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A molecular phylogeny of eurytomid wasps inferred from DNA sequence data of 28S, 18S, 16S, and COI genes

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Abstract

Using partial DNA sequence data from nuclear 28S and 18S genes and mitochondrial 16S and COI genes, we reconstructed a phylogeny of the family Eurytomidae. Both maximum parsimony and Bayesian methods were employed. The analysis revealed a significant incongruence between the mitochondrial genes and the nuclear genes, and we chose the results from the nuclear genes as our preferred hypothesis. Our phylogeny suggested that the family Eurytomidae is not a monophyletic group; neither are the genera *Eurytoma* and *Bruchophagus*. The monophyly of genera *Sycophila* and *Plutarchia* was well supported, as was the close association of the genera *Aiolomorphus*, *Tenuipetiolus*, *Bephratelloides*, and *Phylloxeroxenus*. Our phylogeny also revealed an anticipated pattern, in which species groups from the genera *Eurytoma* and *Bruchophagus* are often more closely related to other small genera than to other species groups of the same genus. Subsequent taxonomic revisions include elevating the subfamily Rileyinae to a family status and the divisions of the genera *Eurytoma* and *Bruchophagus*.

Keywords: 28S rDNA; 18S rDNA; 16S rDNA; COI; Nuclear gene; Mitochondrial gene; Molecular phylogeny; Eurytomidae

1. Introduction

A solid taxonomy is fundamental to all biology, and phylogenies provide a sound foundation for establishing taxonomy. The lack of a reliable taxonomy of the family Eurytomidae (Hymenoptera: Chalcidoidea) has retarded our understanding of the evolutionary history of this economically important group. The family consists of over 1420 described species, which have been classified into approximately 87 genera (Noyes, 2002). Eurytomids have a wide range of lifestyles; most species are parasitic, while a considerable number of them are phytophagous. This family, together with families Leucospidae, Chalcididae, Torymidae, and Ormyridae, forms the chalcidoid complex (Zerova, 1988).

The monophyly of the family Eurytomidae has always been in question. Bouček (1988a) noted that although the family has been established and in use for more than 150 years, tangible synapomorphic characters are lacking. Wijesekara (1997) proposed two morphological characters that support the monophyly of the family Eurytomidae, but he only examined two genera (*Eurytoma* and *Tetramesa*). The difficulty in finding synapomorphies for the family is largely due to the prevalent convergent evolution in the chalcidoid complex. A number of the eurytomid species share similar life history traits with the family Torymidae, especially in the relatively high degree of phytophagy and predilection for gall-forming hosts, which resulted in much morphological similarity between the two families (DiGiulio, 1997; Zerova, 1988). On the other hand, many eurytomids share similar habitats with the family Chalcididae, which resulted in several similar morphological features between these two families (Zerova, 1988). Recently, Campbell et al. (2000) sequenced the D2 regions of the 28S gene for five eurytomid species. They found that the five species did not form a monophyletic group. The study also found that the subfamily Rileyinae of the family Eurytomidae is more closely

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related to other members of Chalcidoidea than to the subfamily Eurytominae.

Unsurprisingly, the classification within the family Eurytomidae is extremely controversial, and the monophyly of its subfamilies and many genera are questionable. Ashmead (1904) first designated five tribes (Aximini, Isosomini, Eurytomini, Rileyini, and Decatomini), all of which were elevated to subfamilies by Ferrière (1950) and maintained by most subsequent studies (Burks, 1971, 1979; Claridge, 1961; Peck, 1963). Burks (1971) added three new subfamilies to this classification (Heimbrinae, Prodecatominae, and Philolemninae), but did not provide synapomorphies for these groups. He also remarked that it was impossible to characterize the eight subfamilies since none possessed a unique set of features. Zerova (1988) consolidated the eight subfamilies into seven, but did not propose synapomorphic characters, although a lot of diagnoses were presented. A more widely accepted consensus recognized three subfamilies: Rileyinae (cosmopolitan), Eurytominae (cosmopolitan), and Heimbrinae (New World) (Bouček, 1988b; Grissell and Schauff, 1997; Stage and Snelling, 1986). Among the three subfamilies, Rileyinae, which includes 12 genera and 50 species (Noyes, 2002), is clearly defined by a 13-segmented antenna (Ashmead, 1904; Bouček, 1988b). The subfamily Heimbrinae is also well-defined by two synapomorphic characters (Stage and Snelling, 1986), but it only includes two genera and seven species (Noyes, 2002). The third subfamily, Eurytominae, became the "trash can," which contains all the remaining taxa (more than 1363 species and 73 genera). The classification at genus level is also problematic. Among the 73 genera of Eurytominae, 33 are monotypic (Noyes, 2002). On the other hand, there are more than 699 species in the genus *Eurytoma* and approximately 121 species in the genus *Bruchophagus* (Noyes, 2002).

There is little understanding about the phylogeny of the family Eurytomidae. Zerova (1988) postulated a phylogenetic relationship of the seven subfamilies based on morphological data. However, the tree was not derived from rigorous phylogenetic analysis, and was merely an educated guess. Using DNA sequence data, Campbell et al. (2000) intended to resolve the relationship of Chalcidoidea at the family level. In their study, five eurytomid species from three genera were included, and the results rejected the monophyly of the family Eurytomidae.

It is clear that morphology alone will not be able to clarify these controversies, and molecular data will provide additional needed information. Molecular data have been proven to be very powerful at resolving difficult problems, particularly questions associated with morphological convergent evolution (e.g. Campbell et al., 1993; Sibley and Ahlquist, 1984). In this study, we explored the possibility of using DNA sequence data to reconstruct a phylogeny of the family Eurytomidae and to evaluate the current taxonomy. Our objectives include (1) testing the monophyly of the family; (2) testing the monophyly of the subfamilies and the genera; (3) reconstructing an overall phylogeny of the family.

2. Materials and methods

2.1. Taxon sampling

Twenty-four species from eleven genera of the family Eurytomidae were selected as ingroup taxa. Three species from the families Chalcididae (*Brachymeria lasus*) and Torymidae (*Megastigmus dorsalis* and *Monodontomerus minor*), which are closely related to the family Eurytomidae (Zerova, 1988), were chosen as outgroups. All specimens were identified based on their morphological characters prior to DNA extraction. We only sampled species that have at least two morphologically identical specimens from the exact same location. One of the identical specimens was used for DNA extraction, in which the whole body was ground up due to the small size of the specimen. The other identical specimen(s) were designated as the reference specimen(s). All reference specimens were deposited in the Institute of Zoology, Chinese Academy of Sciences (Beijing), and in the Royal Ontario Museum (Toronto). Appendix lists all specimens used in this study, including species names, location data, and catalogue numbers of the reference specimens.

2.2. Laboratory protocols

Genomic DNA was extracted from the whole specimen with head removed in advance using the Wizard genomic DNA isolation Protocols (Promega). Most specimens were initially preserved in 100% ethanol in the field, and subsequently processed with critical-point drying methods or transferred in 70% ethanol for long-term preservation.

Fragments from two nuclear genes, 18S and 28S, and from two mitochondrial genes, 16S and COI, were amplified with standard polymerase chain reaction (PCR) protocols and directly sequenced after purification. Primers used for PCR and sequencing are as following: D2 forward primer, 5′-AGT CGT GTT GCT TGA TAG TGC AG-3′ and D2 reverse primer, 5′-TTG GTC CGT GTT TCA AGA CGG G-3′ (28S, Campbell et al., 1993); 18S.up1, 5′-TGG TTG ATC CTG CCA GTA G-3′ and 18SV4.lo1, 5′-CRT HYT YGG CAA ATG CTT TCG C-3′ (18S, Carmean et al., 1992; White et al., 1990); LR-J-12887, 5′-TCG ATT TGA ACT CAA ATC ATG T-3′ and LR-N-13398, 5′-CAC CTG TTT ATC AAA AAC AT-3′ (16S, Simon et al., 1994); C1-J-1751, 5′-GGA TCA CCT GAT ATA GCA TTC CC-3′ and

C1-N-2191, 5′-CCC GGT AAA ATT AAA ATA TAA ACT TC-3′ (COI, Simon et al., 1994). Standard 50 μl PCRs were performed using 0.3 μl *Taq* polymerase (4.0 U, Promega), 5.0 μl *Taq* buffer (1.5 mM $MgCl_2$), 1.0 μl dNTPs (12.5 nmol), 2.0 μl primers (10 pmol), 2.0 μl template DNA and 37.7 μl dH_2O. PCR product purification was accomplished with Qiaquick protocols (Qiagen) and sequencing was conducted with ABI Big Dye protocols or CEQ 8000 terminator chemistries.

2.3. Sequence alignment

All sequences were manually checked and edited using Sequencher (version 3.1.1). Sequence alignment was conducted using Clustal X (Thompson et al., 1997) with parameters set to default. The computer-generated alignment of COI gene sequences was manually adjusted by comparison with the published complete sequence of *Drosophila yakuba* (Clary and Wolstenholme, 1985), at both nucleotide and amino acid levels. Alignments of the rRNA genes (16S, 18S, and 28S) were also edited by visual inspection using MacClade 4.0 (Maddison and Maddison, 2000). Minor changes were made to the alignment and hyper-variable regions were excluded from further analysis due to the ambiguity of the alignment. Such exclusion will increase the reliability of the phylogenetic analysis, as suggested by Swofford et al. (1996).

2.4. Phylogenetic analysis

Sequence data of different genes were analyzed both separately and combined. Both parsimony and likelihood criteria were used to infer the best phylogenetic hypotheses.

Parsimony analyses were implemented using PAUP 4.0b10 (Swofford, 2002). A heuristic search with 50 random stepwise addition replicates via TBR was conducted. All characters were weighted equally and unordered. All parsimony-uninformative characters were excluded from the analysis. Bootstrapping proportions (BSP; Felsenstein, 1985) with 1000 replicates were used for nodal evaluation. The partition homogeneity test (incongruence-length difference test; Farris et al., 1995) was conducted using PAUP to test the congruence between genes and gene combinations. We calculated 1000 replicates with invariant rates excluded (Cunningham, 1997). Dolphin et al. (2000) suggested that noise in one or both partitions might cause artificial incongruence, and developed a method for investigating this possibility. Therefore, an extended homogeneity test using the method of Dolphin et al. (2000) was also conducted.

Bayesian analyses were performed with MrBayes (Huelsenbeck and Ronquist, 2001). A likelihood ratio test (Goldman, 1993) was first conducted to select an evolutionary model that best fits the observed data. The general time-reversible model (GTR; Rodríguez et al., 1990; Yang et al., 1994) was chosen based on the results from the computer program Modeltest (version 3.06; Posada and Candall, 1998). The site-specific rates were estimated during the run. Four Markow chains were used and the dataset was run for 10^6 generations to allow for adequate time of convergence. Trees were sampled every 100 generations. After approximately 20,000 generations, the log-likelihood values of each sampled tree stabilized, and therefore, we used the last 5001 sample trees to estimate the 50% majority rule consensus tree and the Bayesian posterior probabilities (BPP).

Kishino–Hasegawa test, which was performed by PAUP, was employed to test the monophyly/non-monophyly of the major groups. The test was conducted within both the maximum likelihood and maximum parsimony framework.

3. Results

3.1. Sequence divergence

Not all PCR and sequence reactions were successful. From the 27 samples, we obtained 27 sequences for 28S gene, 22 for 18S gene, 17 for 16S gene and 23 for COI gene. After the alignment, a total of 629 base pairs of 28S gene were resolved. Two regions with 23 base pairs in total [corresponding positions in the *Drosophila melanogaster* sequence (Tautz et al., 1988) 698–705, 721–835], were removed from further analysis due to ambiguous alignment. Among the remaining 607 base pairs, 148 were variable and 89 were parsimony informative. The alignment of 18S gene sequences produced 890 base pairs of sequences, of which 43 sites were variable, 23 sites were parsimony informative. The alignment of 16S gene sequences produced 532 base pairs in length, of which six regions with 84 base pairs in total [corresponding positions in the *Drosophila yakuba* sequence (Clary and Wolstenholme, 1985) 13,045–13,046, 13,053–13,056, 13,064–13,069, 13,114–13,173, 13,143–13,250, 13,358–13,361], were removed from further analysis due to ambiguous alignment. Among the remaining 449 base pairs, 151 were variable and 39 were parsimony informative. A total of 442 base pair sequences of COI were obtained. The alignment produced one 3-bp gap; *Eurytoma verticillata* has a three base pair insertion. Among the sites, 193 were variable and 151 were parsimony informative. All sequences were deposited in GenBank and Accession numbers are listed in the Appendix. Aligned sequences are also available upon request.

The pairwise distances among the sequences were highly variable between genes. The 28S gene ranged from 1.69 to 13.5%; the 18S gene ranged from 0.1 to 2.9%; the COI gene ranged from 0.2 to 25.8% and the

16S gene ranged from 1.1 to 16.1%. These data are concordant with other studies of Hymenoptera (Ana et al., 2000; Belshaw et al., 1998; Campbell et al., 1993; Downton and Austin, 1998; Mardulyn and Whitefield, 1999; Schmitz and Moritz, 1994).

3.2. Phylogenetic analysis of the nuclear genes

Although initially we intended to analyze each nuclear gene separately, the limited informative characters of the 18S gene prevented us from getting any reliable results. In addition, the partition homogeneity test revealed no conflict between the 18S and 28S genes. Therefore, we analyzed the 28S gene alone and performed a separate analysis of the combined data of 28S and 18S genes. Predictably, both analyses produced almost identical results. Therefore, here we only report the results from the combined analysis.

Maximum parsimony analysis of the combined data of 28S and 18S produced 112 parsimony informative characters and resulted in two equally most parsimonious trees, each with 276 steps, a consistency index (CI) of 0.5543, and a retention index (RI) of 0.6317 (excluding uninformative characters). The strict consensus tree is shown in Fig. 1. Bootstrap analysis revealed relatively low nodal support; only five nodes received BSP greater than 70 (Fig. 1). The 50% majority rule consensus tree inferred from the Bayesian analysis is very similar to the results of the parsimony analysis (Fig. 1). There is no conflict between the Bayesian trees and the parsimony trees, and they merely differ in resolutions. The Bayesian posterior probabilities (BPP) offered a slightly higher nodal support in comparison with the bootstrapping; eight nodes received BPP greater than 90 (Fig. 1).

The phylogeny revealed several interesting features. First, the ingroup is not monophyletic. Although the parsimony tree and the Bayesian tree placed *Neorileya meridionalis* differently, both suggested that *Neorileya meridionalis* is distantly related to other ingroup members compared to the outgroup members. However, the monophyly of the ingroup excluding *Neorileya meridionalis* is strongly supported (100% BSP and 100% BPP). Second, the monophyly of the genera *Sycophila* and *Plutarchia* is well supported (91 and 100% BSP respectively, and 100% BPP). Third, the phylogeny also suggested that the genera *Bruchophagus* and *Eurytoma* are not monophyletic. This is not surprising considering the sizes of the genera (approximately, 121 and 699 species, respectively). Lastly, the tree revealed several other interesting associations, i.e., the close relationship among genera *Aiolomorphus*, *Tenuipetiolus*, *Bephratelloides*, and *Phylloxeroxenus*, which were well supported by the Bayesian analysis (BPP = 94); and the sister group relationship of genera *Plutarchia* and *Sycophila*.

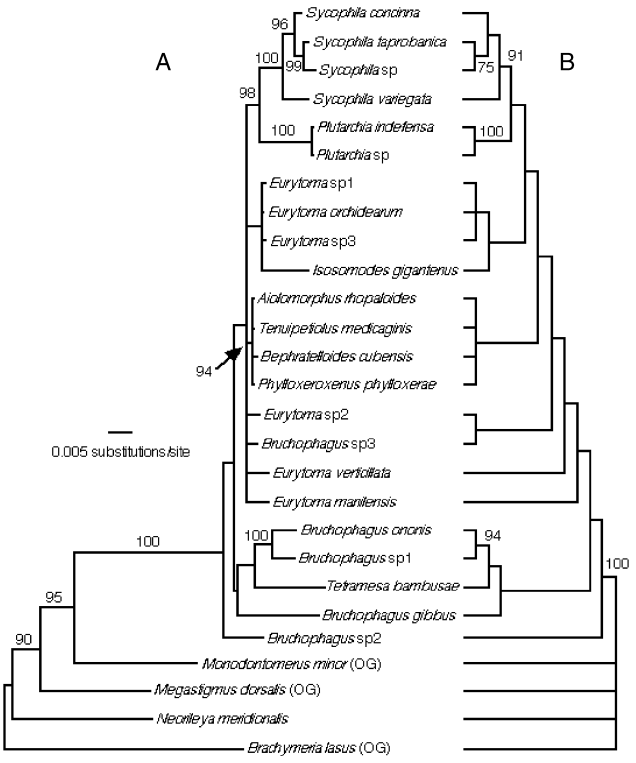

Fig. 1. The phylogenetic hypothesis derived from partial DNA sequences of the nuclear gene 18S and 28S. *Brachymeria lasus*, *Megastigmus dorsalis*, and *Monodontomerus minor* are outgroups. (A) The 50% majority rule consensus from the Bayesian analysis. The numbers above the lines or beside the nodes are Bayesian posterior probabilities. Branch lengths are calculated using likelihood methods with parameters estimated by ModelTest. (B) The strict consensus tree from the parsimony analysis. Numbers above the lines or beside the nodes are bootstrap proportions calculated with 1000 replicates.

3.3. Phylogenetic analysis of the mitochondrial genes

Since the mitochondrial genome usually evolves as a single locus, we analyzed the mitochondrial genes together. Maximum parsimony analysis revealed 262 informative characters and resulted in six equally most parsimonious trees, with 953 steps, a CI of 0.4124 and a RI 0.5118 (excluding uninformative characters). Fig. 2 shows the strict consensus tree. Bootstrap analyses revealed low nodal support; only five nodes received BSP greater than 70. The 50% majority consensus tree from the Bayesian analysis revealed a similar topology with a slightly higher nodal support (Fig. 2). The most striking feature of the resulting trees is that they differ dramatically from the results of the nuclear genes. The best-supported node from the parsimony analysis associated species from five different genera (*Bephratelloides*, *Phylloxeroxenus*, *Tenuipetiolus*, *Megastigmus*, and *Bruchophagus*) together, among which *Megastigmus* is an outgroup member. Although the first three genera also grouped in the same clade on the nuclear gene tree,

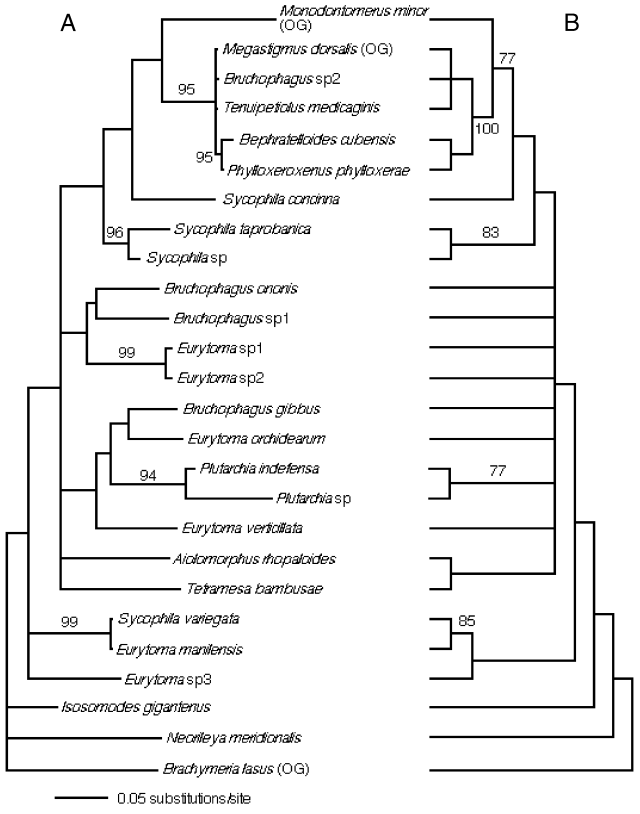

Fig. 2. The phylogenetic hypothesis derived from partial DNA sequences of the mitochondrial gene 16S and COI. *Brachymeria lasus*, *Megastigmus dorsalis*, and *Monodontomerus minor* are outgroups. (A) The 50% majority rule consensus from the Bayesian analysis. The numbers above the lines or beside the nodes are Bayesian posterior probabilities. Branch lengths are calculated using likelihood methods with parameters estimated by ModelTest. (B) The strict consensus tree from the parsimony analysis. Numbers above the lines or beside the nodes are bootstrap proportions calculated with 1000 replicates.

the last two were quite distantly related to the first three. Another outgroup member, *Monodontomerus minor*, is also deeply nested in the ingroup. Only two sister group relationships are shared by both the nuclear gene trees and the mitochondrial gene trees (*Sycophila taprobanica* and *Sycophila* sp.; *Plutarchia indefensa* and *Plutarchia* sp.).

3.4. The incongruence between the nuclear and the mitochondrial genes

The phylogenetic trees resulted from the nuclear genes and mitochondrial genes showed striking different topologies. The results from the partition homogeneity test confirmed the observation: the two data sets were significantly incongruent with each other ($P = 0.001$). To rule out the possibility that the incomplete sequences of several taxa might cause the incongruence, we removed the taxa with incomplete sequences. The test again revealed significant incongruence between the two partitions ($P = 0.001$). We also tested different combinations of genes. Both combinations of (18S + 28S) genes with COI gene or with 16S gene resulted in significant conflict ($P = 0.001$).

Following the suggestions of Dolphin et al. (2000), the extended homogeneity test clearly demonstrated that the level of conflict between the nuclear and mitochondrial genes was significantly higher than levels of conflict observed when the nuclear or the mitochondrial genes were randomized ($p = 0.001$). The average tree length difference was 72 steps without randomization, and ranged from 3 to 12 steps ($n = 30$) when the nuclear data were randomized and 10–21 steps ($n = 30$) when the mitochondrial data were randomized. Clearly, the differences of the two topologies are not an artifact of different random associations, but are determined by substantial conflict of the phylogenetic information nested in the two partitions.

3.5. The monophyly test for the major groups

We tested the monophyly of the subfamily Eurytominae, genus *Sycophila*, and genus *Plutarchia*, the non-monophyly of the family Eurytomidae, genus *Eurytoma*, and genus *Bruchophagus*. When all data were pooled together, the monophyly of the subfamily Eurytominae and the genera *Sycophila* and *Plutarchia*, and the non-monophyly of the family Eurytomidae and the genera *Eurytoma* and *Bruchophagus* were significantly supported by the Kishino–Hasegawa test ($p < 0.05$). The mitochondrial genes alone reached the same conclusion. However, using 28S and 18S alone, only the monophyly of the subfamily Eurytominae and the genus *Plutarchia* was supported with statistical significance. The supports for the monophyly or non-monophyly of the other groups were not statistically significant. We attributed the insignificant supports of the Kishino–Hasegawa test to an insufficient amount of character covariance, due to the extreme conservativeness of the 28S and 18S genes. Nevertheless, other statistical evaluations significantly supported the monophyly of the genera *Sycophila* and *Plutarchia*, and the non-monophyly of the family. For example, the association of the outgroup member, *Monodontomerus minor*, with the ingroup excluding *Neorileya meridionalis* has the Bayesian posterior probability of 95%, which significantly supports the non-monophyly of the family.

4. Discussion

4.1. The preferred phylogenetic hypothesis of the family

We chose the phylogeny produced from the nuclear genes as our preferred hypothesis (Fig. 1). Clearly, the mitochondrial genes tell a quite different story. Such

discrepancy could have resulted from many possible evolutionary processes, such as historical mitochondrial gene horizontal transfer, ancient polymorphism, or different inheritance pathways. Because it is maternally inherited, the ability of mitochondrial DNA to reflect has been discussed extensively in the literature (e.g., Avise, 1991, 1994; Simon et al., 1994), although cases of statistically significant conflict between nuclear and mitochondrial genes are rare (e.g. Palumbi et al., 2001). On the other hand, nuclear ribosomal RNA genes are conservative and reliable markers for inferring phylogeny (e.g. Hillis and Dixon, 1991). Our study presented a clear case of significant conflict between the nuclear gene history and mitochondrial gene history, and we believe that the nuclear gene history is more likely reflect the species history in our case. At present time, we do not have an explanation for the conflict due to a lack of information regarding the general reproductive biology of these parasitic wasps.

This is the first phylogenetic hypothesis to be proposed for the family Eurytomidae. The phylogeny is not fully resolved, and many resolved nodes are not well supported. Clearly, more data are needed. In this regard, we suggest that further studies should focus on nuclear genes, not mitochondrial genes. We only have a limited sampling of taxa, considering that there are more than 1420 species in the family. However, several patterns are clearly demonstrated on our phylogenetic trees. With the phylogeny, a taxonomic revision is in order.

4.2. Taxonomic revision

The family Eurytomidae is not monophyletic. Our phylogenetic hypothesis clearly demonstrated that the subfamily Eurytominae is more closely related to the family Torymidae (genera of *Monodontomerus* and *Megastigmus*) than to subfamily Rileyinae (*Neorileya meridionalis*, Fig. 1). The subfamily Eurytominae is monophyletic. This conclusion is strongly supported by several statistical tests and is concordant with Campbell et al. (2000). Based on our phylogeny, we propose to elevate the subfamily Rileyinae to family Rileyidae. We do not have representatives from the subfamily Heimbrinae. On the basis of taxonomic stability, we suggest that it remains in the family Eurytomidae.

The two largest genera in the family, *Eurytoma* and *Bruchophagus* are clearly not monophyletic. Although this conclusion did not receive statistical support in some occasions, it is the best explanation of our data. In addition, a preliminary morphological study by Chen et al. (unpublished data) corroborated this conclusion. As taxonomy should reflect historical relationships, these non-monophyletic genera should be divided. Three *Bruchophagus* species (*ononis*, *gibbus*, and sp1) are more closely related with genus *Tetramesa* than other *Bruchophagus* species. The genus *Tetramesa* is a large genus and includes more than 188 species. We only have one representative species of the genus in our study, and therefore, we tentatively place the three *Bruchophagus* species in the genus *Tetramesa*. Three *Eurytoma* species (*orchidearum*, sp1, and sp 3) are more closely related to the genus *Isosomodes* than to other *Eurytoma* species. We suggest that these three species be transferred to the genus *Isosomodes*. We anticipate that future phylogenetic studies will discover a similar pattern, in which portions of the "trash can" genera (e.g. *Eurytoma* and *Bruchophagus*) are closely associated with different small genera.

The current taxonomy of the family Eurytomidae is primarily based on the concepts of "synthetic systematics" (Mayr, 1969), and it does not reflect the genealogy of the groups. Most genera, particularly the monotypic genera, were defined by unique lifestyle or occupancy of unique habitats, and many of them have probably evolved from within the current genus *Eurytoma* or genus *Bruchophagus*. This situation is similar to the well-known case of birds versus reptiles. Future taxonomic revision should focus on transferring species from the two "trash can" genera to other small genera. Our results corroborate the appeal by Bouček (1988b), who suggested that the most pressing questions at present are the limits of *Eurytoma*, *Bruchophagus*, and the genera closely related to them.

Our phylogeny strongly supports the monophyly of genera *Sycophila* and *Plutarchia* as well as the close association of the genera *Aiolomorphus*, *Tenuipetiolus*, *Bephratelloides*, and *Phylloxeroxenus*. Furthermore, our phylogeny reveals a high inconsistency with the current classification. For example, Zerova (1988) placed the genera *Tetramesa*, *Aiolomorphus*, and *Isosomodes* in the subfamily of Harmolitinae, but the three genera locate on different clades on our tree. Clearly, substantial revision of the classification of the family Eurytomidae based on a more comprehensive phylogeny is needed, and we hope that this study will serve as a catalyst in drawing more attention to this little known but economically very important group.

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Appendix. Specimens examined. IOZ=Institute of Zoology, Chinese Academy of Sciences, Beijing; ROM = Royal Ontario Museum, Toronto

Species	Locality	Reference specimens voucher number	GenBank Accession numbers			
			28S	18S	16S	COI
Ingroup						
Family Eurytomidae						
Subfamily Eurytominae						
Aiolomorphus rhopaloides	China: Fanjing Mt., Guizhou	IOZ 020001	AY317172	AY317185	AY317212	N/A
Bephratelloides cubensis	Indonesia: East of Kalimantan, Kutai	ROM 920118	AY317173	AY317237	N/A	N/A
Bruchophagus ononis	China: Fanjing Mt., Guizhou	IOZ 020003	AY317170	AY317203	N/A	AY317223
Bruchophagus sp1	China: Riwoqê Tibet	IOZ 010002	AY317164	AY317199	N/A	AY317226
Bruchophagus sp2	Guyana: Rupununi district: Kurupukari	ROM 905047	AY317169	N/A	N/A	AY317239
Bruchophagus sp3	China: Oamdo Tibet	IOZ 010001	AY317160	AY317189	N/A	N/A
Bruchophagus gibbus	China: Dayao Mt., Guangxi	IOZ 990001	AY317155	AY317196	AY317210	AY317227
Eurytoma manilensis	China: Beijing	IOZ 020005	AY317171	AY317191	AY317216	N/A
Eurytoma orchidearum	China: Kangding, Sichuan	IOZ 010003	AY317163	AY317187	AY317213	AY317233
Eurytoma verticillata	China: Baoxing, Sichuan	IOZ 010004	AY317178	AY317194	AY317218	AY317225
Eurytoma sp1	China: Fanjing Mt., Guizhou	IOZ 020004	AY317175	AY317186	AY317209	N/A
Eurytoma sp2	China: Riwoqê, Tibet	IOZ 010005	AY317166	AY317188	AY317214	AY317236
Eurytoma sp3	China: Tianmu Mt., Zhejiang	IOZ 980001	AY317162	AY317190	AY317220	AY317224
Isosomodes gigantenus	Guyana: Mazaruni-Potaro district: Amatuk fall	ROM 905032	AY317168	AY317193	N/A	AY317235
Phylloxeroxenus phylloxerae	Indonesia: East of Kalimantan, Kutai	ROM 920116	AY317176	N/A	N/A	AY317238
Plutarchia indefensa	China: Dayao Mt., Guangxi	IOZ 990002	AY317179	N/A	AY317207	AY317228
Plutarchia sp1	China: Napo, Guangxi	IOZ 200001	AY317181	AY317192	AY317211	AY317222
Sycophila concinna	China: Fanjing Mt., Guizhou	IOZ 020008	AY317167	AY317202	AY317219	AY317232
Sycophila taprobanica	China: Fanjing Mt., Guizhou	IOZ 020009	AY317159	AY317200	AY317208	AY317230
Sycophila sp1	China: Xinhe, Xinjiang	IOZ 020010	AY317158	AY317201	N/A	AY317231
Sycophila variegata	China: Napo, Guangxi	IOZ 200002	AY317157	AY317183	AY317206	AY317234
Tenuipetiolus medicaginis	Indonesia: East of Kalimantan, Kutai	ROM 920118	AY317174	N/A	N/A	AY317242
Tetramesa bambusae	China: Liuba, Shaanxi	IOZ 990003	AY317180	AY317198	AY317217	AY317229
Subfamily Rileyinae						
Neorileya meridionalis	Guyana: Mazaruni-Potaro District: Kaieteur fall	ROM 905018	AY317156	AY317195	AY317215	AY317243
Outgroup						
Family Torymidae						
Megastigmus dorsalis	China: Fanjing Mt., Guizhou	IOZ 020006	AY317161	AY317197	AY317205	AY317240
Monodontomerus minor	China: Fanjing Mt., Guizhou	IOZ 020007	AY317165	AY317182	N/A	AY317241
Family Chalcididae						
Brachymeria lasus	China: Fanjing Mt., Guizhou	IOZ 020002	AY317177	AY317184	AY317204	AY317221

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