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Phylogeny of parasitic wasps of Torymidae (Hymenoptera: Chalcidoidea) and evolution of their life-strategies

Fylogeneze parazitických vosiček čeledi Torymidae (Hymenoptera: Chalcicoidea) a evoluce jejich parazitických životních strategií

Ph.D. thesis

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Declaration

I declare, that except for the individual papers in which the authorship and the copyright are mentioned, this Ph.D. thesis is exclusively my own work. The coauthors approved with the inclusion of all individual papers and relevant references are cited. I have used neither the whole thesis nor its substantial part to obtain any other equal academic degree. I agree with the public release of all parts of the thesis except of those bound by the copyright rights elsewhere.

Prague, 12th December 2013 Petr Janšta

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Abstract

The thesis is focused on phylogeny of the family Torymidae (Hymenoptera: Chalcidoidea) and evolution of their life-strategies. The study consists of general introduction to the phylogeny and classification of the family Torymidae chapter, four published papers in international journals and one manuscript prepared for submission. Firstly, our aim was to figure out the phylogenetic position of Torymidae as well as the position of other chalcidoid families inside superfamily Chalcidoidea (paper I and II). The supermatrix of sequencies of two ribosomal genes (18S rDNA and 28S rDNA) were developed for 649 species of chalcidoid taxa. However, family Torymidae was considered as polyphyletic group with the subfamily Megastigminae unrelated to the subfamily Toryminae (paper I). Monophyly of Torymidae was corroborated in another study (paper II) focused on molecular and morphological characters. We used a web-based, systematics workbench mx database for scoring 233 characters of 300 members of all chalcidoid families. Contrary to our previous only DNA-based study, we revealed also potential sister relationships of Torymidae with Ormyridae+Colotrechninae or Cerocephalinae+Diparinae respectively. Other paper (paper V) was focused on detailed study of Torymidae phylogeny. A total of 5 genes (18S rDNA, 28S rDNA, EF1α, COI and Wg) of altogether 226 ingroup taxa representing 45 of the 67 recognized genera from two accepted subfamilies (Megastigminae and Toryminae) of Torymidae were used to reconstruct Torymidae phylogeny. The monophyly of Torymidae was not confirmed again. We recovered only all known tribes and classified two new tribes (i.e. Boucekini, trib. nov. and Glyphomerini trib. nov.) of subfamily Toryminae. Mapping of selected characters onto phylogenetic tree postulated the larvae of Toryminae originally as exoparasitoids of gall-forming insects in Palaearctic region with several derived traits throughout the Toryminae phylogeny. The life strategy, hosts and distribution of the common ancestor of Megastigminae is still uncertain. Besides the phylogenetical studies, the thesis also contains two taxonomic papers (paper III and IV) where two new genera (*Boucekinus* Janšta & Hanson, 2011 and *Chileana* Janšta & Křížková, 2013) and 6 new species from South America are described. These new taxa represent phylogenetically and evolutionarily very interesting and important lineages of Torymidae.

Abstrakt

Dizertační práce se zabývá fylogenezí parazitických vosiček z čeledi Torymidae (Hymenoptera: Chalcidoidea) a evolucí jejich parazitických životních strategií. Celkově práce obsahuje obecný úvod do fylogeneze a klasifikace čeledi Torymidae, čtyři články publikované v mezinárodních vědeckých periodicích a jeden rukopis. Cílem práce bylo navrhnout fylogenetickou pozici Torymidae a ostatních čeledí v rámci nadčeledi Chalcidoidea (článek I a II). Proto byla sestavena supermatice sekvenčních (molekulárních) znaků dvou ribozomálních genů (18S rDNA a 28S rDNA) pro 649 druhů chalcidek. Nicméně bylo zjištěno, že čeleď Torymidae je pravděpodobně polyfyletickou skupinou, kdy podčeleď Megastigminae se jeví jako nepříbuzná podčeledi Toryminae (článek I). Monofylie Torymidae byla potvrzena až v navazující studii (článek II) zaměřené na společnou analýzu jak molekulárních, tak morfologických znaků. Pro tuto publikaci byla využita online databáze pro skórování 233 znaků u 300 zástupců ze všech čeledí Chalcidoidea. Na rozdíl od předešlé studie (článek I) byly také naznačeny možné příbuzenské vztahy čeledi Torymidae s čeleděmi/podčeleděmi Ormyridae+ Colotrechninae nebo Cerocephalinae+Diparinae. Další článek (článek V) byl zaměřen na detailní studii fylogeneze v rámci čeledi Torymidae. Celkem bylo sekvenováno 5 genů (18S rDNA, 28S rDNA, EF1α, COI a Wg) u 226 zástupců reprezentujících 45 ze 67 popsaných rodů ze dvou podčeledí (Megastigminae a Toryminae) čeledi Torymidae. V rámci studie byly uvnitř podčeledi Toryminae potvrzeny všechny dosud uznávané triby a navíc stanoveny dva nové triby (tj. Boucekini, trib. nov. a Glyphomerini trib. nov.). Nicméně monofylii čeledi Torymidae se opět nepodařilo prokázat. Namapováním vybraných znaků na fylogenetický strom bylo zjištěno, že larvy společného předka podčeledi Toryminae byly pravděpodobně původně ektoparazitoidi hálkotvorného hmyzu v palearktické oblasti. Životní strategie, hostitelé a rozšíření společného předka Megastigminae zůstaly nerozřešené. Mimo fylogenetické studie obsahuje dizertační práce také dva taxonomické články (článek III a IV), ve kterých jsou popsány dva nové rody (*Boucekinus* Janšta & Hanson, 2011 a *Chileana* Janšta & Křížková, 2013) a 6 nových druhů z čeledi Torymidae z Jižní Ameriky. Tyto nové taxony představují velmi zajímavé a důležité linie čeledi Torymidae.

Content

1. Aims of the study and list of papers

1. Despite Chalcidoidea importance, our knowledge of phylogeny and evolution of the family Torymidae as well as other families within this superfamily was very insufficient. There was lack of more comprehensive phylogenetical studies of Chalcidoidea and their families using DNA data and broader morphological comparison for centuries. To acomplish this aim we decided to publish two papers:

Paper I: Munro J. B., Heraty J. M., Burks R. A., Hawks D., Mottern J., Cruaud A., Rasplus J.-Y. & **Jansta P.** 2011: A Molecular Phylogeny of the Chalcidoidea (Hymenoptera). *PLOS ONE* 6(11): e27023.

Paper II: Heraty J. M., Burks R. A., Cruaud A., Gibson G. A. P., Liljeblad J., Munro J., Rasplus J.-Y., Delvare G., **Janšta P.**, Gumovsky A., Huber J., Woolley J. B., Krogmann L., Heydon S., Polaszek A., Schmidt S., Darling D. C., Gates M. W., Mottern J., Murray E., Dal Molin A., Triapitsyn S., Baur H., Pinto J. D., van Noort S., George J. & Yoder M. 2013: A phylogenetic analysis of the megadiverse Chalcidoidea (Hymenoptera). *Cladistics* 29(5): 466-542.

2. Second aim of the study is to provide the first molecular study of the family Torymidae. This family is well known as ectoparasitoids of various larvae of gall-forming insects, however, any of relevant phylogenetical reasearch has not been done to compare morphology-based phylogeny and to figure out possible evolution of life strategies inside family. The results are presented in following manuscript:

Paper V: **Janšta P.**, Cruaud A., Delvare G., Křížková B., Heraty J., Rasplus J.-Y: Molecular phylogeny of the family Torymidae (Hymenoptera: Chalcidoidea). Manuscript prepared for submission to *Cladistics*.

3. Although many scientists worked on Torymidae the taxonomy of the family is still very poorly known. Many species and genera remain undescribed. As a results of our foregoing studies, we discovered some new and phylogenetically important taxa. The last aim was to provide descriptions of these peculiar taxa. We described two new genera and several species in following papers:

Paper III: **Janšta P.**, Vilímová J. and Hanson P. 2011: Description of a new genus, *Boucekinus* (Hymenoptera: Chalcidoidea: Torymidae), with two new species and a discussion of its possible phylogenetic placement. *Zootaxa* 2762: 49-55.

Paper IV: **Janšta P.**, Křížková B., Vilímová J., Rasplus J.-Y. 2013: Description of a new genus, *Chileana* (Hymenoptera: Chalcidoidea: Torymidae), with four new species. *Zootaxa* 3745: 49-63.

2. Introduction

Phylogenetic position of superfamily Chalcidoidea within the Hymenoptera

Chalcidoidea (commonly named as chalcidoids or chalcids) is extremely diverse superfamily within order Hymenoptera. More than 22500 species have been described and more than 500000 are estimated to exist (Heraty et al. 2013; Noyes 2013). Without any doubt, superfamily Chalcidoidea belongs to apocritan Hymenoptera and within clade Proctotrupomorpha (Heraty et al. 2011; Sharkey et al. 2012).

Rasnitsyn (1988) devided Apocrita, based on morfological and fossil characters, into four lineages (Ichnemonomorpha – today´s Ichneumonoidea, Vespomorpha – today´s Aculeata, Proctotrupomorpha and Evanimorpha). Later, based also on paleontological evidence, Evanimorpha were divided into three monophyletic lineages – Stephanomorpha, Ceraphronomorpha and Evanimorpha (Rasnitsyn and Zhang 2010). The most recent molecular or molecular and morphology combined studies (Sharanowski et al. 2010; Heraty et al. 2011; Sharkey et al. 2012) support monophyly of only some of morphological clades sensu Rasnitsyn (1988), i.e. Aculeata and Ichneumonoidea, which is sister to monophyletic Proctotrupomorpha. Superfamily Chalcidoidea was repeatedly included as a part of Proctotrupomorpha, together with Platygastroidea, Cynipoidea, Proctotrupoidea, Mymarommatoidea and Diaprioidea (Castro and Dowton 2006; Sharkey 2007; Heraty et al. 2011; Sharkey et al. 2012).

There are several opinions on sister relationships of Chalcidoidea and the rest of Proctotrupoidea lineages. First phylogenetic hypothesis (Dowton and Austin 1994; Dowton et al. 1997) which were reconstructed based on molecular data (16S rRNA) postulated sister relationships of Chalcidoidea and Platygastridae (Platygasteroidea respectively). Also morphology and anatomy evidence showed similar results (Heraty et al. 1994).

Importantly, Gibson et al. (1999) and Ronquist et al. (1999) evaluated family Mymarommatidae as superfamily Mymarommatoidea and postulated it sister clade of Chalcidoidea. Heraty et al. (2011) were the first who sequenced representatives of superfamily Mymarommatoidea. Molecular analysis nested Chalcidoidea in one monophyletic clade as sister either Mymaromatoidea or Diaprioidea. Chalcidoidea as monophyletic clade together with Mymarommatoidea and Diaprioidea within Proctotrupomorpha based on molecular data were corroborated by Munro et al. (2011). Strongly supported monophyly of Mymarommatoidea as sister group of Chalcidoidea was confirmed when combination of molecular and morphology evidences were used (Sharkey et al. 2012).

Biology and history of classification of Chalcidoidea and position of the family Torymidae

As mentioned above, Chalcidoidea are very diverse group of insects. They range in size from 20 mm (42 mm including ovipositor) – *Doddifoenus* Bouček, 1988 (Krogmann and Burks 2009) to minute, only 0.13 mm long male of *Dicopomorpha echmepterygis* Mockford, 1997, the smallest insect known at all (Mockford 1997). Chalcid wasps are very plastic morphologically and many family and subfamily characters are not disjunctive (Gibson et al. 1999). Many morphological characters are strongly convergent and therefore enlarged femora, reduction of wings, antennal and tarsal segments and metallic coloration can be found in many very distantly related taxonomic groups (Bouček 1988).

Morphological diversity and enormous number of species of chalcidoids are reflected also by their biological and feeding strategies. The animal host range includes all life-history stages of 13 insects ordes as well as mites (genus *Ixodiphagus* Howard, 1907 attacks larvae of *Ixodes rhicinus* Linneaus, 1758), egg-sacs of spiders, cocoons of pseudoscorpions, and gall-forming Anguinidae (Nematoda) (Heraty et al. 2013). Moreover, phytophagous chalcidoids from 6 extant families are known: Agaonidae, Eulophidae, Eurytomidae, Pteromalidae, Tanaostigmatidae and Torymidae (Austin et al. 1998; Gibson et al. 1999).

Females and males of some species of Agaonidae, known as fig wasps, represent extreme case of adaptation to phytophagous life. Most females have mesosternal pollen pockets and mandibular combs for collecting of figs pollen grains and males are adapted to life inside fig syconium, i. e. they can be nearly blind with various apendages reduced, very different from females of Agaonidae and hardly recognizable as chalcidoids (Cruaud et al. 2010).

Regardless to relatively numerous paleontological evidence of chalcids in mid- and lower Cretaceous amber including Alaskan, Campanian, Siberian, Charantes, and Burmese, only few have been examined critically. Therefore Chalcidoidea is one of the most extensively studied lineage in Mesozoic records (McKellar and Engel 2012). First Mymaridae are known from Lebanese amber, 120 – 125 Ma (Schmidt et al. 2010), both Mymaridae and Mymarommatoidea occured in Albian amber, 97 – 110 Ma (Poinar Jr. and Huber 2011), Eulophidae taxa have been found later, in mid Cretaceous Ethiopian amber

(Schmidt et al. 2010). The only known extinct family, the Khutelchalcididae (Chalcidoidea), was described from an impression fossil from lowermost Cretaceous or uppermost Jurassic deposits of Khutel-Khara in East Mongolia (Rasnitsyn et al. 2004), but Gibson et al. (2007) showed that it does not belong to the superfamily Chalcidoidea at all. According to Heraty and Darling (2009), greater diversity of Chalcidoidea does not appear until the Eocene. The most of extant families inluding Eucharitidae, Perilampidae, Pteromalidae and Torymidae appeared in that time. Ot the contrary, McKellar and Engel (2012) published records of 'Eupelmidae?' and 'Torymidae?' from Late Cretaceous amber. However, it is generally accepted that Chalcidoidea have undergone rapid post-Cretaceous diversification as well as angiosperms and other insects (Heraty and Darling 2009; Heraty et al. 2013).

The classification of superfamily Chalcidoidea into families has been very unstable during a history probably due to enormous morphological variability and convergence of chalcidoids characters. Number of recognized families varied from 9 to 24. First comprehensive critical evaluations of morphological characters have not been proposed till Bouček (1988) and Gibson et al. (1999). Second author established 20 families of Chalcidoidea but stated that there are hardly any reliable morphological synapomorphies for any of the recognized families. Later, Elasmidae has been added to Eulophidae (Gauthier et al. 2000). Finally, there are 22 extant families of Chalcidoidea generally accepted now (Agaonidae, Aphelinidae, Azotidae, Chalcididae, Cynipencyrtidae, Encyrtidae, Eriaporidae, Eucharitidae, Eulophidae, Eupelmidae, Eurytomidae, Leucospidae, Mymaridae, Ormyridae, Perilampidae, Pteromalidae, Rotoitidae, Signiphoridae, Tanaostigmatidae, Tetracampidae, Torymidae, and Trichogrammatidae) (Aguiar et al. 2013; Heraty et al. 2013).

Campbell et al. (2000) were first to publish preliminary phylogeny of Chalcidoidea based on molecular characters. They studied 28S rDNA of relatively small number (109 species) of chalcid wasps from 18 families and confirm only some of morphologicaly established groups of the superfamily Chalcidoidea. Much more comprehensive molecular analysis of Chalcidoidea has been published by Munro et al. (2011) using sequencies of two gene regions of ribosomal DNA (18S rDNA and 28S rDNA) of 666 chalcidoid taxa and 56 outgroups. They confirmed monophyly of several families (Agaonidae, Encyrtidae, Eucharitidae, Leucospidae, Mymaridae, Ormyridae, Signiphoridae and Trichogrammatidae), but others were postulated as paraphyletic (Perilampidae) or polyphyletic (Aphelinidae, Chalcididae, Eupelmidae, Eurytomidae, Pteromalidae, Tetracampidae and Torymidae). Mymaridae as sister group to remaining Chalcidoidea was very strongly supported.

In corcordance with Gibson and Huber (2000), the other most basal family after Mymaridae was nested Rotoitidae, a family known only from species *Rotoita basalis* Bouček & Noyes, 1987 endemic to New Zealand and *Chiloe micropteron* Gibson & Huber, 2000 known only from Chile.

First phylogenetic analysis based on both morphological and molecular data has been published recently (Heraty et al. 2013). Using web based content management mx database (Yoder at al. 2006), they scored 233 morphological characters for 300 chalcidoid taxa and 4 outgroups. With some minor exclusions or inclusions, they indicated monophyly of nearly all of previously recognized families sensu Noyes (2013). Aphelinidae, Perilampidae, Pteromalidae and Trichogrammatidae were postulated as polyphyletic and families Azotidae, Eriaporidae and Cynipencyrtidae were suggested as newly recognized monophyletic groups/families. Mymaridae as sister group of Rotoitidae plus remaining Chalcidoidea were also supported based on combined characters analyses (Fig. 1) as well as Rotoitidae following Mymaridae as sister group to the rest of Chalcidoidea. Synapomorphies (usually present in most of the members) which supporting monophyly of Chalcidoidea are multiporous plate sensilla on flagellar segments, presence of an exposed prepectus between lateral sides of pronotum and mesonotum and position of mesothoracic spiracle.

Taxonomy of Torymidae

As well as of many Chalcidoidea families, the taxonomy of Torymidae has changed since the family was described (Walker 1833) and genera *Megastigmus* Dalman, 1820, *Priomerus* Waker, 1833 (synonymum of *Podagrion* Spinola, 1811), *Torymus* Dalman, 1820 and *Callimome* Spinola, 1811 (synonymum of *Torymus*), *Ormyrus* Westwood, 1832 and *Perilampus* Latreille, 1809 were included. Förster (1856) described some other genera of Torymidae and excluded *Ormyrus* and *Perilampus* as members of family Ormyridae and Perilampidae, respectively.

Thomson (1876) divided Torymidae into three subtribes – Megastigmines, Torymides and again Ormyrides and characterised family based on presence of occipital carina, and some other characters. Ashmead (1899, 1904) upgraded Thomson´s subtribes to subfamily levels, reclassified some groups and distinguished Monodontomerinae, Idarninae [Agaonidae: Sycophaginae sensu Cruaud et al. (2011) and Heraty et al. (2013)], Toryminae, Megastigminae, Podagrioninae and Ormyrinae [Ormyridae sensu Bouček (1957)]. Few years

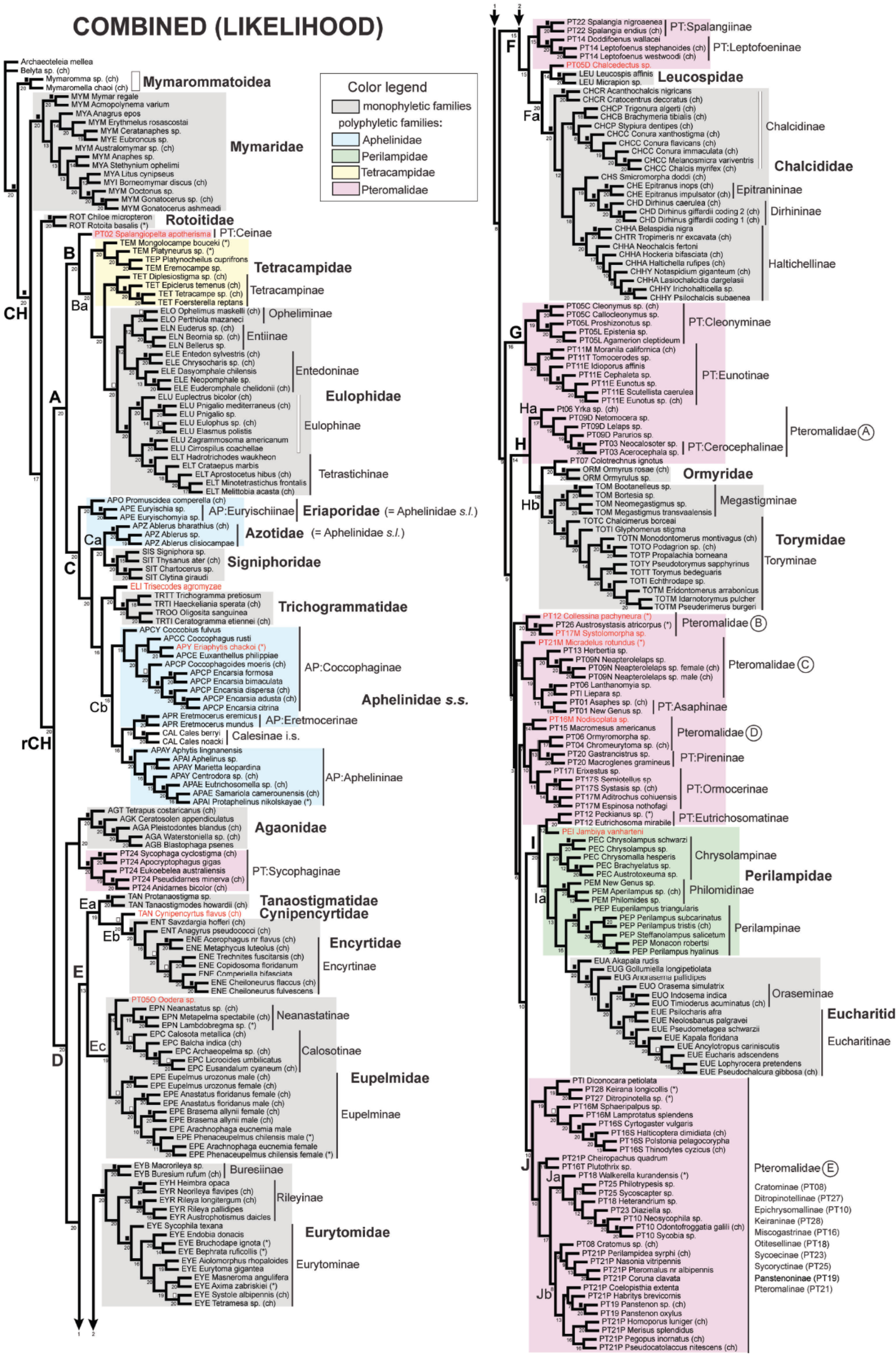

Fig. 1. The most recent phylogeny of superfamily Chalcidoidea based on morphological characters and 18S rDNA and 28S rDNA genes (from Heraty et al. 2013).

later, Crawford (1914) proposed Erimerinae, Peck et al. (1964) Thaumatoryminae, and Hill (1967) Epichrysomallinae as other new subfamilies of Torymidae.

Twenty four years later, Bouček (1988) postulated new classification of Torymidae and suggested synapomorphies of his concept of family. Idarninae and Epichrysomallinae excluded of family Torymidae and accepted only Monodontomerinae, Megastigminae, Toryminae and Thaumatoryminae. He delimited Torymidae by the following synapomorphies: (1) presence of horseshoe like occipital carina; (2) dorsally very short seventh female tergite [(eighth sensu Grissell (1995)] and separated from the eighth [(ninth sensu Grissell (1995)] tergite by a membraneous line; (3) the membrane (membraneous line) on sides expanded and bearing the mobile elongate cercus with long sensorial setae; (4) tergite eight [(nine sensu Grissell (1995)] short, flap-like and weakly sclerotised and (5) the postgenae expanded to the median line so that the hypostomal margins are medially strongly constricted or even fused below occipital foramen [not an exclusive character, because Bouček (1988) found it as synapomorphy of Agaonidae and Torymidae].

Detailed history of classification of the family Torymidae is summarized in Bouček (1988) and later in Grissell (1995), who also critically discussed all synapomorphies used previously (Bouček 1988):

Presence of occipital carina (in Bouček sense synapomorphy No. 1) – the character is homoplasy, having been lost in some Torymidae and is found in some Pteromalidae, some Agaonidae, all Ormyridae and *Echthrodape* Burks, 1969 [*Echthrodape* is one of the mysterious genus of Chalcidoidea and before Grissell (1995) it was assigned to different families repeteadly];

Dorsally very short eighth female tergite, separated from the ninth tergite by a membraneous line (in Bouček sense synapomorphy No. 2) – could be exclusive synapomorphy of Torymidae, but same structure was found also in genus *Chromeurytoma* Cameron, 1912 (Pteromalidae) and *Echthrodape*;

Elongated (excerted) cercus with long sensorial setae in females (in Bouček sense synapomorphy No. 3) – the same structure can be found in *Echthrodape*, few Pteromalidae (*Chromeurytoma*, Sycoryctinae, Epichrysomalinae, Sycoecinae), Eulophidae, Eupelmidae and Agaonidae (Sycophaginae);

Ninth female tergite is short and flap-like (in Bouček sense synapomorphy No. 4) – this state was recorded also in few Agaonidae (Sycophaginae), Pteromalidae (*Chromeurytoma* and Ceinae) and in *Echthrodape*;

Postgenae expanded to the median line so that the hypostomal margins are medially strongly constricted or even fused below occipital foramen (in Bouček sense synapomorphy No. 5) – defined as synapomorphy of Torymidae and Agaonidae (Bouček 1988), but Grissell (1995) found out this state also in all Ormyridae, some Pteromalidae and in *Echthrodape*.

As showed on information mentioned above, Grissell (1995) doubted all of previously respected single synapomorphies. But he believed in monophyly of family in sense of combination of all five states. Combination of these characters as characteristic of Torymidae was adopted also by Gibson et al. (1999).

In molecular studies, Torymidae has never been recovered as one monophyletic group. Campbell et al. (2000) recognized even subfamily Toryminae within Torymidae as polyphyletic group. However, this study was made using very limited taxon sampling (6 species – 2 species of Megastigminae and 4 species of Toryminae) and only one gene (28S rDNA). Also Munro et al. (2011) failed to find Torymidae as monophylum, but Toryminae and Megastigminae resulted each monophyletic with very strong support.

Later, Heraty et al. (2013) have not doubted monophyly of family in any of combined analysis. Monophyly of the family was supported by combination of the following morphological characters: sulci extended from tentorial pits, mesepimeron with posterior margin notched, fore wing with basal lobe present and Rs absent, basitarsus with ordinary setae, metasomal tergites 8 and 9 articulating, cercus arising from membraneous area and valvifers without sclerotized bridge between them.

Phylogenetic position Torymidae within Chalcidoidea

Historicaly, several groups have been added as sister clade to the family Torymidae more or less intuitively (Noyes 1990) or based on convergencies (Cruaud et al. 2010, 2011) but without any certain morphological support (i.e. Ormyridae, Agaonidae, Epichrysomalinae, Sycophaginae).

Bouček (1988) mentioned that Ormyridae could be very close relative to the Torymidae based on shape of occipital carina in corcondance with an intuitive cladogram of Noyes (1990), who put together Torymidae plus Ormyridae and Agaonidae plus Ormocerinae (Pteromalidae) as sister clade. Grissell (1995) added only species from the family Pteromalidae to his phylogenetic analysis of Toryminae without any knowledge of their phylogenetic position, just based on his and of his colleague Steve Heydon intuitive knowledge. Gibson et al. (1999) have not commented any potencially sister groups of Torymidae, just stated that relationships and proper classification of Torymidae, Ormyridae and Agaonidae are one of the more perplexing issues of chalcidoid classification. No logical sister group, neither for Megastigminae nor Toryminae, have been revealed even by first more comprehensive molecular characters analysis (Munro et al. 2011). However, combined morphological and molecular evidence (Heraty et al. 2013) repeatedly settled Torymidae in one clade with Ormyridae plus Colotrechninae (Pteromalidae). Ormyridae has never been supported as a group within Torymidae.

Suprageneric classification of Torymidae (based on morphology only)

Generally respected concept of suprageneric classification of Torymidae has followed Bouček (1988) for long time. As it was mentioned above, he accepted subfamilies Monodontomerinae, Megastigminae, Toryminae and Thaumatoryminae within the family. More comprehensive study was done by Grissell (1995) who made phylogenetic analysis of 24 morphological characters. It was first such study of family Torymidae (respectively Toryminae) and the first comprehensive one in the superfamily Chalcidoidea.

Grissell (1995) assumed the monophyly of family Torymidae although he doubted Bouček´s (1988) synapomorphies of family. He hypothesized monophyly of Megastigminae based on enlarged stigma of fore wing and bilobed clypeus [few genera deviate in having clypeus with one median tooth – *Bortesia* Pagliano & Scaramozzino, 1990 and *Bootanelleus* Girault, 1915 (Bouček 1988)] while the subfamily Toryminae was defined only by the absence of apomorphic features, i.e. by symplesiomorphies. Later, the group was divided into seven tribes based on morphology – Chalcimerini, Microdontomerini, Monodontomerini, Palachiini, Podagrionini, Torymini, and Torymoidini and some genera were left classified as incertae sedis within Toryminae (i.e. *Cryptopristus* Förster, 1856, *Echthrodape*, *Exopristus* Ruschka, 1923, *Glyphomerus* Förster, 1856, *Stenotorymus* Masi, 1938, *Thaumatorymus* Ferrière and Novicky, 1954 and *Zaglyptonotus* Crawford, 1914).

Subfamily Megastigminae

Altogether 198 species in 12 genera (*Bootanelleus* – 13 spp., *Bootania* Dala Torre, 1897 – 12 spp., *Bootanomyia* Girault, 1915 – 22 spp., *Bortesia* – 3 spp., *Ianistigmus* Bouček, 1988 – 1 sp., *Macrodasyceras* Kamijo, 1962 – 2 spp., *Malostigmus* Bouček, 1988 – 1 sp., *Mangostigmus* Bouček, 1986 – 3 spp., *Megastigmus* Dalman, 1820 – 131 spp., *Neomegastigmus* Girault, 1915 – 7 spp., *Paramegastigmus* Girault, 1915 – 1 sp., *Westralianus*

Bouček, 1988 – 2 spp.) are recognized in this subfamily (Bouček 1988; Grissell and Desjardins 2002).

Megastigminae are distributed worldwide, but they are most diverse in Australian region (Bouček 1988; Noyes 2013). Bouček (1988) listed 8 genera distributed only there and two genera (*Mangostigmus* and *Bootania*) occur in both, Australian and Oriental regions. *Macrodasyceras* is restricted only to Japan (Grissell and Desjardins 2002) and genus *Megastigmus* shows worldwide distribution (Bouček 1988; Noyes 2013). Recently, all metalic coloured palaearctic species of *Megastigmus* were tranfered to the genus *Bootanomyia* Doğanlar (2011a) sensu Bouček (1988) and also new species of *Westralianus* from Turkey has been described (Doğanlar 2011b).

Majority of species of the genus *Megastigmus* has phytophagous larvae feeding in coniferous seeds. There are only few species reported from angiosperm seeds (Auger-Rozenberg et al. 2006, Grissell 1999). All species of the genus *Megastigmus* which has been previously mentioned as parasitoids of various gallmakers (mostly larvae of Cynipidae), are now synonymized with *Bootanomyia* (Doğanlar 2011a). As it is known (Bouček 1988), there are only seedseaters within members of the genus *Bootania* and *Macrodasyceras*. *Bootanelleus* has some species which are either parasioids of gall makers or seedeaters. It is supposed that *Bortesia*, *Mangostigmus* and *Neomegastigmus* are also parasitoids of cecidomyiid larvae in galls. *Westralianus altinoezus* Doğanlar, 2011 were reared from unknown Lepidoptera genus galling on *Crataegus monogyna*, thus this genus is considered as zoophagous (Doğanlar 2011b). Biology of any of species from genera *Malostigmus* and *Paramegastigmus* is not known.

Grissell (1999) speculated that phytophagous strategy could be most primitive condition for Megastigminae and wasp species could arose first through gymnosperm, radiated to angiosperm seed, then to insects in seed pods and finally to insects in galls. Similar studies were not performed, only Auger-Rozenberg et al. (2006) discovered (based on cytb and 28S rDNA genes) that species of *Megastigmus* associated with coniferous seeds are monophyletic group, sister to angiosperm feeding group and entomophagous species. The only morphological characters of adults, which separate phytophagous genera from entomophagous, is non-metallic (respectively metallic) coloration (Bouček 1988).

Subfamily Toryminae sensu Grissell (1995)

As mentioned above, Grissell (1995) made the most comprehensive study on Toryminae based on morphology and merged several subfamilies (Erimerinae, Monodontomerinae, Toryminae and Thaumatoryminae) into one subfamily Toryminae and divided the latter to seven tribes: Chalcimerini, Microdontomerini, Monodontomerini, Palachiini, Podagrionini, Torymini, and Torymoidini. However, some genera were left as *incertae sedis*. Today, we distinguish altogether 55 extant genera and 3 genera which are known only from fossil records (Grissell 1995; Janšta et al. 2011, 2013; Noyes 2013).

Tribe Microdontomerini contains 104 species described in nine genera (Grissell 1995, 2005; Askew et al. 2007; Doğanlar and Doğanlar 2008; Narendran et al. 2012; Noyes 2013). Grissell (1995) suggested tribe Microdontomerini as monophyletic and more closely related to Torymoidini and Torymini than to Monodontomerini. It is defined by marginal vein 2 to 4.5X as long as length of stigmal vein and 1.5 to 2.5X as long as postmarginal vein; occipital carina is absent or vaguely indicated. In this tribe following genera are classified: *Adontomerus* Nikolskaja, 1955 – 9 spp., *Ditropinotus* Crawford, 1907 – 2 spp., *Eridontomerus* Crawford, 1907 – 12 spp., *Erimerus* Crawford, 1914 – 1 sp., *Idarnotorymus* Masi, 1916 – 2 spp., *Idiomacromerus* Crawford, 1914 – 42 spp., *Microdontomerus* Crawford, 1907 – 24 spp., *Ophiopinotus* Husain & Kudesia, 1987 – 1 sp., *Pseuderimerus* Gahan, 1919 – 11 spp. But since some characters used to separate genera of Microdontomerini (Grissell 1995) tend to be symplesiomorphic, status of few genera is questioned [i.e. *Adontomerus* and *Idiomacromerus* (Askew et al. 2007) and *Microdontomerus* and *Idiomacromerus* (Grissell 2005)].

The tribe has mostly holarctic distribution with some genera being only palaearctic (*Adontomerus*, *Idarnotorymus* and *Idiomacromerus*). Strictly nearctic genus is *Erimerus*. There are some records of *Eridontomerus biroi* Ruschka, 1923 from Argentina, but most probably it has been introduced by european colonialists (Janšta et al. – in prep.).

Questionable are that of *Idiomacromerus gallicola* (Risbec, 1952) from Afrotropical region and *I. insuetus* (Gahan, 1917) from neotropics, which are most probably either introduced or misidentified (Grissell 1995). *Ophiopinotus* is a controversal genus described probably from India, but there is no locality and no type depository mentioned in description and probably nobody had seen this taxon since the genus was described (Husain and Kudesia 1987). Grissell (1995) assigned this genus to the Microdontomerini based on the wing venation, no occipital carina and angulate hind femur.

Generally, Microdontomerini has very broad host range. *Adontomerus* and *Microdontomerus* parasitise mostly larvae of bees, caterpillars and larvae of gall wasps (Hymenoptera: Cynipidae) in galls (Askew et al. 2004, 2006; Grissell 2005). With some exceptions, larvae of *Idiomacromerus* are feeding on immature stages of gall-forming insects (Nieves-Aldrey et al. 2007; Noyes 2013). There is only one host record of *I. gregarius* (Silvestri, 1943), which recognize it as endoparasitoid of *Mengenilla quaesita* (Strepsiptera: Mengenillidae) (Silvestri 1943). *Idiomacromerus iridis* (Picard, 1930) is commonly reared from egg cases of *Iris* spp. (Mantodea) in Mediterranean (Janšta pers. obs., Delvare – pers. comm). Genera *Ditropinotus*, *Eridontomerus*, *Erimerus*, *Pseuderimerus* and *Idarnotorymus* are commonly mentioned as parasitoids of gall-forming Eurytomidae (Hymenoptera: Chalcidoidea) or Cecidomyiidae (Diptera) in grass stems (Grissell 1995; Burks and Redak 2004; Janšta and Bouček 2006; Noyes 2013).

Altogether six genera and 111 species are known in tribe Torymoidini (Grissell 1995; Xiao et al. 2007; Xiao and Zhao 2010; Janšta et al. 2011). The tribe is considered being monophyletic (Grissell 1995) based on straight anterior metapleural margin and relatively long marginal vein (6-12 times longer than stigmal vein and 3-7 times longer than postmarginal vein). Grissell (1995) stated that phylogenetic position of this tribe is unclear. Characters of some species could shift Torymoidini close to Microdontomerini (to the genera *Microdontomerus* and *Idiomacromerus*), but the shape of marginal vein and occipital carina classify Torymoidini closer to Torymini. Currently, genera *Aloomba* Girault, 1921 – 1 sp., *Boucekinus* Janšta & Hanson, 2011 – 2 spp., *Platykula* Huber, 1927 – 1 sp., *Pseudotorymus* Masi, 1921 – 50 spp., *Torymoidellus* Bouček, 1988 – 1 sp. and *Torymoides* Walker, 1871 – 56 spp. are recognized.

Species of genera *Pseudotorymus* and *Torymoides* have mostly palaearctic or holarctic distribution, but some of them are known from Australian region (half of known species of the genus *Torymoides*), Afrotropical, Neotropical and Oriental regions. Genus *Aloomba* and *Torymoidellus* are known exclusivelly from Australian region and *Boucekinus* and *Platykula* from neotropics, respectively from Neotropical and Nearctic regions (Bouček 1988; Janšta et al. 2011).

Host range of nearly of all known genera is very wide. However, as it is known most of them are parasitoids of larvae of gall-inducing insects from Diptera (mostly Cecidomyiidae and Tephritidae) and Hymenoptera (Cynipidae and Eurytomidae). Some species attack larvae of Lepidoptera (Grissell 1995).

There has been described 11 genera and 78 species in tribe Monodontomerini (Sureshan and Narendran 1996; Grissell 2000; Zerova and Grissell 2000; Tarla et al. 2010;

Janšta et al. 2013). Following genera are classified in this tribe: *Amoturoides* Girault, 1932 – 2 spp., *Anneckeida* Bouček, 1978 – 6 spp., *Chileana* Janšta & Křížková, 2013 – 4 spp., *Chrysochalcissa* Girault, 1915 – 5 spp., *Monodontomerus* Westwood, 1833 – 44 spp., *Oopristus* Steffan, 1968 – 3 spp., *Perissocentrus* Crawford, 1910 – 6 spp., *Pradontomerus* Bouček, 1978 – 1 sp, *Rhynchodontomerus* Novicky & De Santis, 1961 – 1 sp., *Rhynchoticida* Bouček, 1978 – 5 spp. and *Zdenekius* Grissell, 1993 – 1 sp.

The genus *Monodontomerus* is mostly distributed in Holarctic region. *Amoturoides*, *Anneckeida*, *Chrysochalcissa* and *Rhychoticida* are spread through Afrotropical, Australian and Oriental regions. *Oopristus* is known from Oriental and Palaearctic region. *Pradontomerus* is distributed only in Afrotropical region. The rest of genera is known only from New World. *Zdenekius* is genus endemic to Nearctic region, while *Perissocentrus*, *Rhynchodontomerus* and *Chileana* are distributed only in neotropics (Janšta et al. 2013; Noyes 2013).

The tribe is morphologically defined by marginal vein 4 to 6.5 times the length of stigmal vein and 1.5 to 3 times length of postmarginal vein and the occipital carina dorsally flat, closer to the occipital foramen than to hind ocelli and touching or nearly touching the hypostomal carina at its median point (Grissell 1995, 2000).

The most of species of the tribe Monodontomerini (particularly genera *Monodontomerus*, *Perissocentrus*, *Rhychodontomerus*, *Pradontomerus*, part of *Amoturoides*) are exoparasitoids of larvae of solitary aculeate bees, wasps, sawflies and moths including their tachinid and ichneumonid parasitoids. Genera like *Chrysochalcissa*, *Oopristus*, *Rhynchoticida* and part of *Amoturoides* are egg parasitoids of various Heteroptera (Coreidae, Pentatomidae) or Orthoptera (Tettigonidae). Biology of the genus *Anneckeida* is still unknown (Bouček 1978; Grissell 1992, 1995, 2000).

Tribe Palachini was desigated by Bouček (1976) and includes 30 species in three genera (Grissell 1995; Bouček 1998; Narendran and Peter 2009). Genus *Palachia* Bouček, 1970 (25 spp.) and genus *Propalachia* Bouček, 1978 (3 spp.) are known mostly from Afrotropical and Oriental regions while *Palachia pulchra* Bouček, 1970 is the only species known from Palaearctic region. Genus *Neopalachia* (2 spp.) is distributed in neotropics.

Biology of species of this tribe is almost unknown. There is only one host records that *Palachia mangalae* Narendran, 1984 was reared from *Mantis* sp. egg case (Mantodea) (Narendran 1984).

Grissell (1995) defined this tribe based on hind femur either narrow with 2 or more prominent teeth or enlarged with a row of teeth, hind tibia slightly to noticeably curved and with 2 apical spurs, hind-tibial apex truncate or diagonal, propodeum without spiracular sulcus or, if present, oriented towards the lateral corner, metasternal shelf present and metasomal terga not laterally emarginated.

The tribe Chalcimerini, firstly proposed by Bouček (1978), is monotypic tribe including only single genus with single species – *Chalcimerus borceai* Steffan & Andriescu, 1962. Grissell (1995) confirmed his tribal status and defined its following autapomorphies – shortened marginal vein subequal in length to the stigmal vein and 0.5 times as long as the postmarginal vein, the hind femur enlarged with a single row of ventral teeth, and hind tibia greatly curved with apex diagonally truncate and ventrally prolonged into spine with 1 thickened and truncate spur. *Chalcimerus borceai* is ectoparasitoid of cynipids wasps (*Aylax papaveris* and *Barbotinia oraniensis*) in seed capsules of *Papave*r *dubium* and *P. rhoeas* (Bouček 1978; Nieves-Aldrey and Askew 2002; Askew et al. 2006).

In tribe Podagrionini seven genera and 139 of extant species are classified in total (Grissell 1995; Sureshan 2003; Narendran and Sudheer 2004; Delvare 2005; Zhao et al. 2007; Doğanlar and Doğanlar 2009; Narendran and Peter 2009; Narendran and Mercy 2010). Most of the species from genera of the tribe Podagrionini are spread mainly in tropics except for genus *Podagrion* Spinola, 1811 (96 spp.), which has some species distributed also in Holarctic region. Genus *Propachytomoides* Girault, 1914 (3 spp.) is distributed only in Australian region, *Mantiphaga* Ferrière, 1955 (6 spp.) and *Micropodagrion* Ferrière, 1955 (1 sp.) are known only from Afrotropical region, *Podagriomicron* Narendran & Mercy, 2010 (1 sp.) only from Oriental region (India), *Palmon* Dalman, 1825 (20 spp.) has pantropical distribution, and *Podagrionella* Girault, 1913 (12 spp.) is mentioned from paleotropics plus few species known from southern Palaearctic region and Australian region.

Grissell´s (1995) morfological diagnosis of this tribe is as follows: enlarged hind femur with numerous ventral teeth, the greatly curved hind tibia with apex diagonally truncate and ventrally prolonged into a spine, and a single hind-tibial spur; the long marginal vein 3 to 9 times longer than the stigmal vein and 4 to 9 times longer than the postmarginal vein; the venter of the antennal club with an area of micropilosity; and metasomal terga 2-4 laterally and dorsomedially emarginate.

Species of Podagrionini have been repeatedly quoted as parasitoids on mantid eggs (Grissell 1995; Delvare 2005). Two host records for Lepidoptera are also mentioned but they seem to represent marginal cases compared with the hundreds of available data pointing out a specialisation to mantid eggs (Grissell, 1995).

Up to now, there is ten genera and 416 species included in tribe Torymini (Grissell 1995; Sureshan 2007, 2010; Xiao et al. 2012; Noyes 2013). But most of them are assigned to the genus *Torymus* Dalman, 1820, where 390 species are presently described (Noyes 2013). Two genera, *Austorymus* Bouček, 1988 (1 sp.) and *Ovidia* Girault, 1924 (1 sp.) are known only from Australia, *Allotorymus* Huber, 1927 (1 sp.) from Nearctic region, *Mesodiomorus* Strand, 1911 (1 sp.) from Oriental region, *Physothorax* (10 spp.) and *Plesiostigmodes* (1 sp.) only from neotropics and *Lissotorymus* Kamijo, 1961 (1 sp.) only from Japan. *Ecdamua* Walker, 1862 (6 spp.) and *Odopoia* Walker, 1871 (7 spp.) have pantropical distribution with one exception, *E. nambui* Kamijo, 1979, which is known from Palaearctic region (Zavada 2005; Stojanova and Ghahari 2009). Genus *Torymus* is distributed worldwide, but most of the species are restricted to Holarctic region (Grissell 1995).

Genera *Diomorus* Walker, 1834 and *Nannocerus* Mayr, 1885 were also treated as genera close to *Torymus*, i.e. classified in tribe Torymini sensu Grissell (1995) historically. However, both of these genera were synonymized with *Torymus* (Bouček 1993; Graham and Gijswit 1998), because there was not any morphological synapomorphy to support them as separate genera.

Genus *Diomorus* was distinguished from *Torymus* based only on single morphological synapomorphy, the tooth on hind femur, and biology. All species belonged formerly to the genus *Diomorus* are parasitoids of larvae of various aculeates in stems or old galls (Graham and Gijswit 1998). However, Bouček (1996) described *T. pulcher* [later synonymized by Zerova et al. (2000) with *T. kononovae* (Zerova & Seryogina, 1991)] which has intermediate morphological characters between genus *Torymus* and *Diomorus*. Therefore Graham and Gijswit (1998) synonymized *Diomorus* with *Torymus*.

Bouček (1993) also downgraded *Nannocerus* as subgenus of *Torymus* which differs only by host specifity and no even by any of morphological synapomorphies. All of known species of *Nannocerus* are associated with New World *Ficus* spp. fruits, which is unique strategy within species of the genus *Torymus*.

Biology of most of the genera (i.e. *Allotorymus*, *Austorymus*, *Mesodiomorus*, *Odopoia*, *Ovidia*, *Plesiostigmodes*) from tribe Torymini is unknown. Major part of species belonging to the genus *Torymus* is assocìtated with galls, where they attack larvae of gall wasps (Hymenoptera: Cynipidae) or migde flies (Diptera: Cecidomyiidae) (Graham and Gijswit 1998; Zavada 2003; Noyes 2013). Some species of the genus *Ecdamua* are associated with nests of wood-boring aculeate wasps (Williams 1928; Zerova and Seryogina 2003), and species of *Physothorax* are associated with *Ficus* spp. fruits in New World, with only *P. bidentulus* has been confirmed as parasitoid of cecidomyiid larvae (*Ficiomyia perarticulata*) in galls inside *Ficus citrifolia* fruit (Bouček 1993).

Torymini is defined only by one morphological character, the forward projecting anterior metapleural margin (Grissell 1995).

Incertae sedis

Some taxa are treated as *incertae sedis* because they share synapomorphies with more than one tribe (Grissell 1995). Altogether eight genera and 22 species are placed as *incertae sedis* within Toryminae (Grissell 1995; Zerova and Seryogina 1999, 2000; Zerova et al. 2004, 2008; Stojanova 2005).

The genera *Cryptopristus* Förster, 1856 (3 spp.) and *Glyphomerus* Förster, 1856 (9 spp.) have holarctic distribution with the majority of species known only from Palaearctic region. Larvae of species of the genus *Cryptopristus* develope as hyperparasitoids on larvae of gall-forming Eurytomidae (Chalcidoidea) on grass stems or stem galling Cynipidae (Hymenoptera) (Zerova et al. 2008). The most of species of *Glyphomerus* have been reared from galls of Cynipidae associated with Asteraceae, Rosaceae, and Lamiaceae. Some species are known also as hyperparasitoids on larvae of gall-forming Eurytomidae.

Exopristus Ruschka, 1923 (1 sp.), *Exopristoides* Bouček, 1982 (2 spp.) and *Thaumatorymus* Ferrière & Novicky, 1954 (1 sp.) are strictly palaearctic genera. *Exopristoides* was synonymized by Grissell (1995) as *Exopristus*, but latter regarded as a valid genus which differs in structures on antennae, scutellum and gaster tergites (Zerova et al. 2004). The only one species in the genus, *Exopristus trigonomerus* (Masi, 1916), is known as parasitoid in various galls on *Papaver* sp. (Papaveraceae), *Verbascum* sp. (Scrophulariaceae) and *Centaurea* spp. or *Cirsium* sp. (Asteraceae) (Grissell 1995; Zerova et al. 2008). The genus *Exopristoides* is reported from Aylacini galls (Hymenoptera: Cynipidae) on stems of *Phlomis tuberosa* (Lamiaceae) and *Hypecoum imberbe* (Papaveraceae) (Askew et al. 2004; Zerova et al. 2004; Stojanova 2005). The only known host of *Thaumatorymus notanisoides* is *Phanacis hypochoeridis* (Cynipidae: Aylacini) on *Hypochaeris* sp. (Bouček 1977; Askew et al. 2004).

Genus *Echthrodape* Burks, 1969 (2 spp.) is distributed in Australian and Afrotropical regions with one species reported as developing on larvae of *Braunsapis* sp. (Apidae) in dead stems (Burks 1969). Genus *Stenotorymus* Masi, 1938 (1 sp.) is known from Afrotropical and Oriental regions. There is not any host record on that genus (Grissell 1995). Genus *Zaglyptonotus* Crawford, 1914 (3 species) has New World distribution. Some species of this genus were reported from galls of Tephritidae (Diptera) in flower heads of Asteraceae (Sharkey et al. 1987).

Classification mentioned above is after Grissell (1995) and is generally accepted (Janšta et al. 2011, 2013; Munro et al. 2011; Heraty et al. 2013; Noyes 2013). However, Zerova and Seryogina (2003) distinguished only three tribes based on Ukrainian fauna (Podagrionini, Monodontomerini and Torymini) instead of six published before (Grissell 1995). Tribe Podagrionini (sensu Zerova and Seryogina 2003) includes genera *Podagrion* and *Chalcimerus* based on curved hind tibia and hind femur with a row of teeth on hind margin, tribe Monodontomerini includes *Ameromicrus* [*Torymoides* sensu Grissell (1995)], *Cryptopristus*, *Eridontomerus*, *Exopristus*, *Glyphomerus*, *Idarnotorymus*, *Idiomacromerus*, *Microdontomerus*, *Monodontomerus*, *Pseuderimerus*, *Pseudotorymus*, and *Torymoides* [i.e. tribes Microdontomerini, Monodontomerini, Torymoidini and 2 genera placed as *incertae sedis* after Grissell (1995)] and to the tribe Torymini were assigned genera *Torymus* and *Diomorus* [previously synonymized with *Torymus* (Graham and Gijswit 1998; Zerova et al. 2000)]. Morphological synapomorphies supporting these tribes used by Zerova and Seryogina (2003) are the straigth margin of metapleuron for Monodontomerini versus forward projected anterior metapleural margin for Torymini.

Immature stages of Torymidae and its phylogenetic implications

There exists little information about larval morphology of Torymidae and its potential use for phylogenetical studies. First more comprehensive studies were provided by Parker (1924) and Parker and Thompson (1925), who described eggs and larval stages for several various Chalcidoidea species including *Podagrion pachymerum*, *Ditropinotus aureoviridis*, *Torymus* nr. *phyllyreae*, *T. druparum*, *Megastigmus dorsalis* (all Torymidae). Latter, a general description of larval morphology of the family Torymidae (Finlayson and Hagen 1977) and then more detailed with some physiological notes on several species from different tribes of Torymidae (Sellenschlo 1982, 1983, 1984, 1989) were provided. Moreover, there is several studies dealing with larval – different stages (and sometimes eggs) morfology of individual species (Askew 1961, 1966, 2002; Skrzypczyńska and Roques 1987; Askew et al. 2004, 2007; Nieves-Aldrey et al. 2007). But none of these authors used larval morphological data for phylogeny.

However, Gómez et al. (2008) provided first comparative morphological study and reconstructed phylogeny based on terminal-instar larvae of 22 species of Torymidae belonging to two subfamilies (Toryminae and Megastigminae) and four tribes (Microdontomerini, Torymoidini, Torymini and Chalcimerini). Larval morphology did not confirm monophyly of the family Torymidae as was postulated (Grissell 1995; Heraty et al. 2013) and proposed it as two non-related but monophyletic subfamilies.

Larvae of Toryminae differs at least in three unique chracters from Megastigminae (Gómez et al. 2008). Furthermore, zoophagous species of *Megastigmus* have an autapomorphic character state, the presence of a medial frontal pit, which was mentioned before (Askew 1966; Nieves-Aldrey et al. 2008) and is not found in any of zoophagous species of Chalcidoidea. Larval morphology is useful for generic classification, neverthless only the tribe Microdontomerini and Chalcimerini were supported as monophyletic.

Fossil evidence

One of the first fossils had been mentioned by Brues (1910) from Miocene Florissant shale in Colorado. He described four species in genus *Paleotorymus* (*P. aciculatus* Brues, 1910, *P. laevis* Brues, 1910, *P. striatus* Brues, 1910 and *P. typicus* Brues, 1910) and *Torymus bruesi* (Brues, 1910) – transfered from the preoccupied species *T. sackeni* (Grissell 1976). But Grissell (1995), who re-examined more carefully all type specimens, distinguished that some specimens of *P. typicus* appear not to be the same taxon. At least holotypes of *P. typicus* and *P. laevis* seem to be genus *Monodontomerus* or some *Diomorus* species (now synonymum of the genus *Torymus*) according to wing venation. The two other species could be considered as members of family Pteromalidae upon wing venation (Grissell 1995). Heraty and Darling (2009) transferred one more species formerly described from compression fossils (Rubielos de Mora Basin, Spain) by Peñalver and Engel (2006) as *Perilampus renzii* Peñalver & Engel, 2006 to this genus. According to Heraty and Darling (2009) the wing venation of *P. renzii* is consistent with Grissell´s (1995) concept of Monodontomerini and *Paleotorymus*.

Latter, Brues (1923) described *Monodontomerus primaveus* Brues, 1923 from Baltic amber. This is only one fossil species in that genus which includes 44 extant species.

Two other genera are known from Dominican amber (Grissell 1980). *Gummilumpus bouceki* (Grissell, 1980) is assigned to the tribe Palachiini and *Zophodetus woodruffi* Grissell, 1980 seems to be very similar to *Microdontomerus* and tribe Microdontomerini (Grissell 1995).

Species *Palmon bellator* Dalman, 1825 and *Podagrion capitellatum* (Dalman, 1825) and *P. clavellatum* (Dalman, 1825) were described from Gum copal inclusion (Dalman 1835), but unfortunately without type locality specified (Grissell 1995) and therefore does not allow consequent studies.

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3. Summary of papers

Paper I

MUNRO J. B., HERATY J. M., BURKS R. A., HAWKS D., MOTTERN J., CRUAUD A., RASPLUS J.-Y. & **JANSTA P.** 2011: A Molecular Phylogeny of the Chalcidoidea (Hymenoptera). *PLOS* One 6: e27023. DOI: 10.1371/journal.pone.0027023.

Chalcidoidea (Hymenoptera) are extremely diverse with more than 23,000 species described and over 500,000 species estimated to exist. This is the first comprehensive phylogenetic analysis of the superfamily based on a molecular analysis of 18S and 28S ribosomal gene regions for 19 families, 72 subfamilies, 343 genera and 649 species. The 56 outgroups are comprised of Ceraphronoidea and most proctotrupomorph families, including Mymarommatidae. Data alignment and the impact of ambiguous regions are explored using a secondary structure analysis and automated (MAFFT) alignments of the core and pairing regions and regions of ambiguous alignment. Both likelihood and parsimony approaches are used to analyze the data. Overall there is no impact of alignment method, and few but substantial differences between likelihood and parsimony approaches. Monophyly of Chalcidoidea and a sister group relationship between Mymaridae and the remaining Chalcidoidea is strongly supported in all analyses. Either Mymarommatoidea or Diaprioidea are the sister group of Chalcidoidea depending on the analysis. Likelihood analyses place Rotoitidae as the sister group of the remaining Chalcidoidea after Mymaridae, whereas parsimony nests them within Chalcidoidea. Some traditional family groups are supported as monophyletic (Agaonidae, Eucharitidae, Encyrtidae, Eulophidae, Leucospidae, Mymaridae, Ormyridae, Signiphoridae, Tanaostigmatidae and Trichogrammatidae). Several other families are paraphyletic (Perilampidae) or polyphyletic (Aphelinidae, Chalcididae, Eupelmidae, Eurytomidae, Pteromalidae, Tetracampidae and Torymidae). Evolutionary scenarios discussed for Chalcidoidea include the evolution of phytophagy, egg parasitism, sternorrhynchan parasitism, hypermetamorphic development and heteronomy.

Paper II

HERATY J. M., BURKS R. A., CRUAUD A., GIBSON G. A. P., LILJEBLAD J., MUNRO J., RASPLUS J.-Y., DELVARE G., **JANŠTA P.**, GUMOVSKY A., HUBER J., WOOLLEY J. B., KROGMANN L., HEYDON S., POLASZEK A., SCHMIDT S., DARLING D. C., GATES M. W., MOTTERN J., MURRAY

E., DAL MOLIN A., TRIAPITSYN S., BAUR H., PINTO J. D., VAN NOORT S., GEORGE J. & YODER M. 2013: A phylogenetic analysis of the megadiverse Chalcidoidea (Hymenoptera). *Cladistics* **29(5)**: 466-542.

Chalcidoidea (Hymenoptera) is extremely diverse with an estimated 500 000 species. We present the first phylogenetic analysis of the superfamily based on both morphological and molecular data. A web-based, systematics workbench mx was used to score 945 character states illustrated by 648 figures for 233 morphological characters for a total of 66 645 observations for 300 taxa. The matrix covers 22 chalcidoid families recognized herein and includes 268 genera within 78 of 83 subfamilies. Morphological data were analysed alone and in combination with molecular data from ribosomal 18S (2105 bp) and 28S D2–D5 expansion regions (1812 bp). Analyses were analysed alone and in combined datasets using implied-weights parsimony and likelihood. Proposed changes in higher classification resulting from the analyses include: (i) recognition of Eriaporidae, revised status; (ii) recognition of Cynipencyrtidae, revised status; (iii) recognition of Azotidae, revised status; (iv) inclusion of Sycophaginae in Agaonidae, revised status; (v) reclassification of Aphelinidae to include Aphelininae, Calesinae, Coccophaginae, Eretmocerinae and Eriaphytinae; (vi) inclusion of Cratominae and Panstenoninae within Pteromalinae (Pteromalidae), new synonymy; (vii) inclusion of Epichrysomallinae in Pteromalidae, revised status. At a higher level, Chalcidoidea was monophyletic, with Mymaridae the sister group of Rotoitidae plus the remaining Chalcidoidea. A eulophid lineage was recovered that included Aphelinidae, Azotidae, Eulophidae, Signiphoridae, Tetracampidae and Trichogrammatidae. Eucharitidae and Perilampidae were monophyletic if Eutrichosomatinae (Pteromalidae) was included, and Eupelmidae was monophyletic if *Oodera* (Pteromalidae: Cleonyminae) was included. Likelihood recovered a clade of Eupelmidae + (Tanaostigmatidae + (*Cynipencyrtus* + Encyrtidae). Support for other lineages and their impact on the classification of Chalcidoidea is discussed. Several life-history traits are mapped onto the new phylogeny.

Paper III

JANŠTA P., VILÍMOVÁ J. AND HANSON P. 2011: Description of a new genus, *Boucekinus* (Hymenoptera: Chalcidoidea: Torymidae), with two new species and a discussion of its possible phylogenetic placement. *Zootaxa* **2762**: 49-55.

Boucekinus gen. nov. and two new species, *B. masneri* sp. nov. from Ecuador and *B. tatianae* sp. nov. from Costa Rica, are described; *B. tatianae* is designated as the type species. *Boucekinus tatianae* was reared from cecidomyiid galls on *Anemopaegma chrysoleucum* (Bignoniaceae) and *B. masneri* was collected by canopy fogging. The placement of this new genus within Torymidae is discussed.

Paper IV

JANŠTA P., KŘÍŽKOVÁ B., VILÍMOVÁ J., RASPLUS J.-Y. 2013: Description of a new genus, *Chileana* (Hymenoptera: Chalcidoidea: Torymidae), with four new species. *Zootaxa* 3745: 49-63.

Chileana Janšta & Křížková gen. nov. and four new species, *C. cyanea* Janšta & Křížková sp. nov., *C. maculata* Janšta & Křížková sp. nov., *C. tricarinata* Janšta & Křížková sp. nov. And *C. penai* Janšta & Křížková sp. nov., all from Chile, are described. The placement of this new genus within the tribe Monodontomerini is discussed and several characters suggest a close relationship to *Zaglyptonotus*.

Paper V

JANŠTA P., CRUAUD A., DELVARE G., HERATY J., RASPLUS J.-Y.: Molecular phylogeny of the family Torymidae (Hymenoptera: Chalcidoidea). Manuscript prepared for submission to *Cladistics.*

We present the first molecular phylogenetic analysis of the family Torymidae (Hymenoptera: Chalcidoidea) using 4809 nucleotides from 5 genes (18S rDNA, 28S rDNA, EF1α, COI and Wg). Ten outgroups and 226 ingroup taxa were used, representing 45 of the 67 recognized genera in the 2 known subfamilies of Torymidae (Megastigminae and Toryminae). All analyses produced similar topologies, and based on only molecular data, Torymidae is not a monophyletic group and Megastigminae is sister to Ormyridae ((Ormyridae + Megastigminae) + Toryminae). Most of the tribes recognized by morphological characters within the subfamily Toryminae were supported by our results (i.e. Chalcimerini, Microdontomerini, Monodontomerini, Torymini, Torymoidini and Palachiini + Podagrionini). Two new tribes of Torymidae are erected: Boucekini, **trib. nov.** and Glyphomerini, **trib. nov.** As presently understood, the genus *Glyphomerus* is paraphyletic and 7 other genera classified as *incertae sedis* based on earlier studies of morphology were assigned to specific tribes (i.e. *Cryptopristus*, *Echthrodape*, *Exopristoides*, *Exopristus* and part of *Glyphomerus* to Microdontomerini; *Thaumatorymus* to Chalcimerini; *Zaglyptonotus* to Monodontomerini).

Life-strategies, hosts records and distribution of all taxa were mapped onto the reconstructed phylogeny. The larvae of Toryminae are ectoparasitoids of gall-forming insects in the Palaearctic region with several derived traits throughout the Toryminae phylogeny. The life strategy, hosts and distribution of the common ancestor of Megastigminae remains uncertain.

PAPER I

MUNRO J. B., HERATY J. M., BURKS R. A., HAWKS D., MOTTERN J., CRUAUD A., RASPLUS J.-Y. & **JANSTA P.** (2011)

A Molecular Phylogeny of the Chalcidoidea (Hymenoptera).

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To whom it may concern:

This is to declare that Petr Janšta contributed significantly to the paper "Munro J. B., Heraty J. M., Burks R. A., Hawks D., Mottern J., Cruaud A., Rasplus J.-Y. & **Jansta P.** 2011: A Molecular Phylogeny of the Chalcidoidea (Hymenoptera). *PLOS ONE* 6(11): e27023" co-authored by us and included in his PhD thesis. We hereby agree that this paper may be included in his PhD thesis.

On behalf of the authors

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A Molecular Phylogeny of the Chalcidoidea (Hymenoptera)

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Abstract

Chalcidoidea (Hymenoptera) are extremely diverse with more than 23,000 species described and over 500,000 species estimated to exist. This is the first comprehensive phylogenetic analysis of the superfamily based on a molecular analysis of 18S and 28S ribosomal gene regions for 19 families, 72 subfamilies, 343 genera and 649 species. The 56 outgroups are comprised of Ceraphronoidea and most proctotrupomorph families, including Mymarommatidae. Data alignment and the impact of ambiguous regions are explored using a secondary structure analysis and automated (MAFFT) alignments of the core and pairing regions and regions of ambiguous alignment. Both likelihood and parsimony approaches are used to analyze the data. Overall there is no impact of alignment method, and few but substantial differences between likelihood and parsimony approaches. Monophyly of Chalcidoidea and a sister group relationship between Mymaridae and the remaining Chalcidoidea is strongly supported in all analyses. Either Mymarommatoidea or Diaprioidea are the sister group of Chalcidoidea depending on the analysis. Likelihood analyses place Rotoitidae as the sister group of the remaining Chalcidoidea after Mymaridae, whereas parsimony nests them within Chalcidoidea. Some traditional family groups are supported as monophyletic (Agaonidae, Eucharitidae, Encyrtidae, Eulophidae, Leucospidae, Mymaridae, Ormyridae, Signiphoridae, Tanaostigmatidae and Trichogrammatidae). Several other families are paraphyletic (Perilampidae) or polyphyletic (Aphelinidae, Chalcididae, Eupelmidae, Eurytomidae, Pteromalidae, Tetracampidae and Torymidae). Evolutionary scenarios discussed for Chalcidoidea include the evolution of phytophagy, egg parasitism, sternorrhynchan parasitism, hypermetamorphic development and heteronomy.

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Introduction

Chalcidoidea (Hymenoptera) are minute wasps that generally range in size from 1-4 mm, with the smallest only 0.11 mm and the largest up to 45 mm. With an estimated diversity of up to 500,000 morphologically distinct species and an even larger number of cryptic species possible [1,2,3,4], this superfamily is likely the most diverse group of insects. While several families are phytophagous (e.g. all Agaonidae; some Eurytomidae, Eulophidae, Pteromalidae, Tanaostigmatidae and Torymidae), most chalcid wasps are parasitoids. They attack immature and adult stages of virtually all insect orders, but have their greatest diversification on the Hemiptera and Holometabola. Because the individual host is killed as a result of parasitoid development, many chalcid species are successfully used as biological control agents of agricultural and ornamental pests (e.g. Aphelinidae and Encyrtidae) [3]. Both economically and ecologically Chalcidoidea have tremendous importance in both natural and managed ecosystems.

Despite their importance, our understanding of their taxonomy and evolutionary relationships is clearly wanting. Partly because of their small size, they are difficult to collect and study, and only about 23,000 species have been described [4]. Nineteen families are currently recognized, with their diversity spread across as many as 80-89 subfamilies, in many cases without consensus on their higher-level placement.

Chalcidoidea and their proposed sister group Mymarommatoidea first appear in mid Cretaceous amber deposits (Mymaridae) [5,6,7]. Most extant lineages do not appear until the Eocene, suggesting an extremely rapid post-Cretaceous radiation [6]. However, the presence of Eulophidae and Trichogrammatidae in Late Cenomanian amber from Ethiopia pushes chalcidoid diversification back to the mid Cretaceous, about 93–95 Mya [8].

Synapomorphies uniting most of the members of Chalcidoidea include an exposed prepectus, positioning of the mesothoracic spiracle on the lateral margin of the mesoscutum, wing venation reduced to submarginal, marginal, stigmal, and postmarginal veins, and the presence of multiporous plate sensilla on one or more of the antennal flagellomeres [9,10]. Molecular evidence places Chalcidoidea as a monophyletic group nested within a monophyletic Proctotrupomorpha and as the sister group to either Diaprioidea or Mymarommatoidea [11,12,13], but see Sharanowski et al. [14] for an alternate proposal for Ceraphronoidea as the sister group.

Both morphological and molecular evidence place Mymaridae as the sister group of the rest of Chalcidoidea [10,11,13]. A few intuitive hypotheses of relationships within the superfamily have been proposed based on limited morphological justification [5,15,16]. However, for relationships within Chalcidoidea, there has not been a morphology-based cladistic analysis across more than just a few inclusive families [9]. A few molecular analyses have addressed relationships broadly across the superfamily, but these have used relatively few taxa to represent such a diverse group [17,18].

Herein we present the first comprehensive phylogenetic analysis of relationships within the Chalcidoidea using 18S rDNA and the 28S rDNA D2–D5 expansion regions sampled across 722 taxa. The diversity of the superfamily is addressed by the inclusion of 72 subfamilies and 343 genera. Data were aligned according to a secondary structural model, which allows for the unambiguous partitioning of data into conserved regions and regions of ambiguous alignment [19,20,21]. Different optimizations of the alignment using MAFFT [22] are analyzed to compensate for potential alignment artifacts and increase phylogenetic resolution. Our analysis provides a new framework for evaluating the composition and relationships of major groups and hopefully will lead to a better understanding of their evolution.

Materials and Methods

Taxonomic sampling and specimen vouchering

Sequences were obtained for 722 taxa, with 56 outgroups and 666 ingroups (Table S1). Chalcidoidea are represented by all 19 families, 72 subfamilies, 343 genera and 649 species. Most species are represented by a single specimen; however, to remove any doubt of sequencing error, additional individuals of some species that were difficult to place within any expected grouping (e.g., *Idioporus, Cynipencyrtus* and *Diplesiostigma*) were sequenced. Outgroup taxa included exemplars of Ceraphronoidea (Ceraphronidae and Megaspilidae), Cynipoidea (Cynipidae, Figitidae, Ibaliidae and Liopteridae), Diaprioidea (Diapriidae, Maamingidae and Monomachidae), Mymarommatoidea (Mymarommatidae), Platygastroidea (Platygastridae) and Proctotrupoidea (Heloridae, Pelecinidae, Proctotrupidae, Roproniidae and Vanhorniidae). In the present manuscript we follow the family and subfamily classification of Chalcidoidea of Noyes [4], with additional resolution from the following: Agaonidae follows Cruaud et al. [23], Aphelinidae follows Hayat [24], Chalcididae follows Bouček and Delvare [25] and Narendran [26]; Cleonyminae follows Gibson [27], Eucharitidae follows Heraty [28], Eulophidae follows Burks et al. [29]; Pteromalidae follows Bouček [30], Delucchi [31], Graham [32] and Hedqvist [33], Toryminae follows Grissell [34], and Trichogrammatidae follows Owen et al. [35].

The majority of taxa were sequenced and vouchered at the University of California Riverside (UCR). Additional sequences were provided by co-authors (AC and JYR: Agaonidae and some Pteromalidae; PJ: Torymidae), the HymAToL project (various outgroup taxa), Matt Yoder (NC State University; various outgroup taxa), and Andy Austin (University of Adelaide; various outgroup taxa). See Table S1 for a complete listing of contributed sequences and voucher locations. Taxa sequenced at UCR are represented by either a primary (remains of actual specimen sequenced) or secondary (compared specimen from same collection series) specimen voucher. UCR voucher specimens were each assigned a unique UCRC_ENT Museum identification number and barcode. Additional voucher information is housed in a FileMaker Pro database at UCR developed by JM, and is available on request. UCR vouchers were imaged using a GT-Vision automontage system, with images deposited on MorphBank 4.0 (http://www.morphbank.net/).

DNA Extraction, Amplification and Sequencing

Genomic DNA extraction at UCR followed a modified version of the Chelex® protocol [36]. Primer sequences for PCR amplification of 18S rDNA and the 28S rDNA D2, D3 and D4+D5 expansion regions are provided in Table 1. Herein, the amplified regions shall be referred to simply as 18Sa-c, D2, D3 and D4+D5. In some cases, a shorter version of 18Sb was amplified with internal primers (18Si, Table 1). Amplification and sequencing followed established protocols at UCR [37]. UCR sequencing was conducted at the San Diego State University Microchemical Core Facility or the UCR Genomics Core Facility. Protocols for the Rasplus lab sequences follow Cruaud et al. [23]. Sequence verification was conducted by comparing forward and reverse sequences. All sequences are deposited on Genbank (Table S1).

Secondary structure alignment

Sequences were manually aligned using secondary structure models following Deans et al. [38] and Gillespie et al. [20,21,39,40]. The 18Sa fragment began three bases (TAC) prior to the core helix H9 and included the variable regions V1 and V2 and ended with helix H39'. Fragment 18Sb began four bases (AUAA) prior to the core helix H406a (CGAUACGGGACUC), and included the variable regions V3, V4 (expansion region E23-1 through E23-14) and V5, and ended with core helix H960', just prior to V6. 18Sc began with a conserved loop (AAACCTCA), which preceded H984 and ended with the conserved loop (TGA) between H1506 and H1506', and included regions V6–V9. Amplification of the 28S rDNA D2, D3 and D4+D5 expansion regions began a single base (C) prior to helix H375 (GGGUUGC) in the core region preceding D2 and terminated 2 bases following helix H976 (UGG), subsequent to D5. The final alignment contained 545 blocks of data, which accounted for base-pairing helices and their prime, ambiguously-pairing regions of expansion and contraction (REC), ambiguously-pairing regions of slipped-strand compensation (RSC), non-pairing yet highly conserved loops, and non-pairing and variable loop regions of ambiguous

Table 1. Primer sequences.

Primer Name	Primer Sequence	Reference
28S D2-3551 F	5′ - CGT GTT GCT TGA TAG TGC AGC - 3′	[17]
28S D3-4046 F	5′ - GAC CCG TCT TGA AAC ACG GA - 3′	[134]
28S D2-4057 R	5′ - TCA AGA CGG GTC CTG AAA GT - 3′	[37]
28S D3-4413 R	5′ - TCG GAA GGA ACC AGC TAC TA - 3′	[134]
28S D5-4625 R	5′ - CCC ACA GCG CCA GTT CTG CTT ACC - 3′	[135]
18Sa-1 F	5′ - TAC CTG GTT GAT CCT GCC AGT AG - 3′	[135]
18Sb-441 F	5′- AAA TTA CCC ACT CCC GGC A -3′	[11]
18Sa-591 R	5′- G AAT TAC CGC GGC TGC TGG -3′	[135]
18Si-673 F	5′- ATC GCT CGC GAT GTT TAA CT -3′	[11]
18Si-905 R	5′- AGA ACC GAG GTC CTA TTC CA -3′	[11]
18Sc-1204 F	5′ - ATG GTT GCA AAG CTG AAA C - 3′	[135]
18Sb-1299 R	5′- TGG TGA GGT TTC CCG TGT T - 3′	[11]
18Sc-1991 R	5′ - GAT CCT TCC GCA GGT TCA CCT AC - 3′	[135]

28S primers are named for the relative structural position of the primer (next expansion region in direction of primer), for 18S and 28S their complementary 5′ start position in *D. melanogaster* [131,132,133], and whether designated as a forward (F) or reverse (R) primer.

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alignment (RAA). For the purposes of this paper, we treat all three of these regions together as RAA regions.

Comparison between secondary structure and algorithmically generated alignments

Two important aspects of the dataset led us to compare the results obtained with various alignment strategies. First, we are confident of the alignment in the conserved stem-based and core regions; however vagaries of the secondary structure model lead to some local alignments that might not be optimal based on exact pairing of compensatory base changes. Second, distribution and size of RAAs are variable across Chalcidoidea. For such a large matrix, by-eye alignment of these highly-variable ambiguous regions from distantly related taxa is hard to justify. However, these RAAs can be locally informative [11,29] and we prefer not to exclude them from our analyses. To test different optimizations of our secondary structure alignment and the impact of RAAs, we created two submatrices: one including the conserved stem-based and core regions and another including the regions of ambiguous alignment.

The core secondary structure-derived (SS) submatrix was created by manually removing regions of ambiguous alignment (RAAs), leaving only the structurally aligned helices, core regions, and conserved blocks. As alluded to previously, not all loops are 'highly variable' and conserved non-pairing regions, including some loops found in the core, were retained in the SS submatrix.

The second submatrix (RAAs) included the regions of ambiguous alignment *sensu lato* (RAAs, REC, RSCs, and unnamed blocks). An initial 77 regions of ambiguous alignment were identified. Where RECs and their pairing primes bounded an RAA, the blocks were concatenated. Additionally, REC 4 H3q, RAA 24 loop 9, REC 4' H3q', and RAA 25 were concatenated into a single block. Concatenation reduced the number of isolated RAA regions from 77 to 55. Each of these regions was aligned independently and re-included in the corresponding gene region for each of the following datasets.

Sixteen datasets were constructed from these submatrices (Table 2) that can be grouped into four categories: 1) SS submatrix without RAAs; 2–7) SS combined with algorithm-aligned RAAs; 8–10) algorithm-aligned SS submatrix without RAAs; 11–13) algorithm-aligned SS submatrix and algorithm-aligned RAAs, and 14–16) algorithm-aligned dataset in which the SS and RAA submatrices were not treated separately, but with each of the 6 gene regions individually isolated and independently algorithm-aligned.

Automated alignments were performed with MAFFT [22,41,42]. Both the online server (v.6) and the downloadable program (v.6.244b) were used to create initial alignments that utilized the following MAFFT algorithms: E-INS-i, G-INS-i and L-INS-i. Alignments for each partition (core region and each of the 55 regions of ambiguous alignment taken independently) were generated using the default settings (gap opening penalty = 1.53 and offset value = 0.00).

The RAAs were aligned both with and without a guide tree that was generated using the SSNR (core with no RAA) dataset. Our purpose for using a guide tree was to optimize local alignments for each of the RAAs within terminal clusters of independently recognized taxa grouped through analysis of the SSNR, thus aligning nearest neighbors, as opposed to aligning disparate taxa across the entire dataset without any prior grouping. Maximum likelihood (ML) analyses of this dataset were conducted with RAxML v.7.2.7 using a partitioned GTR+Γ model [43] on the Teragrid cluster, Abe [44] via the CIPRES portal V2.2 [45]. We used 1000 rapid bootstrap (BS) replicates for each run, with initial tests using the autoMRE criterion [46] showing 350 BS to be adequate. A GTRCAT approximation of models was used for ML bootstrapping [47]. Ten RAxML analyses utilizing different starting seeds were executed, followed by ML optimization to find the best-scoring tree. The 10 resulting trees were used to generate a strict consensus tree that was converted to a MAFFT-readable guide tree with the script newick2mafft.rb (http://mafft.cbrc.jp/alignment/software/treein.html). This guide tree was implemented in the MAFFT alignments of the isolated RAAs utilizing the E-INS-i, G-INS-i and L-INS-i algorithms (SSGE, SSGG and SSGL, Table 2).

The secondary structure-derived matrix with MAFFT-aligned RAA regions (SSME) is deposited on Texas A&M's Parasitic Hymenoptera Research Labs' jRNA Secondary Structure and its Phylogenetic Implications website (available through http://hymenoptera.tamu.edu/rna/) and as Supplemental Nexus File S1. The 15 remaining datasets, with and without RAA regions, are available from JMH upon request.

Dataset partitioning

Sequences were partitioned into six gene regions 18Sa, 18Sb, 18Sc, D2, D3, and D4+D5, with each partition including their respective aligned RAA regions. The 18Sa-c partitions were defined simply as the region sequenced, inclusive of the primers used. The 28S rDNA expansion regions are also contiguous, being bounded on either side by core sequence, which was amplified in the PCR reaction. The decision as to where to define the end of D2 and start of D3 and likewise, the end of D3 and start of D4+D5, was arbitrarily made to fall within the core regions between the expansion regions. The helix H1a' (UUUCAGG), was assigned to mark the end of D2; while the un-named, non-pairing block of sequence (AC), which follows helix H1a' and proceeds helix H563 (CCGU) marked the start of D3. Helix H812 (CCCUCC) was assigned to mark the end of D3, while the un-named, non-pairing block of sequence (GAAG), which follows helix H812 and precedes helix H822 (UUUCC), marks the start of D4+D5.

Phylogenetic analyses

Maximum Likelihood (ML) analyses and associated bootstrapping (BS) were conducted on the 16 datasets with RAxML v.7.2.7 using a partitioned GTR+Γ model [43] on the Teragrid cluster, Abe [44] via the CIPRES portal V2.2 [45]. A GTRCAT approximation of models was used for ML bootstrapping [47]. To accommodate parameter variation in separate runs [48], 10 analyses were conducted using different seed numbers and 1000 rapid bootstrap (BS) replicates, with the tree with the best known likelihood (BKL) score chosen from among these sets. For comparison of alignments strategies, we examined the number of parsimony informative and uninformative sites, overall length, and the number of step changes mapped with PAUP 4.0* [49] onto each tree using the SSME dataset. The SSME dataset was chosen for the Parsimony analysis, because it provided what we considered to be the optimal results in terms of clade retention and used both the SS and RAA submatrices.

The parsimony analysis of the SSME dataset was conducted with TNT v.1.1 [50,51]. Heuristic searches were performed using a New Technology Search with default settings, except for using a sectorial search, ratchet weighting probability of 5% with 50 iterations, tree-drifting of 50 cycles, tree-fusing of 5 rounds, and best score hit of 10 times, followed by swapping to completion on all trees found. Nodal supports were calculated using 1000 standard bootstrap replicates.

Table 2. Alignment strategies for use of secondary structure and MAFFT alignments of both core/stem (SS) and ambiguous (RAA) regions.

dataset	core/stem	RAA	length	inform.	uninfo.	18Sa	18Sb	18Sc	28S	28S	28S	RAxML	No. of steps
	alignment	alignment							D2	D3	D4-5	best score	SSME data
SSNR	SS	no RAA	2996	853	356	500	757	633	591	333	182	-85277.62	32461
SSGE	SS	guide tree+E-INS-i	4369	1675	566	507	969	701	1302	519	371	-144234.60	32236
SSGL	SS	guide tree+L-INS-i	4369	1676	565	507	969	701	1302	519	371	-144255.37	32223
SSGG	SS	guide tree+G-INS-i	4536	1773	550	507	963	697	1451	531	387	-144123.77	32220
SSME	SS	no guide+E-INS-i	3917	1408	483	506	906	693	993	450	369	-150220.93	31951
SSML	SS	no guide+L-INS-i	3917	1408	487	506	906	693	993	450	369	-150223.77	31957
SSMG	SS	no guide+G-INS-i	3906	1433	468	506	906	694	1023	450	327	-147954.87	31951
MENR	E-INS-i	no RAA	3024	861	375	507	758	634	605	337	183	-85889.86	32522
MLNR	L-INS-i	no RAA	3024	861	374	507	758	634	605	337	183	-85852.51	32483
MGNR	G-INS-i	no RAA	3025	859	380	507	758	634	606	337	183	-85953.75	32527
MEME	E-INS-i	no guide+E-INS-i	3944	1415	502	513	907	694	1007	453	370	-150774.64	32247
MLML	L-INS-i	no guide+L-INS-i	3944	1415	501	513	907	694	1007	453	370	-150775.39	32236
MGMG	G-INS-i	no guide+G-INS-i	3934	1438	492	513	907	695	1038	453	328	-148553.26	32254
MESR	E-INS-i (all data by partition)		4133	1536	553	506	901	693	1196	531	306	-145056.78	31983
MLSR	L-INS-i (all data by partition)		4099	1507	545	506	901	693	1162	531	306	-145084.06	32187
MGSR	G-INS-i (all data by partition)		4139	1519	551	506	901	694	1201	531	306	-145293.59	31997

The guide tree was generated from a RAxML analysis of the SSNR dataset (no RAA). Except for the all data alignments (no submatrix partition), each of the 55 RAA blocks were aligned independently and reinserted into the appropriate gene partition for analysis. E-INS-i, G-INS-i and L-LINS-i are MAFFT alignment options. The RAxML best score was obtained from 10 independent runs using CIPRES v.2.0. The number of informative and uninformative sites and parsimony steps were calculated in PAUP 4.0* for each resulting tree using the SSME dataset.

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To be consistent with our interpretation of bootstrap percentage (BP), we use the following scale: a bootstrap percentage of ≥90% is considered very strong, 80–89% means strong, 70–79% means moderate, and 50–70% means low bootstrap support.

To better track relationships, each taxon includes a prefix which is an abbreviation of it family-group (c.f. Table 3, S1), and the suffix includes the DNA voucher code and letters correponding to the gene regions sequenced, corresponding to the three regions of 18S (tuv), 28S-D2 (x), D3 (y) and D4-5 (z).

Results

Alignment models, tree length and clade support

Summaries of the 16 datasets generated from the two submatrices are presented in Table 2. The core region (SS) was 2996 bp in length and only slightly shorter than the MAFFT alignment of the same data (3,024–3,025 bp), with the differences accumulated mostly in the 28S D2 region. The application of the guide tree to the RAAs produced the longest alignment (4,369–4,536 bp) with the greatest impact on the length of the 28S D2 and D3 regions. Application of the guide tree greatly increased the number of parsimony informative sites (1,675–1,773 bp), the number of uninformative (autapomorphic) sites (550–565 bp), and had the greatest impact on tree length using the SSME dataset as a metric (32,220–32,236 steps) (Table 2). The MAFFT aligned RAAs without a guide tree were added to both the core region (SSME, SSMG and SSML) and to the MAFFT alignment of the core region (MEME, MGMG and MLML). Using mapped state changes and the SSME metric, the core + no guide tree RAAs datasets produced the shortest tree topologies (31,951–31,957 steps). Both the alignment length, and the RAxML best score differed very little within the different MAFFT variants of each alignment model. The MAFFT alignment of all data without regard to partition (MESR, MGSR and MLSR) produced an alignment of intermediate length (4,099–4,139 bp).

Phylogenetic Analyses. A summary of supported clades across six of the 16 analyses is presented in Tables 3 and 4, along with a summary of the >50% majority rule consensus support (MJR) across all 16 best known likelihood (BKL) RAxML trees. We present the BKL tree from the SSME RAxML result (Figs 1–7), with the caveat that this represents only one summary of relationships found within Chalcidoidea. The clade support tables are a better representation of the support for traditional subfamily and family groups (Table 3) and for some higher-level relationships (Table 4). When present, bootstrap support on Figures 1–7 generally corresponds with support across all analyses. Surprisingly, there was little impact of alignment strategy (SS or MAFFT) on the results, except for a slight increase in support for various clades at all levels with the inclusion of RAAs (core and RAA, Tables 3, 4).

Interestingly, the automated (MAFFT) alignments of all data were comparable in clade support to any of the divided alignment strategies based on recognizing the core and stem data. There was slightly better clade support using G-INS-i when applied to data that included RAAs.

Informativeness of RAAs

Within 28S and 18S, distinct structural differences occur between RAA regions for the outgroups, Mymaridae, and the remaining Chalcidoidea taxa. For example, RAA(11) shows a pattern of increase in the number of bases and an associated decrease in degree of conservation for Chalcidoidea in comparison to the outgroup taxa (Fig. 8). Alternatively, RAA(15) reduces to a single nucleotide for Chalcidoidea, with the exclusion of

Table 3. Summary of traditional clades within Chalcidoidea, diversity sampled, and support from various datasets and analyses.

				core only		core and RAA				RAxML	TNT
Code	**Taxonomy**	**gen**	**spp**	**SSNR**	**MENR**	**SSGE**	**SSME**	**MGMG**	**MGSR**	**MJR***	**SSME**
AG	**Agaonidae (76/757)**	19	104	100	100	100	100	100	100	100	97
AGA	'Agaoninae'[a]	12	48	–	–	–	–	–	–	–	–
AG4	'Agaonidae group 4'	2	3	–	*par*	70	75	86	92	75	–
AGB	'Blastophaginae'	3	24	–	–	–	–	–	–	–	–
AGK	Kradibiinae	2	25	–	*par*	–	–	–	–	–	–
AGT	Tetrapusinae	1	4	100	100	100	100	100	100	100	100
AP	**Aphelinidae (33/1168)**	21	87	–	–	–	–	–	–	–	–
API	Aphelinidae *incertae sedis*	4	4	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
APA	Aphelininae	7	22	88[b]	88[b]	97[b]	96[b]	91[b]	86[b]	100[b]	56[b]
APAY	Aphytini	3	12	*par*	*par*	*par*	53	*par*	*par*	*par*	+
APZ	Azotinae	1	12	99	100	100	100	100	100	100	99
APC	Coccophaginae	6	43	+	+	81	+	+	+	94	–
APCP	Pteroptricini	5	31	par	par	par	par	par	par	par	–
APE	Eretmocerinae	1	5	100	100	100	100	100	100	100	100
APR	Euryischiinae	2	2	100	100	100	89	100	100	100	100
CAL	**Calesinae (1/4)**	1	3	100	100	100	100	100	100	100	100
CH	**Chalcididae (87/1464)**	20	37	–	–	–	–	–	–	–	–
CHC	Chalcidinae	8	19	–	–	–	–	–	–	–	–
CHCB	Brachymeriini	1	6	100	100	100	100	100	100	100	100
CHCC	Chalcidini	2	8	100	100	100	100	100	100	100	100
CHCR	Cratocentrini	3	3	–	–	–	–	–	–	–	–
CHCP	Phasgonophorini	2	2	98	100	100	100	100	99	100	100
CHD	Dirhininae	1	5	100	100	100	100	100	100	100	100
CHE	Epitranininae	1	3	+	90	99	95	94	98	100	56
CHH	Haltichellinae	8	12	88	90	100	98	98	97	100	+
CHHA	Haltichellini	5	9	+	+	+	*par*	–	56	+	–
CHHY	Hybothoracini	3	3	*par*	*par*	*par*	93	–	*par*	*par*	*par*
CHS	Smicromorphinae	1	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
EN	**Encyrtidae (460/3735)**	12	14	+	50	81	72	73	78	100	+
ENE	Encyrtinae	8	9	*par*	*par*	*par*	+	72	+	89	+
ENT	Tetracneminae	4	5	72	69	87	77	97	par	65	+
EU	**Eucharitidae (55/423)**	22	46	100[c]	100[c]	100[c]	100[c]	100[c]	100[c]	100[c]	100[c]
EUE	Eucharitinae	16	27	100	100	100	100	100	100	100	96
EUG	Gollumiellinae	2	3	80	93	98	76	86	99	100	par
EUO	Oraseminae	4	16	par	+	71	+	+	+	75	+
EL	**Eulophidae (297/4472)**	27	28	89[d]	92[d]	99[d]	98[d]	97[d]	98[d]	100[d]	+[d]
ELI	Eulophidae *i.s.*	1	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
ELE	Entedoninae	8	8	–	+	50	+	74	59	88	+
ELN	Entiinae	5	6	–	–	67	*par*	+	58	81	+
ELU	Eulophinae	9	10	66	+	96	95	91	85	100	–
ELO	Opheliminae	1	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
ELT	Tetrastichinae	3	3	98	98	100	100	100	100	100	99
EP	**Eupelmidae (45/907)**	19	25	–	–	–	–	–	–	–	–
EPC	Calosotinae	5	7	–	–	–	–	–	–	–	–
EPE	Eupelminae	12	14	+	+	+	–	+	–	–	–
EPN	Neanastatinae	2	4	–	–	–	+	–	–	–	–
EY	**Eurytomidae (88/1424)**	14	28	–	–	–	–	–	–	–	–
EYE	Eurytominae	9	14	100[e]	99e	100[e]	100[e]	100[e]	100[e]	100[e]	100[e]
EYH	Heimbrinae	1	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

Table 3. Cont.

				core only		core and RAA				RAxML	TNT
Code	**Taxonomy**	**gen**	**spp**	**SSNR**	**MENR**	**SSGE**	**SSME**	**MGMG**	**MGSR**	**MJR***	**SSME**
EYR	Rileyinae	2	7	+	+	97	90	87	87	100	+
LEU	**Leucospidae (4/134)**	2	6	98	90	100	100	98	98	100	98
MY	**Mymaridae (103/1424)**	13	15	98	95	100	99	98	97	100	61
MYI	Mymaridae *i.s.*	1	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
MYA	Alaptinae	3	3	–	–	–	–	–	–	–	–
MYE	Eubronchinae	1	2	99	100	98	99	100	87	100	84
MYM	Mymarinae	8	9	–	–	–	–	–	–	–	–
ORM	**Ormyridae (3/125)**	2	3	66	56	67	+	61	52	100	+
PE	**Perilampidae (15/277)**	14	34	+[f]	+[f]	–	–	–	–	–	–
PEI	Perilampidae *i.s.*	1	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
PEA	Akapalinae	1	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
PEM	Philomidinae	3	3	99	98	100	100	100	100	100	97
PEC	Chrysolampinae	4	9	73	67	88	72	68	80	100	–
PEP	Perilampinae	5	20	96	98	100	100	100	99	100	76
PT	**Pteromalidae (588/3506)**	111	130	–	–	–	–	–	–	–	–
PTI	Pteromalidae *i.s.*	2	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
PT01	Asaphinae	3	3	–	–	–	–	–	–	+	–
PT02	Ceinae	1	2	93	93	100	98	98	99	100	98
PT03	Cerocephalinae	3	3	99	99	100	100	100	100	100	100
PT04	Chromeurytominae	1	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
PT05	Cleonyminae	10	10	–	–	–	–	–	–	–	–
PT05D	Chalcedectini	1	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
PT05C	Cleonymini	3	3	68	56	84	54	+	52	100	+
PT05L	Lyciscini	5	5	+	+	92	55	+	+	100	+
PT05O	Ooderini	1	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
PT06	Coelocybinae	4	4	–	–	–	–	–	–	–	–
PT07	Colotrechninae	2	2	–	–	–	–	–	–	–	–
PT08	Cratominae	1	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
PT09	Diparinae	6	8	–	–	–	–	–	–	–	–
PT09D	Diparini	4	4	–	–	–	–	–	–	–	–
PT09N	Neapterolelapini	1	2	57	55	96	73	63	+	81	–
PT10	Epichrysomallinae	16	28	100	100	100	100	100	100	100	93
PT11	Eunotinae	6	7	–	–	–	–	–	–	–	–
PT11E	Eunotini	4	5	52[g]	75[g]	90[g]	86[g]	93[g]	98[g]	100[g]	61[g]
PT11M	Moranilini	1	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
PT11T	Tomocerodini	1	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
PT12	Eutrichosomatinae	1	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
PT13	Herbertiinae	1	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
PT14	Leptofoeninae	2	3	–	–	–	–	–	–	–	–
PT15	Macromesinae	1	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
PT16	Miscogasterinae	9	10	–	–	–	–	–	–	–	–
PT16M	Miscogasterini	5	6	–	–	–	–	–	–	–	–
PT16S	Sphegigasterini	2	2	–	–	–	–	–	–	–	–
PT16T	Trigonoderini	2	2	–	–	–	–	–	–	–	–
PT17	Ormocerinae	6	5	–	–	–	–	–	–	–	–
PT17M	Melanosomellini	3	3	–	–	*par*	–	+	–	–	–
PT17S	Systasini	1	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
PT18	Otitesellinae	3	4	par	–	–	–	–	–	–	–
PT19	Panstenoninae	1	2	96	89	98	98	84	77	100	96

Table 3. Cont.

				core only		core and RAA				RAxML	TNT
Code	**Taxonomy**	**gen**	**spp**	**SSNR**	**MENR**	**SSGE**	**SSME**	**MGMG**	**MGSR**	**MJR***	**SSME**
PT20	Pireninae	4	4	–	–	–	–	–	–	–	–
PT21	Pteromalinae	17	18	–	–	–	–	–	–	–	–
PT21P	Pteromalini	4	4	–	–	–	–	–	–	*par*	–
PT22	Spalangiinae	1	3	100	100	100	100	100	100	100	100
PT23	Sycoecinae	1	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
PT24	Sycophaginae	5	6	82	94	91	81	77	91	100	+
PT25	Sycoryctinae	2	2	–	–	–	–	–	–	–	–
ROT	**Rotoitidae (2/2)**	1	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
SI	**Signiphoridae (4/76)**	8	26	81	80	95	98	97	97	100	52
SIS	Signiphorinae	1	9	100	100	100	100	100	100	100	99
SIT	Thysaninae	3	12	*par*	*par*	*par*	*par*	*par*	*par*	*par*	*par*
TAN	**Tanaostigmatidae (9/92)**	4	5	98[h]	95[h]	99[h]	100[h]	99[h]	100[h]	100[h]	77[h]
TE	**Tetracampidae (15/50)**	6	7	–	–	–	–	–	–	–	
TEM	Mongolocampinae	1	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
TEP	Platynocheilinae	1	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
TET	Tetracampinae	4	5	100[i]	100[i]	100[i]	100[i]	100[i]	100[i]	100[i]	97[i]
TO	**Torymidae (68/986)**	29	41	–	–	–	–	–	–	–	–
TOM	Megastigminae	3	6	66	67	99	99	97	97	100	92
TOT	Toryminae	28	37	–	+	67	+	+	62	86	+
TOTI	Toryminae *i.s.*	3	4	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
TOTM	Microdonteromerini	6	8	–	–	–	*par*	*par*	–	*par*	*par*
TOTN	Monodontomerini	6	8	80	*par*	100	91	89	81	100	97
TOTP	Palachiini	2	2	–	–	–	–	–	–	–	–
TOTO	Podagrionini	4	4	*par*	57	*par*	90	*par*	55	62	+
TOTT	Torymini	3	6	75	74	66	87	68	66	100	–
TOTY	Torymoidini	4	5	*par*	–	–	–	–	–	88	–
TR	**Trichogrammatidae (83/839)**	12	21	–	+	61	65	64	+	94	+
TRO	Oligositinae	9	10	98	100	97	96	95	93	100	+
TROI	Oligositinae *i.s.*	3	4	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
TROC	Chaeotostrichini	2	3	99	100	100	100	100	100	100	100
TROO	Oligositini	1	2	100	100	100	100	100	100	100	100
TROP	Paracentrobiini	1	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
TRT	Trichogrammatinae	3	11	+	*par*	*par*	*par*	*par*	*par*	*par*	*par*
TRTI	Trichogrammatinae *i.s.*	3	5	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
TRTT	Trichogrammatini	2	6	100	100	100	100	100	100	100	100
Number of clades with positive support:				**56**	**59**	**60**	**58**	**58**	**59**	**62**	**52**

Dataset abbreviations explained in Table 4. RAxML majority rule (MJR) is a consensus across all 16 submatrices. Support values are bootstrap percentages. The number of clades with positive support is summed for all clades with either a + (presence) or numerical support; *par* = paraphyletic; – = not monophyletic. Estimated diversity (genera/species) after family group names from Noyes [4]. Taxa represented by a single OTU or *incertae sedis* (*i.s.*) were considered not applicable (n/a) for clade support.

[a] = without Agaonidae Group 4 (*Wiebesia* and *Blastophaga* R1757);
[b] = without Azotinae or *Eretmocerus*;
[c] = excluding Akapalinae and Philomidinae;
[d] = without *Trisecodes*;
[e] = excluding *Buresium*;
[f] = including *Idioporus*;
[g] = excluding *Idioporus*;
[h] = not including *Cynipencyrtus*;
[i] = excluding *Diplesiostigma*.

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Mymaridae. Within the same region, RAA(4) shows a slight but more subtle increase for Chalcidoidea excluding Mymaridae. RSC(4) and RSC(4′) both show support for Chalcidoidea excluding Mymaridae based on a respective increase to a 4 base motif (RSC 4), and an increase to a consistent AT or GT pattern (RSC 4′; not shown). These structural changes support both

Table 4. Higher group relationships supported across various analyses.

	core only		core and RAA				RAxML	TNT
Group Relationships	**SSNR**	**MENR**	**SSME**	**SSGE**	**MGSR**	**MGMG**	**MJR**	**SSME**
Pantolytomiya + Chalcidoidea	−	+	−	+	−	+	62	−
Diaprioidea (part) + Chalcidoidea	−	−	+[a]	−	−	−	56	−
'Diapriidae' + Chalcidoidea	+	−	−	−	−	−	−	−
Mymarommatoidea + Chalcidoidea	−	−	−	−	+	−	−	−
(Proctotrupoidea + Diaprioidea) sister to Chalcidoidea	−	−	−	−	−	−	−	+
Chalcidoidea	99	95	100	100	98	98	100	100
remaining Chalcidoidea minus Mymaridae	91	55	97	95	55	85	94	+
remaining Chalcidoidea minus Rotoitidae and Mymaridae	+	+	+	76	+	+	94	−
Mymaridae: 4−segmented taxa	74	78	75	87	57	80	88	+
Mymaridae: 5-segmented taxa	+	+	76	62	83	+	88	+
Eulophidae: (Opheliminae + *Perthiola*) + Entiinae	−	−	+	+	−	−	56	+
Eucharitidae + Perilampidae	−	−	+	+	+	+	−	+
Perilampidae (with Akapalinae, Philomidinae and *Idioporus*)	+	+	*par*	+	+	+	−	−
Jambiya + Eucharitidae	−	−	+	+	+	+	−	+
Jambiya + Perilampidae	−	+	−	−	−	−	−	−
pteromaloid complex[b]	+	+	+	+	+	+	−	+[c]
Spalangiinae + Agaonidae	−	−	+	−	−	−	−	−
Sycophaginae + Agaonidae	+	−	−	−	−	−	−	−
remaining Agaonidae minus Tetrapusinae	+	55	+	−	−	+	−	+
Aphelininae + Coccophaginae	+[d]	−	−	−	−	−	−	−
Azotinae + Trichogrammatidae	+	+	+	−	+	+	62	−
Azotinae + Signiphoridae	−	−	−	−	−	−	−	+
Agaoninae + Blastophaginae (excluding group 4)	+	+	65	61	+	+	62	+

a = Monomachidae + Diapriidae as sister groups;
b = includes Cratominae, Miscogastrinae, Otitesellinae, Panstenoninae, Pteromalinae and Sycoryctinae;
c = without *Heterandrium* (Otitesellinae);
d = including *Platygerrhus* (Microgasterinae: Trigonoderini).
Dataset abbreviations explained in Table 4. RAxML majority rule (MJR) is a consensus across all 16 submatrices. Support values are bootstrap percentages.
Abbreviations: + refers to presence of clade but without numerical support; *par* = paraphyletic.
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monophyly of Chalcidoidea and a sister group relationship between Mymaridae and the remaining Chalcidoidea. No RAA patterns were observed that would add support for relationships in the outgroup taxa. However within Chalcidoidea, additional structural changes within variable regions add support to some relationships (i.e., an increase in 18S loop(4) size in Perilampidae and Eucharitidae; and deletion of a contiguous variable region (RAAs 23-25) in Eulophinae + Tetrastichinae). Six variable regions in Agaonidae demonstrate substantial growth in size, both across and within the family, that distinguish them from all other Chalcidoidea. The different sizes of the variable regions might be expected to have the greatest impact on results from datasets contrasting the inclusion or exclusion of RAAs, or the MAFFT alignment without reference to the SS core structure; however, overall there appeared to be no impact, with all results consistently supporting monophyly of Chalcidoidea and a sister group relationship between Mymaridae and the remaining Chalcidoidea.

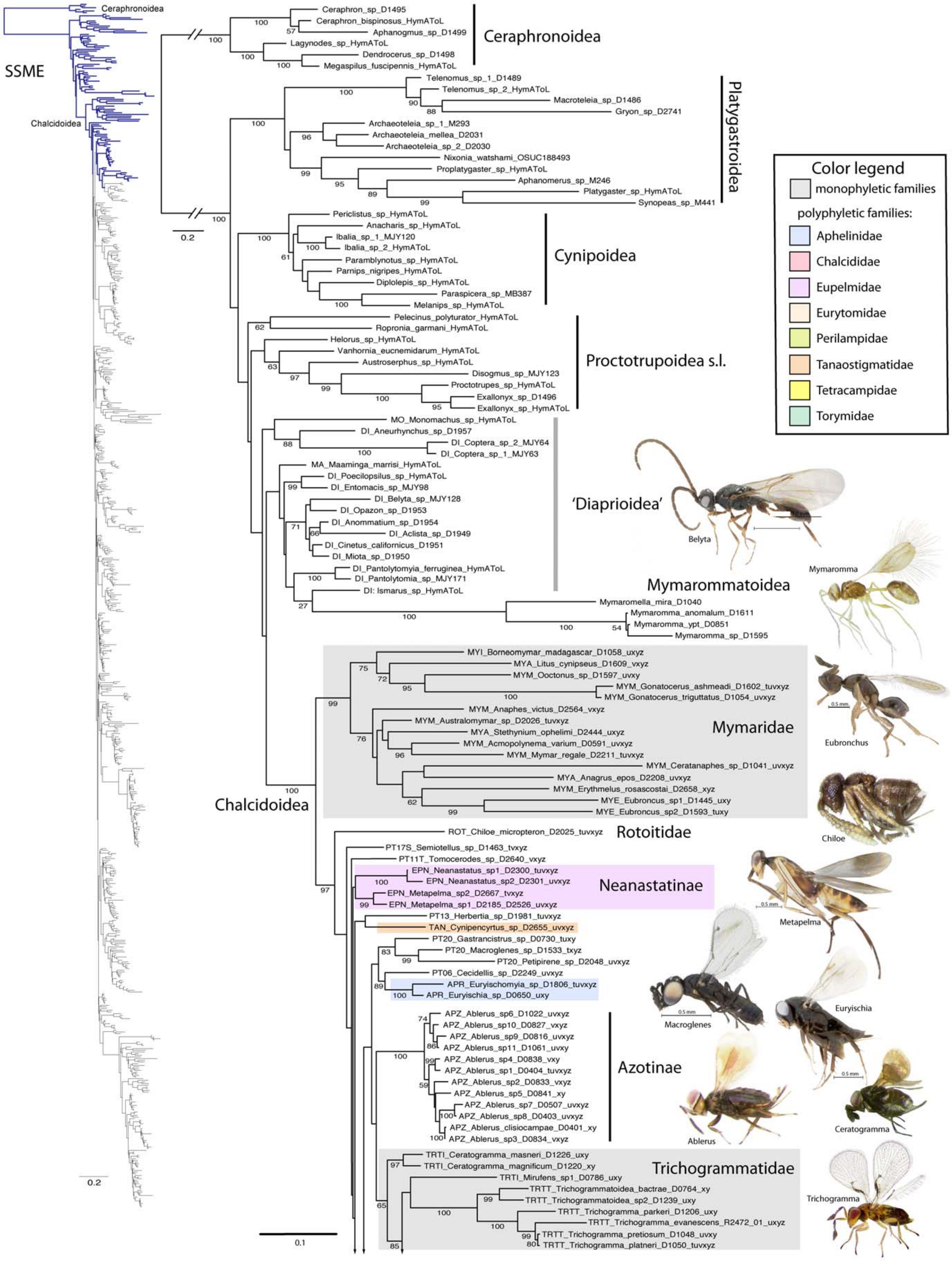
Inclusion of the RAAs contributed to the monophyly of Encyrtinae, Entedoninae and Entiinae (Table 3). Their inclusion increased the BS support for a number of clades, including Agaoninae group 4, Encyrtidae, Eulophinae, Rileyinae, Lyciscini, Eunotini, Signiphoridae and Megastigminae (Tables 3, 4). At a higher group level, the inclusion of the RAA regions provided a greater amount of support for Eucharitidae + Perilampidae, and the genus *Jambiya* as the sister group of Eucharitidae. In no cases did the inclusion of RAAs result in a substantial decrease in support for a clade.

Phylogenetic Relationships

Relationships across the 16 ML analyses overall were the same regardless of alignment method or the inclusion or exclusion of RAAs (Figs. 1–7, Tables 3, 4). The parsimony analysis of the SSME dataset produced more than 10,000 most parsimonious trees of 31,607 steps (RI = 0.62); however the strict consensus was well resolved (Supplementary Fig. S1) and in general accord with the likelihood results.

Outgroup relationships generally favored a paraphyletic Diaprioidea as sister group to Chalcidoidea (Fig. 1), but in a few cases Mymarommatoidea were the proposed sister group. A core Proctotrupomorpha clade of Proctotrupoidea *sensu stricto*, Diaprioidea, Mymarommatoidea and Chalcidoidea were supported in all results. Both Ceraphronoidea and Platygastroidea were distantly related in all analyses.

Chalcidoidea were always monophyletic with strong support, as was a sister group relationship between Mymaridae and the remaining Chalcidoidea (Table 4). *Chiloe micropteron* (Rotoitidae)



Ceraphronoidea
SSME
Chalcidoidea
0.2
Ceraphron_sp_D1495
Ceraphron_bispinosus_HymAToL
Aphanogmus_sp_D1499
Lagynodes_sp_HymAToL
Dendrocerus_sp_D1498
Megaspilus_fuscipennis_HymAToL
Ceraphronoidea
Telenomus_sp_1_D1489
Telenomus_sp_2_HymAToL
Macroteleia_sp_D1486
Gryon_sp_D2741
Archaeoteleia_sp_1_M293
Archaeoteleia_mellea_D2031
Archaeoteleia_sp_2_D2030
Nixonia_watshami_OSUC188493
Proplatygaster_sp_HymAToL
Aphanomerus_sp_M246
Platygaster_sp_HymAToL
Synopeas_sp_M441
Platygastroidea
Periclistus_sp_HymAToL
Anacharis_sp_HymAToL
Ibalia_sp_1_MJY120
Ibalia_sp_2_HymAToL
Paramblynotus_sp_HymAToL
Parnips_nigripes_HymAToL
Diplolepis_sp_HymAToL
Paraspicera_sp_MB387
Melanips_sp_HymAToL
Cynipoidea
Pelecinus_polyturator_HymAToL
Ropronia_garmani_HymAToL
Helorus_sp_HymAToL
Vanhornia_eucnemidarum_HymAToL
Austroserphus_sp_HymAToL
Disogmus_sp_MJY123
Proctotrupes_sp_HymAToL
Exallonyx_sp_D1496
Exallonyx_sp_HymAToL
Proctotrupoidea s.l.
MO_Monomachus_sp_HymAToL
DI_Aneurhynchus_sp_D1957
DI_Coptera_sp_2_MJY64
DI_Coptera_sp_1_MJY63
MA_Maaminga_marrisi_HymAToL
DI_Poecilopsilus_sp_HymAToL
DI_Entomacis_sp_MJY98
DI_Belyta_sp_MJY128
DI_Opazon_sp_D1953
DI_Anommatium_sp_D1954
DI_Aclista_sp_D1949
DI_Cinetus_californicus_D1951
DI_Miota_sp_D1950
DI_Pantolytomyia_ferruginea_HymAToL
DI_Pantolytomia_sp_MJY171
DI: Ismarus_sp_HymAToL
'Diaprioidea'
Belyta
Mymarommatoidea
Mymaromella_mira_D1040
Mymaromma_anomalum_D1611
Mymaromma_ypt_D0851
Mymaromma_sp_D1595
Mymaromma
MYI_Borneomymar_madagascar_D1058_uxyz
MYA_Litus_cynipseus_D1609_vxyz
MYM_Ooctonus_sp_D1597_uvxy
MYM_Gonatocerus_ashmeadi_D1602_tuvxyz
MYM_Gonatocerus_triguttatus_D1054_uvxyz
MYM_Anaphes_victus_D2564_vxyz
MYM_Australomymar_sp_D2026_tuvxyz
MYA_Stethynium_ophelimi_D2444_uxyz
MYM_Acmopolynema_varium_D0591_uvxyz
MYM_Mymar_regale_D2211_tuvxyz
MYM_Ceratanaphes_sp_D1041_uvxyz
MYA_Anagrus_epos_D2208_uvxyz
MYM_Erythmelus_rosascostai_D2658_xyz
MYE_Eubroncus_sp1_D1445_uxy
MYE_Eubroncus_sp2_D1593_tuxy
Mymaridae
Eubronchus
0.5 mm
Chalcidoidea
ROT_Chiloe_micropteron_D2025_tuvxyz
Rotoitidae
Chiloe
PT17S_Semiotellus_sp_D1463_tvxyz
PT11T_Tomocerodes_sp_D2640_vxyz
EPN_Neanastatus_sp1_D2300_tuvxyz
EPN_Neanastatus_sp2_D2301_uvxyz
EPN_Metapelma_sp2_D2667_tvxyz
EPN_Metapelma_sp1_D2185_D2526_uvxyz
Neanastatinae
Metapelma
PT13_Herbertia_sp_D1981_tuvxyz
TAN_Cynipencyrtus_sp_D2655_uvxyz
PT20_Gastrancistrus_sp_D0730_tuxy
PT20_Macroglenes_sp_D1533_txyz
PT20_Petipirene_sp_D2048_uvxyz
PT06_Cecidellis_sp_D2249_uvxyz
APR_Euryischomyia_sp_D1806_tuvxyz
APR_Euryischia_sp_D0650_uxy
Macroglenes
Euryischia
APZ_Ablerus_sp6_D1022_uvxyz
APZ_Ablerus_sp10_D0827_vxyz
APZ_Ablerus_sp9_D0816_uvxyz
APZ_Ablerus_sp11_D1061_uvxy
APZ_Ablerus_sp4_D0838_vxy
APZ_Ablerus_sp1_D0404_tuvxyz
APZ_Ablerus_sp2_D0833_vxyz
APZ_Ablerus_sp5_D0841_xy
APZ_Ablerus_sp7_D0507_uvxyz
APZ_Ablerus_sp8_D0403_uvxyz
APZ_Ablerus_clisiocampae_D0401_xy
APZ_Ablerus_sp3_D0834_vxyz
Azotinae
Ablerus
Ceratogramma
TRTI_Ceratogramma_masneri_D1226_uxy
TRTI_Ceratogramma_magnificum_D1220_xy
TRTI_Mirufens_sp1_D0786_uxy
TRTT_Trichogrammatoidea_bactrae_D0764_xy
TRTT_Trichogrammatoidea_sp2_D1239_uxy
TRTT_Trichogramma_parkeri_D1206_uxy
TRTT_Trichogramma_evanescens_R2472_01_uxyz
TRTT_Trichogramma_pretiosum_D1048_uvxy
TRTT_Trichogramma_platneri_D1050_tuvxyz
Trichogrammatidae
Trichogramma
0.1
Color legend
monophyletic families
polyphyletic families:
Aphelinidae
Chalcididae
Eupelmidae
Eurytomidae
Perilampidae
Tanaostigmatidae
Tetracampidae
Torymidae

Figure 1. Phylogenetic tree from secondary structure alignment of stem data and E-INS-i alignment of RAAs (3917 aligned; SSME). RAxML analysis with seed 38652 and 1000 rbs bootstrap replicates (support >50% above branches). Phylogram of entire tree on left colored to match inset. Taxon names with prefix indicating classification (see Table 3) and suffix indicating DNA voucher number and gene regions included for 18Sa-c (tuv) and D2 (x), D3 (y) and D4-5 (z). Monophyletic families indicated by gray shading; polyphyletic families other than Pteromalidae indicated according to inset color scheme.
doi:10.1371/journal.pone.0027023.g001

was consistently supported in the likelihood results as the sister group of the remaining Chalcidoidea excluding Mymaridae (94% MJR), but with bootstrap support only in the SSGE results (BS 76). However, in the parsimony results *Chiloe* was deeply nested within Chalcidoidea (Supplementary Fig. S1).

Relationships within Chalcidoidea were highly variable along the backbone of the tree and should be regarded as a broad polytomy, but with consistent and sometimes strong support for many traditional taxon groupings at the family, subfamily, and tribe levels (Table 3). There is sometimes a lack of support for families that can be defined by several justifiable synapomorphies such as Chalcididae, and there is consistent support for some other families such as Eulophidae that are founded on what might be considered as weak loss or reductive features [9].

Discussion

Comparison of alignment strategies

Overall, there was little impact of the application of different MAFFT alignments to either the RAA regions, the core secondary structure data, or to the different gene regions without reference to secondary structure. This is optimistic for the future inclusion of new taxa to our data set where we can avoid the labor-intensive approach of having to align new taxa to our existing secondary structure model. Inclusion of the RAAs contributed to monophyly and clade support for a number of taxa, and also increased support at higher levels. Furthermore, structural differences found in various RAAs (Fig. 8) provide clear support for Chalcidoidea, a sister-group relationship between Mymaridae and other Chalcidoidea, and for some of the higher-level groups within Chalcidoidea. Clearly, RAAs do provide some phylogenetic signal and their inclusion in analyses is warranted despite some authors recommending complete [52] or partial [19] deleting of these regions.

Outgroup relationships

We found either Mymarommatoidea or Diaprioidea as the sister group of Chalcidoidea. These equivocal results were similar to results from a recent analysis of Hymenoptera that used more extensive molecular data from four gene regions and nearly complete 28S and 18S data [11]. Molecular data from both studies clearly support a monophyletic group of Diaprioidea, Mymarommatoidea and Chalcidoidea within the Proctotrupomorpha. With the inclusion of morphological data in a combined analysis, Mymarommatoidea is the sister group of Chalcidoidea [13], as hypothesized by Gibson [10]. Unfortunately, the biology of Mymarommatoidea remains unknown, making it difficult to compare with Chalcidoidea.

Phylogenetic relationships within Chalcidoidea

Chalcidoidea are well supported as monophyletic. Mymaridae are strongly supported as monophyletic and the sister group of the remaining Chalcidoidea. This hypothesis was first proposed by Gibson [10] based on morphology, and substantiated by Heraty et al. [11] and Sharkey et al. [13]. *Chiloe micropteron* (Rotoitidae) was the sister group of the remaining Chalcidoidea in all of the likelihood results, but not using parsimony. With more extensive gene sampling, Heraty et al. [11] recovered the same relationships in likelihood analyses of the eye-aligned data, and with parsimony only in the data aligned by eye. Mymaridae and Mymarommatidae are both common in early to mid Cretaceous amber deposits [5,6,8], which support their early origin and sister group relationships. Rotoitidae is unknown in any fossil deposits, but has a potentially archaic pattern of distribution, with genera known only in New Zealand and southern Chile [6], suggesting a late cretaceous origin [53].

After Rotoitidae, the relationships within Chalcidoidea become vague. The backbone of the chalcidoid tree has little support, with taxonomic groups shifting in different analyses from the base to somewhere more apical in the topology. As well, there are few consistent sister group relationships supported among the higher-level groups. One of the few relationships that can be substantiated based on larval morphology, Eucharitidae + Perilampidae [54], occurs in some but not all results, and never has bootstrap support. This is not simply an artifact of our ribosomal dataset; similar results with poor backbone support were also found by Desjardins et al. [18] using 4 nuclear protein coding genes and far fewer taxa. We do recover support for many of the traditional higher-level groups within Chalcidoidea, mostly at the subfamily and tribe level, but also for a few diverse family groups such as Agaonidae, Eulophidae, Eucharitidae and Trichogrammatidae. We also recovered consistent support for a novel pteromaloid complex that is a mix of morphologically very distinct subfamily groups. For some of the traditionally well-supported groups such as Chalcididae, the majority of the included taxa were monophyletic in only one analysis. A similar rare grouping was also found for a monophyletic Signiphoridae + Azotinae.

We found some taxa that could not be placed within any traditional higher-level group. There were also a few singleton taxa that defied placement, including *Diplesiostigma*, *Cynipencyrtus* and *Idioporus*. Interestingly, *Idioporus* is also difficult to place based on morphology, although neither Perilampidae (likelihood) or Rotoitidae (parsimony) were ever suggested as being related based on a morphological study by LaSalle et al. [55]. Calesinae are currently *incertae sedis* within Chalcidoidea [56], and our results to not offer any potential sister groups for this clade. Pteromalidae, as expected, is polyphyletic and affects greatly the composition and relationships of other taxa. Our results will be reevaluated in a combined morphological analysis, which is currently underway (Heraty et al. in prep), but it is clear that the family level relationships of Chalcidoidea are in need of major revision.

For the discussions below, some historical information on relationships is presented for each family group followed by the results of the current study. A more detailed review of classification history and biology can be found in Gibson et al. [9] and Hanson & Gauld [57]. We try not to discuss relationships of taxa within supported clades, but most often species within the same genera and species groups were monophyletic, and relationships within a clade were generally the same across different analyses (Figs 1–7).

Agaonidae. Agaoninae and Sycophaginae (as Idarninae), once included in Torymidae, were moved to Agaonidae by Bouček [30]. Agaonidae *sensu lato* were comprised of Agaoninae, Epichrysomallinae, Otitesellinae, Sycoecinae, Sycophaginae and

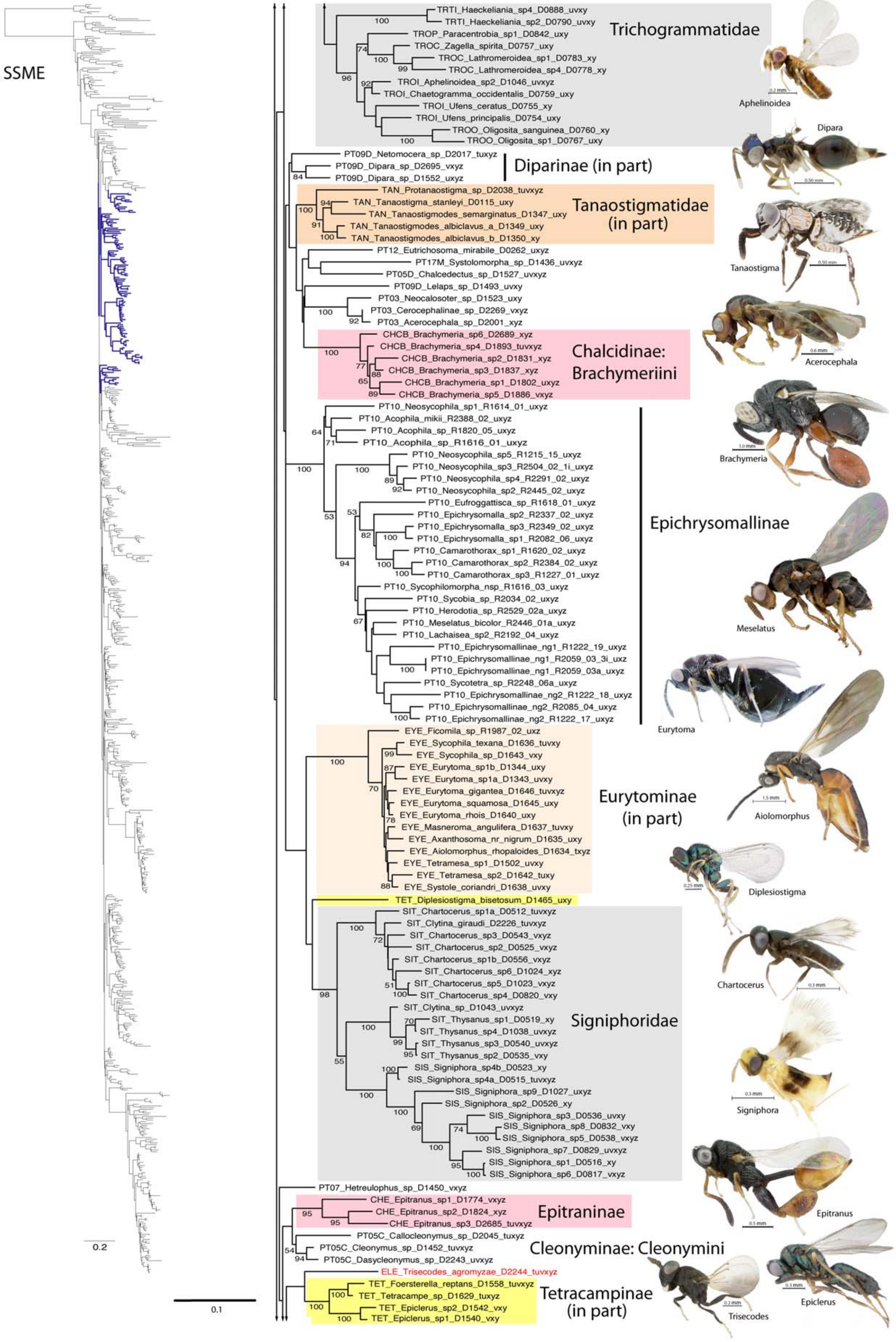


Figure 2. Phylogenetic tree of Chalcidoidea (continued).
doi:10.1371/journal.pone.0027023.g002

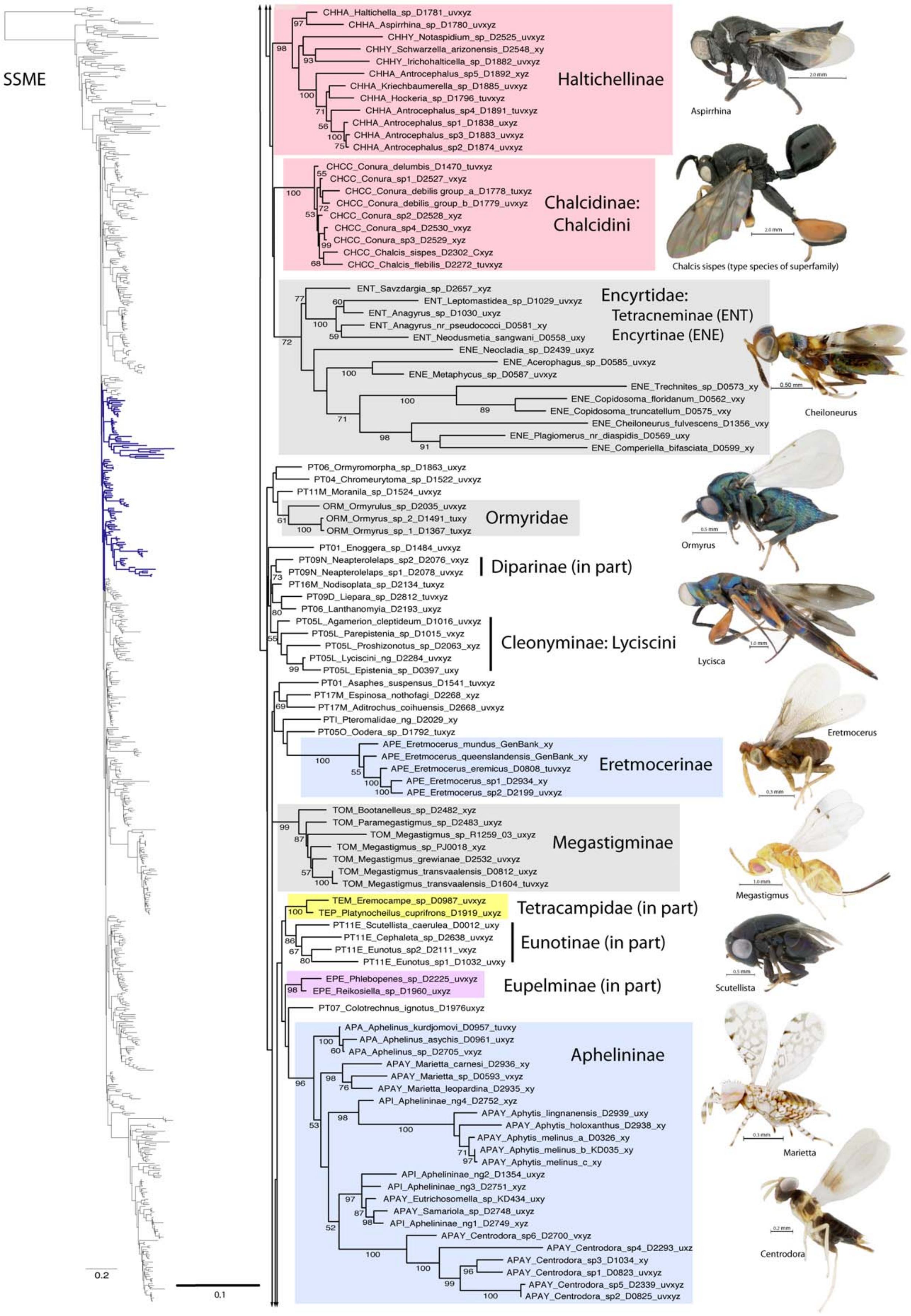


Figure 3. Phylogenetic tree of Chalcidoidea (continued).
doi:10.1371/journal.pone.0027023.g003

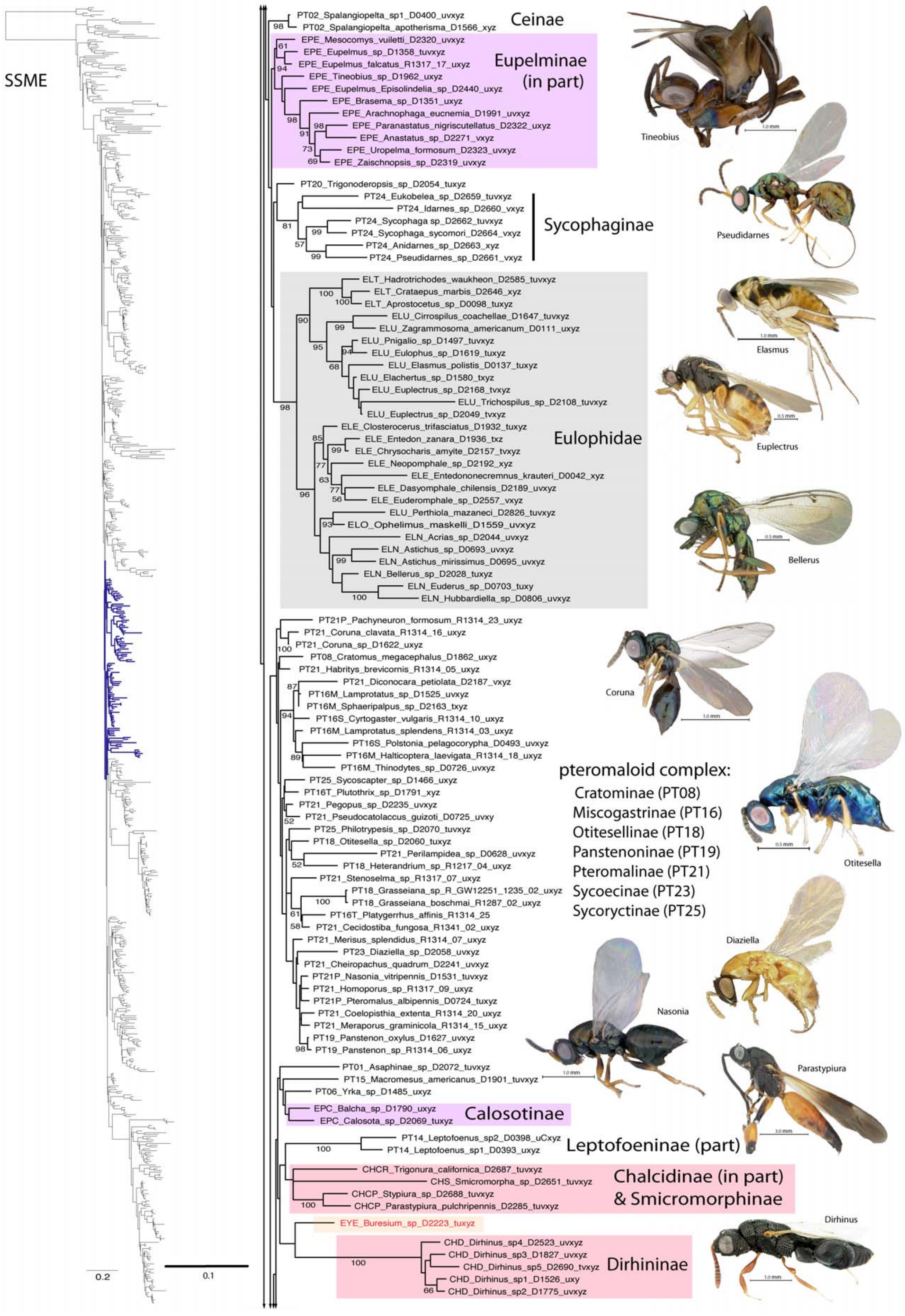


Figure 4. Phylogenetic tree of Chalcidoidea (continued).
doi:10.1371/journal.pone.0027023.g004

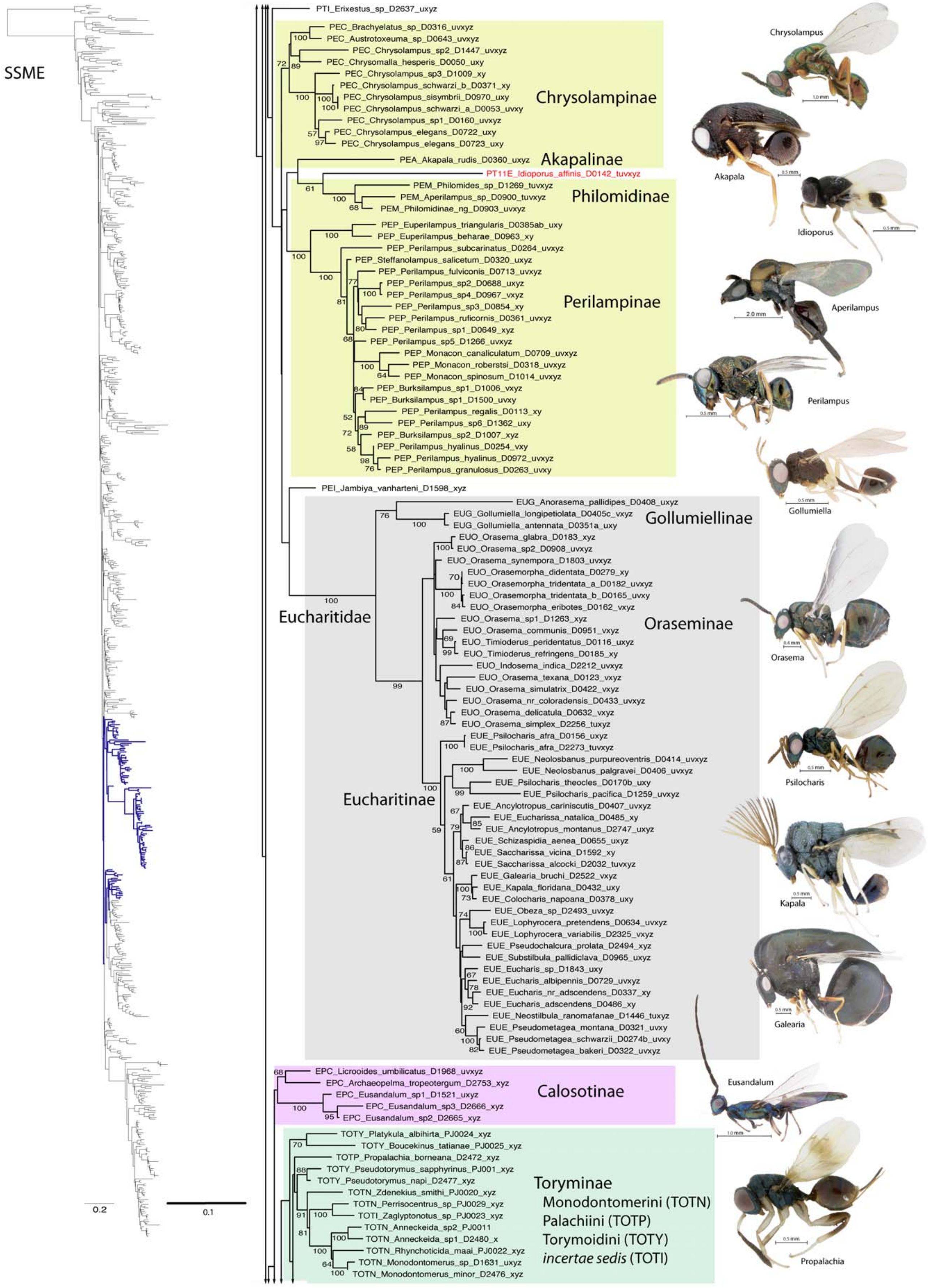


Figure 5. Phylogenetic tree of Chalcidoidea (continued).
doi:10.1371/journal.pone.0027023.g005

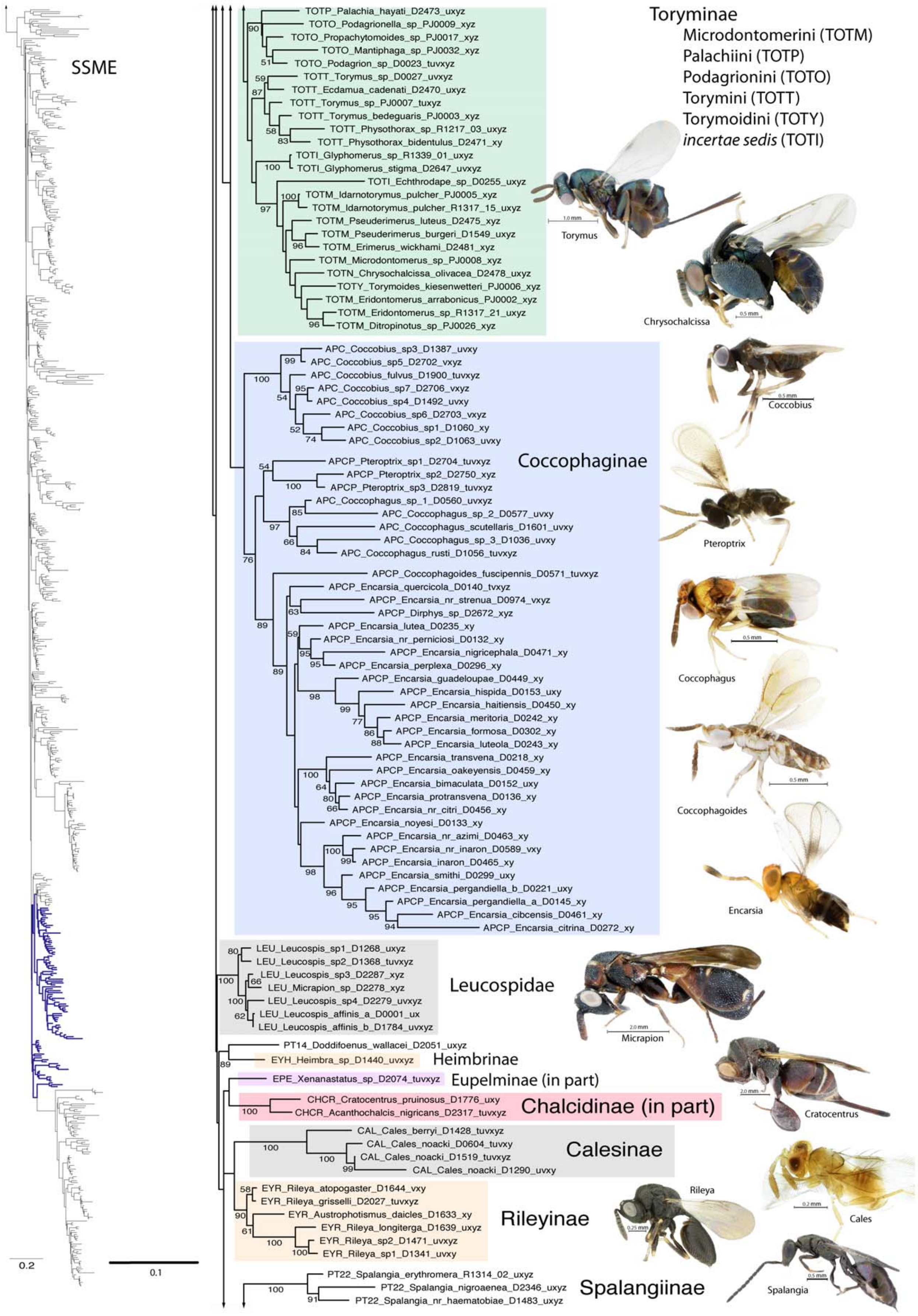


Figure 6. Phylogenetic tree of Chalcidoidea (continued).
doi:10.1371/journal.pone.0027023.g006

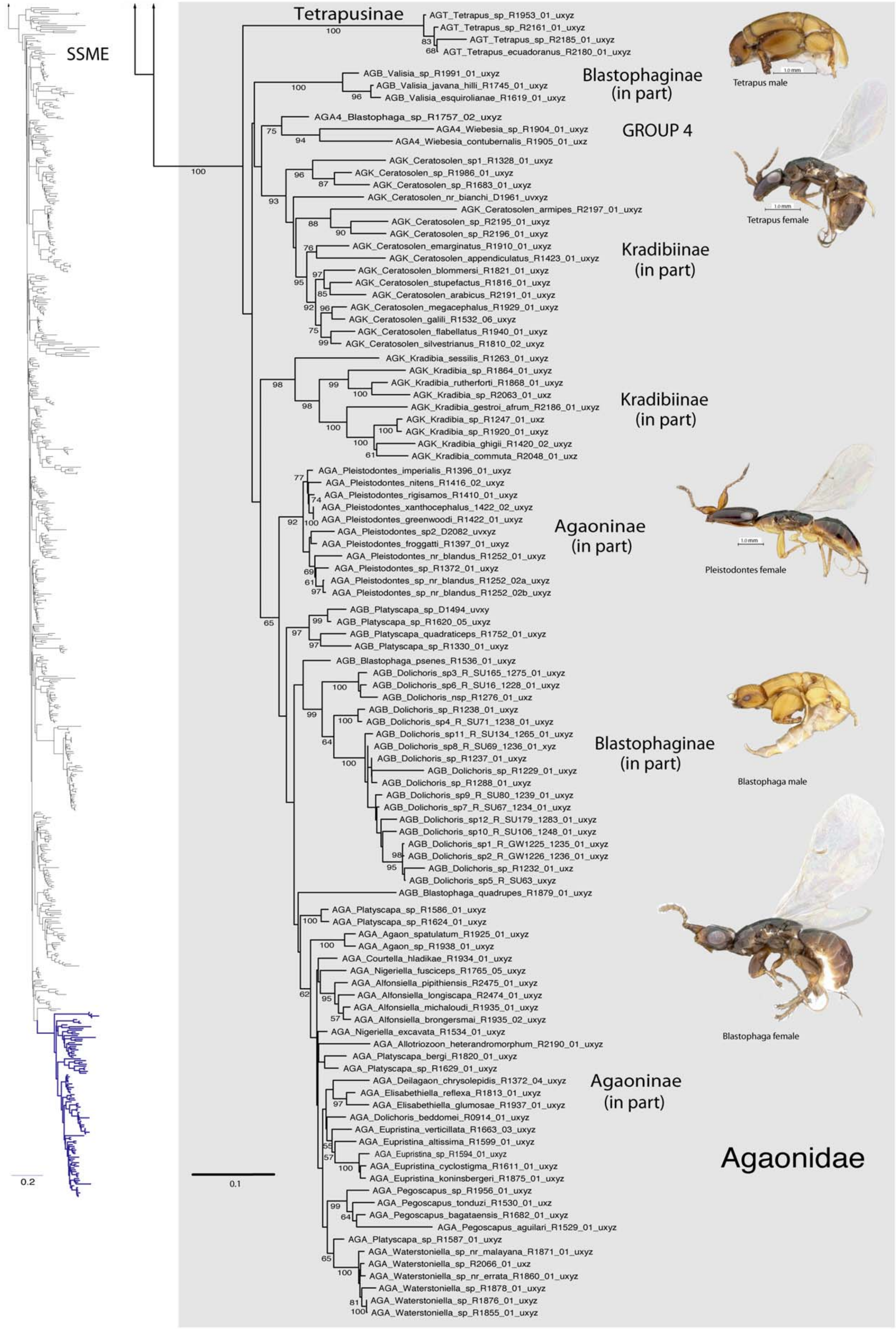
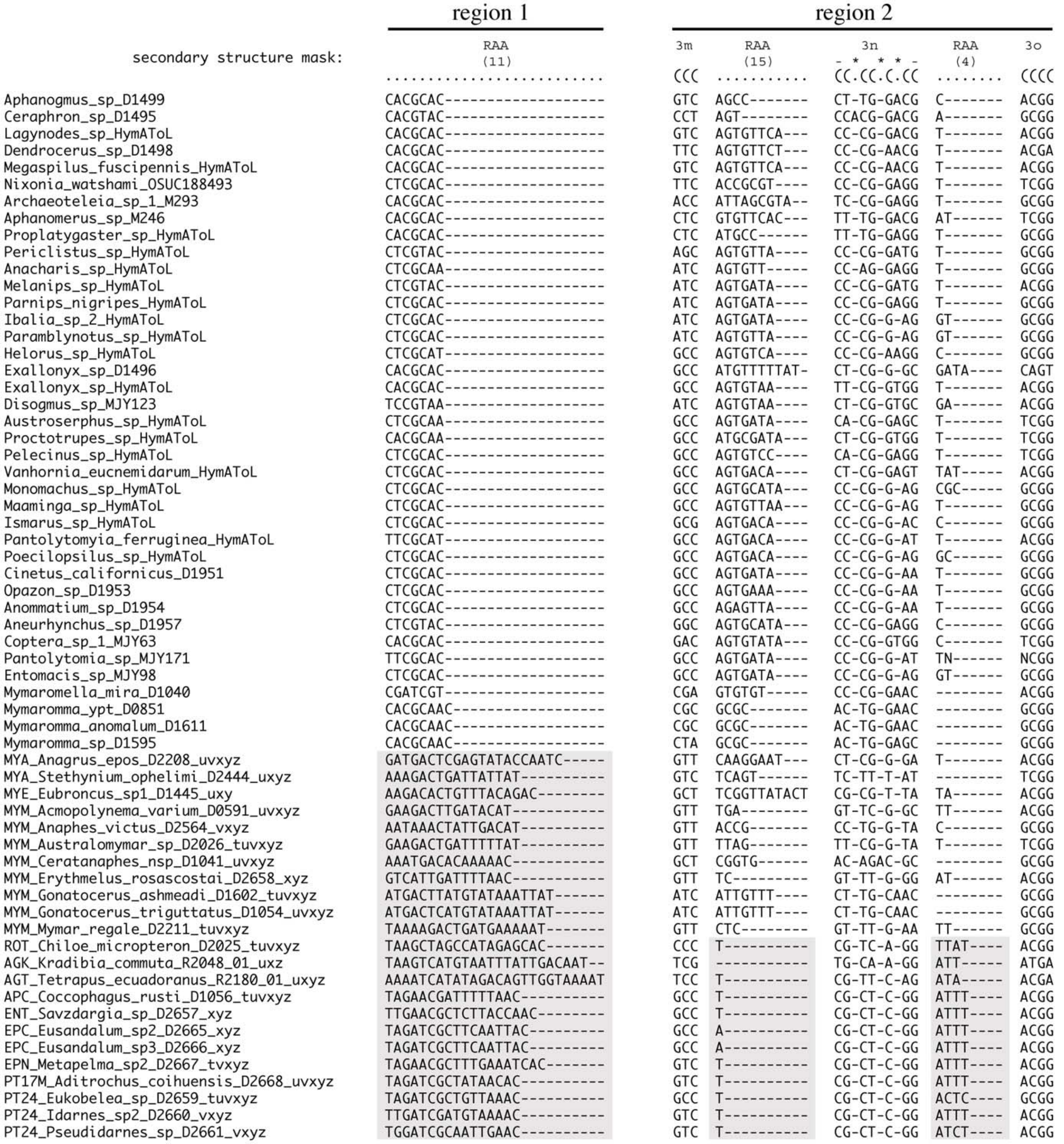


Figure 7. Phylogenetic tree of Chalcidoidea (continued).
doi:10.1371/journal.pone.0027023.g007



	region 1	region 2				
	RAA (11)	3m	RAA (15)	3n - * * * -	RAA (4)	3o
secondary structure mask:	....	(((	....	((.((.(.((	....	((((
Aphanogmus_sp_D1499	CACGCAC-------------------	GTC	AGCC-------	CT-TG-GACG	C-------	ACGG
Ceraphron_sp_D1495	CACGTAC-------------------	CCT	AGT--------	CCACG-GACG	A-------	GCGG
Lagynodes_sp_HymAToL	CACGCAC-------------------	GTC	AGTGTTCA---	CC-CG-GACG	T-------	ACGG
Dendrocerus_sp_D1498	CACGCAC-------------------	TTC	AGTGTTCT---	CC-CG-AACG	T-------	ACGA
Megaspilus_fuscipennis_HymAToL	CACGCAC-------------------	GTC	AGTGTTCA---	CC-CG-AACG	T-------	ACGG
Nixonia_watshami_OSUC188493	CTCGCAC-------------------	TTC	ACCGCGT----	CC-CG-GAGG	T-------	TCGG
Archaeoteleia_sp_1_M293	CACGCAC-------------------	ACC	ATTAGCGTA--	TC-CG-GAGG	T-------	GCGG
Aphanomerus_sp_M246	CACGCAC-------------------	CTC	GTGTTCAC---	TT-TG-GACG	AT------	TCGG
Proplatygaster_sp_HymAToL	CACGCAC-------------------	CTC	ATGCC------	TT-TG-GAGG	T-------	GCGG
Periclistus_sp_HymAToL	CTCGTAC-------------------	AGC	AGTGTTA----	CC-CG-GATG	T-------	GCGG
Anacharis_sp_HymAToL	CTCGCAA-------------------	ATC	AGTGTT-----	CC-AG-GAGG	T-------	GCGG
Melanips_sp_HymAToL	CTCGTAC-------------------	ATC	AGTGATA----	CC-CG-GATG	T-------	ACGG
Parnips_nigripes_HymAToL	CTCGCAC-------------------	ATC	AGTGATA----	CC-CG-GAGG	T-------	GCGG
Ibalia_sp_2_HymAToL	CTCGCAC-------------------	ATC	AGTGATA----	CC-CG-G-AG	GT------	GCGG
Paramblynotus_sp_HymAToL	CTCGCAC-------------------	ATC	AGTGTTA----	CC-CG-G-AG	GT------	GCGG
Helorus_sp_HymAToL	CTCGCAT-------------------	GCC	AGTGTCA----	CC-CG-AAGG	C-------	GCGG
Exallonyx_sp_D1496	CACGCAC-------------------	GCC	ATGTTTTTAT-	CT-CG-G-GC	GATA----	CAGT
Exallonyx_sp_HymAToL	CACGCAC-------------------	GCC	AGTGTAA----	TT-CG-GTGG	T-------	ACGG
Disogmus_sp_MJY123	TCCGTAA-------------------	ATC	AGTGTAA----	CT-CG-GTGC	GA------	ACGG
Austroserphus_sp_HymAToL	CTCGCAA-------------------	GCC	AGTGATA----	CA-CG-GAGC	T-------	TCGG
Proctotrupes_sp_HymAToL	CACGCAA-------------------	GCC	ATGCGATA---	CT-CG-GTGG	T-------	TCGG
Pelecinus_sp_HymAToL	CTCGCAC-------------------	GCC	AGTGTCC----	CA-CG-GAGG	T-------	TCGG
Vanhornia_eucnemidarum_HymAToL	CTCGCAC-------------------	GCC	AGTGACA----	CT-CG-GAGT	TAT-----	ACGG
Monomachus_sp_HymAToL	CTCGCAC-------------------	GCC	AGTGCATA---	CC-CG-G-AG	CGC-----	GCGG
Maaminga_sp_HymAToL	CTCGCAC-------------------	GCC	AGTGTTAA---	CC-CG-G-AG	T-------	GCGG
Ismarus_sp_HymAToL	CTCGCAC-------------------	GCG	AGTGACA----	CC-CG-G-AC	C-------	GCGG
Pantolytomyia_ferruginea_HymAToL	TTCGCAT-------------------	GCC	AGTGACA----	CC-CG-G-AT	T-------	ACGG
Poecilopsilus_sp_HymAToL	CTCGCAC-------------------	GCC	AGTGACA----	CC-CG-G-AG	GC------	GCGG
Cinetus_californicus_D1951	CTCGCAC-------------------	GCC	AGTGATA----	CC-CG-G-AA	T-------	GCGG
Opazon_sp_D1953	CTCGCAC-------------------	GCC	AGTGAAA----	CC-CG-G-AA	T-------	GCGG
Anommatium_sp_D1954	CTCGCAC-------------------	GCC	AGAGTTA----	CC-CG-G-AA	T-------	GCGG
Aneurhynchus_sp_D1957	CTCGTAC-------------------	GGC	AGTGCATA---	CC-CG-GAGG	C-------	GCGG
Coptera_sp_1_MJY63	CACGCAC-------------------	GAC	AGTGTATA---	CC-CG-GTGG	C-------	TCGG
Pantolytomia_sp_MJY171	TTCGCAC-------------------	GCC	AGTGATA----	CC-CG-G-AT	TN------	NCGG
Entomacis_sp_MJY98	CTCGCAC-------------------	GCC	AGTGATA----	CC-CG-G-AG	GT------	GCGG
Mymaromella_mira_D1040	CGATCGT-------------------	CGA	GTGTGT-----	CC-CG-GAAC	--------	ACGG
Mymaromma_ypt_D0851	CACGCAAC------------------	CGC	GCGC-------	AC-TG-GAAC	--------	GCGG
Mymaromma_anomalum_D1611	CACGCAAC------------------	CGC	GCGC-------	AC-TG-GAAC	--------	GCGG
Mymaromma_sp_D1595	CACGCAAC------------------	CTA	GCGC-------	AC-TG-GAGC	--------	GCGG
MYA_Anagrus_epos_D2208_uvxyz	GATGACTCGAGTATACCAATC-----	GTT	CAAGGAAT---	CT-CG-G-GA	T-------	ACGG
MYA_Stethynium_ophelimi_D2444_uxyz	AAAGACTGATTATTAT----------	GTC	TCAGT------	TC-TT-T-AT	--------	TCGG
MYE_Eubroncus_sp1_D1445_uxy	AAGACACTGTTTACAGAC--------	GCT	TCGGTTATACT	CG-CG-T-TA	TA------	ACGG
MYM_Acmopolynema_varium_D0591_uvxyz	GAAGACTTGATACAT-----------	GTT	TGA--------	GT-TC-G-GC	TT------	ACGG
MYM_Anaphes_victus_D2564_vxyz	AATAAACTATTGACAT----------	GTT	ACCG-------	CC-TG-G-TA	C-------	GCGG
MYM_Australomymar_sp_D2026_tuvxyz	GAAGACTGATTTTTAT----------	GTT	TTAG-------	TT-CG-G-TA	T-------	TCGG
MYM_Ceratanaphes_nsp_D1041_uvxyz	AAATGACACAAAAAC-----------	GCT	CGGTG------	AC-AGAC-GC	--------	GCGG
MYM_Erythmelus_rosascostai_D2658_xyz	GTCATTGATTTTAAC-----------	GTT	TC---------	GT-TT-G-GG	AT------	ACGG
MYM_Gonatocerus_ashmeadi_D1602_tuvxyz	ATGACTTATGTATAAATTAT------	ATC	ATTGTTT----	CT-TG-CAAC	--------	GCGG
MYM_Gonatocerus_triguttatus_D1054_uvxyz	ATGACTCATGTATAAATTAT------	ATC	ATTGTTT----	CT-TG-CAAC	--------	GCGG
MYM_Mymar_regale_D2211_tuvxyz	TAAAAGACTGATGAAAAAT-------	GTT	CTC--------	GT-TT-G-AA	TT------	GCGG
ROT_Chiloe_micropteron_D2025_tuvxyz	TAAGCTAGCCATAGAGCAC-------	CCC	T----------	CG-TC-A-GG	TTAT----	ACGG
AGK_Kradibia_commuta_R2048_01_uxz	TAAGTCATGTAATTTATTGACAAT--	TCG	-----------	TG-CA-A-GG	ATT-----	ATGA
AGT_Tetrapus_ecuadoranus_R2180_01_uxyz	AAAATCATATAGACAGTTGGTAAAAT	TCC	T----------	CG-TT-C-AG	ATA-----	ACGA
APC_Coccophagus_rusti_D1056_tuvxyz	TAGAACGATTTTTAAC----------	GCC	T----------	CG-CT-C-GG	ATTT----	ACGG
ENT_Savzdargia_sp_D2657_xyz	TTGAACGCTCTTACCAAC--------	GCC	T----------	CG-CT-C-GG	ATTT----	ACGG
EPC_Eusandalum_sp2_D2665_xyz	TAGATCGCTTCAATTAC---------	GCC	A----------	CG-CT-C-GG	ATTT----	ACGG
EPC_Eusandalum_sp3_D2666_xyz	TAGATCGCTTCAATTAC---------	GCC	A----------	CG-CT-C-GG	ATTT----	ACGG
EPN_Metapelma_sp2_D2667_tvxyz	TAGAACGCTTTGAAATCAC-------	GTC	T----------	CG-CT-C-GG	ATTT----	ACGG
PT17M_Aditrochus_coihuensis_D2668_uvxyz	TAGATCGCTATAACAC----------	GTC	T----------	CG-CT-C-GG	ATTT----	ACGG
PT24_Eukobelea_sp_D2659_tuvxyz	TAGATCGCTGTTAAAC----------	GCC	T----------	CG-CT-C-GG	ACTC----	GCGG
PT24_Idarnes_sp2_D2660_vxyz	TTGATCGATGTAAAAC----------	GTC	T----------	CG-CT-C-GG	ATTT----	ACGG
PT24_Pseudidarnes_sp_D2661_vxyz	TGGATCGCAATTGAAC----------	GTC	T----------	CG-CT-C-GG	ATCT----	ACGG

Figure 8. Examples of structural support from two sections of 28S-D2 (indicated by bar) for outgroups and a sampling of Chalcidoidea. RAA(11) shows an increase in the number of nucleotides and a decrease in the degree of conservation for Chalcidoidea including Mymaridae (highlighted). In all Chalcidoidea excluding Mymaridae, RAA(15) undergoes a dramatic decrease to either 1 or no nucleotides and RAA(4) shows a slight increase in size. The bordering alignment around RAA(15) demonstrates compensatory changes in helices 3m, 3n and 3o.

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Sycoryctinae [58]. Bouček noted that there were no unique morphological characters to define Agaonidae *sensu lato*, yet argued against limiting the family to the pollinating group (Agaoninae) and suggested a sister-group relationship of at least Agaoninae + Sycophaginae. Grissell [34] suggested that Agaonidae (*sensu lato*) may form a derived clade within the Torymidae. Rasplus et al. [59] revised the Agaonidae, having determined that it was not monophyletic, limiting the family to include only Agaoninae (Agaonidae *sensu stricto*). Cruaud et al. [23] analyzed relationships within Agaonidae *s.s.* and proposed up to four subfamilies, Tetrapusinae, Agaoninae group 4 (potential subfamily), 'Blastophaginae' and 'Agaoninae', but with the latter two groups likely collapsing into a single subfamily Agaoninae.

Agaonidae (*sensu stricto*) was monophyletic in all analyses with likelihood BS values of 100% and parsimony support of 97%. Tetrapusinae were recovered with 100% BS in all analyses (Table 3), and were either sister group to the remaining Agaoninae, as reported in [23], or nested within Agaonidae (Table 4). Agaonidae Group 4 was monophyletic in all of the likelihood results, but not parsimony. Kradibiinae were never recovered as monophyletic, although both genera, *Kradibia* and *Ceratosolen*, were each monophyletic. Agaoninae were rendered paraphyletic in all analyses by Blastophaginae, but a monophyletic group of Agaonidae + Blastophaginae, excluding Agaonidae Group 4, was recovered in most results with low support (Table 4).

None of the other subfamilies previously placed in Agaonidae were placed near to Agaonidae, although in the SSNR dataset (core only), Sycophaginae were placed as the sister group of Agaonidae but without bootstrap support.

Aphelinidae. Woolley [60] suggested that monophyly of Aphelinidae was not certain, and noted the historical tendency to group all parasitoids of adult and nymphal Hemiptera into Aphelinidae without an understanding of relationships. Presently, most authors recognize that Aphelinidae may be paraphyletic if not polyphyletic [9,17,61]. Characters uniting the Aphelinidae may also not be apomorphic [24,62]. Based on only a few taxa, Aphelinidae were paraphyletic in the molecular analysis of Campbell et al. [17]. Previous authors have placed aphelinids within various families, including Eulophidae [63,64], Encyrtidae [65,66], Pteromalidae [62] or as a distinct family [67]. Rosen and DeBach [68] noted that Aphelinidae share morphological affinities with both Encyrtidae (shape of the mesopleura and structure of the pro- and mesotibial spurs) and Eulophidae (thoracic sclerite morphology and antennal segmentation). Gibson [69] hypothesized an Aphelinidae + Signiphoridae relationship on the basis of the structure of the mesotrochantinal plate and metasternum, a relationship also proposed by Domenichini [70]. Woolley [71] found strong morphological evidence uniting Azotinae + Signiphoridae. Compere and Annecke [67] and Rosen and DeBach [68] considered Aphelinidae to be more closely related to Signiphoridae and Encyrtidae. Viggiani and Battaglia [72] proposed that Aphelinidae were morphologically allied with Eulophidae and Trichogrammatidae. Relationships within Aphelinidae are just as, if not more, complex [24,63,73,74,75,76,77,78,79,80,81,82]. The most recent treatment of Aphelinidae [24] recognizes the following subfamilies and tribes; Aphelininae (tribes Aphelinini, Aphytini, Eretmocerini and Eutrichosomellini), Eriaphytinae, Azotinae, Coccophaginae (tribes Coccophagini, Physcini and Pteroptricini), Eriaporinae and Euryischiinae. Noyes [4] uses Eretmocerinae, which we follow herein. Calesinae were excluded from Aphelinidae by Hayat [24].

Our results lend support to the idea that Aphelinidae are not monophyletic (Figs 1–6). At best, the two subfamilies Aphelininae (excluding *Eretmocerus*) + Coccophaginae were monophyletic in the SSNR analysis. Aphelininae, Azotinae (*Ablerus*), Eretmocerinae (*Eretmocerus*) and Euryischiinae were each recovered with very strong BS support in all analyses (Table 3). Coccophaginae were monophyletic in the majority (94%) of likelihood analyses, but *Coccobius* was excluded from the other taxa in the parsimony results (Table 3). In the majority of cases, the aphelinine tribes Aphelinini (*Aphelinus*), Aphytini, and Eutrichosomellini (all Aphelininae) are monophyletic, although Eutrichosomellini often renders Aphytini paraphyletic. Within Coccophaginae, *Coccophagus* consistently rendered Pteroptricini paraphyletic. Within Pteroptricini, *Encarsia* is consistently rendered paraphyletic by *Dirphys*.

There was no consistent or plausible sister group taxon for Aphelininae or Coccophaginae. In the majority of analyses, Euryischiinae is sister to *Cecidellis* sp. (Coelocybinae: Pteromalidae), which can be justified morphologically (RGB). The monogeneric Eretmocerinae is monophyletic with strong support in all results, but has no association with other aphelinid taxa. Azotinae were always monophyletic, with 100% bootstrap support, with former members of *Azotus* rendering *Ablerus* paraphyletic, which is an expected result. Azotinae were the sister group to Trichogrammatidae in the likelihood results, but without bootstrap support (Table 4). Monophyly of Azotinae + Signiphoridae is supported by several morphological synapomorphies [71], but this group was recovered only in the parsimony results (Table 4).

Calesinae (unplaced to family)

Cales (Calesinae) were excluded from Aphelinidae and left unplaced in Chalcidoidea by Hayat [83]. Mottern et al. [56] recently reviewed the Calesine, and discussed its unique morphology and potential relationships with various taxa, including Aphelinidae, Eretmocerinae, Eulophidae, Mymaridae and Trichogrammatidae.

Calesinae were monophyletic with 100% BS support in all analyses (Fig. 6). Included in our analysis are two morphological and geographically distinct species, *Cales berryi* from New Zealand, and *Cales noacki* from South America, including Chile. This same pattern of distribution was used as an argument for the archaic placement of Rotoitidae. Although *Cales* was intermediate between Mymaridae and other Chalcidoidea in Campbell et al. [17], it was always well nested within Chalcidoidea in all of our results. No consistent outgroups were identified in any of our results.

Chalcididae. Bouček and Halstead [84] noted that the classification of Chalcididae has changed little over the years. A sister-group relationship with Leucospidae or even the inclusion of Leucospidae within Chalcididae was suggested by Gibson [16,85]. Monophyly of Chalcididae has not been previously doubted, largely based on four morphological synapomorphies [86,87]. Traditional classifications have included Chalcidinae with the tribes, Chalcidini, Cratocentrini, Phasgonophorini and sometimes Brachymeriini, with other subfamilies including Dirhininae, Epitraninae, Haltichellinae and Smicromorphinae [30,88]. In a phylogenetic analysis of the family, Wijesekara [86] proposed that Smicromorphinae were nested within Chalcidinae, with Chalcidinae including Smicromorphinae sister to the remaining chalcidids, followed by a sequence of Cratocentrinae, Brachymeriinae (Brachymeriini + Phasgonophorini), and finally Dirhininae (Dirhinini + Epitranini) + Haltichellinae (Haltichellini + Hybothoracini). Noyes [4] did not recognize Brachymeriinae, which is the convention followed herein.

Chalcididae were not monophyletic in any of our analyses. The MENR analysis produced the closest approximation to a monophyletic Chalcididae, with a grouping of *Dirhinus* (Dirhininae), *Epitranus* (Epitraninae), Chalcidinae, *Brachymeria* (Brachymeriinae), Phasgonophorini and *Trigonura* (Cratocentrini). However, this group surprisingly also included two pteromalid subfamilies (Macromesinae and Leptofoeninae) and excluded *Cratocentrus* and *Acanthochalcis* (Cratocentrini). Otherwise, the various groups were inconsistent in their grouping in the other analyses. At the subfamily level, Epitraninae, Dirhininae and Haltichellinae were all monophyletic with very strong BS support (Table 3). Smicromorphinae included only a single taxon, and was either independent from other chalcidids or it grouped with Cratocentrini or Phasgonophorini, but never with Chalcidini as proposed by Wijesekara. The subfamily Chalcidinae were never monophyletic, but the tribes Brachymeriini, Chalcidini and Phasgonophorini all had very high BS support across all analyses (Table 3). Interestingly, our Old World representatives of *Chalcis* (the type genus of the superfamily; occurring Worldwide) render the widespread New World genus *Conura* paraphyletic in all analyses. While monophyly of Haltichellinae was supported in all analyses, monophyly of the two tribes, Haltichellini and Hybothoracini, varied.

Our results do not offer much resolution for the relationships within Chalcididae, but do offer support for recognition of Brachymeriinae, Dirhininae, Epitraninae, Chalcidinae (as Chalcidini), Haltichellinae and Smicromorphinae. Both Phasgonophorini and Cratocentrini are less easily placed, and we could not recover the monophyly of the Cratocentrini (*Trigonura* and *Acanthochalcis* + *Cratocentrus*) in any of our analyses. Leucospidae never grouped with any of the chalcidid families, which contradicts hypotheses that they are the sister group of Chalcididae, or that they might render Chalcididae paraphyletic.

Encyrtidae. The monophyly of Encyrtidae is not questioned and there is strong morphological evidence to support this family [89]. An Encyrtidae + Tanaostigmatidae sister-group relationship has often been proposed, with this clade in turn being sister to Eupelmidae [69,89,90,91]. Noyes et al. [89] followed the division of Encyrtidae into the subfamilies Tetracneminae and Encyrtinae [92,93,94] and noted that while Tetracneminae is undoubtedly monophyletic, Encyrtinae may represent a paraphyletic assemblage.

Encyrtidae were monophyletic across all analyses, with moderate to very strong BS support from the likelihood analyses with RAAs included (Table 3). Tetracneminae were monophyletic with moderate to very strong support across most analyses, with Encyrtinae forming either a paraphyletic or monophyletic sister group. The extraordinary branch lengths found within Encyrtidae (Fig. 3) occur in the results of both SS and SS + RAA analyses, and thus are not simply the result of having several taxa with long RAA inserts. Our results never supported a close relationship with *Cynipencyrtus,* Tanaostigmatidae or any of the eupelmid subfamilies.

Eucharitidae. Several morphological features support the monophyly of Eucharitidae [28]. Largely on the basis of the highly sclerotized first instar larva (planidium), Heraty and Darling [54] proposed a sister-group relationship for Eucharitidae and Perilampidae. Based on molecular and morphological evidence, Gollumiellinae form the sister group of Oraseminae + Eucharitinae [6,37]. Akapalinae and Philomidinae were proposed as belonging to Eucharitidae by Bouček [30]. Philomidinae share planidial larvae with Eucharitidae [95], but immatures of Akapalinae are unknown.

Eucharitidae *sensu stricto* (Gollumiellinae, Oraseminae and Eucharitinae) were monophyletic with 100% BS support across all analyses. Akapalinae were grouped with Perilampinae in all of the likelihood results, but as the sister group of Eucharitidae *s.s.* in the parsimony analysis. Philomidinae were never grouped with Eucharitidae.

While Eucharitinae were always very strongly supported, Oraseminae was occasionally paraphyletic to Eucharitinae. Gollumiellinae was paraphyletic only in the parsimony analysis. Monophyly of Psilocharitini (*Psilocharis* and *Neolosbanus*) is not supported, which is similar to results from other molecular studies [37].

A Eucharitidae + Perilampidae sister group was retrieved in most of the likelihood analyses that included RAAs, and also in the parsimony analysis (Table 4); however, without bootstrap support. Morphological support for this group rests on the presence of a sclerotized planidial first-instar larvae [54,95], and we place some degree of confidence in results that support their monophyly. With the inclusion of Philomidinae in this clade, it would support a single origin of planidia larvae within Chalcidoidea (Fig. 9). However, parsimony results supported a monophyletic Perilampidae + Eucharitidae, without Philomidinae, which was grouped instead with some Phasgonophorini (Chalcididae) and Rileyinae (Eurytomidae).

Eulophidae. Monophyly of Eulophidae generally has not been challenged, although morphological support is based almost entirely on character reduction [29]. Based largely on molecular evidence, Elasmidae was synonymized with Eulophidae by Gauthier et al. [96]. At a higher level, Schauff et al. [97] suggested a grouping of Eulophidae, Elasmidae and Trichogrammatidae, but made note that there was no strong evidence for such a relationship. Eulophinae were suggested to be the most basal of the four subfamilies due to their "less-specialized features" [97]. In a combined analysis, Burks et al. [29] proposed that Eulophinae + Tetrastichinae were the sister group of (Opheliminae + Entiinae) + Entedoninae. The only eulophid with three-segmented tarsi, *Trisecodes*, was removed from Entedoninae and placed as *incertae sedis* within Eulophidae [29]. The whitefly parasitoid group Euderomphalini were sister group to Entedonini in Entedoninae, which was contrary to their placement in Entiinae by Gumovsky [98].

Eulophidae were monophyletic with strong to very strong support in all of our analyses (Fig. 4, Table 3), but with the exclusion of *Trisecodes*, which in all analyses was sister group to taxa outside Eulophidae. Support was consistently very high for Tetrastichinae, and increased with the inclusion of RAAs for Entedoninae, Entiinae and Eulophinae. As proposed by Gauthier et al. [96], *Elasus* (formerly Elasmidae) was always nested within Eulophinae. As well, Tetrastichinae and Eulophinae (including *Elasmus*) have a unique deletion of a contiguous variable region (RAAs 23-25). *Perthiola* (Anselmellini) was always the sister group *Ophelimus* with high bootstrap support. Anselmellini were placed outside of Eulophinae by Gauthier et al. [96]. With added resolution from the RAAs, *Perthiola* + Opheliminae grouped either with Entiinae (54% of likelihood trees and parsimony; Table 4) or with Entedoninae. Without the RAAs, these four groups were monophyletic but unresolved. Our results support the hypothesis of relationships suggested by Burks et al. [29], and substantiate the potential inclusion of Anselmellini within Opheliminae.

The exclusion of *Trisecodes* from Eulophidae as proposed by Burks et al. [29] is justified. This genus was usually placed (81% of likelihood analyses and parsimony), but without strong support, as the sister group of Tetracampinae (excluding *Diplesiostigma*), and was never grouped with other Eulophidae.

Importantly, there was no relationship supported for Eulophidae with any of the aphelinid subfamilies, including Calesinae, which have many similar reductive features [56]. The analyses without RAAs (SSNR, MENR) did support a Eulophidae + (Azotinae + Trichogrammatidae) clade, but otherwise there were no consistent outgroups, and never any groups that have been previously proposed in the literature.

Eupelmidae. While there is strong morphological support for the monophyly of each of the three subfamilies of Eupelmidae, it has been proposed that the family might represent a grade rather than a clade [9,69,99,100]. The grade was implicated to include Encyrtidae and Tanaostigmatidae, and potentially Aphelinidae, which all share an expanded acropleuron and other associated


Chalcidoidea
Tanaostigmatidae
Epichrysomallinae
Eurytomidae
(*Systole* & *Tetramesa*)
Megastigminae
Sycophaginae
Tetrastichinae
Opheliminae
Sycoryctinae
Otitesellinae
Sycoecinae
Toryminae
Agaonidae
Platygastridae
?
Mymaridae
Trichogrammatidae
Aphelininae
Eupelminae
Eulophidae
Coccophaginae
Rileyinae
Perilampidae
Eucharitidae
Platygastrinae
Euryischiinae
Azotinae
Signiphoridae
Encyrtidae
Eretmocerini
Eunotinae
Aphelininae
Euderomphalini
Coccophaginae
Calesinae
Coccophaginae
Phytophagy
Egg parasitism
Planidial larvae
Sternorrhyncha
parasitism
Heteronomy

Figure 9. Five life history traits mapped onto SSME likelihood tree. Colored squares refer to presence of a trait in a clade, but not in a member sampled in this study.
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features; however, there is also a possibility of closer relationships of one or more subfamilies to Cleonyminae (Pteromalidae) [69].

Eupelmidae were never monophyletic. Also, its subfamilies Calosotinae, Eupelminae, and Neanastatinae were almost never monophyletic. The SSME dataset was one of the rare instances in which Neanastatinae were monophyletic (Fig. 1), but in the same results both Calosotinae and Eupelminae occur twice in very different parts of the tree (Figs 3–6). Eupelminae were monophyletic in some analyses, including both datasets that did not include the RAAs (Table 3). Calosotinae were never monophyletic, with *Calosota* and *Balcha* grouping distantly from *Archaeopelma*, *Licrooides* and *Eusandalum*. None of the Eupelmidae ever grouped with Tanaostigmatidae or Encyrtidae.

Eurytomidae. The monophyly of Eurytomidae was recently questioned as no synapomorphies defining the family are known [101]. Indeed, the molecular analyses of Campbell et al. [17] and Chen et al. [102] and the morphological analyses of Lotfalizadeh et al. [103] failed to recover a monophyletic Eurytomidae. Stage & Snelling [104] recognized Heimbrinae, Rileyinae and Eurytominae, with the latter including the previously recognized Buresiinae. Chen et al. [102] proposed elevating Rileyinae to family status, while Lotfalizadeh et al. [103] found Rileyinae to consist of two clades of unrelated taxa (*Rileya* and *Macrorileya* + *Buresium*). Both molecular and morphological investigations found *Eurytoma* to be polyphyletic [102,103].

Eurytomidae was never recovered as monophyletic in any of our analyses. However, Eurytominae (excluding *Buresium*) were monophyletic in all of our analyses with very high support (Table 3). *Rileya* (Rileyinae) were monophyletic in all analyses, but with very high support only in the likelihood analyses when RAAs were included. Both *Heimbra* (Heimbrinae) and *Buresium* (Eurytominae) never grouped with the other eurytomid genera. No logical outgroups were identified.

Leucospidae. Leucospidae are generally recognized as a monophyletic group of four genera closely related to Chalcididae [86,105]. However, characters proposed to support the monophyly of this combined lineage are all problematic and potentially convergent [9,86].

Leucospidae were monophyletic and had greater than 90% support across all analyses. Our one species of *Micrapion* (South Africa) consistently rendered *Leucospis* (worldwide representation) paraphyletic. No close association with Chalcididae was found.

Mymaridae. Although there was some early doubt about the monophyly of Mymaridae [106], the family has been well substantiated based on morphology and molecular evidence [17,107,108]. Huber [108] noted that the higher classification of Mymaridae is unstable, and as per the advice of Huber and Triapitsyn (personal communication) Mymaridae subfamilies have been abandoned and genera grouped according to their number of tarsal segments. Gibson [10] was the first to propose morphological evidence that Mymaridae might be the sister group of the remaining Chalcidoidea, but without firm resolution.

Mymaridae were found to be monophyletic in all analyses with very strong support (Fig. 1, Table 3). The 4-segmented tarsi group, represented by the genera *Borneomymar*, *Gonatocerus*, *Litus* and *Ooctonus*, were consistently monophyletic across all analyses with moderate to strong support (Table 4). The remaining genera, *Acmopolynema*, *Anagrus*, *Anaphes*, *Australomymar*, *Ceratanaphes*, *Erythmelus*, *Eubroncus*, *Mymar* and *Stethynium*, formed the 5-segmented tarsi group. This group is supported in most analyses (88% of likelihood analyses), with moderate to strong BS support only when RAAs were included. There was no support for Mymarinae or Alaptinae. Eubronchinae were monophyletic, but these were represented by only a single genus. Mymaridae were strongly supported as the sister group of the remaining Chalcidoidea in all analyses.

Ormyridae. Hanson [109,110] noted that the status and relationships of Ormyridae are uncertain. Members of the family have been included as a subfamily in Pteromalidae [111], Torymidae [112], or as their own family [30].

The two genera, *Ormyrus* and *Ormyrulus*, were monophyletic in all of our analyses but with low to very strong BS support (Fig. 3). In 56% of the likelihood analyses, all based on use of the core SS alignment and with or without RAAs, supported a sister-group relationship with *Moranila* (Pteromalidae: Eunotinae: Moranilini), but otherwise there were no consistent outgroup associations, and never any close association with either of the torymid subfamilies.

Perilampidae. The limits of Perilampidae are not clear, with variable inclusion of the subfamilies Chrysolampinae, Philomidinae and Perilampinae, and treatment of each or all groups as a separate family or subfamily of Pteromalidae [9,100,113]. *Akapala* (Akapalinae) were initially placed in Perilampidae, but later transferred to Eucharitidae [30]. More recently, *Jambiya* was described and included within Perilampidae, but an association with either Chrysolampinae or Perilampinae could not be made [114]. *Jambiya* has an enlarged ovipositor, which is also a feature of basal lineages of Eucharitidae, and a relationship with that family cannot be rejected. A proposed relationship between Perilampidae, Philomidinae and Eucharitidae is based on presence of a planidial larva [54,95].

In likelihood results, Perilampidae *sensu stricto* (Chrysolampinae + Perilampinae) was never recovered. With RAAs excluded, a monophyletic 'Perilampidae' was recovered with low support that included Chrysolampinae (67-73% BS), Perilampinae (96-98% BS), Akapalinae, Philomidinae and *Jambiya*. This group also included the pteromalid genus *Idioporus* (Pteromalidae: Eunotinae: Eunotini). In these analyses, Eucharitidae and Perilampidae were not monophyletic. With the inclusion of RAAs, the results are more variable, but often recover Perilampidae and Eucharitidae as monophyletic, *Jambiya* as sister group to Eucharitidae, but again with Philomidinae, Akapalinae and *Idioporus* nested within a paraphyletic or monophyletic Perilampidae, but still with Chrysolampinae and Perilampinae each monophyletic (Fig. 5). A monophyletic Perilampidae *s.s.* (Chrysolampinae + Perilampinae) was recovered only in the parsimony analysis. These results also supported *Jambiya* as the sister group of Akapalinae + Eucharitidae. Philomidinae were distantly placed with Phasgonophorini (Chalcididae) and Rileyinae (Eurytomidae). Thus, while Eucharitidae *s.s.* is well supported, there is conflicting support for the definition of Perilampidae and a definitive association with Eucharitidae.

Pteromalidae. Pteromalidae are essentially a dumping-ground for presumably monophyletic groups that cannot be assigned to established families and which lack family status in their own right [9]. Herein, we recognize the 30 subfamilies of Noyes [4], as well as the three non-pollinator fig-wasp associated subfamilies assigned to Pteromalidae (Otitesellinae, Sycoecinae and Sycoryctinae) or placed as *incertae sedis* (Epichrysomallinae and Sycophaginae) by Rasplus et al. [59]. Historically, many pteromalid subfamilies were elevated to family status, only to once again resume subfamily status within Pteromalidae [9]. There has been no comprehensive morphological analysis of the family. Molecular analyses have supported the concept of a polyphyletic assemblage, but even the most comprehensive studies have sampled relatively few taxa across the spectrum of the family [17,18]. We were able to sample 25 of these 36 subfamilies, and where possible sample more extensively within groups (Table 3). We limit our discussion below to significant groupings or results. Notably, many of the taxa are 'almost' monophyletic, often with the exclusion of one or more taxa, and many of these cases will need to be evaluated elsewhere.

Pteromalidae were expected to be polyphyletic [9,15], and were never retrieved as monophyletic. Several subfamilies were monophyletic and very strongly supported across all analyses including Ceinae (*Spalangiopelta*), Cerocephalinae, Epichrysomallinae, Panstenoninae (*Panstenon*), Pteromalinae, Spalangiinae (*Spalangia*) and Sycophaginae. In no case did support increase with the addition of RAAs. Of interest is the a novel grouping of the pteromalid subfamilies Cratominae (*Cratomus*), Miscogastrinae (except *Nodisoplata*), Otitesellinae, Panstenoninae, Pteromalinae, Sycoecinae (*Diaziella*) and Sycoryctinae. This grouping occurs in all analyses, including parsimony, but without bootstrap support. A clade of Miscogastrinae and Pteromalinae was strongly supported by Desjardins et al. [18], but none of these other subfamilies were included as part of that study. This 'pteromalid complex' is peculiar for its small amount of molecular divergence and high degree of morphological complexity, especially for the non-pollinating fig wasps Otitesellinae and Sycoryctinae. The low divergence and stability across various analyses suggest that the subfamilies in this group might eventually be synonymized under Pteromalinae. The taxononic placement of *Nodisoplata*, which was placed outside of this complex, needs to be reconsidered. The two other two fig-wasp associated subfamilies, Epichrysomallinae and Sycophaginae, were monophyletic but not associated with any consistent outgroup taxon. In one analysis without RAAs (SSNR), Sycophaginae were the sister group of Agaonidae, but without BS support. This result was proposed by Copland and King [115].

Coelocybinae, Ormocerinae, Pireninae and Pteromalinae were never monophyletic. Cleonyminae were polyphyletic. In all analyses, Cleonymini and Lyciscini were each monophyletic with low support in all analyses, with Lyciscini gaining increased support from the inclusion of RAAs. Chalcedectini (*Chalcedectus*) had variable relationships, but never with other Cleonyminae. Ooderini (*Oodera*) had sister-group relationships that varied from Leucospidae to Encyrtidae, and on two occasions, Lyciscini. Cratominae (*Cratomus*) had variable relationships throughout the analyses, but often occurred in the pteromalid complex as suggested by its morphology. Diparinae were never monophyletic, as also found by Desjardins et al. [18]. Eunotinae were never retrieved as monophyletic, and the tribes Moranilini and Tomocerodini, each represented by a single taxon, were inconsistently allied with other families. Eunotini were monophyletic and strongly supported in all of the analyses. Surprisingly, Leptofoeninae, which have strong morphological support, were never monophyletic. Ormocerinae were never monophyletic. Sycoryctinae and Otitesellinae were consistently polyphyletic which is a result supported by morphology [59]. Within Otitesellinae, the two *Grasseiana* species form a monophyletic group, while *Heterandrium* sp. and *Otitesella* sp. were inconsistently allied with other taxa. Panstenoninae were nested within Pteromalinae. Pireninae and Pteromalinae were never monophyletic. Spalangiinae were always monophyletic, but were never recovered with a consistent sister group.

For Pteromalidae, our results are similar to those of Desjardins et al. [18] based on an analysis of four protein coding genes. The family is polyphyletic with respect to most Chalcidoidea and few of the higher-level assemblages can be consistently grouped with other pteromalid or chalcidoid groups.

Rotoitidae. In their description of the family, Bouček and Noyes [116] noted that Rotoitidae may be the sister group of Tetracampidae and Eulophidae. Other potential associations have included Eulophidae, Mymaridae, Trichogrammatidae and Tetracampidae [15,16]. Based on an analysis of both distribution and ovipositor morphology, Gibson & Huber [117] concluded that Rotoitidae might be the second most ancestral lineage of Chalcidoidea after Mymaridae, but noted that features of the antenna and mesosoma conflict with this conclusion.

Rotoitidae were represented by one species, *Chiloe micropteron*. In all but one of the likelihood analyses, it was basal and sister to the remaining Chalcidoidea after Mymaridae, with BS support for a monophyletic Chalcidoidea after Rotoitidae only in the SSGE results. The alternate likelihood result placed it as the sister group of Mymaridae, thus still basal within the superfamily. Parsimony results have *Chiloe* nested within Chalcidoidea as the sister group of *Idioporus* (Eunotinae: Eunotini) in a clade with *Systolomorpha* (Pteromalidae: Ormocerinae: Melanosomellini) and Trichogrammatidae. No morphological features would support this alternative hypothesis.

Signiphoridae. There is little doubt over the monophyly of Signiphoridae; however, Thysaninae may be paraphyletic with respect to Signiphorinae [71,118]. Gibson [69] suggested a relationship between Signiphoridae and Aphelinidae, or members within Aphelinidae. Woolley [71] proposed a Signiphoridae + Azotinae sister group based on an unsegmented antennal club, presence of an epiproct [70] posterior to the syntergum in all female Azotinae and Signiphoridae, and apodemes projecting forward from the anterolateral angles of sterna 3 to 6 of the metasoma of females. Pedata and Viggiani [119] alluded to an azotine + signiphorid relationship with the discovery of tubercles above the spiracles of third instar *Ablerus perspeciosus* and *Signiphora flavella* larvae.

Signiphoridae and Signiphorinae (*Signiphora*) both monophyletic with very strong support across all analyses (Table 3). Thysaninae were paraphyletic in all of our results. The placement of *Clytina* was puzzling, with *C. giraudi* rendering *Chartocerus* paraphyletic in all analyses, while *Clytina* sp. D1023 was consistently the sister group of *Thysanus*.

Signiphoridae were not placed with Azotinae, or any logical outgroup, in any of the likelihood analyses. In these analyses, Azotinae was consistently the sister group of Trichogrammatidae. However, in the parsimony analysis, Azotinae and Signiphoridae were monophyletic and did not group with Trichogrammatidae.

Tanaostigmatidae. Tanaostigmatidae *sensu* LaSalle [90] is a distinct monophyletic group. LaSalle and Noyes [91] transferred *Cynipencyrtus* from Encyrtidae to Tanaostigmatidae, yet noted that this genus was morphologically and biologically distinct from other members of the family. It has been argued that *Cynipencyrtus* could be sister to Encyrtidae, sister to Tanaostigmatidae + Encyrtidae, or sister to Tanaostigmatidae alone [9,69,99]. There is strong morphological support for monophyly of the Tanaostigmatidae + Encyrtidae clade, but weaker support for the inclusion of Eupelmidae within this group [9].

Tanaostigmatidae *sensu stricto* (without *Cynipencyrtus*) was always monophyletic with strong support. *Cynipencyrtus* was variously allied with other taxa throughout the different analyses, and tanaostigmatids were never the sister group of Encyrtidae. This disparate grouping may be an artifact of the larger analysis, as we have been able to recover Tanaostigmatidae + (*Cynipencyrtus* + Encyrtidae) in a study with a smaller and more selective sampling of taxa (Mottern & Heraty, unpublished).

Tetracampidae. Tetracampidae probably represents a polyphyletic assemblage with three extant subfamilies [120]. There is considerable argumentation for placement of the different subfamilies as Aphelinidae, Eulophidae or Pteromalidae [9,30,55].

Tetracampidae were never monophyletic in our analyses. Excluding *Diplesiostigma*, Tetracampinae were monophyletic and very strongly supported. *Diplesiostigma* varied in placement in every analysis, but never occurred with other Tetracampidae. The two representatives of Mongolocampinae and Platynocheilinae were clustered in a monophyletic group in all analyses with very high support, and most likelihood results grouped them with Eunotini (Pteromalidae: Eunotinae; excluding *Idioporus*), however with low support.

Torymidae. Placement of Torymidae is uncertain, and it was proposed that the family arose from within the pteromalid lineage [121]. Historically, Torymidae have included Agaoninae and Sycophaginae (= Idarninae), which were removed by Bouček [30]. Torymidae were revised by Grissell [34] and include only two subfamilies, the largely phytophagous Megastigminae and the mostly parasitic Toryminae, with the latter divided into seven tribes that encompassed the previously recognized Erimerinae, Monodontomerinae and Thaumatotoryminae and several taxa as *incertae sedis*. Campbell et al. [17] failed to find a monophyletic group, despite what they and Gibson et al. [9] noted to be strong morphological support for the family.

Torymidae were never monophyletic, but Megastigminae and Toryminae were each monophyletic with very strong support (Table 3). Support for tribes within Toryminae was variable. Torymini were monophyletic with low to very strong support in all analyses except parsimony, and Podagrionini were either monophyletic mostly with low support (62% of likelihood analyses) or paraphyletic. Monodontomerini were monophyletic with strong bootstrap support in all analyses, but with the inclusion of the unplaced *Zaglyptonotus* and exclusion of *Chrysochalcissa* which clusters deep within Microdontomerini. *Echthrodape* (Toryminae *incertae sedis*) was previously placed in Eucharitidae and Perilampidae and then Torymidae by Grissell [34]. This genus was recovered as the sister group of Microdontomerini. The unplaced *Glyphomerus* exemplars remained unplaced within Toryminae with no particular association with other tribes. The two representatives of Palachiini grouped either with Torymoidini or Podagrionini, but never together. None of the groups seemed to be impacted by the inclusion or exclusion or RAAs. No logical sister groups were identified for either subfamily.

Trichogrammatidae. Trichogrammatidae are well defined and according to Bouček and Noyes [116], are possibly the only monothetic family of Chalcidoidea. Owen et al. [35] assessed higher-level groups and generic relationships based on molecular and morphological evidence and recognized a paraphyletic Trichogrammatinae and monophyletic Oligositinae. Of the groups sampled herein, *Ceratogramma* (Trichogrammatinae; unplaced to tribe) were recognized as the sister group of the remaining Trichogrammatidae.

Trichogrammatidae were monophyletic in nearly all of our analyses (94% of the MJR consensus trees), but with low BS support in likelihood analyses only after the inclusion of RAAs. *Ceratogramma* was sister to the remaining Trichogrammatidae in all results, except for one analysis when it was excluded from the family (Table 3, SSNR). Our internal relationships mirror those of Owen et al. [35]. Trichogrammatidae were sister to Azotinae in all but the parsimony analysis, which placed them as a sister group of *Idioporus*, *Rotoita* and *Systolomorpha*.

Conclusions

Is the diverse and unsupported backbone of Chalcidoidea the product of a rapid radiation event [48,122]? Mymaridae first appear in the early to mid Cretaceous [6]. Based on what appear to be valid fossils of Eulophidae and Trichogrammatidae, there are records of higher-level chalcidoids in only one mid-Cretaceous deposit [8], with records of the same age other than Mymaridae more questionable [6]. The diversification of chalcidoid families does not appear until the Eocene, with modern genera common in Oligocene and Miocene amber deposits [6]. Chalcidoids are mostly parasitoids, and their host groups in the Hemiptera and Holometabola were all undergoing an explosive radiation during the same period at the end of the Cretaceous [123], and a similar tracking of host diversification is not unexpected.

Using an array of nuclear protein coding genes but with fewer taxa, Desjardins et al. [18] found similar results that showed a weak backbone of relationships across their chalcidoid groups sampled. Given a scenario of explosive radiation of Chalcidoidea during a relatively short time period, it may be difficult to resolve higher group relationships with confidence [122]. However, the trees that we have recovered can help to evaluate some scenarios within a context of which groups are consistently supported and their relationships on the various tree topologies. These molecular results provide a unique perspective for examining relationships and hypotheses of chalcidoid evolution, especially in a group prone to morphological convergence.

What is the ancestral mode of host association for Chalcidoidea? Bouček [124] proposed Cleonyminae or some other wood-beetle parasitoids as having the most ancestral forms, but hypothesized that phytophagy could be plesiomorphic for the superfamily. This latter assumption was based on his observation that phytophagous species tend to be primitive within their respective groups. The placement of Chalcidoidea as sister group to either Diaprioidea or Proctotrupoidea *sensu stricto* and the basal sister group placement of Mymaridae argue against Bouček's hypothesis of a phytophagous ancestor. As well, the phytophagous groups are scattered across the tree and almost never basal within a particular lineage, as in with gall-forming Opheliminae derived from within Eulophidae, or seed-feeding Megastigminae, which are distantly placed from their proposed sister group, the Toryminae (Fig. 9).

Noyes [15] argued for a monophyletic Mymaridae + (Rotoitidae + Tetracampidae) as the sister group of the remaining Chalcidoidea. Our results somewhat support his hypothesis, placing Mymaridae and Rotoitidae at the base of the chalcidoid tree (Fig. 1), but with a different phylogenetic ordering, and with Tetracampidae both polyphyletic and placed more distally on the various topologies. Morphological evidence supports a sister group relationship between Mymaridae and the remaining Chalcidoidea [10,16,61]. Our results and more comprehensive analyses of Hymenoptera [11,13] strongly support this hypothesis. Likelihood results place Rotoitidae as the sister group of the remaining Chalcidoidea after Mymaridae.

Mymaridae are virtually all egg parasitoids, primarily of Auchenorrhyncha, Heteroptera and Coleoptera [125]. The only known exception is for two species of *Stethynium* attacking larvae of *Ophelimus* (Eulophidae) [126]. We included *S. ophelimi* in our analysis, and its derived placement within the family suggests a secondary derivation of larval parasitism (Fig. 1). Egg parasitism is likely the ancestral trait for Mymaridae. Within the remaining Chalcidoidea, egg parasitism occurs in all Trichogrammatidae and a few other scattered taxa (Fig. 9). None of our results placed these chalcidoid egg parasitoids close to the root of Chalcidoidea. Is it possible for egg parasitism to be ancestral for the superfamily? Mymarommatoidea may be egg parasitoids of Psocoptera [127].

The small body size of Rotoitidae suggests that they also might be egg parasitoids, but there is not even a suspected host for this group [9]. Diaprioidea are primarily larval parasitoids of fly larvae or pupae with a few taxa hyperparasitic on Dryinidae or Formicidae [128]; none are egg parasitoids. Even if Mymarommatoidea are resolved as the sister group of Chalcidoidea (only in some of our results), the biology of these and Rotoitidae will need to be resolved before we can confidently consider egg parasitism as a basal trait for the superfamily.

Associated with an extreme diversity of host use, larval morphology is extremely diverse in Chalcidoidea [129]. Two types of hypermetamorphic development occur in Hymenoptera [130]. Type II involves deposition away from the host of a sclerotized planidiform first-instar larva that transforms in later instars to a typical weakly sclerotized sac-like hymenopteriform larva. Within Hymenoptera, this occurs only in one genus of Ichneumonidae (*Euceros*) and in Perilampidae (including Philomidinae) and Eucharitidae [95]. Although not recovered across all analyses, our results offer support for the single development of this trait within Chalcidoidea (Fig. 9).

Another important trait is the use of sessile Sternorrhyncha as hosts within Chalcidoidea, which ultimately leads to their importance in biological control programs. Mapping sternorrhynchan parasitism, either as primary parasitoids or hyperparasitoids, onto our current 'best' hypothesis shows a general scattering of host use that suggests multiple independent host shifts to this group. Probably most significant is the lack of grouping in any of our analyses of Encyrtidae and the aphelinid subfamilies Aphelininae, Azotinae, Coccophaginae, Eretmocerinae and Euryischiinae, which have in the past been treated as a single family [66]. Our results suggest that any traits associated with successful host use of Sternorrhyncha are independent events, and especially within Aphelinidae, should not be considered as phylogenetically linked. This is also important when we consider the single origin of heteronomy, or alternate host use by different sexes, which occurs only in the monophyletic Coccophaginae (Fig. 9).

Our results present the most comprehensive phylogenetic analysis of relationships Chalcidoidea based only on molecular data.. While not robust across the backbone of relationships within Chalcidoidea, they offer some firm insights into the origin and evolution of this important and highly diverse group of insects. Monophyly of many of the traditional groups is supported, and the secondary structure alignment and data set will be useful for future studies. Many changes in the higher classification of taxa within Chalcidoidea are suggested by these results. However, we reserve any judgment on these changes until our combined morphological and molecular analyses are complete.

Supporting Information

Figure S1 Parsimony analysis of SSME dataset using TNT (31,607 steps; r.i. 0.62, strict consensus of >10,000 trees). Bootstrap values plotted to nodes with values greater than 95% represented by dot.
(PDF)

Table S1 Specimens sequenced and deposition information for specimen data and genebank accession numbers.
(XLS)

Nexus File S1 Chalcidoidea SSME dataset.
(NEX)

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Author Contributions

Conceived and designed the experiments: JMH JBM. Performed the experiments: JMH JBM RAB DH JM AC J-YR PJ. Analyzed the data: JMH JBM. Wrote the paper: JMH JBM. Designed voucher database: JM.

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Paper II

HERATY J. M., BURKS R. A., CRUAUD A., GIBSON G. A. P., LILJEBLAD J., MUNRO J., RASPLUS J.-Y., DELVARE G., **JANŠTA P.**, GUMOVSKY A., HUBER J., WOOLLEY J. B., KROGMANN L., HEYDON S., POLASZEK A., SCHMIDT S., DARLING D. C., GATES M. W., MOTTERN J., MURRAY E., DAL MOLIN A., TRIAPITSYN S., BAUR H., PINTO J. D., VAN NOORT S., GEORGE J. & YODER M. (2013)

A phylogenetic analysis of the megadiverse Chalcidoidea (Hymenoptera).

Cladistics **29(5)**: 466-542.

Original paper on pages 73-149 available at:

http://onlinelibrary.wiley.com/doi/10.1111/cla.12006/abstract

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To whom it may concern:

This is to declare that Petr Janšta contributed significantly to the paper "Heraty J. M., Burks R. A., Cruaud A., Gibson G. A. P., Liljeblad J., Munro J., Rasplus J.-Y., Delvare G., **Janšta P.**, Gumovsky A., Huber J., Woolley J. B., Krogmann L., Heydon S., Polaszek A., Schmidt S., Darling D. C., Gates M. W., Mottern J., Murray E., Dal Molin A., Triapitsyn S., Baur H., Pinto J. D., van Noort S., George J. & Yoder M. 2013: A phylogenetic analysis of the megadiverse Chalcidoidea (Hymenoptera). *Cladistics* 29(5): 466-542" co-authored by us and included in his PhD thesis. We hereby agree that this paper may be included in his PhD thesis.

On behalf of the authors

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Paper III

Janšta P., Vilímová J. and Hanson P. (2011)

Description of a new genus, *Boucekinus* (Hymenoptera: Chalcidoidea: Torymidae), with two new species and a discussion of its possible phylogenetic placement.

Zootaxa **2762**: 49-55.

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To whom it may concern:

This is to declare that Petr Janšta contributed significantly to the paper "**Janšta P.**, Vilímová J. and Hanson P. 2011: Description of a new genus, *Boucekinus* (Hymenoptera: Chalcidoidea: Torymidae), with two new species and a discussion of its possible phylogenetic placement. *Zootaxa* 2762: 49-55" co-authored by us and included in his PhD thesis. We hereby agree that this paper may be included in his PhD thesis.

On behalf of the authors

Paul Hanson

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Paper IV

JANŠTA P., KŘÍŽKOVÁ B., VILÍMOVÁ J., RASPLUS J.-Y. 2013:

Description of a new genus, *Chileana* (Hymenoptera: Chalcidoidea: Torymidae), with four new species.

Zootaxa **3745**: 49-63.

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UMR CENTRE DE BIOLOGIE POUR LA GESTION DES POPULATIONS

A l'attention de :

Montferrier-sur-Lez, le 4 décembre 2013

To whom it may concern:

By this letter, I declare that Petr Janšta contributed significantly to the following publication :

"**Janšta P.**, Křížková B., Vilímová J., Rasplus J.-Y. 2013: Description of a new genus, *Chileana* (Hymenoptera: Chalcidoidea: Torymidae), with four new species. *Zootaxa*, 3745: 49-63."

This paper was co-authored by several researchers from different institutes and is included in his PhD thesis. We hereby agree that this publication may be included in his PhD thesis.

On behalf of the authors

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Paper V

JANŠTA P., CRUAUD A., DELVARE G., HERATY J., RASPLUS J.-Y.

Molecular phylogeny of the family Torymidae (Hymenoptera: Chalcidoidea).

Manuscript prepared for submission to *Cladistics.*

UMR CENTRE DE BIOLOGIE POUR LA GESTION DES POPULATIONS

A l'attention de :

Montferrier-sur-Lez, le 8 décembre 2013

To whom it may concern:

By this letter, I declare that Petr Janšta contributed significantly to the following publication :

"Janšta P., Cruaud A., Delvare G., Heraty J., Rasplus J.-Y: Molecular phylogeny of the family Torymidae (Hymenoptera: Chalcidoidea). Manuscript prepared for submission to *Cladistics*"

This paper was co-authored by several researchers from different institutes and is included in his PhD thesis. We hereby agree that this publication may be included in his PhD thesis.

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Molecular phylogeny of the family Torymidae (Hymenoptera: Chalcidoidea)

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Abstract

We present the first molecular phylogenetic analysis of the family Torymidae (Hymenoptera: Chalcidoidea) using 4809 nucleotides from 5 genes (18S rDNA, 28S rDNA, EF1α, COI and Wg). Ten outgroups and 226 ingroup taxa were used, representing 45 of the 67 recognized genera in the 2 known subfamilies of Torymidae (Megastigminae and Toryminae). All analyses produced similar topologies, and based on only molecular data, Torymidae is not a monophyletic group and Megastigminae is sister to Ormyridae ((Ormyridae + Megastigminae) + Toryminae). Most of the tribes recognized by morphological characters within the subfamily Toryminae were supported by our results (i.e. Chalcimerini, Microdontomerini, Monodontomerini, Torymini, Torymoidini and Palachiini + Podagrionini). Two new tribes of Torymidae are erected: Boucekini, **trib. nov.** and Glyphomerini, **trib. nov.** As presently understood, the genus *Glyphomerus* is paraphyletic and 7 other genera classified as *incertae sedis* based on earlier studies of morphology were assigned to specific tribes (i.e. *Cryptopristus*, *Echthrodape*, *Exopristoides*, *Exopristus* and part of *Glyphomerus* to Microdontomerini; *Thaumatorymus* to Chalcimerini; *Zaglyptonotus* to Monodontomerini). Life-strategies, hosts records and distribution of all taxa were mapped onto the reconstructed phylogeny. The larvae of Toryminae are ectoparasitoids of gall-forming insects in the Palaearctic region with several derived traits throughout the Toryminae phylogeny. The life strategy, hosts and distribution of the common ancestor of Megastigminae remains uncertain.

Introduction

Chalcidoidea is an extremely diverse superfamily within the order Hymenoptera. More than 22,500 species have been described and their overall diversity is estimated at more than 500,000 species (Heraty 2009; Noyes 2013). Twenty-two families are now recognized (Aguiar et al. 2013; Heraty et al. 2013). Their morphological diversity and enormous number of species are reflected also by their biological and feeding strategies. Most Chalcidoidea are parasitoids and thus are among the most important natural enemies of other insects. Despite their functional importance in natural ecosystems, the taxonomy, biology and phylogeny of most chalcidoid families is poorly known (Huber 2009). Until now, only a few comprehensive phylogenetic studies of entire families/or subfamilies have been published. Most of these studies are based on morphology (Gibson 1995 - Eupelminae; Grissell 1995 - Toryminae; Heraty 2002 - Eucharitidae; Lotfalizadeh et al. 2007 - Eurytominae), and only a few used molecular characters (Owen et al. 2007 - Trichogrammatidae; Cruaud et al. 2010 - Agaonidae, 2011b - Sycophaginae, 2013 - Sycoecinae; Burks et al. 2011 - Eulophidae; Segar et al. 2012 - Sycoryctinae; Murray et al. 2013 - Eucharitidae).

Torymidae is one of the mid-sized families of Chalcidoidea. The family includes 67 valid genera and about 1100 described species (Grissell 1995; Janšta et al. 2013). Monophyly of the family is supported only in analyses that include adult morphological characters (Grissell 1995; Gibson et al. 1999; Heraty et al. 2013). Although, each of these authors acknowledged the monophyly of Torymidae, there is no synapomorphy defining the family. In contrast, the monophyly of Torymidae based only on molecular or larval characters, has never been recovered (Campbell et al. 2000; Gómez et al. 2008; Munro et al. 2011).

The classification of Torymidae has changed several times since the family was described (Walker 1833) and the family also has included members from several other families (Ashmead 1904; Wiebes 1961; Hill 1967). Bouček (1988) summarized the classification history of Torymidae, proposed a new one, and suggested five synapomorphies defining the family. Grissell (1995) questioned all of the proposed synapomorphies, but suggested that the family was monophyletic based on a combination of the five character states.

Historically, several families (i.e. Ormyridae, Agaonidae, Epichrysomallinae, Sycophaginae) have been proposed to be sister to Torymidae based either on intuitive analysis (Noyes 1990) or on convergences (Cruaud et al. 2010, 2011b), but without any morphological support. Bouček (1988) mentioned that Ormyridae could be closely related to Torymidae based on shape of the occipital carina. The same hypothesis was also proposed by Noyes

(1990) in his intuitive cladogram that groups together Torymidae plus Ormyridae and Agaonidae plus Ormocerinae (Pteromalidae). Grissell (1995) added Pteromalidae as the outgroup to his phylogenetic analysis of the Toryminae (Toryminae and Megastigminae are the only recognized subfamilies of Torymidae). Gibson et al. (1999) noted that relationships and the proper classification of Torymidae, Ormyridae and Agaonidae are one of the more perplexing issues of chalcidoid classification. Because of variable results, no sister group was proposed for Torymidae as a result of the comprehensive phylogenetic analyses of Chalcidoidea using molecular (Munro et al. 2011) or combined molecular and morphological data (Heraty et al. 2013). However, this latter analysis repeatedly placed Torymidae in a clade with Ormyridae and Colotrechninae (Pteromalidae).

Grissell (1995) proposed the most comprehensive phylogenetic analysis of the Torymidae (primarily Toryminae), based on 24 morphological characters and 46 taxa. He hypothesized the monophyly of both Megastigminae and Toryminae. Toryminae were divided into seven tribes (Fig. 1): Chalcimerini included 1 genus and 1 species (i.e. 1/1), Microdontomerini (9/104), Monodontomerini (11/68), Palachiini (3/30), Podagrionini (7/139), Torymini (10/416) and Torymoidini (6/111) (Grissell 1995, 2000, 2005; Sureshan and Narendran 1996; Bouček 1998; Zerova and Grissell 2000; Sureshan 2003, 2007, 2010; Narendran and Sudheer 2004; Delvare 2005; Askew et al. 2007; Xiao et al. 2007, 2012; Zhao et al. 2007; Doğanlar and Doğanlar 2008; Narendran and Peter 2009; Doğanlar and Doğanlar 2009; Narendran and Mercy 2010; Tarla et al. 2010; Xiao and Zhao 2010; Janšta et al. 2011, 2013; Narendran et al. 2012; Noyes 2013). Some genera were treated as *incertae sedis* within Toryminae, and remain unplaced: *Cryptopristus* Förster, 1856 (3 species), *Echthrodape* Burks, 1969 (2), *Exopristoides* Bouček, 1982 (2), *Exopristus* Ruschka, 1923 (1), *Glyphomerus* Förster, 1856 (9), *Stenotorymus* Masi, 1938 (1), *Thaumatorymus* Ferrière and Novicky, 1954 (1), and *Zaglyptonotus* Crawford, 1914 (3) (Grissell 1995; Zerova and Seryogina 1999, 2000; Zerova et al. 2004, 2008; Stojanova 2005). The subfamily Megastigminae is comprised of 198 species in 12 genera (Bouček 1988; Grissell and Desjardins 2002; Doğanlar 2011a, 2011b; Noyes 2013). There has not been a rigorous phylogenetic study of that subfamily based on either morphological or molecular characters, although monophyly of the 3–4 included genera was supported by Munro et al. (2011) and Heraty et al. (2013).

Most torymid genera are distributed in the Old World and in Australian region, (Grissell 1995; Janšta et al. 2013; Noyes 2013), with only a few spreading to the New World: *Megastigmus*, *Cryptopristus*, *Glyphomerus*, *Ditropinotus*, *Eridontomerus*, *Idiomacromerus*, 1995). Recently, many new amber fossils (mostly from Baltic amber) of undetermined Torymidae have been recorded (Krogmann – pers. comm.). Surprisingly, the oldest comes from Burmese amber (mid-Cretaceous, latest Albian, ca. 100 Mya) and appears to be a genus belonging to the tribe Monodontomerini (Janšta – pers. observ.).

The main aims of our study are to (i) propose the first phylogenetic hypothesis for Torymidae based on molecular data using broad taxon sampling, (ii) test the monophyly of the family, (iii) compare the phylogeny of Torymidae to previous morphological results and test the current classification, (iv) test the monophyly of most genera, (v) discuss the origin of host specialization, and (vi) assess the biogeography of the family, subfamilies and tribes.

Microdontomerus, *Pseuderimerus*, *Monodontomerus*, *Podagrion*, *Palmon*, *Torymus*. Only a few genera are endemic to the Neotropical and the Nearctic regions, including *Boucekinus* and *Platykula* (Torymoidini); *Erimerus* (Microdontomerini); *Zaglyptonotus* (Toryminae *incertae sedis*); *Chileana*, *Perissocentrus* and *Zdenekius* (Monodontomerini); *Allotorymus*, *Physothorax* and *Plesiostigmodes* (Torymini); and *Neopalachia* (Palachiini).

The biology of torymid species is still largely unknown but a few well defined patterns are apparent. Most larvae of Toryminae (Chalcimerini, most of Microdontomerini, Torymini, Torymoidini) are ectoparasitoids of various gall makers (mainly Cynipidae and Cecidomyiidae). Palachiini and most of Podagrionini are endoparasitoids of mantid eggs or larvae. Most Monodontomerini are ectoparasitoids on larvae of bees or endoparasitoids of eggs of Heteroptera and pupae of Lepidoptera (Grissell 1995). Only a few species are phytophagous (part of Torymini). Within Megastigminae, nearly all genera are ectoparasitoids of various gall makers with the exception of the *Megastigmus* species, which are mostly phytophagous (Grissell 1999).

Little is known about the larval morphology of Torymidae. There are several individual studies concerning larval morphology and descriptions of various immature stages (Askew 1961, 1966, 2002; Sellenschlo 1982, 1983, 1984, 1989; Skrzypczyńska and Roques 1987; Askew et al. 2004, 2007; Nieves-Aldrey et al. 2007), but none have discussed larval characters in a phylogenetic framework. The only phylogenetic analysis based on terminal-instar larval characters was by Gómez et al. (2008). In this study, larval morphology did not support the monophyly of Torymidae as postulated previously (Grissell 1995) but instead suggested that Megastigminae and Toryminae were distantly related. As well, within Megastigminae, the zoophagous species of *Megastigmus* exhibit an autapomorphic character (Askew 1966; Nieves-Aldrey et al. 2008) not found in any of the phytophagous species.

Only a few fossil Torymidae have been documented. The oldest torymid fossils were discovered in Baltic amber – 44 Mya (Brues 1923 – *Monodontomerus primaveus* Brues, 1923) and the Dominican amber (15-45 Mya, Cruaud et al. 2010). Two extinct genera are known from the Dominican amber (Grissell 1980). *Gummilumpus bouceki* (Grissell, 1980) which is assigned to the tribe Palachiini and *Zophodetus woodruffi* Grissell, 1980 that appeared to be closely related to Microdontomerini (Grissell 1995). The extinct genus *Paleotorymus* and extant *Torymus* were documented also from the Eocene-Oligocene boundary of Florissant shales in Colorado (Grissell 1976, 1995) and from Miocene compression fossils in Spain (Peñalver and Engel 2006; Heraty and Darling 2009). However, the assignation of some species of *Paleotorymus* to Torymidae was questioned (Grissell of Heteroptera or Orthoptera. We believed that these genera clustered within one subclade of Monodontomerini closely related to *Monodontomerus* (Bouček 1978; Grissell 1995). This peculiar biology is unique in Monodontomerini and is only shared by Palachiini and Podagrionini.

Since its description (Bouček 1976), Palachiini was considered closely related to Podagrionini. Palachiini differs from Podagrionini in having a shorter hind coxa, slender hind femur bearing weak teeth, and a truncated hind tibia bearing two apical spurs (Bouček 1978). In all our analyses Palachiini is polyphyletic, forming a poorly supported grade that includes some species of *Podagrionella* (Fig. 3c). As discussed by Bouček (1978), *Propalachia* appears more plesiomorphic than *Palachia*. We recovered *Propalachia* as sister to all other Palachiini (only represented by *Palachia*) and *Podagrionini*. Palachiini and Podagrioni share a unique modification of the metasternum (Grissell 1995). The genera *Palachia*, *Palmon* and *Propachytomoides* are monophyletic in our results, and are also well-defined morphologically (Grissell 1995). *Podagrion* is also recovered monophyletic in MP and BA but not ML, but this may also be artefactual.

Within *Podagrion* (subclade 9a), we failed to recover the discrete genera suggested by Delvare (unpublished) (genus *Neogrion* – for New World species, *Afrogrion* for part of African species and *Australgrion* for part of Australasian species). *Podagrionella* appears polyphyletic, but is formed several monophyletic groups that roughly reflect previously accepted genera, i.e. *Iridophagoides*, *Iridophaga* and *Podagrionella* (Bouček 1976, 1988). However, because the backbone support within Podagrionini is low, we prefer not to split *Podagrionella* into distinct genera at this time.

Evolution of life strategies

Many of the most basal genera of Megastigminae (*Bortesia*, *Bootanelleus* or *Bootanomia*) are ectoparasitoids of gall making Cynipidae or Cecidomyiidae (Figs 4a, b), with the phytophagous *Megastigmus* nested within a lineage of parasitoids. Phytophagy was proposed as ancestral for Megastigminae by Grissell (1995). Ancestral reconstruction of states on our results yields an ambiguous evolutionary pattern, probably because our knowledge of the biology of many genera of Megastigminae is still fragmentary. Furthermore, outgroups (*Ficomila*, *Sycophaga* and *Odontofroggatia* all of them are phytophagous) were not chosen

Material and methods

Taxon sampling

A total of 226 ingroup taxa (2/3 of the known genera) were used for this study. The chosen taxa represent the two accepted subfamilies of Torymidae, as well as all tribes of Toryminae recognized prior to our study (Grissell 1995, Fig. 1). To test the monophyly of Torymidae, we included in our analysis species of Ormyridae (*Ormyrus* spp. and *Ormyrulus* sp.) that are considered as closely related to Torymidae (Heraty et al. 2013). We also include species belonging to more distant taxa: Eurytomidae (*Ficomila* sp.); Agaonidae, Sycophaginae (*Sycophaga* sp.); Pteromalidae, Epichrysomallinae (*Odontofroggatia* spp.) that were sometimes historically included into the Torymidae (Bouček 1988; Grissell 1995; Gibson et al. 1999). A total of 10 outgroup species belonging to four families were used (Tab. 1).

Most specimens sampled were initally preserved in 70 or 96% EtOH, although a few extracts were made from dry, card-mounted specimens (Tab. 1). Specimen vouchers are deposited in the Center for Biology and Management of Populations, INRA, Montferrier-sur-Lez, France (CBGP); Charles University, Faculty of Science, Department of Zoology, Prague, Czech Republic (PJ); Department of Entomology, University of California, Riverside, CA, USA (UCRC); and the National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM).

Molecular methods

DNA extraction either followed a modified Chelex protocol (CP) (Walsh et al. 1991) or isolation using the Qiagen DNAeasy ® kit (QP) following the manufacturer's protocol. If we had more than one specimen from a specific taxon and locality, we used destructive DNA extraction (D) to obtain a higher quantity of genomic DNA. We used non-destructive DNA extraction (N) for rare taxa or for taxa that belong to taxonomically difficult groups (Tab. 1).

Five loci were sequenced: two nuclear protein coding genes, the F2 copy of elongation factor-1α (EF-1α, 517 bp) and Wingless (*Wg*, 403bp); two ribosomal nuclear genes, *18S rDNA* (V3-V5 expansion region, ~933bp) and *28S rDNA* (D2-D3 and D4-D5 expansion regions, ~1450bp); and mitochondrial cytochrome c oxidase subunit I (*COI*, 1506bp). Primer sequences and amplification protocols followed Cruaud et al. (2010) for *COI* and *Wg*, Cruaud et al. (2011) for *EF-1α,* and Munro et al. (2011) for *18S rDNA* and *28S rDNA.*

PCR products were either sent as non-purified products to Macrogen Inc. for purification and sequencing, or purified using the QIAquick PCR Purification Kit ®, and then sequenced using the BigDyeTerminator v3.1 ® kit (Applied Biosystems) and an 3130xl Genetic Analyzer at Charles University in Prague, an ABI3730XL sequencer at Genoscope, Evry, France, or sequenced at the San Diego State University Microchemical Core Facility or the UCR Genomics Core Facility. All sequences are deposited in GenBank (!change for NUMBERS!). All regions sequenced for specific taxa are listed in Tab. 1.

Alignment

Contigs were assembled and subsequently edited using the software Geneious version 6.1.6 ® (available from http://www.geneious.com/). All gene regions were aligned using MAFFT (Katoh and Standley 2013) version 7.110 (available from http://mafft.cbrc.jp/alignment/server/) using the E-INS-i strategy for ribosomal genes and L-INS-i strategy for coding genes. Alignments of protein-coding sequences (*EF-1α*, *Wg*, *COI*) were translated into amino acid using Geneious 6.1.6 to detect stop-codons that may indicate pseudogenes or misalignments. Ribosomal ambiguous alignment regions (non-pairing bases) were realigned using secondary structure models (Gillespie et al. 2005; Munro et al. 2011). Possible substitution saturation for each gene and nucleotid position, respectively, was checked by plotting the number of transition (Ts) and transversion (Tv) vs F84 model of distance using DAMBE (Xia 2013).

Phylogenetic analyses

Parsimony and probabilistic methods were used to reconstruct trees under different optimality criteria. Maximum parsimony analysis (MP) were conducted using PAUP* 4.0b10 (Swofford 2003) and 1000 random addition of sequences with TBR swapping. Gaps were treated as missing characters and all substitutions were equally weighted. Nodal supports for MP trees were assessed with 1000 bootstrap replications. Bootstrap percentage (BP) ≥ 70% were regarded as strong nodal support (Farache et al. 2013).

For both Bayesian (BA) and maximum likelihood (ML) analyses, jModelTest 2.1.4 (Posada 2008; Darriba et al. 2012) was used to calculate models of evolution for individual genes or nucleotides position respectively under the Akaike information criteria (AIC). ML and BA analyses were conducted using different partitioning dataset and implementing separate nucleotide substitution models for subsets of the data. ML analyses were implemented in RAxML 7.6.3 using GTRCAT approximation with 1000 bootstrap replicates

(Stamatakis 2006). Several runs with different seeds settings were run to estimate the best likelihood score. Bootstrap percentages (BP) of more than 95% were considered as strong support and BP < 70% as weak (Cruaud et al. 2011b). For Bayesian Analyses (BA), a parallel version of MrBayes 3.2.2 (Huelsenbeck et al. 2001; Ronquist and Huelsenbeck 2003) was used. Parameter values for the partitioning strategy were initiated with default uniform priors and branch lengths estimated using default exponential priors, with the dataset partitioned by genes and nucleotide position (Cruaud et al. 2010). We ran two independent runs of 30 million generations, sampling every 10,000, with the first 25% of samples discarded as burn-in. Posterior probabilities (PP) ≥ 0.95 were considered as strong support, PP < 0.90 as weak (Farache et al. 2013). All ML and BA analyses were conducted on the CIPRES Science Gateway (Miller et al. 2010).

Character mapping

To infer biogeography and the evolution of life-history strategies, we conducted MP ancestral state reconstruction using Mesquite 2.75 (Maddison and Maddison 2011). All reconstruction was performed on the resulting RAxML (ML) tree. We assembled a matrix of three characters from our own collecting data or several literature sources (Hanson 1992; Grissell 1995; Gómez et al. 2008; Janšta et al. 2013; Noyes 2013).

We divided taxa based on life-strategy of larvae (Fig. 4a) – i.e. phytophagous species feeding in plant seeds, zoophagous ectoparasitoids feeding on larvae of gall-forming insects, zoophagous ectoparasitoids feeding on larvae of non-galling insects, zoophagous endoparasitoids feeding on larvae of non-galling insects, endoparasitoids of insect eggs; based on information about larvae host records at higher taxonomic level of hosts – i.e. phytophagous species feeding in plant seeds, zoophagous species feeding on larvae of Cynipidae, Cecidomyiidae, other Diptera, Aculeata, Eurytomidae, Coleoptera, in pupae of Lepidoptera and in eggs of Heteroptera and Mantodea; and based on distribution of taxa added to analysis according to biogeographical regions – i.e. Afrotropical region, Palaearctic region, Oriental region, Australian region, Nearctic region and Neotropical region.

In cases where we did not have enough biological data to summarize life-strategy or host records for specific parasitoid taxa included into analysis (i.e. for species which hosts are unknown or we were not able to determine them to the species level), we generalized it based on genus-level characteristic from literature. Thus, when species of the genus are known as parasitoids of more then one higher taxa mentioned above, we add into analysis all these main hosts possibilities for specific taxa of parasitoid.

Results

Alignment and phylogenetic analyses

A total of 4809 bp was used for our analysis: *18S rDNA* = 933bp, *28S rDNA* = 1450bp, *EF-1α* = 517, *COI* = 1506 and *Wg* = 403. Under parsimony with gaps treated as missing, there were 1347 constant, 1900 variable and 1562 informative characters. Alignment of protein coding genes revealed no stop codons or frame shifts. For the mitochondrial locus (*COI*), the third codon position (nt3) showed a strong bias in base composition (A+T 92%) and saturation (Fig. 2a). *Wingless* had a base composition bias (C+G 72%) and was saturated for the nt3 position (Fig. 2b). Therefore we decided to recode these nucleotides positions as purines (R) or pyrimidines (Y) (Phillips and Penny 2003; Cruaud et al. 2011a).

Prior to testing for an optimal partitioning strategy, we selected sequence evolution model for the entire concatenated dataset, every single gene, stems and loop region in ribosomal genes as well as for each position in coding genes using AIC criterion in jModelTest 2.1.4. An optimal partitioning was then selected after comparing the Bayes factors (BF) of each partitioning strategy (Cruaud et al. 2010). The best BF scores was for separating the dataset into ten partitions as follows – RNA(1) + *EF-1α* nt1(2) + *EF-1α* nt2(3) + *EF-1α* nt3(4) + *COI* nt1RY(5) + *COI* nt2(6) + *COI* nt3RY(7) + *Wg* nt1(8) + *Wg* nt2(9) + *Wg* nt3RY(10). Models choosen for these partitions for BA were GTR+I+Γ (for 1, 2, 4, 5, 6), GTR + Γ (8) and GTR (3, 7, 9, 10).

The parsimony analysis (MP) of the entire dataset resulted in four most parsimonious trees with a tree length of 14,669 steps (CI = 0.17, RI = 0.60). The 50% majority-rule consensus was computed from all best trees. Bayesian analysis (BA) and Maximum Likelihood (ML) results yielded almost the same topology (Fig. 3a-c), but with a slightly different topology from MP analysis. Deviations of topology under different phylogenetic approaches are discussed below.

Monophyly of Torymidae

In all analyses, Torymidae was not recovered as a monophyletic group, with a monophyletic Toryminae recovered as sister to Megastigminae + Ormyridae (node support ML/BA/MP 68/0.97/-). Megastigminae were monophyletic (95/0.99/96) (clade 1), and each of the included genera were monophyletic, but with low support for *Megastigmus*. *Bootanomyia* (Megastigminae) is monophyletic in all topologies, but with no MP support (84/0.97/-).

Phylogenetic position of other genera was congruent in all results with *Bortesia* + *Bootanelleus* sister to all other Megastigminae (95/0.99/96).

Phylogeny of Toryminae

Nodal support for a monophyletic Toryminae was high in all analyses (99/1/70). All analyses recovered a monophyletic *Thaumotorymus* + (*Chalcimerus* + *Exopristoides*) (clade 2) with high support (100/1/92) that was sister group to all other Toryminae. The Torymoidini + Torymini *sensu* Grissell (1995) was recovered sister (95/1/-) to a relatively poorly supported clade (74/1/-) composed of all other Toryminae tribes (Microdontomerini, Monodontomerini, Podagrionini, Palachiini, and genera treated by morphology as *incertae sedis*).

Torymoidini was paraphyletic in all analyses (clade 2 & 3, Fig. 3b), with some Torymoidini (*Boucekinus* + *Platykula* + one undescribed genus from Chile) (clade 3) (100/1/70) sister group to a monophyletic group of other Torymoidini (clade 4) (91/1/-) +Torymini (97/1/57). *Pseudotorymus* was paraphyletic within clade 2 in all analyses. A well supported clade (100/1/83) including all African *Pseudotorymus* species was recovered as sister to a clade (100/1/60) clustering all *Pseudotorymus* species from the Holarctic region (100/1/82) and a monophyletic *Torymoides* (93/1/57) showing Australian species basal to Palaearctic species. Support for Torymoidini + Torymini excluding clade 3 was relatively strong (83/.99/-).

The tribe Torymini (clade 5, Fig. 3b) was recovered as monophyletic in all analyses (97/1/57). *Ecdamua* was sister to all other Torymini but without any support. The genus *Torymus* was polyphyletic, and included species of *Physothorax* and *Plesiostigmodes*. The New World species of *Torymus* plus the New World genera of Torymini (*Physothorax* and *Plesiostigmodes*) plus the subgenus *Nannocerus* of *Torymus* sp. were clustered in a poorly supported clade (79/1/56) (subclade 5a).

The remaining Toryminae were subdivided into two clades with contrasted support (Fig. 3c). A poorly supported clade included Glyphomerini, Microdontomerini (clade 7) and awell supported clade grouping all Podagrionini, Palachiini and Monodontomerini. Glyphomerini (including only the genus *Glyphomerus*) was recovered paraphyletic. Two species of *Glyphomerus* (incl. *G. stigma* from Europe) grouped in a strongly supported clade (clade 6) (100/1/100) while the other species of *Glyphomerus* were nested within all species of Microdontomerini and formed a well supported clade (clade 7) (99/1/77). The position of Glyphomerini was unstable in different analyses. They were recovered neither sister to tribe

Microdontomerini (clade 7) (ML and BA) or to Monodontomerini (clade 8) and Palachiini + Podagrionini (clade 9) (MP). Except for the ML results, the support in both cases of topology was considered as weak (72/-/-).

Within clade 7, all included genera were monophyletic, with the exception of *Eridontomerus* and *Pseuderimerus*. *Eridontomerus* was rendered paraphyletic by *Ditropinotus,* and *Pseuderimerus* was rendered paraphyletic by *Erimerus*. Several genera previously classified as *incertae sedis* were placed in our clade 7 with very high support, namely *Cryptopristus*, *Echthrodape, Exopristus* and *Glyphomerus* in part.

Monodontomerini (clade 8) were monophyletic and included the genera *Zaglyptonotus* (98/1/61) and *Zdenekius*. *Zdenekius* was recovered sister to all other Monodontomerini only by ML (84/-/-). Monodontomerini was subdivided with strong support into South American (93/1/74) and Palaearctic (99/1/95) subclades (subclade 8a and 8b respectively).

Palachiini was recovered as polyphyletic with respect to Podagrionini in all analyses, but was recovered monophyletic in ML and BA (clade 9) (95/1/-). *Propalachia* (Palachiini) was sister to the rest of Palachiini plus Podagrionini in the ML and BA results (94/1/-). Within clade 9, *Propalachia*, *Palachia*, *Propachytomoides* and *Palmon* were monophyletic. *Podagrion* was monophyletic only when *Mantiphaga* (1003 TOTO *Mantiphaga* sp1 UAE) was included. *Podagrionella* was separated into several distantly related clades mostly according to former (now synonymized) subgenera.

Characters mapping

We mapped the life-strategies of larvae (Fig. 4a), information about hosts associations for larvae (Fig. 4b), and the distribution of taxa according to their biogeographical regions (Fig. 5). Despite the noticeable disproportion in taxa from different regions and clades, we tried to include taxa from all regions where they are known (e.g. for genus *Torymus*, which is distributed in all regions, species were included from across its entire range).

Discussion

The monophyly of Torymidae (Megastigminae + Toryminae) was never recovered in our analyses, however this result lacks support and need to be confirmed by future studies. This contradicts earlier studies (e.g., Bouček 1988; Grissell 1995; Heraty et al. 2013; but see Campbell et al. 2000 and Munro et al. 2011). However, the monophyly of the family has not yet been supported by morphology (exclusive synapomorphy).

Our results suggest that 1) Toryminae and Megastigminae should be raised to family rank (Torymidae and Megastigmidae) or 2) Ormyridae must be downgraded to a subfamily of Torymidae (Ormyrinae). Ormyridae and *Colotrechnus* (Pteromalidae: Colotrechninae) were sister to a monophyletic Torymidae in Heraty et al. (2013), but with weak morphological support.

The larval morphology of Megastigminae and Toryminae are very different (Gómez et al. 2008), which also supports treating them as distinct family groups. Synapomorphies based on larval characters for individual subfamilies were postulated as body segments almost bare, without setae or only with very short setae; mandibles with four or five teeth; labrum divided into several small lobes in Megastigminae and body segments strongly hairy; mandibles with a single tooth; labrum undivided for Toryminae (Gómez et al. 2008).

Megastigminae

Phytophagy has been considered as the ancestral feeding strategy for Megastigminae (Grissell 1995). However, our analysis highlighted the derived position of the phytophagous *Megastigmus*. The ancestral feeding strategy remains equivoqual but phytophagy appears to have originated at least twice in the genera *Bootanelleus* and *Megastigmus* (Figs 4a, b).

Our results confirm that the metallic zoophagous species of *Megastigmus* (mostly Palaearctic) were correctly placed within the Australian genus *Bootanomyia,* as advocated by Doğanlar (2011a). Larvae of Australian *Bootanomyia* are not known, nevertheless exclusive autapomorphy (the presence of a medial frontal pit) of zoophagous larvae formerly included into *Megastigmus*, (now *Bootanomyia*) were observed repeatedly (Askew 1966; Nieves-Aldrey et al. 2008). A similar character has not been observed in phytophagous *Megastigmus* (Nieves-Aldrey et al. 2008).

Most genera and species of Megastigminae are restricted to the Australian region (Bouček 1988). However, based on our sampling of taxa and outgroups, the ancestral distribution of Megastigminae remains equivocal (Fig. 5).

Toryminae

The monophyly of Toryminae has been demonstrated by several studies based on both molecular and morphological characters (Grissell 1995; Campbell et al. 2000; Munro et al. 2011; Heraty et al. 2013) and is corroborated by our results. Despite its monophyly, no distinct apomorphy defined the clade, and the group is defined by an exclusive combination of five characters (Bouček 1988; Grissell 1995).

Our analyses corroborated the monophyly of nearly all tribes defined by Grissell (1995), but our relationships differ from what he proposed (Fig. 1).

Clade 2 is sister to all other Toryminae tribes. This clade includes *Chalcimerus* and two previously unclassified genera, *Exopristoides* and *Thaumatorymus*. *Chalcimerus* was formerly treated as the sister group to Podagrionini. This position was supported by several synapomorphies (enlarged hind leg with toothed femora, curved tibiae bearing only one apical tibial spur). However, these putative synapomorphies are homoplastic across Chalcidoidea (Heraty et al. 2013) and erroneously suggested a relationship between Podagrionini and *Chalcimerus*. There is no synapomorphy suggesting a close relationship between *Thaumatorymus*, *Exopristoides* and *Chalcimerus*. The only character shared between *Chalcimerus* and *Exopristoides* is the so called “primitive” wing venation (Grissell 1995). Because of similar biology [all are known to be parasitoids of gallwasps (Cynipidae) of the tribe Aylacini] and similar distribution (all species are restricted to the West Palaearctic region), we treat them as one tribe, Chalcimerini.

Members of the clades 3, 4 and 5 share one synapomorphy, which is the *Torymus*-like wing venation (Grissell 1995; Janšta et al. 2011). Torymini (clade 5) is characterized by the sinuate metapleural shelf (Grissell 1976). However, relationships within Torymini are still unresolved. *Torymus* and few New World genera form a paraphyletic assemblage that was recovered as sister to *Ecdamua*, with species belonging to clade 5 subdivided into two distinct subclades (subclade 5a and 5b).

Subclade 5a included the formerly recognized *Diomorus* (previously synonymized under *Torymus*; Graham and Gijswit 1998; Zerova et al. 2000) and some New World species of *Torymus* including the subgenera *Nannocerus*, *Physothorax* and *Plesiostigmodes*. Within this clade, larvae of *Torymus* (=*Diomorus*) are ectoparasitic on larvae of aculeate wasps, which is a biology also shared with species of *Ecdamua* (Graham and Gijswit 1998; Zerova and Seryogina 2003), with *Ecdamua* the sister group of clade 5a+b. Based on our personal observations, *Physothorax* and *Torymus* (*Nannocerus*) species are associated with figs. Bouček (1993) reported parasitism by *Physothorax bidentulus* on larvae of gall-forming

Cecidomyiidae inside *Ficus* syconia. Thus we assume that most species of *Physothorax*, *Plesiostigmodes* and *Torymus* (*Nannocerus*) develop as gall-maker parasitoids within figs. These species are only known from the neotropics (with a few species reaching Florida, i.e. *P. bidentulus*; Noyes 2013). The morphological differences between these three genera/subgenera are 1) number of ventral teeth on the hind femur: one in *T.* (*Nannocerus*), two in *Plesiostigmodes* and sometimes more than two in *Physothorax*; 2) winged males in *Physothorax* versus non-winged males in *Physothorax* and *T.* (*Nannocerus*), and 3) presence of two annelli (discoidal basal antennal flagellomeres) in *Plesiostigmodes* versus a single anellus in the others (Ashmead 1904; Bouček 1993). Our data do not allow us to question the monophyly of the discussed genera and subgenus, but the number of anneli or anneliform segments is a very labile character within these groups (Bouček 1993). Torymini is the only group of Torymidae containing species with apterous males. Apterism probably evolved as an answer to the association with figs as previously observed in many other fig wasps (Cruaud et al. 2010, 2011b; Segar et al. 2012).

Subclade 5b included the remaining species of *Torymus* and *Allotorymus splendens*. *Allotorymus* differs from *Torymus* by exhibiting an elongated pronotum and a strongly clavate flagellum, but most other characters are also shared with species from the *Torymus laetus* species group (Grissell 1976; Graham and Gijswit 1998; Zavada 2003).

Our sampling of Torymini is not representative of the overall diversity of the tribe (only five of ten known genera), therefore we hesitate to make the taxonomic changes until the relationships are confirmed by an analysis including more genera. Comparing to the other tribes, branch length and genetic distance between *Torymus* species and species-groups (especially those from the Old World) strongly suggest that the genus underwent a rapid diversification.

Torymoidini were subdivided into two clades (clade 3 and clade 4) that form a grade to Torymini. Clade 3 comprises only the New World genera, *Boucekinus*, one undescribed genus from Chile, and *Platykula*. As previously noticed (Janšta et al. 2011), *Boucekinus* appears to be morphologically similar to *Platykula*. Although the biology is only known for *Boucekinus*, we propose clade 3 as a new tribe (Boucekini **trib. nov.**). Torymoidini (clade 4) is now restricted to only *Pseudotorymus* and *Torymoides*. *Pseudotorymus* was paraphyletic in our analyses, with the most basal clade contains all Palaearctic species of *Pseudotorymus*. The second *Pseudotorymus* clade is sister to *Torymoides* and contains only Afrotropical species. Risbec (1951) described the genus *Senegalella* which was only reported from the Afrotropical region and differed from *Pseudotorymus* only by the absence of setae on the dorsal surface of hind coxa. Grissell (1995) also found some undescribed species of *Senegalella* in the Oriental region, northern Africa and southern Europe and based on these distribution records synonymized *Senegalella* with *Pseudotorymus*. According to our results, *Senegalella* (genus with bare hind coxa dorsally) appears to be a valid genus, sister to *Torymoides*.

The torymoid clade (clades 3-5) is sister to Microdontomerini + *Glyphomerus stigma* and Monodontomerini+Palachiini+Podagrionini. The position of *G. stigma* (together with *G.* cf. *stigma* from Canada) was unstable in our analyses, but support values are always low. In the MP analysis, the *Glyphomerus* clade is recovered sister to Monodontomerini + Palachiini + Podagrionini. Grissell (1995) included *Glyphomerus* as *incertae sedis* within Toryminae and placed it in a basal multifurcation. He found no morphological support to allocate *Glyphomerus* to any of the torymid tribes and defined the genus on a combination of several „plesiomorphic characters“. We decide here to classify in the genus as a new tribe (Glyphomerini **trib. nov.**) due to clear genetic distance from other members of the subfamily Toryminae.

We consider the monophyletic clade 7 to be the Microdontomerini ***sensu novum***. *Echthrodape* was not included in previous phylogenetic analyses and was considered as *incertae sedis* within Toryminae by Grissell (1995). In our analyses, *Echthrodape* is sister to all other Microdontomerini. While there is no morphological character to supporting its inclusion in Microdontomerini, we consider it as part of Microdontomerini. Microdontomerini also includes several genera (*Cryptopristus*, *Exopristus*, other *Glyphomerus* species) previously treated as *incertae sedis* by Grissell (1995). The relationships of *Glyphomerus* was discussed above and it is clear from our results that the genus is polyphyletic. *Glyphomerus stigma* differ from other species of the genus by the wing venation and the length of the malar space (Stojanova 2005). There are two other congeneric species with short malar space which are associated with gallwasps on *Rosa* spp. (Noyes 2013) that potentially could be part of clade 6 (tribe Glyphomerini). *Cryptopristus* and *Exopristus* were previously considered as close relatives to either Monodontomerini, Chalcimerini, Palachiini or Podagrionini (Grissell 1995), because they share the same modification of the hind femora, however - as stated above - this character is very homoplastic. Following *Echthrodape*, *Exopristus* is sister to the rest of Microdontomerini but with no morphological support. *Cryptopristus* is sister to the genus *Idarnotorymus.* Both genera have a distinct emargination of the hind margin of the metasomal tergites and are respectively parasitic of gallmaking Eurytomidae in stems of Poaceae or of Aylacini (Cynipidae) in stems of *Salvia syriaca* (Grissell 1995; Zerova et al. 2008).

The genera *Microdontomerus*, *Adontomerus*, and *Idiomacromerus sensu* Grissell (1995) are monophyletic. However *Pseuderimerus* were paraphyletic with respect to *Erimerus* and *Eridontomerus* paraphyletic with respect to *Ditropinotus*. *Pseuderimerus* and *Erimerus* share two common characters: 1) a single apical spur on the hind tibia and 2) males with reduced eyes (Grissell 1995; Burks and Redak 2004). However, the paraphyly of *Pseuderimerus* could be artefactual and linked to the low number of sequenced genes.

Eridontomerus and *Ditropinotus* were considered as distinct genera by Grissell (1995). However, all the characters that separate them appears to be highly homoplastic and there is no synapomorphy that reliably distinguishes these genera (Janšta – pers. observ.). Moreover, all species of these genera share the same biology and are recurrently reported as larval parasitoids of Eurytomidae (Chalcidoidea) in grass stems (Grissell 1995; Janšta and Bouček 2006) (Fig. 4b).

Monodontomerini (clade 8) was monophyletic in all our analyses. The tribe is well defined by the morphological characters proposed by Grissell (1995), although *Chileana* and *Zaglyptonotus* bear an occipital carina that is not known in other Monodontomerini (Janšta et al. 2013). Both *Zdenekius* and the Neotropical genus *Rhynchodontomerus* (not included in our study) were considered to be basal monodontomerines by Grissell (1993, 1995). Our results place *Zdenekius* as sister to all other Monodontomerini.

Beyond *Zdenekius*, the Monodontomerini group into two geographically defined subclades. The first subclade (8a) includes only the New World genera *Chileana*, *Perissocentrus*, *Zaglyptonotus,* and an undescribed genus. All of these genera are well defined by morphological characters. *Zaglyptonotus* was treated as *incertae sedis* by Grissell (1995) because it shares feaures with Torymoidini. However, Janšta et al. (2013) recently described *Chileana*, which shares some characters (long hind tibial spurs, incision of hing margin of metasomal tergites) with *Zaglyptonotus*, but also characters typical of other Monodontomerini. The second subclade (8b) includes mostly Old World genera (*Monodontomerus*, *Anneckeida* and *Rhynchoticida*) and the Holarctic *Monodontomerus*. In our results, *Monodontomerus* is paraphyletic with respect to *Rhynchoticida,* however this may be artefactual as we failed to sequence most genes for *Rhynchoticida* (only 28S rDNA) (Tab 1 and Fig. 3c). Morphologically, *Rhynchoticida* (small bodied with an unusually short ovipositor) is similar to *Anneckeida* and few other Monodontomerini that are egg parasitoids appropriately for that purpose (Figs 4a, b) as phytophagy is not a plesiomorphic state (Heraty et al. 2013) within Chalcidoidea.

Ectoparasitism is considered to be a derived strategy within Chalcidoidea with chalcid larvae of the basal families of Chalcidoidea endoparasitic (Gómez et al. 2008). However, our analyses reveal that the common ancestors of Toryminae, Chalcimerini, Boucekini, Torymoidini, Glyphomerini and Microdontomerini were recovered ectoparasitoids on gallmaker larvae (Figs 4a, b).

The ancestral biology of Torymini is ambiguous. The most basal taxa *Ecdamua* and *Torymus* (formerly *Diomorus*) are ectoparasitoids of Aculeate wasps. Most other Torymini are ectoparasitoids of larvae of Cynipidae or Cecidomyiidae and only a few clades returned to phytophagy (Graham and Gijswit 1998). However, our reconstructed topology within Torymini is poorly supported and may lead to artefactual conclusions.

With the exception of Mymaridae and Trichogrammatidae, oophagy (parasitism of eggs) is rare within Chalcidoidea , occurring only in some species of Eulophidae, Pteromalidae, Torymidae and Eurytomidae (Heraty et al. 2013). Grissell (1995) suggested that oophagy was a derived strategy that evolved several times within Toryminae. As far as known, oophagy occurs only within Podagrionini, Palachiini, Microdontomerini and Monodontomerini (Grissell 1995). The same author also suggested that presence of many oophagous species within Podagrionini, Palachiini and Monodontomerini may indicated close phylogenetic relationships. He also postulated that egg parasitism could be the reason for the highest percentage of zoophagous endoparasitoids in Monodontomerini (most of species *Perissocentrus* and few species of *Monodontomerus* are feeding within Lepidoptera pupae).

The ancestral feeding strategy for the clade Monodontomerini+Podagrioni+Palachiini is ambiguous (Figs 4a, b). These tribes are with no doubts closely related but more studies on the biology of these wasps is needed, especially for Monodontomerini and Palachiini, to better identify their ancestral feeding strategy.

There is only one biological record for *Palachia* and Palachiini (parasitoids of mantid eggs), consequently generalizing this biology to the whole tribe is inadequate. However, *Palachia* is a well supported clade within Podagrionini and almost all genera of Podagrionini are oophagous of mantid eggs. Consequently, oophagy of Dictyoptera (Blattodea

+ Mantodea) could be the ancestral biology of Podagrionini + *Palachia* and probably also *Propalachia*, but there is not host record for that genus (Bouček 1998).

Biogeography

The ancestral area for Megastigminae was ambiguous in our results. We included 7 of 12 known genera of Megastigminae, of which 4 are endemic to Australia. The five genera not included into our analysis are distributed in Australia or in the Oriental region. *Megastigmus*, *Bootanomyiia* and *Westralianus* are the only genera with species distributed outside of the oriental or Australasian regions (Bouček 1988; Doğanlar 2011a, 2011b).

The Palearctic region was proposed as the ancestral area for Toryminae, and several tribes are only or mostly known from the Old World. Chalcimerini is only known from west Palaearctic (Grissell 1995). The common ancestor of tribes Glyphomerini and Microdontomerini are probably of Palaearctic origin. However, the position of Glyphomerini is unstable and not well supported.

The three clades within Torymoidini are distributed mostly in the Old World. *Pseudotorymus* is mostly European with one species reaching the Nearctic region. *Pseudotorymus* (formerly known as *Senegallela*) are distributed in the afrotropics but probably also reach North Africa and the Oriental region (few species not included to our dataset). Many species of *Torymoides* and two genera not included in our analyses (*Allomba* and *Torymoidellus*) reach the Australasian region (Bouček 1988; Grissell 1995).

Microdontomerini are also proposed to have originated in the Old World (Fig. 5). Only a few species within *Cryptopristus*, *Ditropinotus* and *Eridontomerus* are known from both the Old and New World, but they probably spread to the New World with their hosts (Grissell 1995; Janšta and Bouček 2006). All of them could be spread to New World with their hosts as they are mostly parasitoids of larvae of various gallmakers on stems of cereals (Poaceae). In contrast, several species of *Idiomacromerus*, *Microdontomerus*, *Pseuderimerus* and one *Erimerus*, have a derived position inside the generic phylogeny and could be considered as true New World species (Grissell 1995).

Podagrionini and Palachiini have a pantropical distribution but clearly orginate in the Old World probably in the Oriental region. They colonized recurrently and independently several regions of the world.

The ancestral area for Boucekini, Torymoidini and Torymini is ambiguous, but Boucekini is a New World clade that is sister to the others that have genera and species distributed all around the world. Mapping of their distribution on the clade of Torymini support our results from all phylogenetic analysis, i.e. paraphyly of the genus *Torymus*. New World species of *Torymus* probably belong to a different genus. But more comprehensive genetic and morphological studies are needed to confirm our hypothesis. The rest of species of the genus *Torymus* could have probably (but with any bootstrap support) Palaearctic origin with some exeptions of distribution into different areas.

The ancestral distribution of Monodontomerini is ambiguous. The tribe consists of two New World clades and one Old World clade.

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Table 1. Species used in this study, their specimen, family/subfamily/tribal code, gene fragments sequenced, type of extraction, deposit condition prior to DNA extraction, voucher and extract deposit and locality (TOM - Torymidae: Megastigminae, OR - Ormyridae, AS - Agaonidae: Sycophaginae, PTE - Epichrysomallinae, TOTT - Torymidae: Toryminae: Torymini, TOTY - Torymidae: Toryminae: Torymoidini, TOTN - Torymidae: Toryminae: Monodontomerini, TOTO - Torymidae: Toryminae: Podagrionini, TOTP - Torymidae: Toryminae: Palachiini, TOTM - Torymidae: Toryminae: Microdontomerini, TOTC - Torymidae: Toryminae: Chalcimerini, TOTI - Torymidae: Toryminae: incertae sedis; D - destructive, N - non-destructive, QP - Quaigen protocol, CP - Chelex protocol; CBGP - Center for Biology and Management of Populations, INRA, Montferrier-sur-Lez, France, PJ - Charles University in Prague, Faculty of Science, Department of Zoology, Prague, Czech Republic, UCR - Department of Entomology, University of California, Riverside, USNM - National Museum of Natural History, Smithsonian Institution, Washington). Outroups species names are bolded.

Species name	**Specimen code**	**Fam./ subfam./ tribal code**	**18S rDNA**	**28S rDNA D2-D3**	**28S rDNA D4-D5**	**EF-1α**	**COI barcode**	**COI2183-3014**	**Wg**	**Extraxtion**	**Deposit condition**	**Voucher deposit**	**Exctract deposit**	**Locality**
Megastigmus sp.	1259_03	TOM	1259_03_a	1259_03_b	1259_03_c	1259_03_d	1259_03_e	-	-	D, QP	96	CBGP	CBGP	New Caledonia, Maré
***Ormyrus* sp.**	1868_08	OR	1868_08_a	1868_08_b	1868_08_c	1868_08_d	1868_08_e	1868_08_f	-	D, QP	96	CBGP	CBGP	Malaysia, Sarawak, Pa'Lungan
***Sycophaga* sp.**	1940_02	AS	1940_02_a	1940_02_b	1940_02_c	1940_02_d	1940_02_e	-	-	D, QP	96	CBGP	CBGP	Cameroon, 30 km W Yaoundé
***Ficomila* sp.**	1987_02	EU	1987_02_a	1987_02_b	1987_02_c	1987_02_d	1987_02_e	1987_02_f	-	D, QP	96	CBGP	CBGP	Malaysia, Sarawak, Pa'Lungan
***Odontofroggatia* sp.**	2355_03	PTE	2355_03_a	2355_03_b	2355_03_c	2355_03_d	2355_03_e	2355_03_f	-	D, QP	96	CBGP	CBGP	Indonesia, Papua Barat, Tanahaubuh river
Odontofroggatia ishii	2355_05	PTE	2355_05_a	2355_05_b	2355_05_c	2355_05_d	2355_05_e	2355_05_f	-	D, QP	96	CBGP	CBGP	Indonesia, Papua Barat, Tanahaubuh river
Torymus sp. laetus group	31002	TOTT	31002_a	-	31002_c	31002_d	-	31002_f	-	N, QP	96	PJ	CBGP	Italia, Friuli Venezia Giuli, Pontebba
Physothorax sp.1	121703	TOTT	JN623527	JN623859	JN623859	-	-	-	-	D, QP	96	CBGP	CBGP	France, Guadeloupe, Vieux Fort
***Ormyrulus* sp.**	46O	OR	JN623316	JN623706	JN624064	-	-	-	-	N, CP	96	UCR	CBGP	Thailand, Surat Thani Province
Ecdamua cadenati	D2470	TOTT	JN623526	JN623857	JN623857	-	-	-	-	N, CP	D	USNM	UCR	Kenya, Eastern Prov., At Athi River
Physothorax bidentulus	D2471	TOTT	D2471_a	JN623858	JN623858	-	-	-	-	N, CP	D	USNM	UCR	Florida, Florida City
Glyphomerus tibialis	D2474	TOTI	D2474_a	D2474_b	D2474_c	D2474_d	D2474_e	D2474_f	-	N, CP	96	UCR	CBGP	Czech Republic, Budkovice
Bootanelleus sp.	D2482	TOM	D2482_a	JN623841	JN623841	-	-	-	-	N, CP	D	UCR	UCR	Australia, WA, Doubleyung
Paramegastigmus sp.	D2483	TOM	JN623515	JN623844	JN623844	-	-	-	-	N, CP	D	PJ	UCR	Thailand, Trang Pr., Khao Chong
Pseudotorymus sp.1	3001	TOTY	3001_a	-	3001_c	3001_d	-	-	-	N, QP	96	PJ	CBGP	Cameroon, Nord Ouest, Fundong
Torymus (*Diomorus*) sp.	3002	TOTT	3002_a	-	3002_c	3002_d	-	3002_f	3002_g	N, QP	96	PJ	CBGP	Cameroon, Jamga
Podagrion sp.1	3003	TOTO	3003_a	3003_b	3003_c	3003_d	-	3003_f	3003_g	N, QP	96	PJ	CBGP	Cameroon, Nord Ouest, Babungo
Podagrion sp.2	3005	TOTO	3005_a	-	3005_c	3005_d	3005_e	3005_f	-	N, QP	96	PJ	CBGP	Cameroon, Nord Ouest, Fundong
Podagrion sp.3	3006	TOTO	3006_a	-	3006_c	3006_d	3006_e	3006_f	-	N, QP	96	PJ	CBGP	Cameroon, Nord Ouest, Batibo
Podagrion sp.4	3007	TOTO	3007_a	-	3007_c	3007_d	3007_e	3007_f	-	N, QP	96	PJ	CBGP	Cameroon, Adamaoua, Wak
Podagrion sp.5	3008	TOTO	3008_a	-	3008_c	3008_d	3008_e	3008_f	-	N, QP	96	PJ	CBGP	Cameroon, Nord Ouest, Njinikom
Podagrionella (*Iridophagoides*) *tatianae*	3010	TOTO	3010_a	3010_b	3010_c	3010_d	3010_e	3010_f	-	D, QP	96	PJ	CBGP	Spain, Cádiz, Grazalema
Podagrion splendens	3011	TOTO	3011_a	-	3011_c	3011_d	3011_e	3011_f	-	N, QP	96	PJ	CBGP	France, Hérault, Saint-Félix-de-lHéras
Podagrion bouceki	3012	TOTO	3012_a	-	3012_c	3012_d	3012_e	3012_f	-	D, QP	96	PJ	CBGP	Morocco, Mrirt
Palmon sp.1	3014	TOTO		3014_b	3014_c	3014_d	-	3014_f	-	N, QP	96	PJ	CBGP	French Guayna, Kourou
Podagrion sp.6	3015	TOTO	3015_a	-	3015_c	3015_d	3015_e	3015_f	-	N, QP	96	PJ	CBGP	French Guayna, Kourou
Podagrion pachymerum	3017	TOTO	3017_a	3017_b	3017_c	3017_d	3017_e	3017_f	-	D, QP	96	PJ	CBGP	Italy, Lombardia, Vobarno
Podagrion minus	3018	TOTO	3018_a	-	3018_c	3018_d	3018_e	3018_f	-	N, QP	96	PJ	CBGP	Spain, Cádiz, Grazalema
Podagrionella (*Iridophaga*) *korsakowi*	3019	TOTO	3019_a	3019_b	3019_c	3019_d	3019_e	3019_f	-	D, QP	96	PJ	CBGP	Mauritania, 15 km N Nouakchott
Podagrionella (*Iridophaga*) *lichtensteini*	3020	TOTO	3020_a	-	3020_c	3020_d	3020_e	3020_f	-	D, QP	96	PJ	CBGP	France, Hérault, Cournonterral
Mantiphaga bekiliensis	3021	TOTO	3021_a	3021_b	3021_c	3021_d	3021_e	3021_f	-	D, QP	96	PJ	CBGP	Madagascar, Andrika
Podagrion sp.7	3022	TOTO	3022_a	-	3022_c	3022_d	3022_e	3022_f	3022_g	N, QP	96	PJ	CBGP	Vanuatu, Santo
Exopristus trigonomerus	3023	TOTI	3023_a	3023_b	3023_c	3023_d	3023_e	3023_f	-	D, QP	96	PJ	CBGP	France, Hérault, Saint-Pierre-de-Lafage
Idarnotorymus pulcher	3024	TOTM	3024_a	-	3024_c	3024_d	3024_e	3024_f	-	D, QP	96	PJ	CBGP	France, Gard, Beauvoisin
Podagrion bouceki	3025	TOTO	3025_a	-	3025_c	3025_d	3025_e	3025_f	-	D, QP	96	PJ	CBGP	France, Gard, Beauvoisin
Pseuderimerus sp.1	3026	TOTM	3026_a	-	3026_c	3026_d	3026_e	-	-	N, QP	96	PJ	CBGP	France, Ardèche, Les Vans
Pseudotorymus napi	3027	TOTY	3027_a	-	3027_c	3027_d	3027_e	3027_f	3027_g	D, QP	96	PJ	CBGP	France, Hérault, Mauguio
Pseudotorymus napi	3028	TOTY	3028_a	-	3028_c	3028_d	3028_e	3028_f	3028_g	D, QP	96	PJ	CBGP	France, Lozère, Saint-Maurice-du-Ventalou
Torymus sp. laetus group	3029	TOTT	3029_a	3029_b	3029_c	3029_d	3029_e	3029_f	3029_g	N, QP	96	PJ	CBGP	France, Aveyron, Viala-du-Pas-de-Jaux
Megastigmus sp.	3030	TOM		3030_b	3030_c	3030_d	-	-	3030_g	N, QP	96	PJ	CBGP	France, Alpes-Maritimes, La Bollène-Vésubie
Torymus sp.1	3033	TOTT	3033_a	-	3033_c	3033_d	3033_e	3033_f	-	N, QP	96	PJ	CBGP	France, Alpes-Maritimes, Saint-Martin-Vésubie
Torymus sp.2	3035	TOTT	3035_a	-	3035_c	3035_d	3035_e	3035_f	-	N, QP	96	PJ	CBGP	France, Alpes-Maritimes, Saint-Martin-Vésubie
Torymus sp.3	3036	TOTT	3036_a	-	3036_c	3036_d	3036_e	3036_f	3036_g	N, QP	96	PJ	CBGP	France, Alpes-Maritimes, Saint-Martin-Vésubie
Torymus sp.4	3037	TOTT	3037_a	-	3037_c	3037_d	3037_e	3037_f	-	N, QP	96	PJ	CBGP	France, Alpes-Maritimes, Belvédère
Torymus sp.5	3038	TOTT	3038_a	-	3038_c	3038_d	3038_e	3038_f	-	N, QP	96	PJ	CBGP	France, Alpes-Maritimes, Belvédère
Torymus sp.6	3040	TOTT		-	3040_c	3040_d	3040_e	3040_f	3040_g	N, QP	96	PJ	CBGP	France, Alpes-Maritimes, Belvédère
Torymus sp.7	3042	TOTT	3042_a	3042_b	3042_c	3042_d	3042_e	3042_f	-	N, QP	96	PJ	CBGP	France, Alpes-Maritimes, Belvédère
Torymus sp.8	3043	TOTT	3043_a	-	3043_c	3043_d	3043_e	3043_f	-	D, QP	96	PJ	CBGP	France, Alpes-Maritimes, Valdeblore
Microdontomerus sp.1	3044	TOTM	3044_a	3044_b	3044_c	3044_d	3044_e	3044_f	-	N, QP	96	PJ	CBGP	France, Alpes-Maritimes, Valdeblore
Idiomacromerus sp.1	3045	TOTM	3045_a	-	3045_c	3045_d	3045_e	3045_f	3045_g	N, QP	96	PJ	CBGP	France, Alpes-Maritimes, Valdeblore
Torymus sp.9	3047	TOTT	3047_a	-	3047_c	3047_d	3047_e	3047_f	-	N, QP	96	PJ	CBGP	France, Alpes-Maritimes, Valdeblore
Bootanomyia (*M.*) *dorsalis*	3050	TOM	3050_a	3050_b	3050_c	3050_d	-	-	-	D, QP	96	PJ	CBGP	France, Alpes-Maritimes, Sauze
Torymus sp.10	3051	TOTT	3051_a	3051_b	3051_c	3051_d	3051_e	3051_f	-	N, QP	96	PJ	CBGP	France, Alpes-Maritimes, Valdeblore
Monodontomerus sp.1	3052	TOTN	3052_a	3052_b	3052_c	3052_d	-	3052_f	3052_g	N, QP	96	PJ	CBGP	France, Alpes-Maritimes, Valdeblore
Megastigmus sp.2	3053	TOM	3053_a	-	3053_c	3053_d	3053_e	-	3053_g	N, QP	96	PJ	CBGP	France, Alpes-Maritimes, Valdeblore
Megastigmus bipunctatus	3056	TOM		3056_b	3056_c	3056_d	3056_e	-	3056_g	N, QP	96	PJ	CBGP	France, Alpes-Maritimes, Belvédère
Bootanomyia (*M.*)*stigmatizans*	3057	TOM	3057_a	-	3057_c	-	3057_e	-	3057_g	N, QP	96	PJ	CBGP	France, Corse, Olmi-Cappela
Bootanomyia (*M.*) cf. *dorsalis*	3058	TOM	3058_a	-	3058_c	3058_d	3058_e	-	3058_g	N, QP	96	PJ	CBGP	France, Corse, Olmi-Cappela
Megastigmus pistaciae	3059	TOM		3059_b	3059_c	3059_d	3059_e	-	-	D, QP	96	PJ	CBGP	France, Corse, Ghisonacchia
Megastigmus bipunctatus	3060	TOM	3060_a	-	3060_c	-	3060_e	-	-	N, QP	96	PJ	CBGP	Italy, Veneto, Ferrara di Monte Baldo
Microdontomerus annulatus	3064	TOTM	3064_a	3064_b	3064_c	3064_d	-	3064_f	-	D, QP	96	PJ	CBGP	France, Hérault, Saint-Privat
Torymus fagineus	3066	TOTT	3066_a	-	3066_c	3066_d	3066_e	3066_f	-	N, QP	96	PJ	CBGP	France, Gard, Arphy
Torymus sp.11	3068	TOTT	3068_a	-	3068_c	3068_d	3068_e	3068_f	-	N, QP	96	PJ	CBGP	France, Hérault, Saint-Pierre-de-Lafage
Torymus (*Syntomaspis*) sp.	3069	TOTT	3069_a	-	3069_c	3069_d	-	3069_f	-	D, QP	96	PJ	CBGP	France, Vaucluse, Rustrel
Torymus bedeguaris	3070	TOTT	3070_a	3070_b	3070_c	3070_d	-	3070_f	-	D, QP	96	PJ	CBGP	France, Gard, Saint-Martial
Pseudotorymus sp.2	3073	TOTY	3073_a	-	3073_c	3073_d	3073_e	3073_f	3073_g	N, QP	96	PJ	CBGP	France, Hérault, Cournonterral
Glyphomerus tibialis	3074	TOTI	3074_a	3074_b	3074_c	3074_d	3074_e	-	-	N, QP	96	PJ	CBGP	France, Hérault, Saint-Félix-de-lHéras
Eridontomerus arrabonicus	3076	TOTM	3076_a	3076_b	3076_c	3076_d	-	3076_f	-	D, QP	96	PJ	CBGP	France, Aveyron, Lapanouse-de-Cernon
Idiomacromerus sp.2	3077	TOTM	3077_a	3077_b	3077_c	3077_d	3077_e	-	-	N, QP	96	PJ	CBGP	France, Pyrénées-Orientales, Banyuls-sur-Mer
Torymoides kisenwetteri	3078	TOTY	3078_a	3078_b	3078_c	3078_d	-	3078_f	3078_g	D, QP	96	PJ	CBGP	France, Pyrénées-Orientales, Banyuls-sur-Mer
Torymus sp.12	3082	TOTT	3082_a	-	3082_c	3082_d	3082_e	3082_f	-	D, QP	96	PJ	CBGP	France, Gard, Valleraugue
Torymus sp.13	3085	TOTT	3085_a	-	3085_c	3085_d	3085_e	3085_f	-	N, QP	96	PJ	CBGP	France, Hérault, Rosis
Torymus flavipes	3086	TOTT	3086_a	-	3086_c	3086_d	-	3086_f	-	D, QP	96	PJ	CBGP	France, Hérault, Cambon-et-Salbergues
Glyphomerus stigma	3088	TOTI	JN623518	JN623847	JN623847	3088_d	3088_e	3088_f	-	N, QP	96	PJ	CBGP	France, Gard, Saint-Martial
Torymus rubi	3089	TOTT	3089_a	-	3089_c	3089_d	3089_e	-	-	N, QP	96	PJ	CBGP	Österreich, Kärnten, Stinzesteig
Torymus stenus	3090	TOTT	3090_a	3090_b	3090_c	3090_d	3090_e	-	-	N, QP	96	PJ	CBGP	Italia, Friuli Venezia Giuli, along GR 608
Torymus sp.14	3093	TOTT	3093_a	-	3093_c	3093_d	-	3093_f	-	D, QP	96	PJ	CBGP	Italia, Friuli Venezia Giuli, Altiplano del Montasio
Torymus sp.15	3094	TOTT		-	3094_c	3094_d	-	-	-	N, QP	96	PJ	CBGP	Italia, Friuli Venezia Giuli, Altiplano del Montasio
Torymus sp.16	3095	TOTT	3095_a	-	3095_c	3095_d	-	3095_f	-	D, QP	96	PJ	CBGP	Italia, Friuli Venezia Giuli, forest NW Sella nevea
Torymus ventralis	3097	TOTT	3097_a	-	3097_c	3097_d	-	-	-	N, QP	96	PJ	CBGP	Italia, Friuli Venezia Giuli, NE Monte Privat
Torymus laetus	3098	TOTT	3098_a	-	3098_c	3098_d	3098_e	3098_f	-	N, QP	96	PJ	CBGP	Italia, Friuli Venezia Giuli, NE Monte Privat
Torymus sp.17	3099	TOTT	3099_a	-	3099_c	3099_d	3099_e	-	-	N, QP	96	PJ	CBGP	Italia, Friuli Venezia Giuli, NE Monte Privat
Torymus hylesini	3103	TOTT	3103_a	-	3103_c	3103_d	-	3103_f	3103_g	D, QP	96	PJ	CBGP	Italia, Friuli Venezia Giuli, Gamiscen
Torymus sp.18	3106	TOTT		-	3106_c	3106_d	3106_e	3106_f	-	N, QP	96	PJ	CBGP	Slovenia, Goriška, Krnsko jezero
Torymus sp.19	3110	TOTT	3110_a	-	3110_c	3110_d	3110_e	3110_f	-	D, QP	96	PJ	CBGP	Slovenia, Goriška, Soča River trail
Torymus sp.20	3111	TOTT	3111_a	-	3111_c	3111_d	3111_e	3111_f	-	N, QP	96	PJ	CBGP	Slovenia, Goriška, Zadnjica
Torymus sp.21	3112	TOTT	3112_a	-	3112_c	3112_d	3112_e	-	-	N, QP	96	PJ	CBGP	Slovenia, Goriška, Zadnjica
Torymus sp.22	3113	TOTT		-	3113_c	3113_d	3113_e	3113_f	-	N, QP	96	PJ	CBGP	Österreich, Kärnten, Krishnig
Torymus flavipes	3114	TOTT	3114_a	-	3114_c	3114_d	-	3114_f	-	D, QP	96	PJ	CBGP	France, Valgorge
Glyphomerus stigma	3115	TOTI	3115_a	-	3115_c	3115_d	-	3115_f	3115_g	D, QP	96	PJ	CBGP	France, Gard, Saint-Martial
***Ormyrus* sp.1**	3116	OR		3116_b	3116_c	3116_d	-	3116_f	-	D, QP	96	PJ	CBGP	Österreich, Kärnten, Krishnig
Anneckeida maai	JN623854	TOTN		JN623854	JN623854	-	-	-	-	N, CP	D	PJ	PJ	Thailand, Chrang Mai Prov., Pa Hua Kho
Propalachia borneana	JN623856	TOTP		JN623856	JN623856	-	-	-	-	N, CP	D	PJ	PJ	Taiwan, Nantou, Tungpu
Boucekinus tatianae	JN623860	TOTY	1131_a	JN623860	JN623860	1131_d	1131_e	-	-	N, CP	D	PJ	PJ	Costa Rica, La Selva
Platykula albihirta	JN624252	TOTY	1130_a	JN624252	JN624252	1130_d	1130_e	-	-	N, CP	D	PJ	PJ	USA, Cuckoo
Zaglyptonotus sp.	JN624263	TOTI	JN624263	JN624263	JN624263	1129_d	-	-	-	N, CP	D	USNM	PJ	USA, Fairfax
Eridontomerus biroi	JN624264	TOTM	JN624264	JN624264	JN624264	1132_d	-	-	-	N, CP	D	UCR	PJ	Argentina, Mendoza
Anneckeida sp.1	JN624267	TOTN		JN624267	JN624267	-	-	-	-	N, CP	D	PJ	PJ	Kenya, Gembe Hills
Perissocentrus sp.1	JN624270	TOTN	JN624270	JN624270	JN624270	1134_d	1134_e	-	-	N, CP	D	PJ	PJ	Argentina, Neuquén
Rhynchoticida maai	JN624271	TOTN		JN624271	JN624271	-	-	-	-	N, CP	D	PJ	PJ	Thailand, Khao Chong
Zdenekius smithi	JN624272	TOTN		JN624272	JN624272	-	-	-	-	N, CP	D	PJ	PJ	USA, Mathias
Propachytomoides sp.1	JN624275	TOTO		JN624275	JN624275	-	-	-	-	N, CP	70	PJ	PJ	Australia, Karijini NP
***Ormyrus* sp.2**	J1001	OR		J1001_b	J1001_c	J1001_d	-	J1001_f	-	D, QP	96	PJ	CBGP	Taiwan, Taipei
***Ormyrus* sp.3**	J1002	OR	J1002_a	-	J1002_c	J1002_d	-	J1002_f	-	D, QP	96	PJ	CBGP	Taiwan, Taipei
Physothorax sp.2	J1004	TOTT	J1004_a	-	J1004_c	J1004_d	-	-	-	N, QP	96	PJ	CBGP	Brazil?
Plesiostigmodes sp.	J1007	TOTT		-	J1007_c	-	-	-	-	N, QP	96	PJ	CBGP	Brazil?
Physothorax sp.3	J1008	TOTT		J1008_b	J1008_c	J1008_d	-	J1008_f	-	D, QP	96	PJ	CBGP	Brazil?
Torymus (*Nannocerus*) sp.	J1009	TOTT	J1009_a	-	J1009_c	J1009_d	-	-	-	D, QP	96	PJ	CBGP	Brazil?
***Ormyrus* sp.1**	47O	OR	JN623317	JN623707	JN624065	-	-	-	-	N, CP	96	UCR	UCR	Kyrgyzstan, Ozhalal-Abad
Eridontomerus isosomatis	1228	TOTM		-	-	-	1228_e	-	-	N, QP	96	PJ	PJ	France, Hérault, Pass de L'Escalette
Adontomerus sp.1	1231	TOTM		-	-	-	1231_e	-	-	N, QP	96	PJ	PJ	Turkey, Adana, Hamidiye
Eridontomerus biroi	1234	TOTM		-	-	1234_d	1234_e	-	-	N, QP	96	PJ	PJ	Turkey, Adana, Hamidiye
Eridontomerus laticornis	1237	TOTM		-	-	1237_d	1237_e	-	-	N, QP	96	PJ	PJ	Turkey, Adana, Buyuk Sofulu
Adontomerus sp.2	1243	TOTM	1243_a	1243_b	1243_c	1243_d	1243_e	-	-	N, QP	96	PJ	PJ	Iran, Lorestan, Khorrambad
Eridontomerus isosomatis	1247	TOTM		-	-	-	1247_e	-	-	N, QP	96	PJ	PJ	Turkey, Adana, Hamidiye
Eridontomerus biroi	1249	TOTM		-	-	-	1249_e	-	-	N, QP	96	PJ	PJ	Italy, Toscana env.

Eridontomerus fulviventris	1250	TOTM	1250_a	1250_b	1250_c	1250_d	1250_e	-	-	N, QP	96	PJ	PJ	Italy, Toscana env.
Idiomacromerus sp.3	1253	TOTM	1253_a	1253_b	1253_c	1253_d	1253_e	-	-	N, QP	96	PJ	PJ	UAE, Wadi Shawkah
Microdontomerus sp.2	1255	TOTM	1255_a	1255_b	1255_c	1255_d	1255_e	-	-	N, QP	96	PJ	PJ	UAE, Wadi Bih dam
Idiomacromerus sp.4	1257	TOTM	1257_a	1257_b	1257_c	1257_d	-	-	-	N, QP	96	PJ	PJ	UAE, Bithnah
Eridontomerus biroi	1267	TOTM	1267_a	1267_b	1267_c	1267_d	1267_e	-	-	N, QP	96	PJ	PJ	Canada, Ontario
Glyphomerus aylax	1272	TOTI	1272_a	1272_b	1272_c	1272_d	1272_e	-	-	N, QP	96	PJ	PJ	Bulgaria, Dzhendemtepe, Plovdiv
Exopristoides hypecoi	1273	TOTM	1273_a	1273_b	1273_c	1273_d	1273_e	-	-	N, QP	96	PJ	PJ	Bulgaria, Dzhendemtepe, Plovdiv
Thaumatorymus notanisoides	1274	TOTI	1274_a	1274_b	1274_c	1274_d	1274_e	-	-	N, QP	96	PJ	PJ	Turkey, Adana, Buyuk Sofulu
Adontomerus impolitus	1277	TOTM	1277_a	1277_b	1277_c	1277_d	1277_e	-	-	N, QP	96	PJ	PJ	Spain, Arino
Echthrodape sp.2	12801	TOTI		12801_b	12801_c	-	12801_e	-	-	N, QP	D	PJ	PJ	Madagascar, Fianarantdsoa prov., Vohiparara
Microdontomerus sp.3	1285	TOTM	1285_a	1285_b	1285_c	1285_d	1285_e	-	-	N, QP	96	PJ	PJ	USA, Arizona, Barry Goldwater
Idiomacromerus sp.5	1286	TOTM	1286_a	1286_b	1286_c	1286_d	1286_e	-	-	N, QP	96	PJ	PJ	USA, Arizona, Barry Goldwater
Monodontomerus sp.2	PJ0010	TOTN		PJ0010_b	PJ0010_c	-	-	-	-	N, CP	96	PJ	PJ	USA, Arizona, Sonoita
Eridontomerus biroi	PJ00271	TOTM	PJ00271_a	PJ00271_b	PJ00271_c	1240_d	1240_e	-	-	N, CP	96	PJ	PJ	Argentina, Mendoza, La Consulta
Torymus sp.23	PJ0028	TOTT	PJ0028_a	PJ0028_b	PJ0028_c	1133_d	1133_e	-	-	N, CP	70	PJ	PJ	Australia, Hollern
Podagrionella (*Iridophaga*) sp.1	1001	TOTO	1001_a	1001_b	1001_c	1001_d	1001_e	-	-	D, QP	70	PJ	CBGP	UAE, Fujairah
Mantiphaga sp.1	1003	TOTO	1003_a	-	1003_c	-	1003_e	-	-	D, QP	70	PJ	CBGP	UAE, Fujairah
Ecdamua indica	1004	TOTT	1004_a	-	1004_c	-	-	-	-	D, QP	70	PJ	CBGP	UAE, Fujairah
Microdontomerus sp.4	1005	TOTM	1005_a	1005_b	1005_c	1005_d	1005_e	-	-	D, QP	70	PJ	CBGP	UAE,Wadi Maidaq
Podagrion sp.8	1006	TOTO	1006_a	-	1006_c	1006_d	1006_e	1006_f	-	D, QP	70	PJ	CBGP	UAE,Wadi Maidaq
Idiomacromerus sp.2	1007	TOTM	1007_a	-	1007_c	1007_d	-	-	-	D, QP	70	PJ	CBGP	UAE, S of Ras al-Khaimah
Torymus (*Diomorus*) cf. *armatus*	1008	TOTT	1008_a	1008_b	1008_c	-	-	-	1008_g	D, QP	70	PJ	CBGP	UAE, Sharjah x Khor Kalba
Mantiphaga sp.2	1013	TOTO	1013_a	-	-	1013_d	-	-	-	N, QP	96	PJ	CBGP	Madagascar
Palmon sp.2	1014	TOTO	1014_a	-	1014_c	1014_d	-	-	-	N, QP	96	PJ	CBGP	Madagascar
Megastigmus sp.3	1015	TOM	1015_a	1015_b	1015_c	1015_d	1015_e	-	1015_g	D, QP	70	PJ	CBGP	Australia
Torymus fulvum	1016	TOTT	1016_a	-	1016_c	1016_d	1016_e	-	1016_g	D, QP	70	PJ	CBGP	Canada
Pseudotorymus sp.3	1017	TOTY	1017_a	-	1017_c	1017_d	-	1017_f	-	D, QP	96	PJ	CBGP	RSA
Podagrion sp.9	1018	TOTO	1018_a	1018_b	1018_c	1018_d	1018_e	1018_f	-	D, QP	96	PJ	CBGP	RSA
Torymus subnudus	1019	TOTT	1019_a	1019_b	1019_c	1019_d	1019_e	1019_f	-	D, QP	96	PJ	CBGP	RSA
Podagrionella (*Iridophaga*) sp.2	1020	TOTO		-	-	1020_d	1020_e	-	-	N, QP	96	PJ	CBGP	RSA
Palachia sp.	1021	TOTP	1021_a	1021_b	1021_c	-	-	-	1021_g	D, QP	96	PJ	CBGP	India
Torymus (*Diomorus*) *orientalis*	1022	TOTT	1022_a	-	1022_c	1022_d	1022_e	1022_f	1022_g	D, QP	96	PJ	CBGP	India
Torymoides sp.1	1023	TOTY	1023_a	1023_b	1023_c	1023_d	-	1023_f	1023_g	D, QP	96	PJ	CBGP	RSA
Chileana cyanea	1024	TOTN	1024_a	1024_b	1024_c	1024_d	1024_e	1024_f	1024_g	D, QP	96	PJ	CBGP	Chile
Pseudotorymus sp.4	1027	TOTY	1027_a	-	1027_c	1027_d	-	1027_f	-	N, QP	96	PJ	CBGP	RSA
Podagrion sp.10	1028	TOTO	1028_a	1028_b	1028_c	1028_d	1028_e	1028_f	-	D, QP	96	PJ	CBGP	India
Perissocentrus sp.1	1029	TOTN	1029_a	1029_b	1029_c	1029_d	1029_e	1029_f	1029_g	N, QP	96	PJ	CBGP	Chile
Torymus sp.24	1030	TOTT	1030_a	1030_b	1030_c	1030_d	1030_e	1030_f	-	D, QP	96	PJ	CBGP	RSA
New genus Torymoidini sp.	1032	TOTY	1032_a	-	-	1032_d	-	1032_f	1032_g	N, QP	96	PJ	CBGP	Chile
Idiomacromerus papaveris	1033	TOTM		1033_b	1033_c	1033_d	1033_e	1033_f	1033_g	N, QP	96	PJ	CBGP	Portugal, Junquieira
Exopristus trigonomerus	1034	TOTI	1034_a	1034_b	1034_c	1034_d	1034_e	1034_f	-	D, QP	96	PJ	CBGP	Portugal, Junquieira
Torymoides kiessenwetteri	1035	TOTY	1035_a	-	1035_c	1035_d	-	1035_f	1035_g	D, QP	96	PJ	CBGP	Portugal, Junquieira
Eridontomerus arrabonicus	1036	TOTM	1036_a	1036_b	1036_c	1036_d	1036_e	1036_f	-	D, QP	96	PJ	CBGP	Czech Republic, Raná
Pseuderimerus luteus	10372	TOTM	10372_a	-	10372_c	10372_d	10372_e	-	-	D, QP	96	PJ	CBGP	Croatia, Srebreno
Pseudotorymus sapphyrinus	1038	TOTY	1038_a	1038_b	1038_c	1038_d	1038_e	1038_f	1038_g	D, QP	96	PJ	CBGP	Hungary, Kunbaracs
Idarnotorymus pulcher	1039	TOTM	1039_a	-	1039_c	1039_d	1039_e	1039_f	-	D, QP	96	PJ	CBGP	Croatia, Srebreno
Cryptopristus caliginosus	1040	TOTI	1040_a	1040_b	1040_c	1040_d	1040_e	-	-	D, QP	96	PJ	PJ	Czech Republic, Klentnice
Podagrionella (*Podagrionella*) sp.1	1041	TOTO	1041_a	-	1041_c	1041_d	1041_e	1041_f	-	N, QP	96	PJ	CBGP	Kenya
Anneckeida sp.2	1042	TOTN	1042_a	1042_b	1042_c	1042_d	1042_e	1042_f	-	D, QP	96	PJ	CBGP	Kenya
Torymus sp.25	1043	TOTT	1043_a	-	1043_c	1043_d	1043_e	1043_f	1043_g	N, QP	96	PJ	CBGP	Costa Rica
Podagrion sp.11	1044	TOTO	1044_a	-	1044_c	-	-	1044_f	-	N, QP	96	PJ	CBGP	Bolivia
Torymus sp.26	1045	TOTT	1045_a	-	1045_c	1045_d	1045_e	1045_f	1045_g	N, QP	96	PJ	CBGP	Costa Rica
Torymus sp.27	1046	TOTT	1046_a	-	1046_c	1046_d	1046_e	1046_f	1046_g	N, QP	96	PJ	CBGP	Costa Rica
Propalachia sp.1	1047	TOTP	1047_a	1047_b	1047_c	-	1047_e	1047_f	-	D, QP	96	PJ	CBGP	Kenya
Podagrion sp.12	1048	TOTO	1048_a	-	1048_c	1048_d	1048_e	1048_f	-	D, QP	70	PJ	CBGP	USA, California, 6km W Perris
Ditropinotus aureoviridis	1049	TOTM	1049_a	1049_b	1049_c	1049_d	1049_e	1049_f	-	D, QP	70	PJ	CBGP	USA, California, 9mi WSW Blakwell´s Corner
New genus Monodontomerini sp.	1052	TOTN	1052_a	1052_b	1052_c	1052_d	1052_e	1052_f	1052_g	N, QP	70	PJ	CBGP	Argentina, Rio Negro, El Bolson
Palmon sp.3	1054	TOTO	1054_a	1054_b	1054_c	1054_d	-	1054_f	-	D, QP	70	PJ	CBGP	Ecuador, Sucumbios, Sacha Lodge
Podagrion sp.13	1055	TOTO	1055_a	-	-	1055_d	1055_e	-	-	N, QP	70	PJ	CBGP	Ecuador, Napo, Yasuni Nat. Park
Podagrion sp.14	1057	TOTO	1057_a	-	1057_c	-	1057_e	1057_f	-	D, QP	70	PJ	CBGP	French Guiana, PK35
Torymus sp.28	1058	TOTT	1058_a	-	1058_c	1058_d	1058_e	1058_f	1058_g	N, QP	70	PJ	CBGP	French Guiana, Patawa Kaw Mountains
Megastigmus sp.4	1059	TOM	1059_a	1059_b	1059_c	1059_d	-	-	1059_g	D, QP	70	PJ	CBGP	Costa Rica, Heredia, Zarquí de Moravia
Torymoides sp.2	1061	TOTY	1061_a	-	1061_c	1061_d	-	1061_f	1061_g	D, QP	70	PJ	CBGP	Australia, QLD, Wooroonoonan NP
Megastigminae gen. sp.	1062	TOM	1062_a	1062_b	1062_c	1062_d	1062_e	-	1062_g	D, QP	70	PJ	CBGP	Australia, QLD, Wooroonoonan NP
Podagrion sp.15	1065	TOTO	1065_a	-	1065_c	1065_d	1065_e	1065_f	-	D, QP	70	PJ	CBGP	USA, Arizona, Huachucha Mts.
Platykula sp.1	1068	TOTY		-	1068_c	-	-	-	-	D, QP	70	PJ	CBGP	USA, Illinois, Dixon Springs
Podagrion sp.16	1069	TOTO	1069_a	1069_b	1069_c	1069_d	-	1069_f	-	D, QP	70	PJ	CBGP	Argentina, Corrientes, Rt.12&Rio Sta. Lucia
Platykula sp.2	1072	TOTY	1072_a	-	1072_c	1072_d	-	1072_f	1072_g	D, QP	70	PJ	CBGP	Chile, Maipo, El Yeso
Pseudotorymus sp.5	1073	TOTY	1073_a	1073_b	1073_c	1073_d	1073_e	1073_f	1073_g	D, QP	70	PJ	CBGP	Canada, Ontario, vic. of Emo Hwy 11
Torymus (*Diomorus*) *armatus*	1074	TOTT	1074_a	1074_b	1074_c	1074_d	-	1074_f	1074_g	D, QP	70	PJ	CBGP	Canada, British Columbia, Vancouver Pacific Spirit Pr. Pk.
Glyphomerus cf. *stigma*	1076	TOTI	1076_a	-	1076_c	1076_d	1076_e	1076_f	1076_g	D, QP	70	PJ	CBGP	Canada, Ontario, 5km NE Almonte
Allotorymus splendens	1077	TOTT	1077_a	-	1077_c	1077_d	1077_e	1077_f	1077_g	D, QP	70	PJ	CBGP	Canada, Manitoba, Tallgrass Prairie Preserve
Podagrion sp.17	1078	TOTO	1078_a	-	1078_c	1078_d	-	-	-	D, QP	70	PJ	CBGP	Australia, WA, Pebble Mouse Creek
Podagrion sp.18	1079	TOTO	1079_a	1079_b	1079_c	1079_d	1079_e	1079_f	-	D, QP	70	PJ	CBGP	Australia, WA, 158km S Newman
Malostigmus sp.1	1080	TOM	1080_a	1080_b	1080_c	1080_d	1080_e	1080_f	-	D, QP	70	PJ	CBGP	Australia, WA, Pebble Mouse Creek
Propachytomoides sp.2	1081	TOTO		1081_b	1081_c	1081_d	-	-	-	D, QP	70	PJ	CBGP	Australia, WA, Mt. Robinson
Podagrion sp.3	1082	TOTO	1082_a	1082_b	1082_c	1082_d	-	1082_f	1082_g	D, QP	70	PJ	CBGP	Australia, WA, 45km S Newman
Torymoides sp.3	1083	TOTY	1083_a	-	1083_c	1083_d	1083_e	1083_f	1083_g	D, QP	70	PJ	CBGP	Australia, WA, 82km E jct Karijini Dr.
Malostigmus sp.2	1084	TOM	1084_a	1084_b	1084_c	1084_d	1084_e	-	-	D, QP	70	PJ	CBGP	Australia, WA, Mt. Augustus Nat. Park
Propalachia sp.2	1085	TOTP		-	1085_c	-	1085_e	1085_f	-	N, QP	70	PJ	CBGP	Laos, Houa Phan prov., Phou Pane Mt.
Physothorax sp.4	1086	TOTT	1086_a	-	1086_c	1086_d	1086_e	-	-	N, QP	70	PJ	CBGP	Venezuela, Aragua, Henri Pittier Nat. Park
Monodontomerus sp.3	1087	TOTN	1087_a	1087_b	1087_c	1087_d	1087_e	1087_f	-	D, QP	70	PJ	CBGP	South Korea, Jirisan Hamyang-gun
Podagrion sp.19	1089	TOTO	1089_a	-	1089_c	1089_d	1089_e	1089_f	-	D, QP	70	PJ	CBGP	Malaysia
Palmon sp.4	1090	TOTO	1090_a	1090_b	1090_c	1090_d	1090_e	1090_f	-	D, QP	70	PJ	CBGP	Taiwan, Pintung Kenting Nat. Park
Neomegastigmus sp.	1092	TOM	1092_a	1092_b	1092_c	1092_d	1092_e	-	1092_g	D, QP	70	PJ	CBGP	Australia, NT, 53km SSW Darwin
Podagrion sp.20	1095	TOTO		-	1095_c	1095_d	1095_e	1095_f	-	N, QP	70	PJ	CBGP	Papua New Guinea, East New Britain, Bainings Mts.
Cryptopristus caliginosus	1096	TOTI	1096_a	1096_b	1096_c	1096_d	1096_e	1096_f	-	D, QP	70	PJ	CBGP	Canada, QC, Belle-Anse
Podagrionella (*Podagrionella*) sp.2	1097	TOTO	1097_a	-	1097_c	1097_d	1097_e	-	-	D, QP	70	PJ	CBGP	Mozambique, Niassa Cuamba, Mituque
Propalachia sp.3	1098	TOTP		1098_b	-	-	1098_e	1098_f	-	D, QP	70	PJ	CBGP	Mozambique, Niassa Cuamba, Mituque
Pogadrionella (*Iridophagoides*) sp.	1099	TOTO	1099_a	1099_b	1099_c	-	1099_e	1099_f	1099_g	D, QP	70	PJ	CBGP	Papua New Guinea, Morobe Prov., Wau
Podagrionella (*Podagrionella*) sp.3	1101	TOTO	1101_a	1101_b	1101_c	1101_d	1101_e	1101_f	-	D, QP	70	PJ	CBGP	Papua New Guinea, East New Britain, Bainings Mts.
Palmon sp.5	1102	TOTO	1102_a	-	1102_c	1102_d	1102_e	1102_f	-	D, QP	70	PJ	CBGP	South Africa, Northwest, Sun City
Bootanomyia sp.	1103	TOM	1103_a	1103_b	1103_c	1103_d	1103_e	1103_f	1103_g	D, QP	70	PJ	CBGP	Australia, ACT Canberra, Black Mts.
Eridontomerus biroi	1108	TOTM		-	1108_c	1108_d	1108_e	-	-	N, QP	70	PJ	CBGP	Canada, Alberta, Magreth Melyture Ranch
Bortesia sp.	1109	TOM	1109_a	1109_b	1109_c	1109_d	1109_e	-	-	D, QP	70	PJ	CBGP	Australia, QLD, Brisbane Forest Park
Torymoides sp.4	1110	TOTY	1110_a	-	1110_c	1110_d	-	-	-	D, QP	70	PJ	CBGP	Australia, QLD, Mount Glorious
Podagrionella (*Iridophaga*) sp.3	1112	TOTO		-	1112_c	1112_d	1112_e	1112_f	-	N, QP	70	PJ	CBGP	Australia, QLD, Mareeba
Physothorax sp.5	1114	TOTT	1114_a	1114_b	1114_c	1114_d	1114_e	-	1114_g	N, QP	70	PJ	CBGP	Venezuela, Aragua, Henri Pittier Nat. Park
Monodontomerus obscurus	1136	TOTN	1136_a	1136_b	1136_c	1136_d	-	1136_f	-	D, QP	96	PJ	CBGP	Czech Republic, Brno, Hády
Idiomacromerus sp.8	1137	TOTM		-	1137_c	1137_d	-	1137_f	1137_g	N, QP	96	PJ	CBGP	USA, Arizona
Torymus sp.29	1138	TOTT	1138_a	1138_b	1138_c	1138_d	1138_e	1138_f	-	D, QP	96	PJ	CBGP	USA, Kansas
Torymus sp.30	1139	TOTT		-	1139_c	1139_d	1139_e	1139_f	-	N, QP	96	PJ	CBGP	USA, Arizona
Torymus sp.31	1140	TOTT		-	1140_c	1140_d	1140_e	1140_f	-	N, QP	96	PJ	CBGP	USA, Arizona
Torymus sp.32	1142	TOTT		-	1142_c	1142_d	1142_e	1142_f	-	N, QP	96	PJ	CBGP	USA, Arizona
Megastigmus sp.6	1144	TOM	1144_a	1144_b	1144_c	1144_d	1144_e	-	1144_g	D, QP	96	PJ	CBGP	RSA
Bootanomyia (*Megastigmus*) *dorsalis*	1145	TOM	1145_a	1145_b	1145_c	-	1145_e	-	-	D, QP	96	PJ	CBGP	Jordan
Pseudotorymus sp.6	1147	TOTY	1147_a	-	1147_c	1147_d	-	1147_f	-	N, QP	96	PJ	CBGP	RSA
Idiomacromerus sp.9	1150	TOTM	1150_a	1150_b	1150_c	1150_d	1150_e	1150_f	1150_g	D, QP	96	PJ	CBGP	Portugal
Torymus sp.33	1151	TOTT	1151_a	-	1151_c	1151_d	-	1151_f	-	N, QP	70	PJ	CBGP	South Korea
Torymus sp.34	1152	TOTT	1152_a	1152_b	1152_c	1152_d	-	1152_f	-	N, QP	70	PJ	CBGP	South Korea
Physothorax sp.6	1153	TOTT	1153_a	1153_b	1153_c	1153_d	1153_e	-	1153_g	N, QP	70	PJ	CBGP	Venezuela
Pseudotorymus sp.7	1154	TOTY	1154_a	-	1154_c	1154_d	1154_e	1154_f	1154_g	N, QP	96	PJ	CBGP	Portugal
Perissocentrus sp.3	1156	TOTN	1156_a	1156_b	1156_c	1156_d	1156_e	1156_f	1156_g	D, QP	70	PJ	CBGP	Chile
Monodontomerus sp.4	1157	TOTN	1157_a	-	1157_c	1157_d	1157_e	1157_f	-	N, QP	96	PJ	CBGP	Tunisia
Torymus sp.35	1158	TOTT	1158_a	-	1158_c	1158_d	1158_e	1158_f	-	N, QP	96	PJ	CBGP	Tunisia
Chalcimerus borceai	1159	TOTC	1159_a	1159_b	1159_c	1159_d	1159_e	-	-	N, QP	96	PJ	PJ	Turkey, Adana, Kamisli
Pseuderimerus burgeri	UCRC299	TOTM	JN623521	JN623852	JN623852	-	-	-	-	N, CP	96	UCR	UCR	USA, California, Riverside Co.
Echthrodape sp.1	UCRC475	TOTI	JN623516	JN623845	JN623845	-	-	-	-	N, CP	D	UCR	UCR	Australia: QLD
Erimerus wickhami	UCRC629	TOTM	D2481_a	JN623850	JN623850	1278_d	-	-	-	N, CP	D	UCR	UCR	USA, California, Wileys Well Road
Palachia hayati	UCRC749	TOTP	JN623525	JN624276	JN624276	-	-	-	-	N, CP	D	PJ	UCR	India, Uttar Pradesh, New Delhi

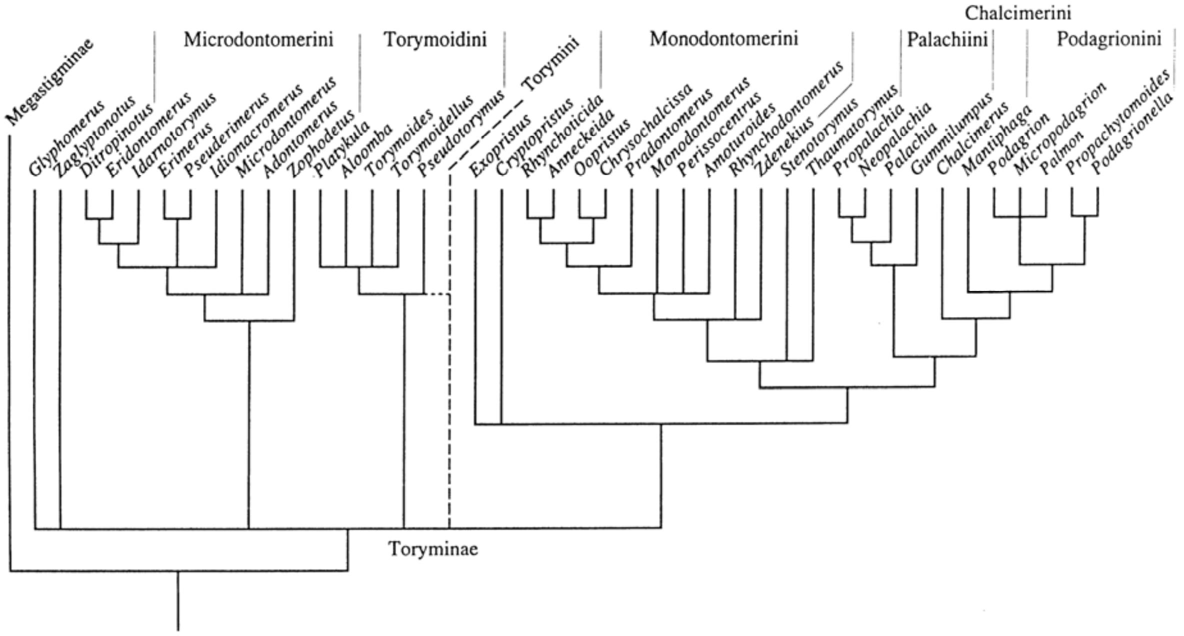
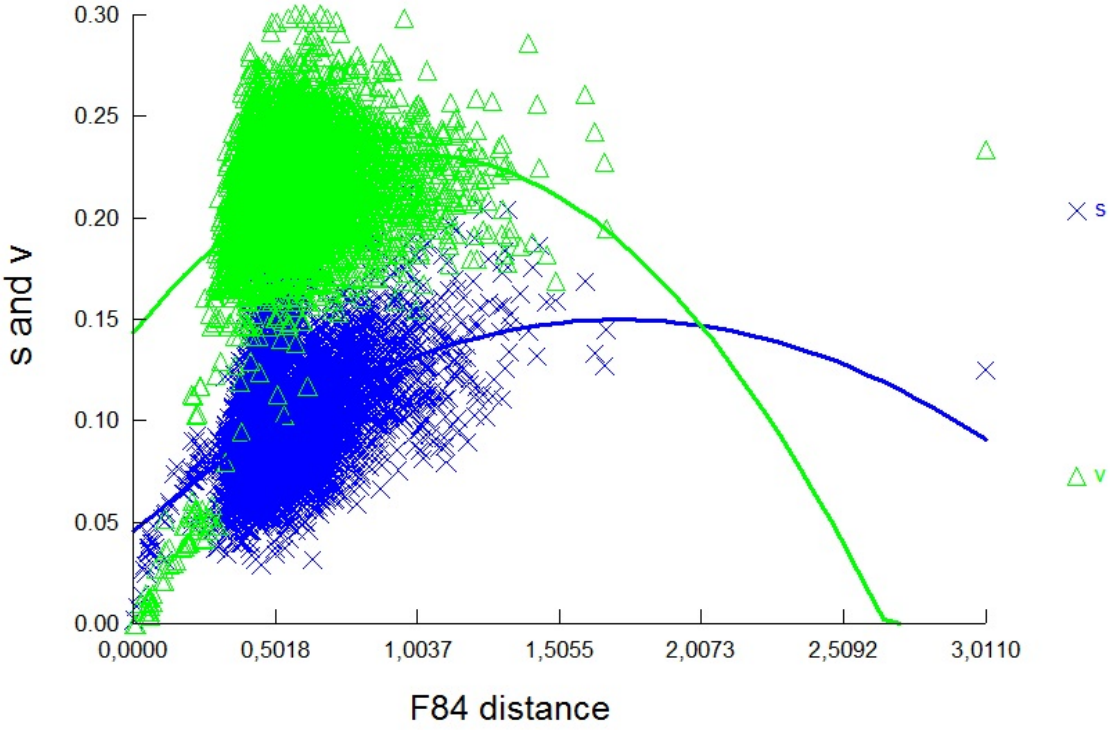
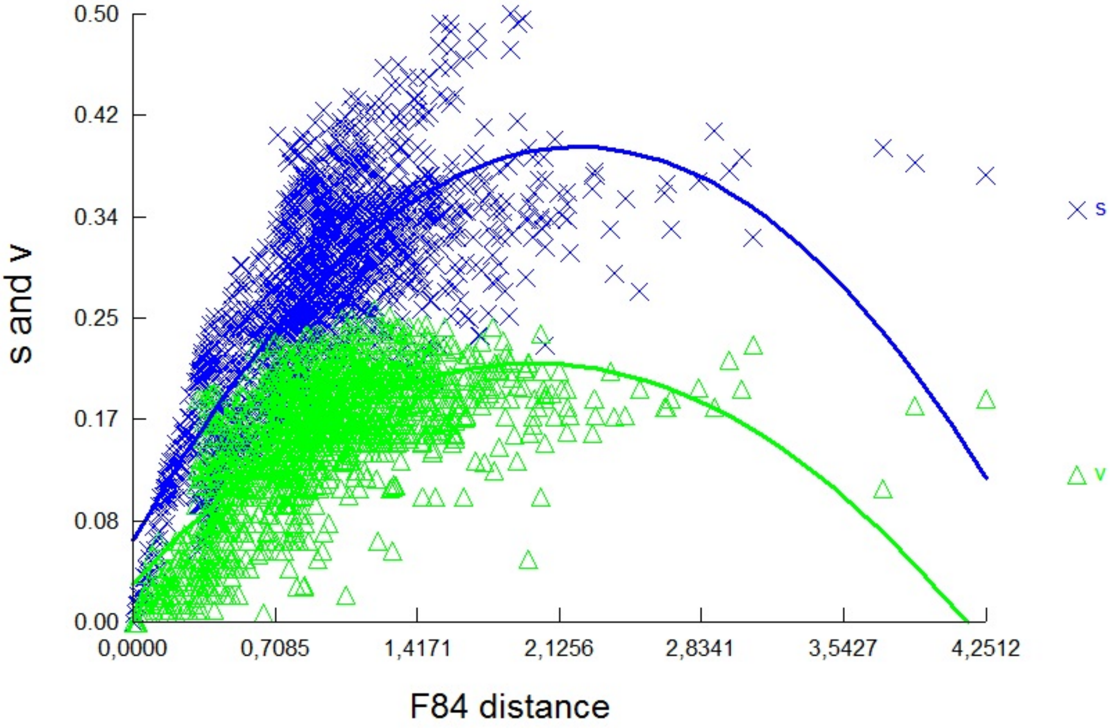


Fig. 1. Phylogeny of Toryminae based on morphological characters (modified from Grissell 1995).



a



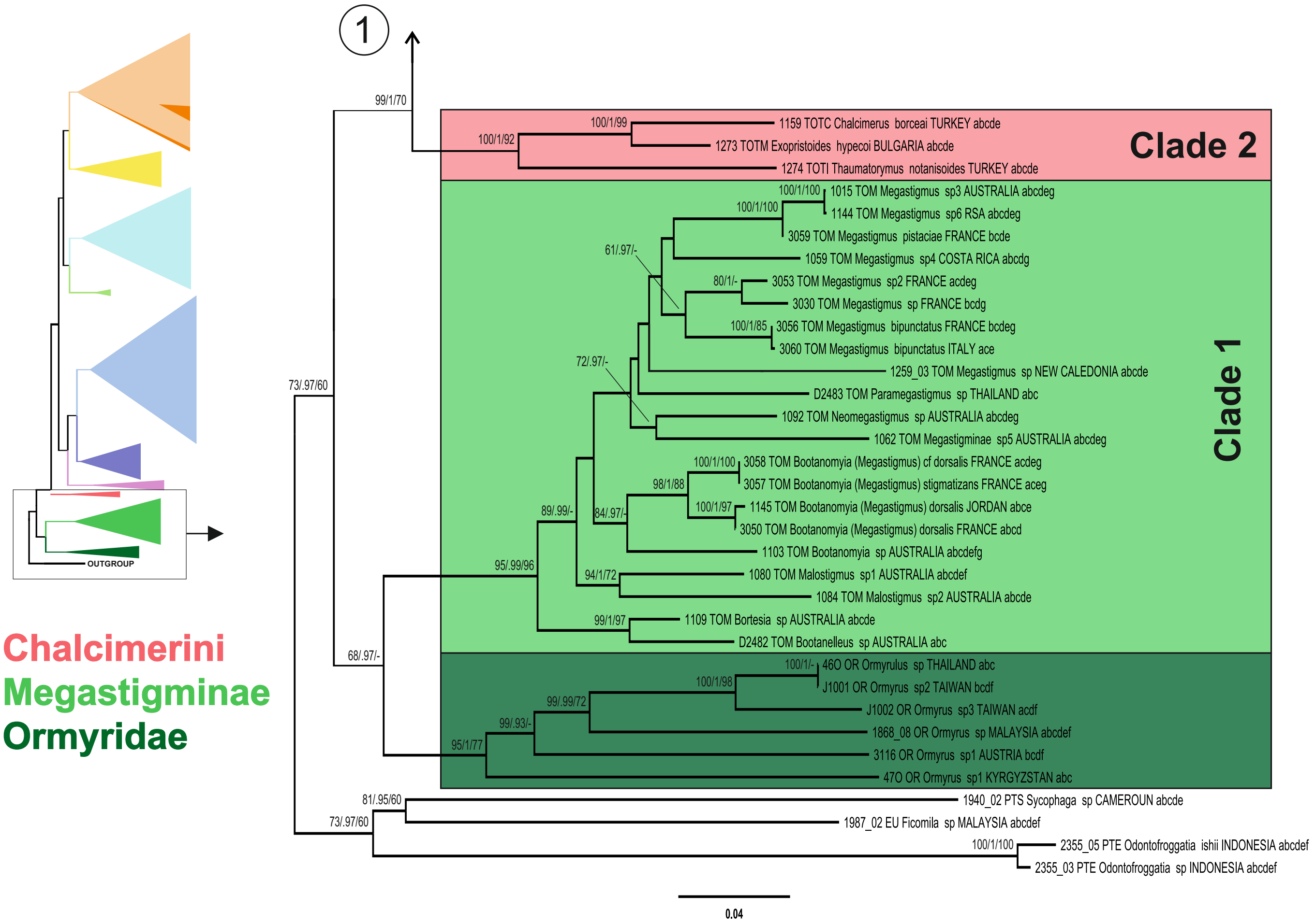
b

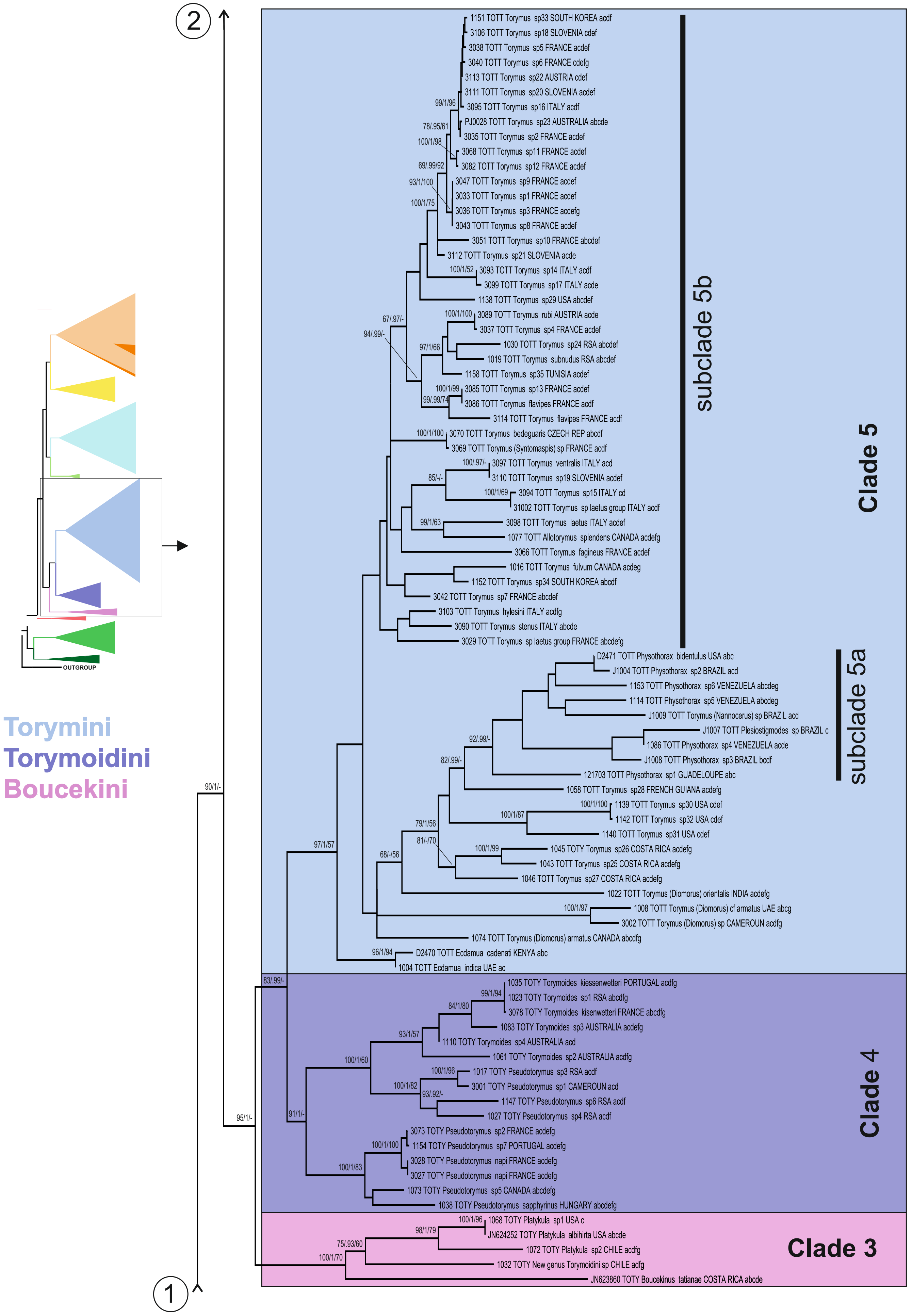
Fig. 2. Saturation plots for transitions (s) and transversions (v) of third codon positions for COI (a) and Wg (b). Uncorrected p-distances on y-axis versus F84 distances on x-axis.

Fig. 3a. Phylogram of Torymidae relationships (outgroups, Ormyridae, Megastigminae and Toryminae: Chalcimerini) based on RAxML with 1000 bootstrap (BP) replications. Support values above branches indicate in order: BP maximum likelihood ≥ 50, posterior probablities (PP) ≥0.90, BP maximum parsimony ≥ 50; stars indicate different topologies for the given analysis. Letters behind name of each taxa specify gene regions sequenced (i.e. 18S, 28SD2, 28SD3-5, EF1a, COI barcode, COI 2183-3014, Wg, respectively).

Fig. 3b. Phylogram of Torymidae relationships (Toryminae: Boucekini, Torymoidini and Torymini) based on RAxML with 1000 boostrap (BP) replications. Support values above branches indicate in order: BP maximum likelihood ≥ 50, posterior probablities (PP) ≥0.90, BP maximum parsimony ≥ 50; stars indicate different topologies for the given analysis. Letters behind name of each taxa specify gene regions sequenced (i.e. 18S, 28SD2, 28SD3-5, EF1a, COI barcode, COI 2183-3014, Wg, respectively).

Fig. 3c. Phylogram of Torymidae relationships (Toryminae: Glyphomerini, Microdontomerini, Monodontomerini and Palachiini + Podagrionini) based on RAxML with 1000 bootstrap (BP) replications. Support values above branches indicate in order: BP maximum likelihood ≥ 50, posterior probablities (PP) ≥0.90, BP maximum parsimony ≥ 50; stars indicate different topologies for the given analysis. Letters behind name of each taxa specify gene regions sequenced (i.e. 18S, 28SD2, 28SD3-5, EF1a, COI barcode, COI 2183-3014, Wg, respectively).


OUTGROUP
Chalcimerini
Megastigminae
Ormyridae
①
99/1/70
73/.97/60
68/.97/-
Clade 2
100/1/99
100/1/92
1159 TOTC Chalcimerus borceai TURKEY abcde
1273 TOTM Exopristoides hypecoi BULGARIA abcde
1274 TOTI Thaumatorymus notanisoides TURKEY abcde
Clade 1
100/1/100
100/1/100
61/.97/-
80/1/-
100/1/85
72/.97/-
1015 TOM Megastigmus sp3 AUSTRALIA abcdeg
1144 TOM Megastigmus sp6 RSA abcdeg
3059 TOM Megastigmus pistaciae FRANCE bcde
1059 TOM Megastigmus sp4 COSTA RICA abcdg
3053 TOM Megastigmus sp2 FRANCE acdeg
3030 TOM Megastigmus sp FRANCE bcdg
3056 TOM Megastigmus bipunctatus FRANCE bcdeg
3060 TOM Megastigmus bipunctatus ITALY ace
1259_03 TOM Megastigmus sp NEW CALEDONIA abcde
D2483 TOM Paramegastigmus sp THAILAND abc
1092 TOM Neomegastigmus sp AUSTRALIA abcdeg
1062 TOM Megastigminae sp5 AUSTRALIA abcdeg
100/1/100
98/1/88
100/1/97
89/.99/-
84/.97/-
95/.99/96
94/1/72
99/1/97
3058 TOM Bootanomyia (Megastigmus) cf dorsalis FRANCE acdeg
3057 TOM Bootanomyia (Megastigmus) stigmatizans FRANCE aceg
1145 TOM Bootanomyia (Megastigmus) dorsalis JORDAN abce
3050 TOM Bootanomyia (Megastigmus) dorsalis FRANCE abcd
1103 TOM Bootanomyia sp AUSTRALIA abcdefg
1080 TOM Malostigmus sp1 AUSTRALIA abcdef
1084 TOM Malostigmus sp2 AUSTRALIA abcde
1109 TOM Bortesia sp AUSTRALIA abcde
D2482 TOM Bootanelleus sp AUSTRALIA abc
100/1/-
100/1/98
99/.99/72
99/.93/-
95/1/77
46O OR Ormyrulus sp THAILAND abc
J1001 OR Ormyrus sp2 TAIWAN bcdf
J1002 OR Ormyrus sp3 TAIWAN acdf
1868_08 OR Ormyrus sp MALAYSIA abcdef
3116 OR Ormyrus sp1 AUSTRIA bcdf
47O OR Ormyrus sp1 KYRGYZSTAN abc
81/.95/60
73/.97/60
100/1/100
1940_02 PTS Sycophaga sp CAMEROUN abcde
1987_02 EU Ficomila sp MALAYSIA abcdef
2355_05 PTE Odontofroggatia ishii INDONESIA abcdef
2355_03 PTE Odontofroggatia sp INDONESIA abcdef
0.04


2
1
OUTGROUP
Torymini
Torymoidini
Boucekini
Clade 5
subclade 5b
subclade 5a
Clade 4
Clade 3
90/1/-
95/1/-
83/.99/-
97/1/57
91/1/-
1151 TOTT Torymus sp33 SOUTH KOREA acdf
3106 TOTT Torymus sp18 SLOVENIA cdef
3038 TOTT Torymus sp5 FRANCE acdef
3040 TOTT Torymus sp6 FRANCE cdefg
3113 TOTT Torymus sp22 AUSTRIA cdef
3111 TOTT Torymus sp20 SLOVENIA acdef
99/1/96
3095 TOTT Torymus sp16 ITALY acdf
78/.95/61
PJ0028 TOTT Torymus sp23 AUSTRALIA abcde
100/1/98
3035 TOTT Torymus sp2 FRANCE acdef
3068 TOTT Torymus sp11 FRANCE acdef
69/.99/92
3082 TOTT Torymus sp12 FRANCE acdef
93/1/100
3047 TOTT Torymus sp9 FRANCE acdef
3033 TOTT Torymus sp1 FRANCE acdef
100/1/75
3036 TOTT Torymus sp3 FRANCE acdefg
3043 TOTT Torymus sp8 FRANCE acdef
3051 TOTT Torymus sp10 FRANCE abcdef
3112 TOTT Torymus sp21 SLOVENIA acde
100/1/52
3093 TOTT Torymus sp14 ITALY acdf
3099 TOTT Torymus sp17 ITALY acde
1138 TOTT Torymus sp29 USA abcdef
100/1/100
3089 TOTT Torymus rubi AUSTRIA acde
67/.97/-
3037 TOTT Torymus sp4 FRANCE acdef
94/.99/-
97/1/66
1030 TOTT Torymus sp24 RSA abcdef
1019 TOTT Torymus subnudus RSA abcdef
1158 TOTT Torymus sp35 TUNISIA acdef
100/1/99
3085 TOTT Torymus sp13 FRANCE acdef
99/.99/74
3086 TOTT Torymus flavipes FRANCE acdf
3114 TOTT Torymus flavipes FRANCE acdf
100/1/100
3070 TOTT Torymus bedeguaris CZECH REP abcdf
3069 TOTT Torymus (Syntomaspis) sp FRANCE acdf
100/.97/-
3097 TOTT Torymus ventralis ITALY acd
85/-/-
3110 TOTT Torymus sp19 SLOVENIA acdef
100/1/69
3094 TOTT Torymus sp15 ITALY cd
31002 TOTT Torymus sp laetus group ITALY acdf
99/1/63
3098 TOTT Torymus laetus ITALY acdef
1077 TOTT Allotorymus splendens CANADA acdefg
3066 TOTT Torymus fagineus FRANCE acdef
1016 TOTT Torymus fulvum CANADA acdeg
1152 TOTT Torymus sp34 SOUTH KOREA abcdf
3042 TOTT Torymus sp7 FRANCE abcdef
3103 TOTT Torymus hylesini ITALY acdfg
3090 TOTT Torymus stenus ITALY abcde
3029 TOTT Torymus sp laetus group FRANCE abcdefg
D2471 TOTT Physothorax bidentulus USA abc
J1004 TOTT Physothorax sp2 BRAZIL acd
1153 TOTT Physothorax sp6 VENEZUELA abcdeg
1114 TOTT Physothorax sp5 VENEZUELA abcdeg
J1009 TOTT Torymus (Nannocerus) sp BRAZIL acd
J1007 TOTT Plesiostigmodes sp BRAZIL c
92/.99/-
1086 TOTT Physothorax sp4 VENEZUELA acde
82/.99/-
J1008 TOTT Physothorax sp3 BRAZIL bcdf
121703 TOTT Physothorax sp1 GUADELOUPE abc
1058 TOTT Torymus sp28 FRENCH GUIANA acdefg
100/1/100
1139 TOTT Torymus sp30 USA cdef
100/1/87
1142 TOTT Torymus sp32 USA cdef
79/1/56
1140 TOTT Torymus sp31 USA cdef
81/-/70
100/1/99
1045 TOTY Torymus sp26 COSTA RICA acdefg
68/-/56
1043 TOTT Torymus sp25 COSTA RICA acdefg
1046 TOTT Torymus sp27 COSTA RICA acdefg
1022 TOTT Torymus (Diomorus) orientalis INDIA acdefg
100/1/97
1008 TOTT Torymus (Diomorus) cf armatus UAE abcg
3002 TOTT Torymus (Diomorus) sp CAMEROUN acdfg
1074 TOTT Torymus (Diomorus) armatus CANADA abcdfg
96/1/94
D2470 TOTT Ecdamua cadenati KENYA abc
1004 TOTT Ecdamua indica UAE ac
1035 TOTY Torymoides kiessenwetteri PORTUGAL acdfg
99/1/94
1023 TOTY Torymoides sp1 RSA abcdfg
84/1/80
3078 TOTY Torymoides kisenwetteri FRANCE abcdfg
1083 TOTY Torymoides sp3 AUSTRALIA acdefg
93/1/57
1110 TOTY Torymoides sp4 AUSTRALIA acd
100/1/60
1061 TOTY Torymoides sp2 AUSTRALIA acdfg
100/1/96
1017 TOTY Pseudotorymus sp3 RSA acdf
100/1/82
3001 TOTY Pseudotorymus sp1 CAMEROUN acd
93/.92/-
1147 TOTY Pseudotorymus sp6 RSA acdf
1027 TOTY Pseudotorymus sp4 RSA acdf
3073 TOTY Pseudotorymus sp2 FRANCE acdefg
100/1/100
1154 TOTY Pseudotorymus sp7 PORTUGAL acdefg
3028 TOTY Pseudotorymus napi FRANCE acdefg
100/1/83
3027 TOTY Pseudotorymus napi FRANCE acdefg
1073 TOTY Pseudotorymus sp5 CANADA abcdefg
1038 TOTY Pseudotorymus sapphyrinus HUNGARY abcdefg
100/1/96
1068 TOTY Platykula sp1 USA c
98/1/79
JN624252 TOTY Platykula albihirta USA abcde
75/.93/60
1072 TOTY Platykula sp2 CHILE acdfg
100/1/70
1032 TOTY New genus Torymoidini sp CHILE adfg
JN623860 TOTY Boucekinus tatianae COSTA RICA abcde

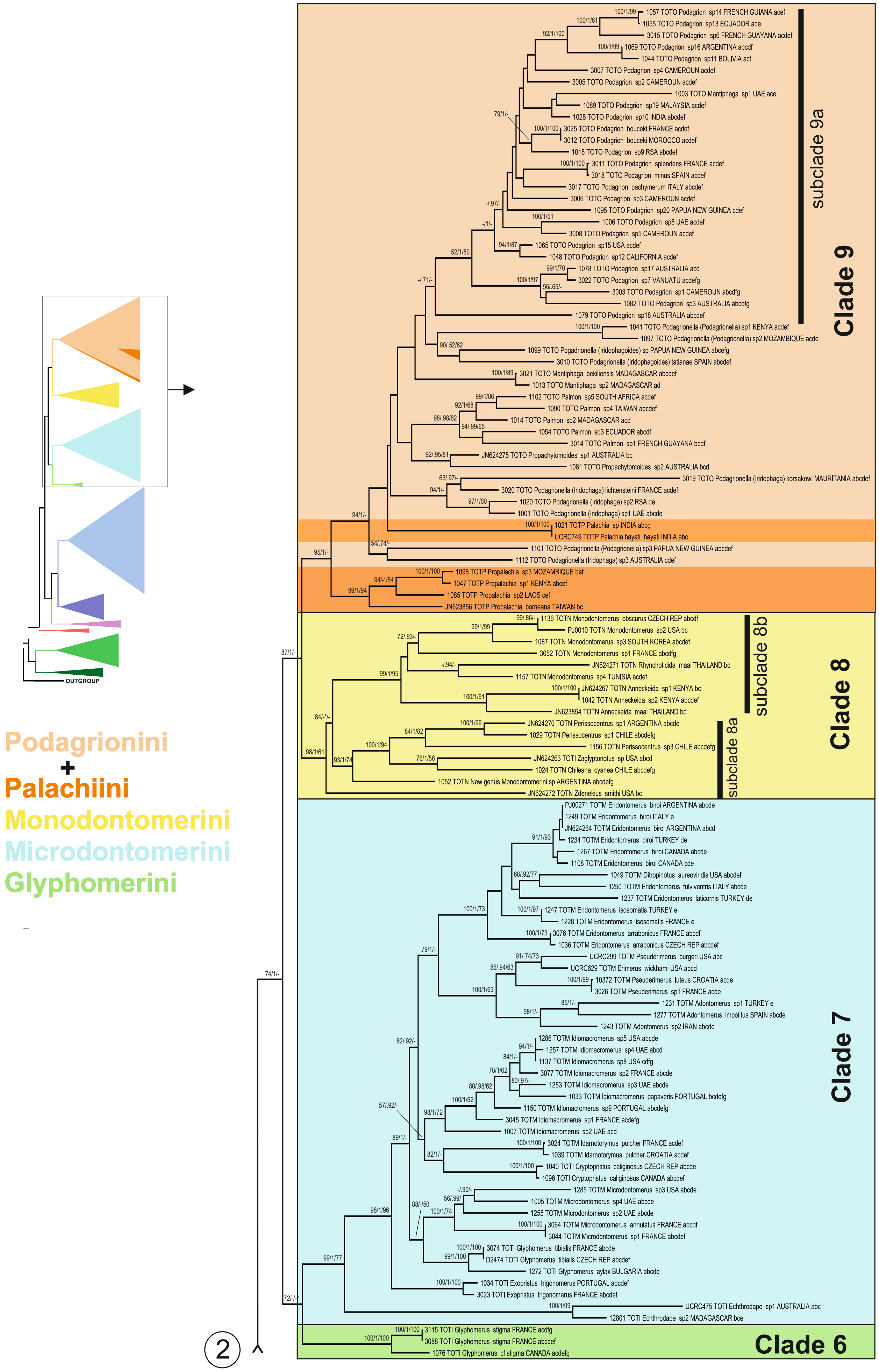

OUTGROUP
Podagrionini
+
Palachiini
Monodontomerini
Microdontomerini
Glyphomerini
100/1/99
1057 TOTO Podagrion sp14 FRENCH GUIANA acef
100/1/61
1055 TOTO Podagrion sp13 ECUADOR ade
92/1/100
3015 TOTO Podagrion sp6 FRENCH GUAYANA acdef
100/1/99
1069 TOTO Podagrion sp16 ARGENTINA abcdf
1044 TOTO Podagrion sp11 BOLIVIA acf
3007 TOTO Podagrion sp4 CAMEROUN acdef
3005 TOTO Podagrion sp2 CAMEROUN acdef
1003 TOTO Mantiphaga sp1 UAE ace
1089 TOTO Podagrion sp19 MALAYSIA acdef
79/1/-
1028 TOTO Podagrion sp10 INDIA abcdef
100/1/100
3025 TOTO Podagrion bouceki FRANCE acdef
3012 TOTO Podagrion bouceki MOROCCO acdef
1018 TOTO Podagrion sp9 RSA abcdef
100/1/100
3011 TOTO Podagrion splendens FRANCE acdef
3018 TOTO Podagrion minus SPAIN acdef
3017 TOTO Podagrion pachymerum ITALY abcdef
3006 TOTO Podagrion sp3 CAMEROUN acdef
-/.97/-
1095 TOTO Podagrion sp20 PAPUA NEW GUINEA cdef
-/1/-
100/1/51
1006 TOTO Podagrion sp8 UAE acdef
3008 TOTO Podagrion sp5 CAMEROUN acdef
94/1/87
1065 TOTO Podagrion sp15 USA acdef
52/1/50
1048 TOTO Podagrion sp12 CALIFORNIA acdef
99/1/70
1078 TOTO Podagrion sp17 AUSTRALIA acd
-/.71/-
100/1/97
3022 TOTO Podagrion sp7 VANUATU acdefg
56/.65/-
3003 TOTO Podagrion sp1 CAMEROUN abcdfg
1082 TOTO Podagrion sp3 AUSTRALIA abcdfg
1079 TOTO Podagrion sp18 AUSTRALIA abcdef
subclade 9a
100/1/100
1041 TOTO Podagrionella (Podagrionella) sp1 KENYA acdef
1097 TOTO Podagrionella (Podagrionella) sp2 MOZAMBIQUE acde
90/.52/62
1099 TOTO Pogadrionella (Iridophagoides) sp PAPUA NEW GUINEA abcefg
3010 TOTO Podagrionella (Iridophagoides) tatianae SPAIN abcdef
100/1/89
3021 TOTO Mantiphaga bekiliensis MADAGASCAR abcdef
1013 TOTO Mantiphaga sp2 MADAGASCAR ad
99/1/86
1102 TOTO Palmon sp5 SOUTH AFRICA acdef
92/1/68
1090 TOTO Palmon sp4 TAIWAN abcdef
98/.98/82
1014 TOTO Palmon sp2 MADAGASCAR acd
94/.99/65
1054 TOTO Palmon sp3 ECUADOR abcdf
3014 TOTO Palmon sp1 FRENCH GUAYANA bcdf
92/.95/81
JN624275 TOTO Propachytomoides sp1 AUSTRALIA bc
1081 TOTO Propachytomoides sp2 AUSTRALIA bcd
63/.97/-
3019 TOTO Podagrionella (Iridophaga) korsakowi MAURITANIA abcdef
94/1/-
3020 TOTO Podagrionella (Iridophaga) lichtensteini FRANCE acdef
97/1/60
1020 TOTO Podagrionella (Iridophaga) sp2 RSA de
94/1/-
1001 TOTO Podagrionella (Iridophaga) sp1 UAE abcde
100/1/100
1021 TOTP Palachia sp INDIA abcg
UCRC749 TOTP Palachia hayati hayati INDIA abc
54/.74/-
1101 TOTO Podagrionella (Podagrionella) sp3 PAPUA NEW GUINEA abcdef
95/1/-
1112 TOTO Podagrionella (Iridophaga) sp3 AUSTRALIA cdef
100/1/100
1098 TOTP Propalachia sp3 MOZAMBIQUE bef
94/-*/54
1047 TOTP Propalachia sp1 KENYA abcef
99/1/94
1085 TOTP Propalachia sp2 LAOS cef
JN623856 TOTP Propalachia borneana TAIWAN bc
Clade 9
99/.86/-
1136 TOTN Monodontomerus obscurus CZECH REP abcdf
99/1/99
PJ0010 TOTN Monodontomerus sp2 USA bc
87/1/-
72/.93/-
1087 TOTN Monodontomerus sp3 SOUTH KOREA abcdef
3052 TOTN Monodontomerus sp1 FRANCE abcdfg
-/.94/-
JN624271 TOTN Rhynchoticida maai THAILAND bc
99/1/95
1157 TOTN Monodontomerus sp4 TUNISIA acdef
100/1/100
JN624267 TOTN Anneckeida sp1 KENYA bc
100/1/91
1042 TOTN Anneckeida sp2 KENYA abcdef
JN623854 TOTN Anneckeida maai THAILAND bc
subclade 8b
84/-*/-
100/1/99
JN624270 TOTN Perissocentrus sp1 ARGENTINA abcde
84/1/82
1029 TOTN Perissocentrus sp1 CHILE abcdefg
100/1/94
1156 TOTN Perissocentrus sp3 CHILE abcdefg
98/1/61
76/1/56
JN624263 TOTI Zaglyptonotus sp USA abcd
93/1/74
1024 TOTN Chileana cyanea CHILE abcdefg
1052 TOTN New genus Monodontomerini sp ARGENTINA abcdefg
JN624272 TOTN Zdenekius smithi USA bc
subclade 8a
Clade 8
PJ00271 TOTM Eridontomerus biroi ARGENTINA abcde
1249 TOTM Eridontomerus biroi ITALY e
JN624264 TOTM Eridontomerus biroi ARGENTINA abcd
91/1/93
1234 TOTM Eridontomerus biroi TURKEY de
1267 TOTM Eridontomerus biroi CANADA abcde
1108 TOTM Eridontomerus biroi CANADA cde
68/.92/77
1049 TOTM Ditropinotus aureovir dis USA abcdef
1250 TOTM Eridontomerus fulviventris ITALY abcde
1237 TOTM Eridontomerus laticornis TURKEY de
100/1/73
100/1/97
1247 TOTM Eridontomerus isosomatis TURKEY e
1228 TOTM Eridontomerus isosomatis FRANCE e
100/1/73
3076 TOTM Eridontomerus arrabonicus FRANCE abcdf
1036 TOTM Eridontomerus arrabonicus CZECH REP abcdef
79/1/-
91/.74/73
UCRC299 TOTM Pseuderimerus burgeri USA abc
85/.94/63
UCRC629 TOTM Erimerus wickhami USA abcd
74/1/-
100/1/99
10372 TOTM Pseuderimerus luteus CROATIA acde
100/1/63
3026 TOTM Pseuderimerus sp1 FRANCE acde
85/1/-
1231 TOTM Adontomerus sp1 TURKEY e
98/1/-
1277 TOTM Adontomerus impolitus SPAIN abcde
1243 TOTM Adontomerus sp2 IRAN abcde
1286 TOTM Idiomacromerus sp5 USA abcde
94/1/-
1257 TOTM Idiomacromerus sp4 UAE abcd
82/.92/-
84/1/-
1137 TOTM Idiomacromerus sp8 USA cdfg
78/1/62
3077 TOTM Idiomacromerus sp2 FRANCE abcde
80/.97/-
1253 TOTM Idiomacromerus sp3 UAE abcde
80/.98/62
1033 TOTM Idiomacromerus papaveris PORTUGAL bcdefg
100/1/62
1150 TOTM Idiomacromerus sp9 PORTUGAL abcdefg
57/.92/-
98/1/72
3045 TOTM Idiomacromerus sp1 FRANCE acdefg
1007 TOTM Idiomacromerus sp2 UAE acd
89/1/-
100/1/100
3024 TOTM Idarnotorymus pulcher FRANCE acdef
82/1/-
1039 TOTM Idarnotorymus pulcher CROATIA acdef
100/1/100
1040 TOTI Cryptopristus caliginosus CZECH REP abcde
1096 TOTI Cryptopristus caliginosus CANADA abcdef
-/.90/-
1285 TOTM Microdontomerus sp3 USA abcde
56/.99/
88/-/50
1005 TOTM Microdontomerus sp4 UAE abcde
98/1/96
100/1/74
1255 TOTM Microdontomerus sp2 UAE abcde
100/1/100
3064 TOTM Microdontomerus annulatus FRANCE abcdf
3044 TOTM Microdontomerus sp1 FRANCE abcdef
100/1/100
3074 TOTI Glyphomerus tibialis FRANCE abcde
99/1/77
99/1/100
D2474 TOTI Glyphomerus tibialis CZECH REP abcdef
1272 TOTI Glyphomerus aylax BULGARIA abcde
100/1/100
1034 TOTI Exopristus trigonomerus PORTUGAL abcdef
72/-/-
3023 TOTI Exopristus trigonomerus FRANCE abcdef
100/1/99
UCRC475 TOTI Echthrodape sp1 AUSTRALIA abc
12801 TOTI Echthrodape sp2 MADAGASCAR bce
Clade 7
100/1/100
3115 TOTI Glyphomerus stigma FRANCE acdfg
100/1/100
3088 TOTI Glyphomerus stigma FRANCE abcdef
1076 TOTI Glyphomerus cf stigma CANADA acdefg
Clade 6
②

Fig. 4. Mapping of life-history strategies and hosts of larvae. The branch color reflects the most parsimonous ancestral state for that branch mapped onto ML tree topology, color of background indicates the tribal/subfamiliar or familiar affiliation. a - life-strategy of larvae, b - information about hosts records of larvae.

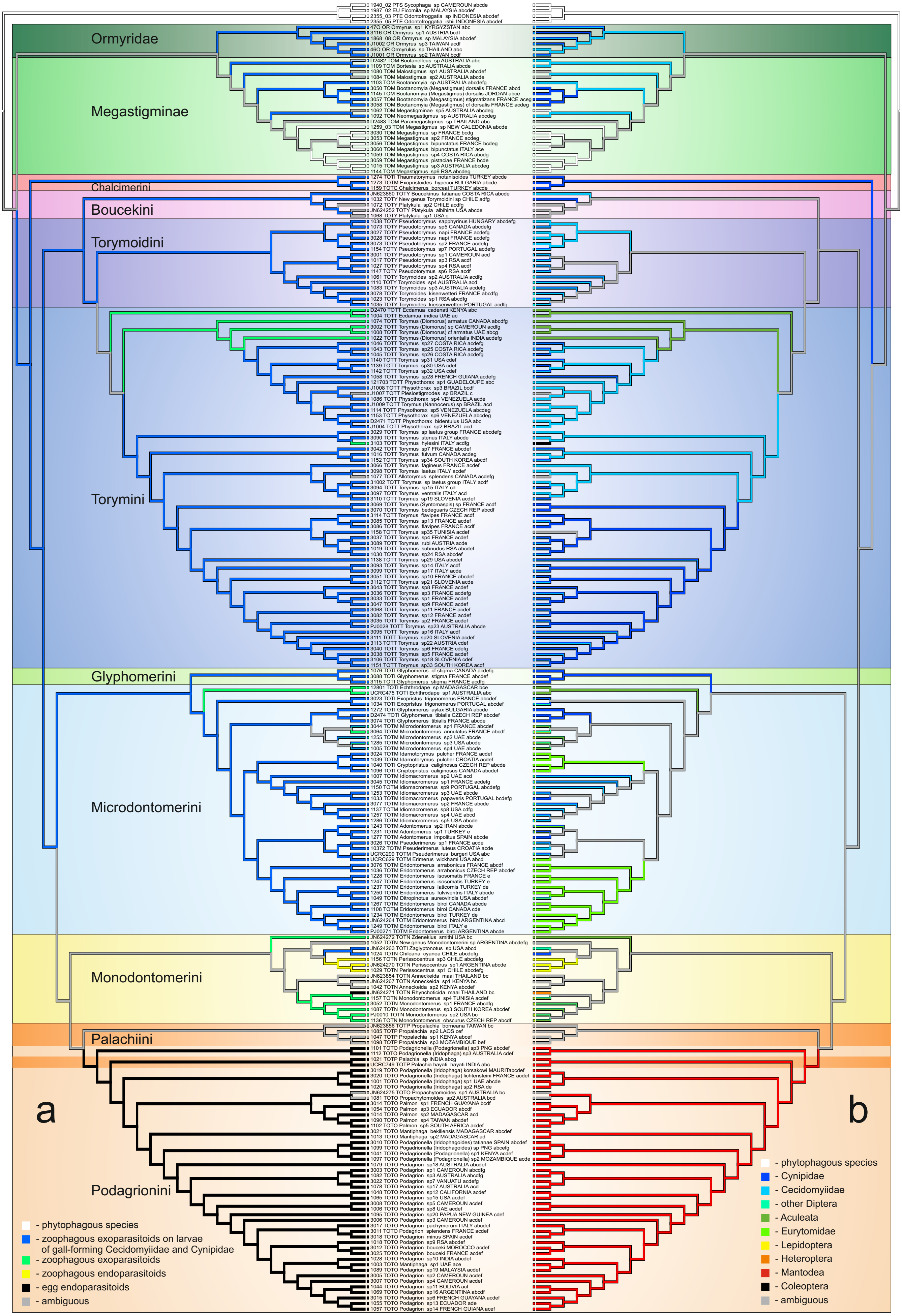
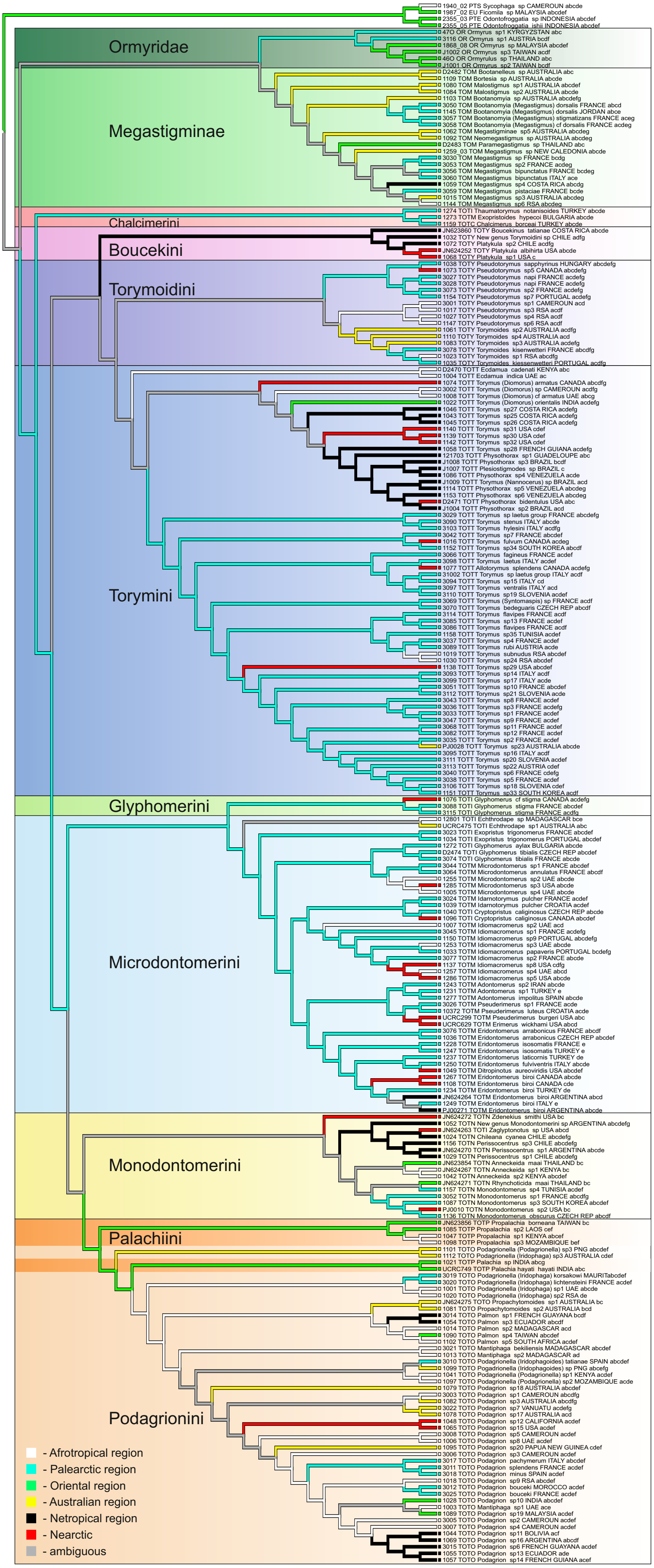

Ormyridae
Megastigminae
Chalcimerini
Boucekini
Torymoidini
Torymini
Glyphomerini
Microdontomerini
Monodontomerini
Palachiini
Podagrionini
1940_02 PTS Sycophaga sp CAMEROUN abcde
1987_02 EU Ficomila sp MALAYSIA abcdef
2355_03 PTE Odontofroggatia sp INDONESIA abcdef
2355_05 PTE Odontofroggatia ishii INDONESIA abcdef
47O OR Ormyrus sp1 KYRGYZSTAN abc
3116 OR Ormyrus sp1 AUSTRIA bcdf
1868_08 OR Ormyrus sp MALAYSIA abcdef
J1002 OR Ormyrus sp3 TAIWAN acdf
46O OR Ormyrulus sp THAILAND abc
J1001 OR Ormyrus sp2 TAIWAN bcdf
D2482 TOM Bootanelleus sp AUSTRALIA abc
1109 TOM Bortesia sp AUSTRALIA abcde
1080 TOM Malostigmus sp1 AUSTRALIA abcdef
1084 TOM Malostigmus sp2 AUSTRALIA abcde
1103 TOM Bootanomyia sp AUSTRALIA abcdefg
3050 TOM Bootanomyia (Megastigmus) dorsalis FRANCE abcd
1145 TOM Bootanomyia (Megastigmus) dorsalis JORDAN abce
3057 TOM Bootanomyia (Megastigmus) stigmatizans FRANCE aceg
3058 TOM Bootanomyia (Megastigmus) cf dorsalis FRANCE acdeg
1062 TOM Megastigminae sp5 AUSTRALIA abcdeg
1092 TOM Neomegastigmus sp AUSTRALIA abcdeg
D2483 TOM Paramegastigmus sp THAILAND abc
1259_03 TOM Megastigmus sp NEW CALEDONIA abcde
3030 TOM Megastigmus sp FRANCE bcdg
3053 TOM Megastigmus sp2 FRANCE acdeg
3056 TOM Megastigmus bipunctatus FRANCE bcdeg
3060 TOM Megastigmus bipunctatus ITALY ace
1059 TOM Megastigmus sp4 COSTA RICA abcdg
3059 TOM Megastigmus pistaciae FRANCE bcde
1015 TOM Megastigmus sp3 AUSTRALIA abcdeg
1144 TOM Megastigmus sp6 RSA abcdeg
1274 TOTI Thaumatorymus notanisoides TURKEY abcde
1273 TOTM Exopristoides hypecoi BULGARIA abcde
1159 TOTC Chalcimerus borceai TURKEY abcde
JN623860 TOTY Boucekinus tatianae COSTA RICA abcde
1032 TOTY New genus Torymoidini sp CHILE adfg
1072 TOTY Platykula sp2 CHILE acdfg
JN624252 TOTY Platykula albihirta USA abcde
1068 TOTY Platykula sp1 USA c
1038 TOTY Pseudotorymus sapphyrinus HUNGARY abcdefg
1073 TOTY Pseudotorymus sp5 CANADA abcdefg
3027 TOTY Pseudotorymus napi FRANCE acdefg
3028 TOTY Pseudotorymus napi FRANCE acdefg
3073 TOTY Pseudotorymus sp2 FRANCE acdefg
1154 TOTY Pseudotorymus sp7 PORTUGAL acdefg
3001 TOTY Pseudotorymus sp1 CAMEROUN acd
1017 TOTY Pseudotorymus sp3 RSA acdf
1027 TOTY Pseudotorymus sp4 RSA acdf
1147 TOTY Pseudotorymus sp6 RSA acdf
1061 TOTY Torymoides sp2 AUSTRALIA acdfg
1110 TOTY Torymoides sp4 AUSTRALIA acd
1083 TOTY Torymoides sp3 AUSTRALIA acdefg
3078 TOTY Torymoides kisenwetteri FRANCE abcdfg
1023 TOTY Torymoides sp1 RSA abcdfg
1035 TOTY Torymoides kiessenwetteri PORTUGAL acdfg
D2470 TOTT Ecdamua cadenati KENYA abc
1004 TOTT Ecdamua indica UAE ac
1074 TOTT Torymus (Diomorus) armatus CANADA abcdfg
3002 TOTT Torymus (Diomorus) sp CAMEROUN acdfg
1008 TOTT Torymus (Diomorus) cf armatus UAE abcg
1022 TOTT Torymus (Diomorus) orientalis INDIA acdefg
1046 TOTT Torymus sp27 COSTA RICA acdefg
1043 TOTT Torymus sp25 COSTA RICA acdefg
1045 TOTT Torymus sp26 COSTA RICA acdefg
1140 TOTT Torymus sp31 USA cdef
1139 TOTT Torymus sp30 USA cdef
1142 TOTT Torymus sp32 USA cdef
1058 TOTT Torymus sp28 FRENCH GUIANA acdefg
121703 TOTT Physothorax sp1 GUADELOUPE abc
J1008 TOTT Physothorax sp3 BRAZIL bcdf
J1007 TOTT Plesiostigmodes sp BRAZIL c
1086 TOTT Physothorax sp4 VENEZUELA acde
J1009 TOTT Torymus (Nannocerus) sp BRAZIL acd
1114 TOTT Physothorax sp5 VENEZUELA abcdeg
1153 TOTT Physothorax sp6 VENEZUELA abcdeg
D2471 TOTT Physothorax bidentulus USA abc
J1004 TOTT Physothorax sp2 BRAZIL acd
3029 TOTT Torymus sp laetus group FRANCE abcdefg
3090 TOTT Torymus stenus ITALY abcde
3103 TOTT Torymus hylesini ITALY acdfg
3042 TOTT Torymus sp7 FRANCE abcdef
1016 TOTT Torymus fulvum CANADA acdeg
1152 TOTT Torymus sp34 SOUTH KOREA abcdf
3066 TOTT Torymus fagineus FRANCE acdef
3098 TOTT Torymus laetus ITALY acdef
1077 TOTT Allotorymus splendens CANADA acdefg
31002 TOTT Torymus sp laetus group ITALY acdf
3094 TOTT Torymus sp15 ITALY cd
3097 TOTT Torymus ventralis ITALY acd
3110 TOTT Torymus sp19 SLOVENIA acdef
3069 TOTT Torymus (Syntomaspis) sp FRANCE acdf
3070 TOTT Torymus bedeguaris CZECH REP abcdf
3114 TOTT Torymus flavipes FRANCE acdf
3085 TOTT Torymus sp13 FRANCE acdef
3086 TOTT Torymus flavipes FRANCE acdf
1158 TOTT Torymus sp35 TUNISIA acdef
3037 TOTT Torymus sp4 FRANCE acdef
3089 TOTT Torymus rubi AUSTRIA acde
1019 TOTT Torymus subnudus RSA abcdef
1030 TOTT Torymus sp24 RSA abcdef
1138 TOTT Torymus sp29 USA abcdef
3093 TOTT Torymus sp14 ITALY acdf
3099 TOTT Torymus sp17 ITALY acde
3051 TOTT Torymus sp10 FRANCE abcdef
3112 TOTT Torymus sp21 SLOVENIA acde
3043 TOTT Torymus sp8 FRANCE acdef
3036 TOTT Torymus sp3 FRANCE acdefg
3033 TOTT Torymus sp1 FRANCE acdef
3047 TOTT Torymus sp9 FRANCE acdef
3068 TOTT Torymus sp11 FRANCE acdef
3082 TOTT Torymus sp12 FRANCE acdef
3035 TOTT Torymus sp2 FRANCE acdef
PJ0028 TOTT Torymus sp23 AUSTRALIA abcde
3095 TOTT Torymus sp16 ITALY acdf
3111 TOTT Torymus sp20 SLOVENIA acdef
3113 TOTT Torymus sp22 AUSTRIA cdef
3040 TOTT Torymus sp6 FRANCE cdefg
3038 TOTT Torymus sp5 FRANCE acdef
3106 TOTT Torymus sp18 SLOVENIA cdef
1151 TOTT Torymus sp33 SOUTH KOREA acdf
1076 TOTI Glyphomerus cf stigma CANADA acdefg
3088 TOTI Glyphomerus stigma FRANCE abcdef
3115 TOTI Glyphomerus stigma FRANCE acdfg
12801 TOTI Echthrodape sp MADAGASCAR bce
UCRC475 TOTI Echthrodape sp1 AUSTRALIA abc
3023 TOTI Exopristus trigonomerus FRANCE abcdef
1034 TOTI Exopristus trigonomerus PORTUGAL abcdef
1272 TOTI Glyphomerus aylax BULGARIA abcde
D2474 TOTI Glyphomerus tibialis CZECH REP abcdef
3074 TOTI Glyphomerus tibialis FRANCE abcde
3044 TOTM Microdontomerus sp1 FRANCE abcdef
3064 TOTM Microdontomerus annulatus FRANCE abcdf
1255 TOTM Microdontomerus sp2 UAE abcde
1285 TOTM Microdontomerus sp3 USA abcde
1005 TOTM Microdontomerus sp4 UAE abcde
3024 TOTM Idarnotorymus pulcher FRANCE acdef
1039 TOTM Idarnotorymus pulcher CROATIA acdef
1040 TOTI Cryptopristus caliginosus CZECH REP abcde
1096 TOTI Cryptopristus caliginosus CANADA abcdef
1007 TOTM Idiomacromerus sp2 UAE acd
3045 TOTM Idiomacromerus sp1 FRANCE acdefg
1150 TOTM Idiomacromerus sp9 PORTUGAL abcdefg
1253 TOTM Idiomacromerus sp3 UAE abcde
1033 TOTM Idiomacromerus papaveris PORTUGAL bcdefg
3077 TOTM Idiomacromerus sp2 FRANCE abcde
1137 TOTM Idiomacromerus sp8 USA cdfg
1257 TOTM Idiomacromerus sp4 UAE abcd
1286 TOTM Idiomacromerus sp5 USA abcde
1243 TOTM Adontomerus sp2 IRAN abcde
1231 TOTM Adontomerus sp1 TURKEY e
1277 TOTM Adontomerus impolitus SPAIN abcde
3026 TOTM Pseuderimerus sp1 FRANCE acde
10372 TOTM Pseuderimerus luteus CROATIA acde
UCRC299 TOTM Pseuderimerus burgeri USA abc
UCRC629 TOTM Erimerus wickhami USA abcd
3076 TOTM Eridontomerus arrabonicus FRANCE abcdf
1036 TOTM Eridontomerus arrabonicus CZECH REP abcdef
1228 TOTM Eridontomerus isosomatis FRANCE e
1247 TOTM Eridontomerus isosomatis TURKEY e
1237 TOTM Eridontomerus laticornis TURKEY de
1250 TOTM Eridontomerus fulviventris ITALY abcde
1049 TOTM Ditropinotus aureoviridis USA abcdef
1267 TOTM Eridontomerus biroi CANADA abcde
1108 TOTM Eridontomerus biroi CANADA cde
1234 TOTM Eridontomerus biroi TURKEY de
JN624264 TOTM Eridontomerus biroi ARGENTINA abcd
1249 TOTM Eridontomerus biroi ITALY e
PJ00271 TOTM Eridontomerus biroi ARGENTINA abcde
JN624272 TOTN Zdenekius smithi USA bc
1052 TOTN New genus Monodontomerini sp ARGENTINA abcdefg
JN624263 TOTI Zaglyptonotus sp USA abcd
1024 TOTN Chileana cyanea CHILE abcdefg
1156 TOTN Perissocentrus sp3 CHILE abcdefg
JN624270 TOTN Perissocentrus sp1 ARGENTINA abcde
1029 TOTN Perissocentrus sp1 CHILE abcdefg
JN623854 TOTN Anneckeida maai THAILAND bc
JN624267 TOTN Anneckeida sp1 KENYA bc
1042 TOTN Anneckeida sp2 KENYA abcdef
JN624271 TOTN Rhynchoticida maai THAILAND bc
1157 TOTN Monodontomerus sp4 TUNISIA acdef
3052 TOTN Monodontomerus sp1 FRANCE abcdfg
1087 TOTN Monodontomerus sp3 SOUTH KOREA abcdef
PJ0010 TOTN Monodontomerus sp2 USA bc
1136 TOTN Monodontomerus obscurus CZECH REP abcdf
JN623856 TOTP Propalachia borneana TAIWAN bc
1085 TOTP Propalachia sp2 LAOS cef
1047 TOTP Propalachia sp1 KENYA abcef
1098 TOTP Propalachia sp3 MOZAMBIQUE bef
1101 TOTO Podagrionella (Podagrionella) sp3 PNG abcdef
1112 TOTO Podagrionella (Iridophaga) sp3 AUSTRALIA cdef
1021 TOTP Palachia sp INDIA abcg
UCRC749 TOTP Palachia hayati hayati INDIA abc
3019 TOTO Podagrionella (Iridophaga) korsakowi MAURITabcdef
3020 TOTO Podagrionella (Iridophaga) lichtensteini FRANCE acdef
1001 TOTO Podagrionella (Iridophaga) sp1 UAE abcde
1020 TOTO Podagrionella (Iridophaga) sp2 RSA de
JN624275 TOTO Propachytomoides sp1 AUSTRALIA bc
1081 TOTO Propachytomoides sp2 AUSTRALIA bcd
3014 TOTO Palmon sp1 FRENCH GUAYANA bcdf
1054 TOTO Palmon sp3 ECUADOR abcdf
1014 TOTO Palmon sp2 MADAGASCAR acd
1090 TOTO Palmon sp4 TAIWAN abcdef
1102 TOTO Palmon sp5 SOUTH AFRICA acdef
3021 TOTO Mantiphaga bekiliensis MADAGASCAR abcdef
1013 TOTO Mantiphaga sp2 MADAGASCAR ad
3010 TOTO Podagrionella (Iridophagoides) tatianae SPAIN abcdef
1099 TOTO Pogadrionella (Iridophagoides) sp PNG abcefg
1041 TOTO Podagrionella (Podagrionella) sp1 KENYA acdef
1097 TOTO Podagrionella (Podagrionella) sp2 MOZAMBIQUE acde
1079 TOTO Podagrion sp18 AUSTRALIA abcdef
3003 TOTO Podagrion sp1 CAMEROUN abcdfg
1082 TOTO Podagrion sp3 AUSTRALIA abcdfg
3022 TOTO Podagrion sp7 VANUATU acdefg
1078 TOTO Podagrion sp17 AUSTRALIA acd
1048 TOTO Podagrion sp12 CALIFORNIA acdef
1065 TOTO Podagrion sp15 USA acdef
3008 TOTO Podagrion sp5 CAMEROUN acdef
1006 TOTO Podagrion sp8 UAE acdef
1095 TOTO Podagrion sp20 PAPUA NEW GUINEA cdef
3006 TOTO Podagrion sp3 CAMEROUN acdef
3017 TOTO Podagrion pachymerum ITALY abcdef
3011 TOTO Podagrion splendens FRANCE acdef
3018 TOTO Podagrion minus SPAIN acdef
1018 TOTO Podagrion sp9 RSA abcdef
3012 TOTO Podagrion bouceki MOROCCO acdef
3025 TOTO Podagrion bouceki FRANCE acdef
1028 TOTO Podagrion sp10 INDIA abcdef
1003 TOTO Mantiphaga sp1 UAE ace
1089 TOTO Podagrion sp19 MALAYSIA acdef
3005 TOTO Podagrion sp2 CAMEROUN acdef
3007 TOTO Podagrion sp4 CAMEROUN acdef
1044 TOTO Podagrion sp11 BOLIVIA acf
1069 TOTO Podagrion sp16 ARGENTINA abcdf
3015 TOTO Podagrion sp6 FRENCH GUAYANA acdef
1055 TOTO Podagrion sp13 ECUADOR ade
1057 TOTO Podagrion sp14 FRENCH GUIANA acef
a
b
- phytophagous species
- zoophagous exoparasitoids on larvae of gall-forming Cecidomyiidae and Cynipidae
- zoophagous exoparasitoids
- zoophagous endoparasitoids
- egg endoparasitoids
- ambiguous
- phytophagous species
- Cynipidae
- Cecidomyiidae
- other Diptera
- Aculeata
- Eurytomidae
- Lepidoptera
- Heteroptera
- Mantodea
- Coleoptera
- ambiguous

Fig. 5. Mapping of distribution by geographical region for specimens included in analysis. Branch color reflects the most parsimonous ancestral state for that branch mapped onto the RAxML tree topology; color of background indicates the tribal/subfamiliar or familiar affiliation.


1940_02 PTS Sycophaga sp CAMEROUN abcde
1987_02 EU Ficomila sp MALAYSIA abcdef
2355_03 PTE Odontofroggatia sp INDONESIA abcdef
2355_05 PTE Odontofroggatia ishii INDONESIA abcdef
Ormyridae
47O OR Ormyrus sp1 KYRGYZSTAN abc
3116 OR Ormyrus sp1 AUSTRIA bcdf
1868_08 OR Ormyrus sp MALAYSIA abcdef
J1002 OR Ormyrus sp3 TAIWAN acdf
46O OR Ormyrulus sp THAILAND abc
J1001 OR Ormyrus sp2 TAIWAN bcdf
Megastigminae
D2482 TOM Bootanelleus sp AUSTRALIA abc
1109 TOM Bortesia sp AUSTRALIA abcde
1080 TOM Malostigmus sp1 AUSTRALIA abcdef
1084 TOM Malostigmus sp2 AUSTRALIA abcde
1103 TOM Bootanomyia sp AUSTRALIA abcdefg
3050 TOM Bootanomyia (Megastigmus) dorsalis FRANCE abcd
1145 TOM Bootanomyia (Megastigmus) dorsalis JORDAN abce
3057 TOM Bootanomyia (Megastigmus) stigmatizans FRANCE aceg
3058 TOM Bootanomyia (Megastigmus) cf dorsalis FRANCE acdeg
1062 TOM Megastigminae sp5 AUSTRALIA abcdeg
1092 TOM Neomegastigmus sp AUSTRALIA abcdeg
D2483 TOM Paramegastigmus sp THAILAND abc
1259_03 TOM Megastigmus sp NEW CALEDONIA abcde
3030 TOM Megastigmus sp FRANCE bcdg
3053 TOM Megastigmus sp2 FRANCE acdeg
3056 TOM Megastigmus bipunctatus FRANCE bcdeg
3060 TOM Megastigmus bipunctatus ITALY ace
1059 TOM Megastigmus sp4 COSTA RICA abcdg
3059 TOM Megastigmus pistaciae FRANCE bcde
1015 TOM Megastigmus sp3 AUSTRALIA abcdeg
1144 TOM Megastigmus sp6 RSA abcdeg
Chalcimerini
1274 TOTI Thaumatorymus notanisoides TURKEY abcde
1273 TOTM Exopristoides hypecoi BULGARIA abcde
1159 TOTC Chalcimerus borceai TURKEY abcde
Boucekini
JN623860 TOTY Boucekinus tatianae COSTA RICA abcde
1032 TOTY New genus Torymoidini sp CHILE adfg
1072 TOTY Platykula sp2 CHILE acdfg
JN624252 TOTY Platykula albihirta USA abcde
1068 TOTY Platykula sp1 USA c
Torymoidini
1038 TOTY Pseudotorymus sapphyrinus HUNGARY abcdefg
1073 TOTY Pseudotorymus sp5 CANADA abcdefg
3027 TOTY Pseudotorymus napi FRANCE acdefg
3028 TOTY Pseudotorymus napi FRANCE acdefg
3073 TOTY Pseudotorymus sp2 FRANCE acdefg
1154 TOTY Pseudotorymus sp7 PORTUGAL acdefg
3001 TOTY Pseudotorymus sp1 CAMEROUN acd
1017 TOTY Pseudotorymus sp3 RSA acdf
1027 TOTY Pseudotorymus sp4 RSA acdf
1147 TOTY Pseudotorymus sp6 RSA acdf
1061 TOTY Torymoides sp2 AUSTRALIA acdfg
1110 TOTY Torymoides sp4 AUSTRALIA acd
1083 TOTY Torymoides sp3 AUSTRALIA acdefg
3078 TOTY Torymoides kisenwetteri FRANCE abcdfg
1023 TOTY Torymoides sp1 RSA abcdfg
1035 TOTY Torymoides kiessenwetteri PORTUGAL acdfg
Torymini
D2470 TOTT Ecdamua cadenati KENYA abc
1004 TOTT Ecdamua indica UAE ac
1074 TOTT Torymus (Diomorus) armatus CANADA abcdfg
3002 TOTT Torymus (Diomorus) sp CAMEROUN acdfg
1008 TOTT Torymus (Diomorus) cf armatus UAE abcg
1022 TOTT Torymus (Diomorus) orientalis INDIA acdefg
1046 TOTT Torymus sp27 COSTA RICA acdefg
1043 TOTT Torymus sp25 COSTA RICA acdefg
1045 TOTT Torymus sp26 COSTA RICA acdefg
1140 TOTT Torymus sp31 USA cdef
1139 TOTT Torymus sp30 USA cdef
1142 TOTT Torymus sp32 USA cdef
1058 TOTT Torymus sp28 FRENCH GUIANA acdefg
121703 TOTT Physothorax sp1 GUADELOUPE abc
J1008 TOTT Physothorax sp3 BRAZIL bcdf
J1007 TOTT Plesiostigmodes sp BRAZIL c
1086 TOTT Physothorax sp4 VENEZUELA acde
J1009 TOTT Torymus (Nannocerus) sp BRAZIL acd
1114 TOTT Physothorax sp5 VENEZUELA abcdeg
1153 TOTT Physothorax sp6 VENEZUELA abcdeg
D2471 TOTT Physothorax bidentulus USA abc
J1004 TOTT Physothorax sp2 BRAZIL acd
3029 TOTT Torymus sp laetus group FRANCE abcdefg
3090 TOTT Torymus stenus ITALY abcde
3103 TOTT Torymus hylesini ITALY acdfg
3042 TOTT Torymus sp7 FRANCE abcdef
1016 TOTT Torymus fulvum CANADA acdeg
1152 TOTT Torymus sp34 SOUTH KOREA abcdf
3066 TOTT Torymus fagineus FRANCE acdef
3098 TOTT Torymus laetus ITALY acdef
1077 TOTT Allotorymus splendens CANADA acdefg
31002 TOTT Torymus sp laetus group ITALY acdf
3094 TOTT Torymus sp15 ITALY cd
3097 TOTT Torymus ventralis ITALY acd
3110 TOTT Torymus sp19 SLOVENIA acdef
3069 TOTT Torymus (Syntomaspis) sp FRANCE acdf
3070 TOTT Torymus bedeguaris CZECH REP abcdf
3114 TOTT Torymus flavipes FRANCE acdf
3085 TOTT Torymus sp13 FRANCE acdef
3086 TOTT Torymus flavipes FRANCE acdf
1158 TOTT Torymus sp35 TUNISIA acdef
3037 TOTT Torymus sp4 FRANCE acdef
3089 TOTT Torymus rubi AUSTRIA acde
1019 TOTT Torymus subnudus RSA abcdef
1030 TOTT Torymus sp24 RSA abcdef
1138 TOTT Torymus sp29 USA abcdef
3093 TOTT Torymus sp14 ITALY acdf
3099 TOTT Torymus sp17 ITALY acde
3051 TOTT Torymus sp10 FRANCE abcdef
3112 TOTT Torymus sp21 SLOVENIA acde
3043 TOTT Torymus sp8 FRANCE acdef
3036 TOTT Torymus sp3 FRANCE acdefg
3033 TOTT Torymus sp1 FRANCE acdef
3047 TOTT Torymus sp9 FRANCE acdef
3068 TOTT Torymus sp11 FRANCE acdef
3082 TOTT Torymus sp12 FRANCE acdef
3035 TOTT Torymus sp2 FRANCE acdef
PJ0028 TOTT Torymus sp23 AUSTRALIA abcde
3095 TOTT Torymus sp16 ITALY acdf
3111 TOTT Torymus sp20 SLOVENIA acdef
3113 TOTT Torymus sp22 AUSTRIA cdef
3040 TOTT Torymus sp6 FRANCE cdefg
3038 TOTT Torymus sp5 FRANCE acdef
3106 TOTT Torymus sp18 SLOVENIA cdef
1151 TOTT Torymus sp33 SOUTH KOREA acdf
Glyphomerini
1076 TOTI Glyphomerus cf stigma CANADA acdefg
3088 TOTI Glyphomerus stigma FRANCE abcdef
3115 TOTI Glyphomerus stigma FRANCE acdfg
Microdontomerini
12801 TOTI Echthrodape sp MADAGASCAR bce
UCRC475 TOTI Echthrodape sp1 AUSTRALIA abc
3023 TOTI Exopristus trigonomerus FRANCE abcdef
1034 TOTI Exopristus trigonomerus PORTUGAL abcdef
1272 TOTI Glyphomerus aylax BULGARIA abcde
D2474 TOTI Glyphomerus tibialis CZECH REP abcdef
3074 TOTI Glyphomerus tibialis FRANCE abcde
3044 TOTM Microdontomerus sp1 FRANCE abcdef
3064 TOTM Microdontomerus annulatus FRANCE abcdf
1255 TOTM Microdontomerus sp2 UAE abcde
1285 TOTM Microdontomerus sp3 USA abcde
1005 TOTM Microdontomerus sp4 UAE abcde
3024 TOTM Idarnotorymus pulcher FRANCE acdef
1039 TOTM Idarnotorymus pulcher CROATIA acdef
1040 TOTI Cryptopristus caliginosus CZECH REP abcde
1096 TOTI Cryptopristus caliginosus CANADA abcdef
1007 TOTM Idiomacromerus sp2 UAE acd
3045 TOTM Idiomacromerus sp1 FRANCE acdefg
1150 TOTM Idiomacromerus sp9 PORTUGAL abcdefg
1253 TOTM Idiomacromerus sp3 UAE abcde
1033 TOTM Idiomacromerus papaveris PORTUGAL bcdefg
3077 TOTM Idiomacromerus sp2 FRANCE abcde
1137 TOTM Idiomacromerus sp8 USA cdfg
1257 TOTM Idiomacromerus sp4 UAE abcd
1286 TOTM Idiomacromerus sp5 USA abcde
1243 TOTM Adontomerus sp2 IRAN abcde
1231 TOTM Adontomerus sp1 TURKEY e
1277 TOTM Adontomerus impolitus SPAIN abcde
3026 TOTM Pseuderimerus sp1 FRANCE acde
10372 TOTM Pseuderimerus luteus CROATIA acde
UCRC299 TOTM Pseuderimerus burgeri USA abc
UCRC629 TOTM Erimerus wickhami USA abcd
3076 TOTM Eridontomerus arrabonicus FRANCE abcdf
1036 TOTM Eridontomerus arrabonicus CZECH REP abcdef
1228 TOTM Eridontomerus isosomatis FRANCE e
1247 TOTM Eridontomerus isosomatis TURKEY e
1237 TOTM Eridontomerus laticornis TURKEY de
1250 TOTM Eridontomerus fulviventris ITALY abcde
1049 TOTM Ditropinotus aureoviridis USA abcdef
1267 TOTM Eridontomerus biroi CANADA abcde
1108 TOTM Eridontomerus biroi CANADA cde
1234 TOTM Eridontomerus biroi TURKEY de
JN624264 TOTM Eridontomerus biroi ARGENTINA abcd
1249 TOTM Eridontomerus biroi ITALY e
PJ00271 TOTM Eridontomerus biroi ARGENTINA abcde
Monodontomerini
JN624272 TOTN Zdenekius smithi USA bc
1052 TOTN New genus Monodontomerini sp ARGENTINA abcdefg
JN624263 TOTI Zaglyptonotus sp USA abcd
1024 TOTN Chileana cyanea CHILE abcdefg
1156 TOTN Perissocentrus sp3 CHILE abcdefg
JN624270 TOTN Perissocentrus sp1 ARGENTINA abcde
1029 TOTN Perissocentrus sp1 CHILE abcdefg
JN623854 TOTN Anneckeida maai THAILAND bc
JN624267 TOTN Anneckeida sp1 KENYA bc
1042 TOTN Anneckeida sp2 KENYA abcdef
JN624271 TOTN Rhynchoticida maai THAILAND bc
1157 TOTN Monodontomerus sp4 TUNISIA acdef
3052 TOTN Monodontomerus sp1 FRANCE abcdfg
1087 TOTN Monodontomerus sp3 SOUTH KOREA abcdef
PJ0010 TOTN Monodontomerus sp2 USA bc
1136 TOTN Monodontomerus obscurus CZECH REP abcdf
Palachiini
JN623856 TOTP Propalachia borneana TAIWAN bc
1085 TOTP Propalachia sp2 LAOS cef
1047 TOTP Propalachia sp1 KENYA abcef
1098 TOTP Propalachia sp3 MOZAMBIQUE bef
1101 TOTO Podagrionella (Podagrionella) sp3 PNG abcdef
1112 TOTO Podagrionella (Iridophaga) sp3 AUSTRALIA cdef
1021 TOTP Palachia sp INDIA abcg
UCRC749 TOTP Palachia hayati hayati INDIA abc
Podagrionini
3019 TOTO Podagrionella (Iridophaga) korsakowi MAURITabcdef
3020 TOTO Podagrionella (Iridophaga) lichtensteini FRANCE acdef
1001 TOTO Podagrionella (Iridophaga) sp1 UAE abcde
1020 TOTO Podagrionella (Iridophaga) sp2 RSA de
JN624275 TOTO Propachytomoides sp1 AUSTRALIA bc
1081 TOTO Propachytomoides sp2 AUSTRALIA bcd
3014 TOTO Palmon sp1 FRENCH GUAYANA bcdf
1054 TOTO Palmon sp3 ECUADOR abcdf
1014 TOTO Palmon sp2 MADAGASCAR acd
1090 TOTO Palmon sp4 TAIWAN abcdef
1102 TOTO Palmon sp5 SOUTH AFRICA acdef
3021 TOTO Mantiphaga bekiliensis MADAGASCAR abcdef
1013 TOTO Mantiphaga sp2 MADAGASCAR ad
3010 TOTO Podagrionella (Iridophagoides) tatianae SPAIN abcdef
1099 TOTO Pogadrionella (Iridophagoides) sp PNG abcefg
1041 TOTO Podagrionella (Podagrionella) sp1 KENYA acdef
1097 TOTO Podagrionella (Podagrionella) sp2 MOZAMBIQUE acde
1079 TOTO Podagrion sp18 AUSTRALIA abcdef
3003 TOTO Podagrion sp1 CAMEROUN abcdfg
1082 TOTO Podagrion sp3 AUSTRALIA abcdfg
3022 TOTO Podagrion sp7 VANUATU acdefg
1078 TOTO Podagrion sp17 AUSTRALIA acd
1048 TOTO Podagrion sp12 CALIFORNIA acdef
1065 TOTO Podagrion sp15 USA acdef
3008 TOTO Podagrion sp5 CAMEROUN acdef
1006 TOTO Podagrion sp8 UAE acdef
1095 TOTO Podagrion sp20 PAPUA NEW GUINEA cdef
3006 TOTO Podagrion sp3 CAMEROUN acdef
3017 TOTO Podagrion pachymerum ITALY abcdef
3011 TOTO Podagrion splendens FRANCE acdef
3018 TOTO Podagrion minus SPAIN acdef
1018 TOTO Podagrion sp9 RSA abcdef
3012 TOTO Podagrion bouceki MOROCCO acdef
3025 TOTO Podagrion bouceki FRANCE acdef
1028 TOTO Podagrion sp10 INDIA abcdef
1003 TOTO Mantiphaga sp1 UAE ace
1089 TOTO Podagrion sp19 MALAYSIA acdef
3005 TOTO Podagrion sp2 CAMEROUN acdef
3007 TOTO Podagrion sp4 CAMEROUN acdef
1044 TOTO Podagrion sp11 BOLIVIA acf
1069 TOTO Podagrion sp16 ARGENTINA abcdf
3015 TOTO Podagrion sp6 FRENCH GUAYANA acdef
1055 TOTO Podagrion sp13 ECUADOR ade
1057 TOTO Podagrion sp14 FRENCH GUIANA acef
- Afrotropical region
- Palearctic region
- Oriental region
- Australian region
- Netropical region
- Nearctic
- ambiguous

4. Conclusions and future prospects

The thesis had three aims – (1) to figure out the phylogenetic position of Torymidae as well as the position of the other chalcidoid families inside superfamily Chalcidoidea based on molecular and morphological characters, (2) to provide the first molecular study of the family Torymidae, to compare the results with known morphological concept of the family and to figure out possible evolution of life strategies inside family and (3) to contribute to the taxonomy of the family which is still very poorly known.

To accomplish the first aim we developed and analysed supermatrix of sequencies of two ribosomal genes (18S rDNA and 28S rDNA) for 649 species of chalcidoid taxa. However, family Torymidae was considered as polyphyletic group with the subfamily Megastigminae unrelated to the subfamily Toryminae and without any logical sister group inside entire superfamily. Therefore we tried to corroborate monophyly of Torymidae in subsequent study focused on molecular and morphological characters of selected taxa. Altogether 233 characters of 300 members of all chalcidoid families were scored. Contrary to our previous DNA only based study, we both confirmed the monophyly of family and revealed also potential sister relationships of Torymidae with Ormyridae+Colotrechninae or Cerocephalinae+Diparinae, respectively. However, we are planning to make much more detailed study of the superfamily phylogeny using preliminary results of transcriptomic studies to obtain more genes and more reliable support of backbone nodes. It will be good starting base for testing various evolutionary hypotheses inside so diverse superfamily as Chalcidoidea is.

Second aim of the study was fullfiled by reconstruction of Torymidae phylogeny based on 5 gene fragments using 226 ingroup taxa representing 45 of the 67 recognized genera from two accepted subfamilies and all known tribes. Based on these data the monophyly of Torymidae was not confirmed. However, we recovered all known tribes and established two new tribes of the subfamily Toryminae. Larvae of the most common ancestor of Toryminae were postulated as exoparasitoids of gall-forming insects in Palaearctic region with several derived traits throughout the Toryminae phylogeny. The life strategy, hosts and distribution of the common ancestor of Megastigminae is still uncertain. In the future, we would like to add some more closer relative outgroups (i.e. Colotrechninae and Diparinae, both Pteromalidae) to reveal monophyly of Toryminae and find out the possible closer sister group of Megastigminae. The detailed study of relevant morphological characters to define morphologicaly the entire family, respectively subfamily, tribes and genera is also needed. We will concentrate also on the critical survey of all known and particularly on undescribed fossil records as well as on verifying published host records and finding new ones for members of the family, respectively two subfamilies. We have to concentrate equally to study of larval characters of more representatives of the Megastigminae and Toryminae as well as of the other chalcidoid taxa. These data are necessary for reconstruction of more robust evolutionary hypothesis of the Torymidae, respectively Megastigminae and Toryminae history.

Contribution to the taxonomy of the family, the third aim, is a by-product of above mentioned studies.We have found and described some new unknown genera (*Boucekinus* Janšta & Hanson, 2011 with two species and *Chileana* Janšta & Křížková, 2013 with four new species) from South America. We included also keys to the species, diagnosis of the genera and discussion of their potential phylogenetic placement within the family. Many new species and still some genera remained undescribed. We will focus on their description in the future. We hope that our consequent studies will contribute to the knowledge of the world biodiversity of this small parasitic wasps as well as to knowledge of their biology.