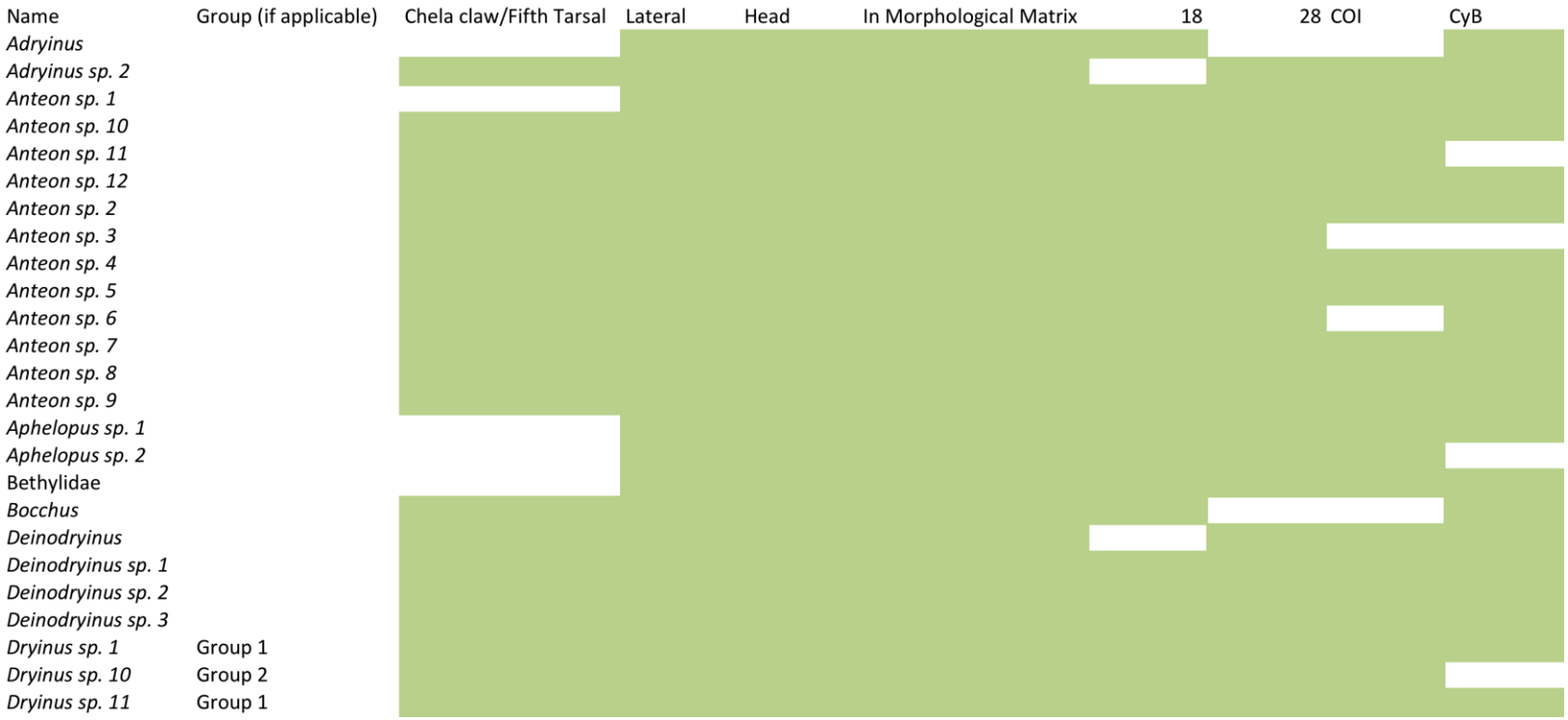


Table 3 .1 Continued

Name	Group (if applicable)	Chela claw/Fifth Lateral	Head	In Morphological Matrix	18	28	COI	CyB
Dryinus sp. 12	Group 1							
Dryinus sp. 13	Group 3							
Dryinus sp. 14	Group 1							
Dryinus sp. 15	Group 4							
Dryinus sp. 2	Group 4							
Dryinus sp. 3	Group 1							
Dryinus sp. 4	Group 1							
Dryinus sp. 5	Group 1							
Dryinus sp. 6	Group 1							
Dryinus sp. 7	Group 1							
Dryinus sp. 8	Group 2							
Dryinus sp. 9	Group 2							
Echthrodelphax sp.								
Embolemidae								
Epigonatopus								
Eucamptonyx								
Fiorianteon sp. 1								
Fiorianteon sp. 2								
Gonatopus cyphotes	Group 7							
Gonatopus peculiaris	Group 6							
Gonatopus sp.	Group 7							
Gonatopus sp. 1	Group 7							
Gonatopus sp. 4	Group 10							
Lonchodryinus sp. 1								
Lonchodryinus sp. 2								
Madecadryinus politus								
Thaumatodryinus								

APPENDIX D

MORPHOLOGICAL CHARACTER CODES FOR CHAPTER III, COURTESY OF TOSHIHARU MITA

1. Host: Coleoptera (0), Cicadomorpha (1), Fulgoromorpha (2)
2. Thylacium: larva do not produce thylacium (0), larva produce thylacium (1)
3. Cocoon: Single (0), double (1)
4. Location of cocoon: inside plant tissue (0), on plant tissue (1), in soil (2)
5. Setae on larvae: absent (0), present (1)
6. Mouth: Prognathous (0), orthognathous (1), hypognathous (2)
7. Location of larvae on host: abdomen (0), thorax (1)
8. Longitudinal median carina on clypeus: absent (0), present (1)
9. Antennae: well separated from each other (0), close together (1)
10. Gena: well exposed in lateral view (0), slightly or not exposed (1)
11. Foretarsus: not chelate (0), chelate (1)
12. Number of claws: Two, with rudimentary claw or both claws present (0), One with rudimentary claw absent (1)
13. Setae or lamellae on enlarged claw: Enlarged claw simple, without row or structure (0), bearing row of bristles or lamellae (1)
14. Modification of the apex of enlarged claw: apex of enlarged claw simple (0), apex of enlarged claw bearing dull subapical tooth (1), apex of enlarged claw bearing acute subapical tooth (2), apex of enlarged claw rounded (3)
15. Position of apex of enlarged claw when closed: apex of enlarged claw touching distal apex of 4^{th} tarsomere (0), apex of enlarged claw beyond apex of 4^{th} tarsomere (1)
16. Size of arolium: arolium normal in size (0), arolium large (1)

17. Shape of arolium: conical (0), flattened (1)
18. Inner margin of 5th tarsomere: simple and without row of lamellae or bristles (0), bearing one row of lamellae (1), bearing more than one row of lamellae (2)
19. Lamellae on apical tip of fifth tarsomere: lamellae absent (0), single row of lamellae (1), more than a single row of lamellae (2)
20. Shape of apical tip of fifth tarsomere: simple (0), sinuate to avoid the apex of enlarged claw (1), forming cup-like structure (claw cup) (2)
21. Remainder of foretarsomere: simple (0), forming hook-like structure (1), expanded (2)
22. Basal part of enlarged claw or simple claw: Simple (0), one bristle present on prominence (1), single bristle present, not located on a basal prominence (2)
23. Relative length of projection of fifth tarsomere: absent (0), less than 0.5 (1), more than 0.5 (2)
24. Relative length of 1st tarsomere with 4th tarsomere longer than 1.5 x TIV (0), shorter than 1.5 x TIV (1)
25. Trochanter: coxa greater than 1.5 x trochanter (0), coxa less than 1.5 x trochanter (1)
26. Coxa: Short (0), elongate (1)
27. Antennae: 10-segmented (0), 12-segmented (1)
28. Rhinarium on apical antennomeres: absent (0), present (1)
29. Tuft of setae arising from rhinarium: absent (0), present (1)
30. Occipital carinae: complete (0), incomplete or absent (1)
31. Subocular carinae: present (0), absent or quite indistinct (1)

32. Number of labial palp segments: Three (0), Two (1), One (2)
33. Number of maxillary palp segments: five – six (0), three – four (1), two (2)
34. Ocelli: Absent (0), present (1)
35. Mesosoma: Composed of fused segments (0), division between the pronotum, scutum, metanotum and propodeum easily visible (1)
36. Notch on distal apex of pronotum: Absent (0), present (1)
37. Anterior transverse impression: Absent (0), present (1)
38. Posterior transverse impression: Absent (0), present (1)
39. Epicnemium: Separated from mesepisternum (0), fused with mesepisternum (1), epicnemium not separated by suture (2)
40. Notaulices: Absent (not obscured by striations) (0), Present (may be obscured by striations in Dryininae) (1),
41. Longitudinal sulcus on ventral-lateral part of mesepisternum: Present (0), absent (1)
42. Sculpture on mesepisternum: Reticulate (0), smooth, granulated, or transversely striate (1)
43. Tegula: Present (0), absent (apterous)
44. Wing size: Wings absent (0), wings present, normal in size (1), Wings much reduced (2)
45. Number of cell(s) on forewing: Three or more (0), two (1), one (1), wing absent (3)
46. Pterothorax: Simple (0), modified; stalk shaped (1)

47. Sculpture on dorsal surface of propodeum: reticulate (0), transversely striate (1), granulate or smooth (2)
48. Longitudinal carinae on posterior surface of propodeum: present (0), absent (1)
49. Pronotum tubercle: touching tegula (0), separated from tegula or tubercle absent (1)
50. Number of tibial spur(s) in midleg: Two (0), one (1), two (0)

APPENDIX E
SPECIMEN DATA FOR CHAPTER IV

Table 4.1: Specimen information and sequencing completion (in green)

Name	Collected by	Country	Institution	16S	18S	28S	COI	CytB
Allobethylus tomoae	Genbank	NA	NA					
Anisepyris sp. 1	C Azevedo	Brazil	UFES					
Anisepyris sp. 2	C Azevedo	Brazil	UFES					
Anisepyris sp. 3	C Azevedo	Brazil	UFES					
Anisepyris sp. 4	C Azevedo	Brazil	UFES					
Anisepyris sp. 5	Carly Tribull	USA	AMNH					
Anisepyris sp. 6	Carly Tribull	USA	AMNH					
Anisepyris sp. 7	Carly Tribull	USA	AMNH					
Anisepyris sp. 8	Carly Tribull	USA	AMNH					
Anisepyris sp. 9	Carly Tribull	USA	AMNH					
Apenesia elegans	Genbank	NA	NA					
Apenesia sp. 1	Carly Tribull	USA	AMNH					
Apenesia sp. 2	Carly Tribull	USA	AMNH					
Bakeriella sp. 1	M Pittier	Venezuela	UFES					
Bakeriella sp. 2	M Pittier	Venezuela	UFES					
Bakeriella sp. 3	C Azevedo	Brazil	UFES					
Bakeriella sp. 4	A Desjardins	French Guiana	CNC					
Bethylus fuscicornis	Genbank	NA	NA					
Cephalonomia sp. 1	Carly Tribull	USA	AMNH					
Cephalonomia sp. 2	Carly Tribull	USA	AMNH					
Chlorepyris/Rhabdepyris sp. 1	A Desjardins	French Guiana	CNC					
Chlorepyris/Rhabdepyris sp. 10	ME Irwin, FD Parker	Australia	UFES					
Chlorepyris/Rhabdepyris sp. 11	C Lambkin, N Starick	Australia	QLD					
Chlorepyris/Rhabdepyris sp. 12	ME Irwin, FD Parker	Australia	UFES					
Chlorepyris/Rhabdepyris sp. 13	ME Irwin, FD Parker	Australia	CNC					
Chlorepyris/Rhabdepyris sp. 14	C Lambkin, N Starick	Australia	QLD					
Chlorepyris/Rhabdepyris sp. 15	C Lambkin, N Starick	Australia	QLD					
Chlorepyris/Rhabdepyris sp. 16	C Lambkin, N Starick	Australia	QLD					
Chlorepyris/Rhabdepyris sp. 17	ME Irwin, FD Parker	Australia	UFES					
Chlorepyris/Rhabdepyris sp. 18	ME Irwin, FD Parker	Australia	UFES					

Table 4.1 Continued

Name	Collected by	Country	Institution	16S	18S	28S	COI	CytB
Chlorepyris/Rhabdepyris sp. 19	ME Irwin, FD Parker	Australia	CNC					
Chlorepyris/Rhabdepyris sp. 2	Adrienn Perrard	French Guiana						
Chlorepyris/Rhabdepyris sp. 20	ME Irwin, FD Parker	Australia	CNC					
Chlorepyris/Rhabdepyris sp. 3	A Desjardins	French Guiana	CNC					
Chlorepyris/Rhabdepyris sp. 4	A Desjardins	French Guiana	CNC					
Chlorepyris/Rhabdepyris sp. 5	Adrienn Perrard	French Guiana						
Chlorepyris/Rhabdepyris sp. 6	C Azevedo	Brazil	UFES					
Chlorepyris/Rhabdepyris sp. 7	M Sharkey	Venezuela	UFES					
Chlorepyris/Rhabdepyris sp. 8	Not Listed	Peru	UFES					
Chlorepyris/Rhabdepyris sp. 9	ME Irwin, FD Parker	Australia	UFES					
Chrysis cembricola	Genbank	NA	NA		GQ410611.1	GQ374718.	GQ374633.1	
Chrysis principalis	Genbank	NA	NA	JX413849.1	JX413894.1	JX413938.1		JX413981.1
Cleptes seoulensis	Genbank	NA	NA	JX413851.1	JX413896.1	JX413940.1		JX413983.1
Dissomphalus sp.	Genbank	NA	NA	JX413844.1	JX413880.1	JX413927.1		JX413975.1
Epyris (staphylinoides group) sp. 1 (male)	A Polaszek	Saba	BNHM/UMS					
Epyris (staphylinoides group) sp. 10, male	T Jaruphan , O. Buswaong	Thailand	UFES					
Epyris (staphylinoides group) sp. 11 (female)	B & J Gill	Uganda	CNC					
Epyris (staphylinoides group) sp. 12 (male)	B & J Gill	Uganda	CNC					
Epyris (staphylinoides group) sp. 13 male	A Polaszek	DR Congo	BNHM					
Epyris (staphylinoides group) sp. 14 (female	B & J Gill	Uganda	CNC					
Epyris (staphylinoides group) sp. 2 (male)	K Tsujii	Laos	UFES					
Epyris (staphylinoides group) sp. 3 (male)	K Tsujii	Laos	UFES					
Epyris (staphylinoides group) sp. 4 (female)	A Polaszek	Saba	BNHM/UMS					
Epyris (staphylinoides group) sp. 5, male	A Polaszek	Saba	BNHM/UMS					
Epyris (staphylinoides group) sp. 6, female	A Polaszek	Saba	BNHM/UMS					
Epyris (staphylinoides group) sp. 7, female	A Polaszek	Saba	BNHM/UMS					
Epyris (staphylinoides group) sp. 8 (male)	A Polaszek	Saba	BNHM/UMS					
Epyris (staphylinoides group) sp. 9	T Jaruphan , O. Buswaong	Thailand	UFES	JX413855.1	JX413900.1	JX413944.1		JX413987.1
Epyris festivus	Genbank	NA	NA					
Epyris idaten	Jongok Lim	South Korea	NA	JX413855.1	JX413900.1	JX413944.1		JX413987.1
Epyris koreanus	Jongok Lim	South Korea	NA					
Epyris niger	P Mason	Switzerland	CNC					
Epyris niwoh	Jongok Lim	South Korea	NA	JX413859.1	JX413904.1	JX413948.1		JX413991.1

Table 4.1 Continued

Name	Collected by	Country	Institution	16S	18S	28S	COI	CytB
Epyris sp. 10	C Villament	Mozambique	PNHM					
Epyris sp. 11	M Olmi	Mozambique	CNC					
Epyris sp. 12	A Polaszek	DR Congo	BNHM					
Epyris sp. 13	B & J Gill	Uganda	CNC					
Epyris sp. 14	Carly Tribull	USA	AMNH					
Epyris sp. 15	J Ascher	Panama						
Epyris sp. 16	C Azevedo	Brazil	UFES					
Epyris sp. 17	M Pittier	Venezuela	UFES					
Epyris sp. 18	M Pittier	Venezuela	UFES					
Epyris sp. 19	A Desjardins	French Guiana	CNC					
Epyris sp. 2	A Smetana, L Robillard	Nicuagra	CNC					
Epyris sp. 20	C Azevedo	Brazil	UFES					
Epyris sp. 21	C Azevedo	Brazil	UFES					
Epyris sp. 22	C Azevedo	Brazil	uFES					
Epyris sp. 23	C Azevedo	Brazil	UFES					
Epyris sp. 24	ME Irwin, FD Parker	Australia	CNC					
Epyris sp. 25	ME Irwin, FD Parker	Australia	UFES					
Epyris sp. 26	M Olmi	Mozambique	CNC					
Epyris sp. 27	S Van Noort	South Africa	UFES/S.Afr					
Epyris sp. 28	ME Irwin, FD Parker	Australia	UFES					
Epyris sp. 29	L Masner	Australia	CNC					
Epyris sp. 3	Carly Tribull	USA	AMNH					
Epyris sp. 30	ME Irwin, FD Parker	Australia	CNC					
Epyris sp. 31	C Lambkin, N Starick	Australia	QLD					
Epyris sp. 32	C Lambkin, N Starick	Australia	QLD					
Epyris sp. 33	A Polaszek	Saba	BNHM/UMS					
Epyris sp. 34	A Polaszek	DR Congo	BNHM					
Epyris sp. 35	A Polaszek	DR Congo	BNHM					
Epyris sp. 36	Not Listed	South Korea	CNC					
Epyris sp. 37	P Mason	Switzerland	CNC					
Epyris sp. 38	R Copeland	Kenya	CNC					
Epyris sp. 39	M Olmi	Mozambique	CNC					
Epyris sp. 4	Carly Tribull	USA	AMNH					
Epyris sp. 40	Not Listed	South Korea	CNC					
Epyris sp. 41	Not Listed	South Korea	CNC					
Epyris sp. 42	A Polaszek	Saba	BNHM/UMS					

Table 4.1 Continued

Name	Collected by	Country	Institution	16S	18S	28S	COI	CytB
Epyris sp. 43	Goulet & Boudreault	USA	CNC					
Epyris sp. 44	S Kimani	Kenya	CNC					
Epyris sp. 45	S Van Noort	South Africa	UFES/S.Afr					
Epyris sp. 46	BL Fisher	Madagascar	UFES/CASC					
Epyris sp. 47	Adrienn Perrard	French Guiana						
Epyris sp. 48	C Azevedo	Brazil	UFES					
Epyris sp. 49	S Van Noort	South Africa	UFES/S.Afr					
Epyris sp. 5	N Eardley	Cananda	CNC					
Epyris sp. 50	S Van Noort	South Africa	UFES/S.Afr					
Epyris sp. 51	S Van Noort	South Africa	UFES/S.Afr					
Epyris sp. 52	S Van Noort	South Africa	UFES/S.Afr					
Epyris sp. 53	S Van Noort	South Africa	UFES/S.Afr					
Epyris sp. 54	Carly Tribull	USA	AMNH					
Epyris sp. 55	Carly Tribull	USA	AMNH					
Epyris sp. 56	Carly Tribull	USA	AMNH					
Epyris sp. 57	S Van Noort	South Africa	UFES/S.Afr					
Epyris sp. 58	J Halada	Central African Republic	CNC					
Epyris sp. 59	S Van Noort	South Africa	UFES/S.Afr					
Epyris sp. 6	K Tsujii	Laos	UFES					
Epyris sp. 60	Carly Tribull	USA	AMNH					
Epyris sp. 61	Carly Tribull	USA	AMNH					
Epyris sp. 62	Carly Tribull	USA	AMNH					
Epyris sp. 63	Carly Tribull	USA	AMNH					
Epyris sp. 7	A Polaszek	Saba	BNHM/UMS					
Epyris sp. 8	A Polaszek	Saba	BNHM/UMS					
Epyris sp. 9	T Jaruphan , O. Buswaong	Thailand	UFES					
Epyris sp.1	Carly Tribull	USA	AMNH					
Epyris yamatonis	Jongok Lim	South Korea	NA	JX413859.1	JX413904.1	JX413948.1		JX413991.1
Epyris/Trachepyris sp. 1 (male)	C Villament	Mozambique	PNHM					
Epyris/Trachepyris sp. 2 (male)	C Villament	Mozambique	PNHM					
Epyris/Trachepyris sp. 3 (male)	S Van Noort	South Africa	UFES/S.Afr					
Epyris/Trachepyris sp. 4 (male)	T Jaruphan , O. Buswaong	Thailand	UFES					
Epyris/Trachepyris sp. 5 (male)	T Jaruphan , O. Buswaong	Thailand	UFES					
Epyris/Trachepyris sp. 6 (male)	K Sa-nog, S Bukaw Adnafai	Thailand	UFES					

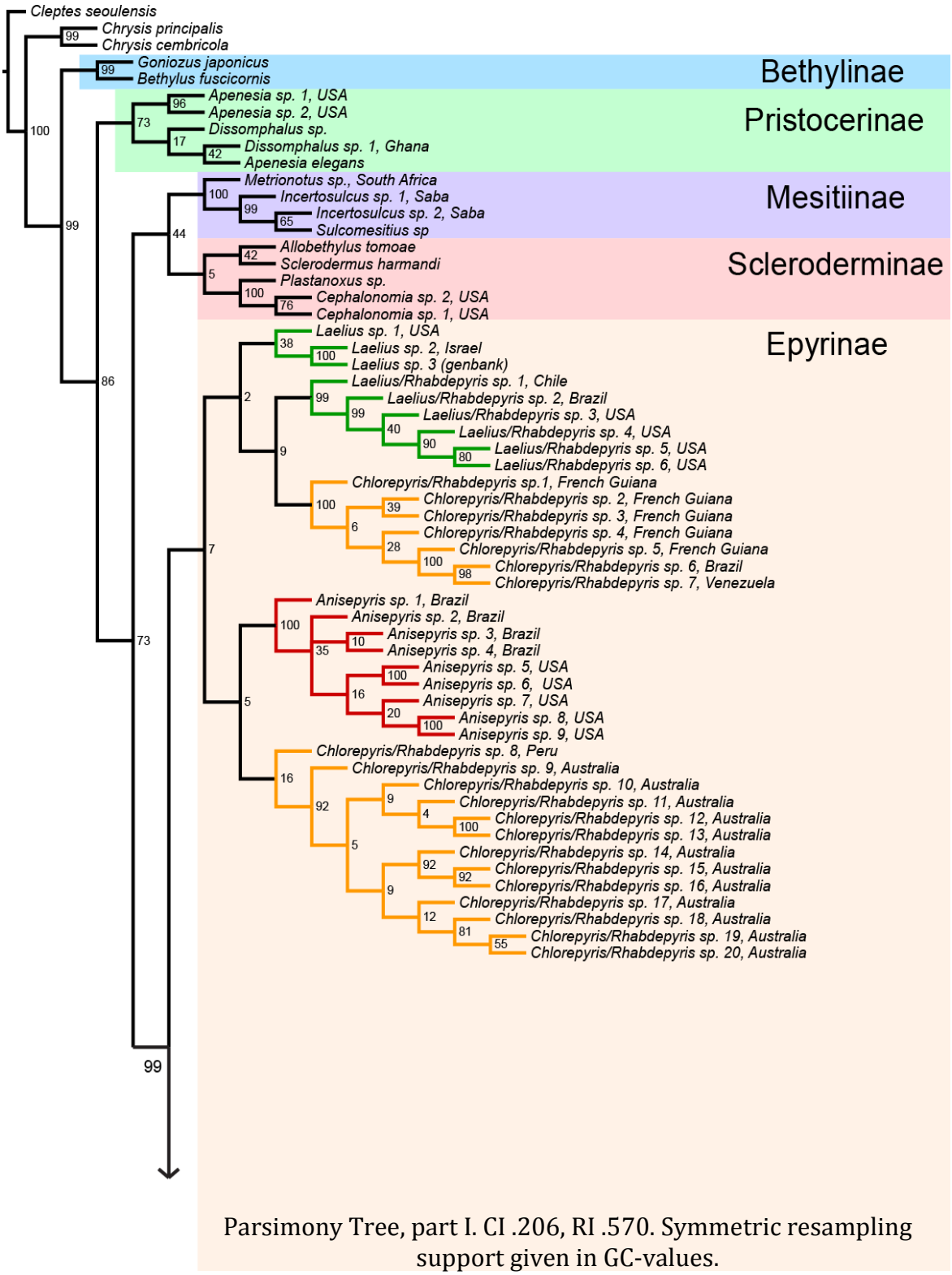
Table 4.1 Continued

Name	Collected by	Country	Institution	16S	18S	28S	COI	CytB
Epyris/Trachepyris sp. 7 (male)	K Sa-nog, S Bukaw Adnafai	Thailand	UFES					
Epyris/Trachepyris sp. 8 (male)	T Jaruphan , O. Buswaong	Thailand	UFES					
Epyris/Trachepyris sp. 9 (male)	T Jaruphan , O. Buswaong	Thailand	UFES					
Goniozus japonicus	Jongok Lim	South Korea	NA	JX413863.1	JX413908.1	JX413952.1		JX413995.1
Holepyris brevicarinatus	Genbank	NA	NA	JX413867.1	JX413913.1	JX413955.1		JX414000.1
Holepyris sp. 1	K Tsujii	Laos						
Holepyris sp. 10	BL Fisher	Madagascar	UFES/CASC					
Holepyris sp. 11	Carly Tribull	USA	AMNH					
Holepyris sp. 12	A Polaszek	DR Congo	BNHM					
Holepyris sp. 13	A Polaszek	Saba	BNHM/UMS					
Holepyris sp. 14	A Polaszek	Saba	BNHM/UMS					
Holepyris sp. 15	L Leblanc, M Kalamen	Papua New Guinea	CNC					
Holepyris sp. 16	O Frimpong	Ghana	CNC					
Holepyris sp. 17	O Frimpong	Ghana	CNC					
Holepyris sp. 18	Carly Tribull	USA	AMNH					
Holepyris sp. 19	Carly Tribull	USA	AMNH					
Holepyris sp. 2	Not Listed	South Korea	CNC					
Holepyris sp. 20	A Lopez Garcia	Mexico	CNC					
Holepyris sp. 21	A Lopez Garcia	Mexico	CNC					
Holepyris sp. 3	A Polaszek	Saba	BNHM/UMS					
Holepyris sp. 4	BL Fisher	Madagascar	UFES/CASC					
Holepyris sp. 5	T Jaruphan , O. Buswaong	Thailand	UFES					
Holepyris sp. 6	L Leblank, M Kalamen	Papua New Guinea	CNC					
Holepyris sp. 7	ME Irwin, FD Parker	Australia	CNC					
Holepyris sp. 8	S Kimani	Kenya	CNC					
Holepyris sp. 9	C Villament	Mozambique	PNHM					
Holepyris susanowo	Genbank	NA	NA	JX413868.1	JX413914.1	JX413956.1		JX414001.1
Incertosulcus sp. 1	A Polaszek	Saba	BNHM/UMS					
Incertosulcus sp. 2	A Polaszek	Saba	BNHM/UMS					
Laelius sp. 1	Carly Tribull	USA	AMNH					
Laelius sp. 2	ME Irwin	Israel	CNC					
Laelius sp. 3	CNC	NA	NA					
Laelius/Rhabdepyris sp. 1	Bernardo Santos	Chile	AMNH					
Laelius/Rhabdepyris sp. 2	C Azevedo	Brazil	UFES					
Laelius/Rhabdepyris sp. 3	Carly Tribull	USA	AMNH					

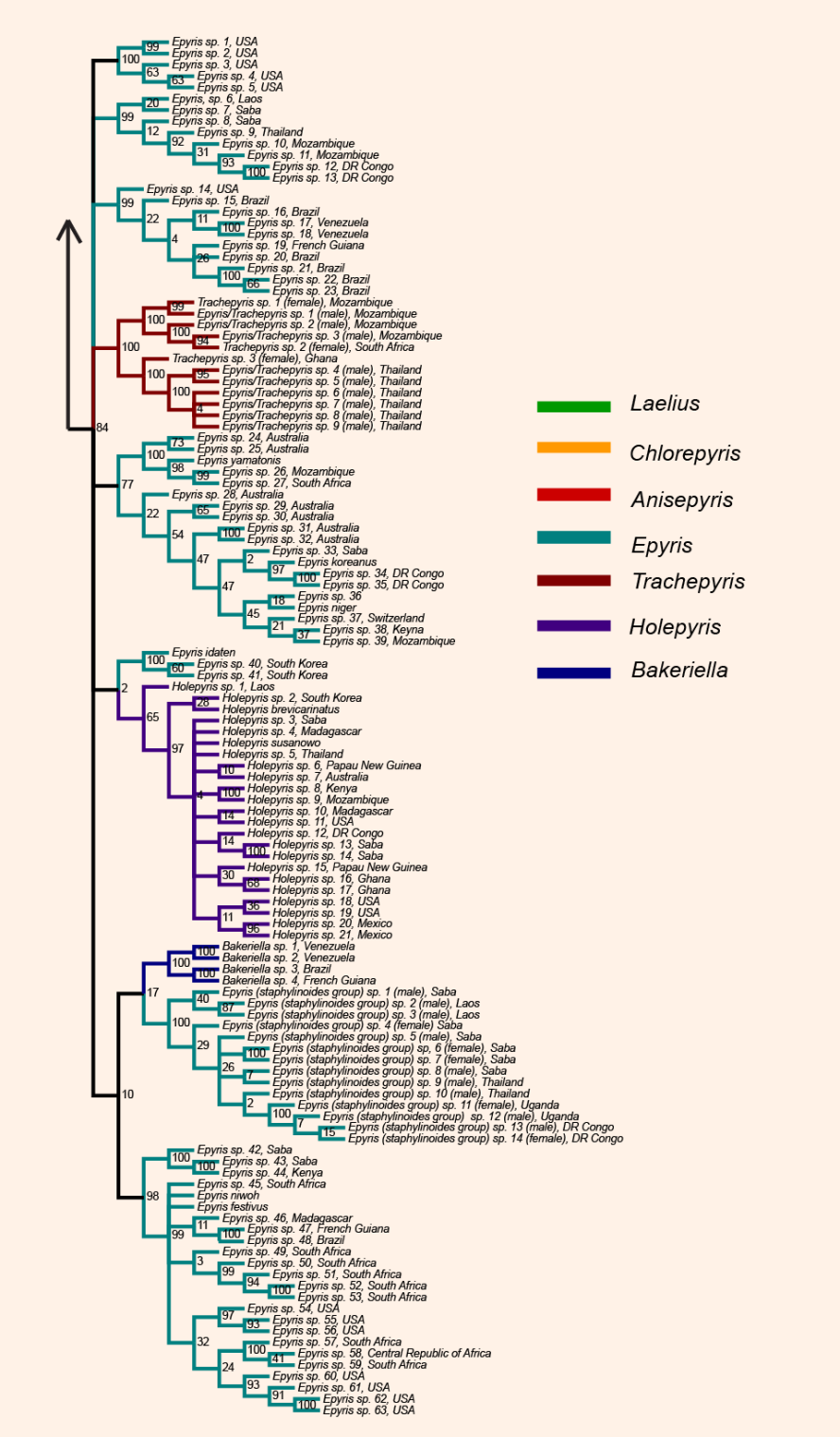
Table 4.1 Continued

Name	Collected by	Country	Institution	16S	18S	28S	COI	CytB
Laelius/Rhabdepyris sp. 4	Carly Tribull	USA	AMNH					
Laelius/Rhabdepyris sp. 5	Carly Tribull	USA	AMNH					
Laelius/Rhabdepyris sp. 6	Carly Tribull	USA	AMNH					
Metrionotus	S Van Noort	South Africa	UFES/S.Afr					
Plastanoxus sp. 1	Genbank	NA	NA	JX413871.1	JX413917.1	JX413958.1		JX414004.1
Dissomphalus sp. 1	R Longair, A Tungbari	Ghana	CNC					
Sclerodermus harmandi	Genbank	NA	NA	JX413873.1	JX413921.1	JX413962.1		JX414008.1
Sulcomesitius sp.	Rosana Nunes	NA	UFES					
Trachepyris sp. 1 (female)	M Olmi	Mozambique	CNC					
Trachepyris sp. 2 (female)	S Van Noort	South Africa	UFES/S.Afr					
Trachepyris sp. 3 (female)	R Longair	Ghana	CNC					

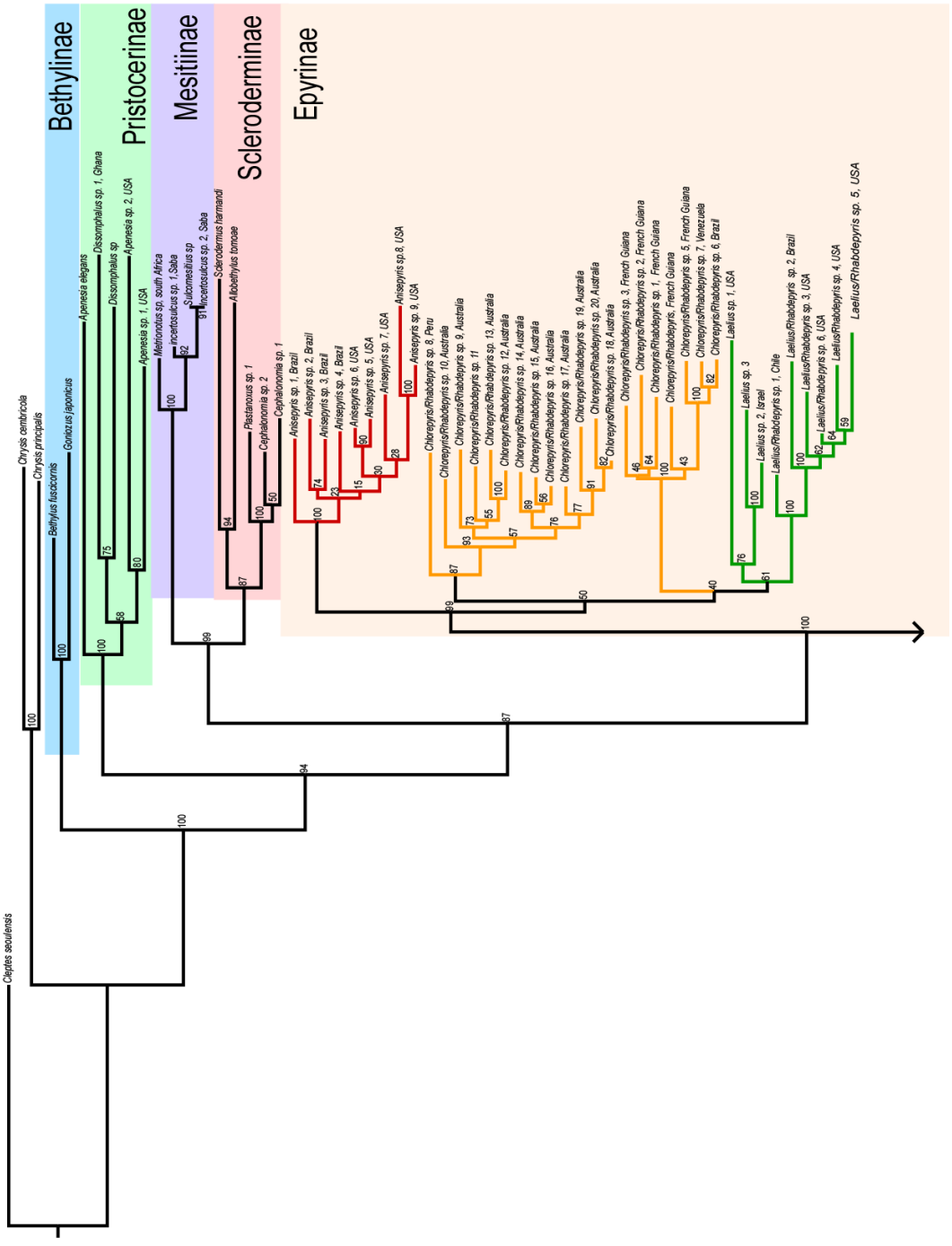
APPENDIX F
EXPANSION PRINTOUTS OF THE PARSIMONY (1), LIKELIHOOD (2), AND BAYESIAN (3) TREES FROM CHAPTER IV



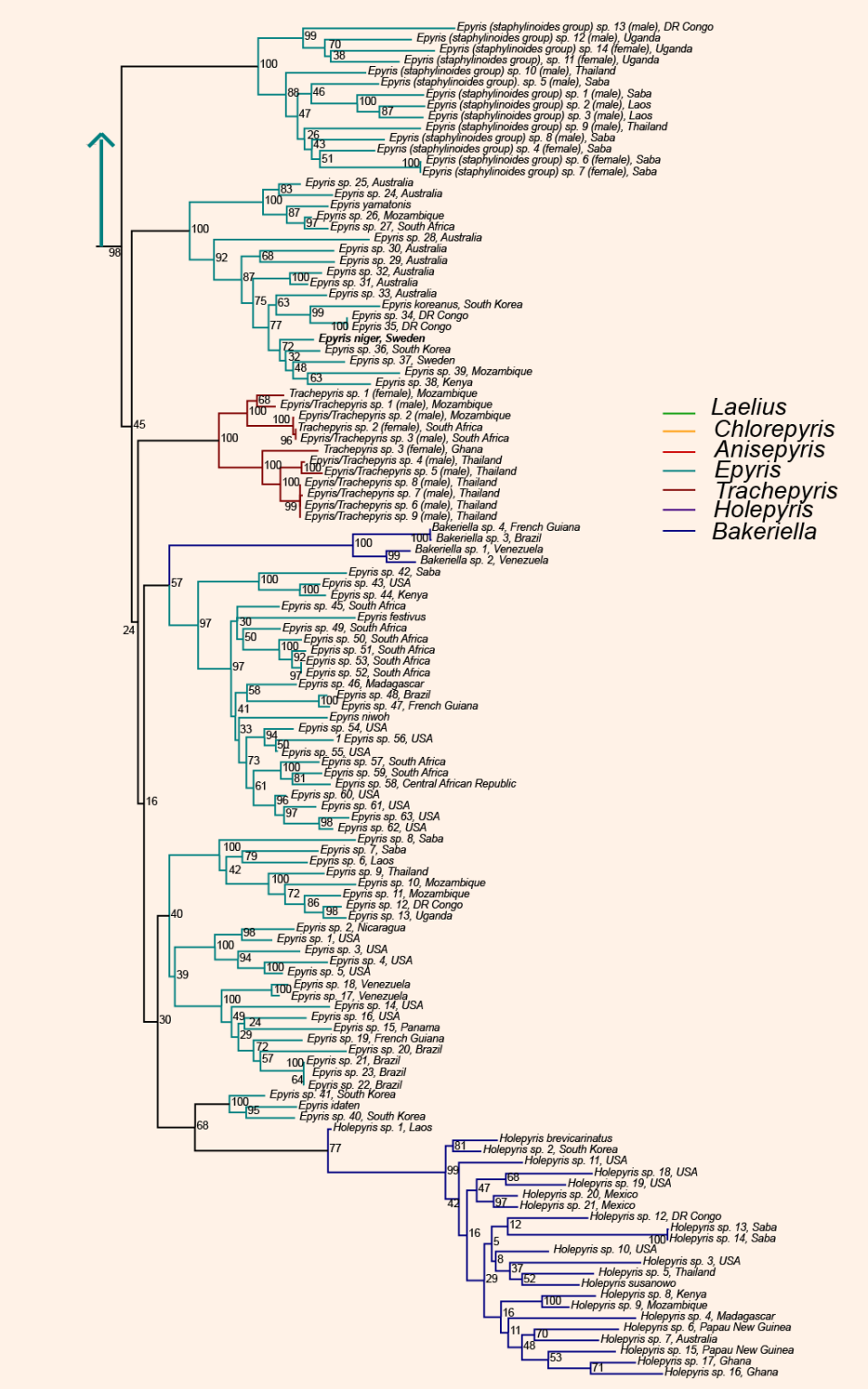
Parsimony Tree, part I. CI .206, RI .570. Symmetric resampling support given in GC-values.



Parsimony Tree, part II. CI .206, RI .570. Symmetric resampling support given in GC-values.

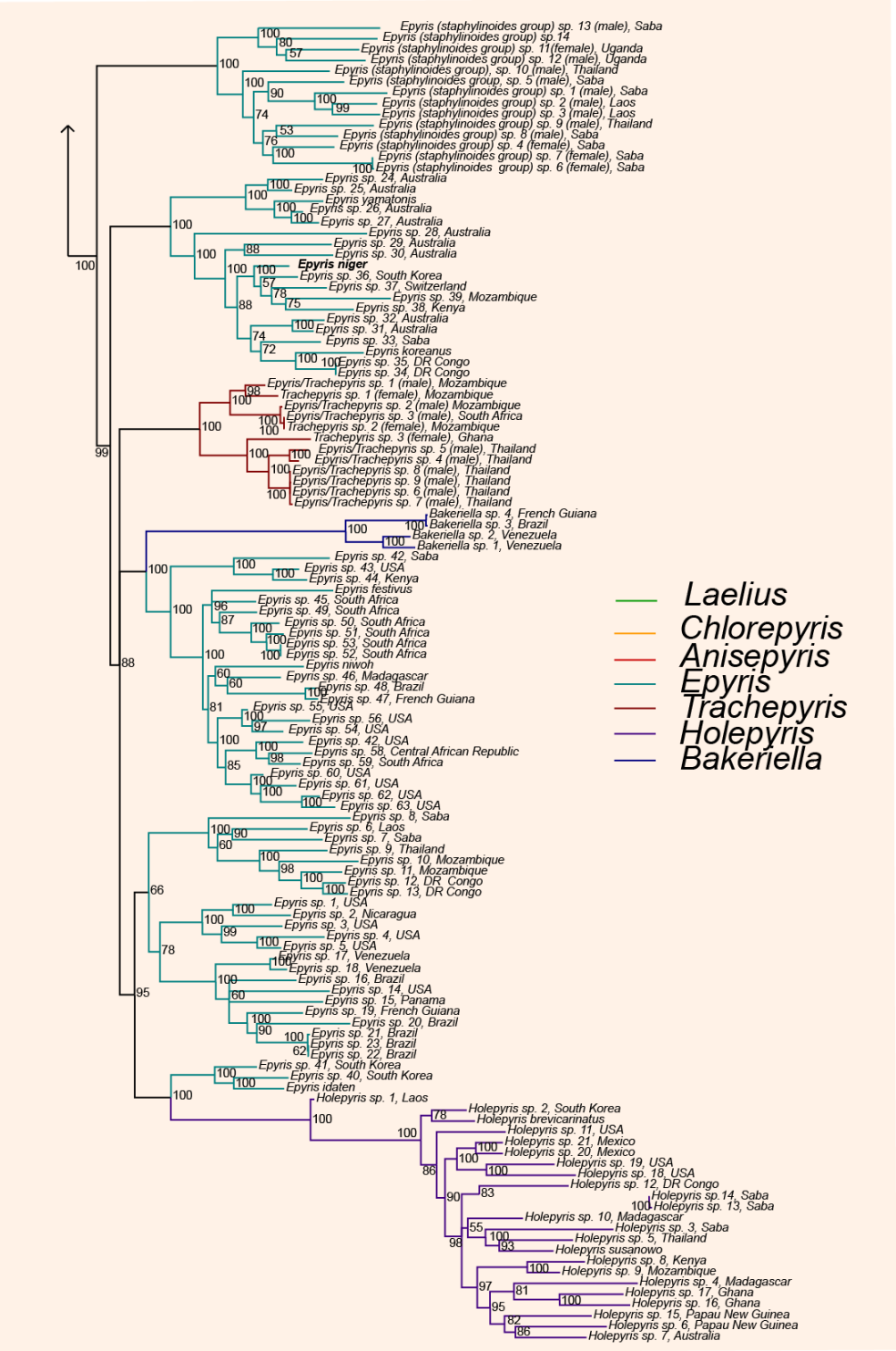


Likelihood tree. Part I. Rapid Bootstrap values shown at nodes.



Likelihood tree. Part II. Rapid Bootstrap values shown at nodes.

Bayesian Tree. Part I. Support probabilities shown at nodes as a percent.



Bayesian Tree. Part II. Support probabilities shown at nodes as a percent.