Evolution of the hymenopteran megaradiation

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ABSTRACT

The Hymenoptera – ants, bees and wasps – represent one of the most successful but least understood insect radiations. We present the ﬁrst comprehensive molecular study spanning the entire order Hymenoptera. It is based on approximately 7 kb of DNA sequence from 4 gene regions (18S, 28S, COI and EF-1α) for 116 species representing all superfamilies and 23 outgroup taxa from eight orders of Holometabola. Results are drawn from both parsimony and statistical (Bayesian and likelihood) analyses, and from both by-eye and secondary-structure alignments. Our analyses provide the ﬁrst ﬁrm molecular evidence for monophyly of the Vespina (Orussioidea + Apocrita). Within Vespina, our results indicate a sister-group relationship between Ichneumonoidea and Proctotrupomorpha, while the stinging wasps (Aculeata) are monophyletic and nested inside Evasiomyrmoidea. In Proctotrupomorpha, our results provide evidence for a novel core clade of proctotrupoids, and support for the recently proposed Diaprioidea. An unexpected result is the support for monophyly of a clade of wood-boring sawﬂies (Xiphydrioidea + Siricoidea). As in previous molecular studies, Orussioidea remain difﬁcult to place and are either sister group to a monophyletic Apocrita, or the sister group of Stephanidae within Apocrita. Both results support a single origin of parasitism, but the latter would propose a controversial reversal in the evolution of the wasp-waist. Generally our results support earlier hypotheses, primarily based on morphology, for a basal grade of phytophagous families giving rise to a single clade of parasitic Hymenoptera, the Vespina, from which predatory, pollen-feeding, gall-forming and eusocial forms evolved.

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1. Introduction

The Hymenoptera represent one of the most successful Meso-zoic radiations of insects (Grissell, 1999). The major groups were established by the Late Jurassic, with 22 superfamilies and many of the 89 extant families appearing by the Mid to Late Cretaceous (Rasnitsyn, 1988, 2002b). The basal hymenopteran lineages are either pollen or shoot feeders in staminate pine cones, or external or internal leaf feeders, with subsequent transitions through stem- and wood-boring habits in living or dead plant tissue (Rasnitsyn, 2002b; reviewed in Sharkey, 2007). Parasitism appears to have evolved only once in the Vespina (Orussioidea + Apocrita), and led to an explosive radiation in the “Parasitica”. The Chalcidoidea alone are estimated to contain more than 500,000 species with the bulk of the diversification occurring after the Cretaceous boundary (Heraty and Darling, 2009). From these parasitic ancestors, novel behavioral shifts to predation, pollen feeding, provisioning and the development of eusociality in the Aculeata occurred, and through gall-making, reversals to phytophagy in several different lineages (Eggerton and Belshaw, 1992; Heraty, 2009). Hymenoptera are pervasive in almost all terrestrial habitats and have tremendous influence as agricultural and human pests, beneﬁcial control agents of other arthropods, and plant pollinators. However, their phylogeny – and hence the origin of this tremendous diversity – has not been well understood.

Hymenopterans are traditionally divided into Symphyta [broad-waisted, mainly phytophagous] and Apocrita (with a wasp-waist, parasitic ancestor) (Gauld and Bolton, 1988). The current consensus view holds that symphytans constitute a paraphyletic grade with Xyeloidea, Tentredinoidea, Pamphilioidea, Cephioidea, Siricoidea (Anaxyelidae + Siricidae), Xiphydrioidea and Orussioidea leading to Apocrita (Sharkey, 2007; Vilhelmsen, 2006; Vilhelmsen...
et al., 2010). Three clades are considered particularly well supported: Unicalcarida (all Hymenoptera except Xyelidae, Tenthredinoidae and Pamphilioidae), Vespsia (Orussidae + Apocrita) and Apocrita (Rasnitsyn and Zhang, 2010; Ronquist et al., 1999; Schulmeister, 2003a,b; Vilselmsen, 2006; Vilselmsen et al., 2010).

Evidence for this scenario is largely derived from morphological analyses (Rasnitsyn and Zhang, 2010; Schulmeister, 2003a,b; Vilselmsen, 1997, 2001, 2006; Vilselmsen et al., 2010), with some of the crucial pieces dating back to classical works by Rasnitsyn (1969, 1980, 1988) and Gibson (1985). Only a few molecular or combined studies address these basal relationships (Schulmeister, 2003b; Schulmeister et al., 2002). In the most comprehensive molecular analysis of symphytan relationships to date, based on 2.9 kb of ribosomal and mitochondrial sequence data, Schulmeister (2003b) reported Bremer support values of only 3–6 for clades basal to the divergence of Cepheoidae, and little resolution beyond that point. Importantly, resolution of Unicalcarida was dependent entirely on morphological data.

Much of the framework for our current understanding of apocritan relationships was established by the groundbreaking contributions of the Russian palaeoentomologist Alexandr Rasnitsyn (1969, 1980, 1988; Rasnitsyn and Zhang, 2010). Based on careful evaluation of morphological and fossil evidence, he divided Apocrita into four lineages (see color legend in Fig. 1): Ichneumonoidea (his Ichneumonomorpha), Aculeata (his Vespomorpha), Proctotrupomorpha and Evaniomorpha (Rasnitsyn, 1988, 2002b). Ichneumonoidea and Aculeata have long been recognized as natural groups, while the latter were novel concepts. Rasnitsyn had further proposed Ichneumonoidea and Aculeata as sister groups (Rasnitsyn and Zhang, 2010). Ronquist et al. (1999) expressed Rasnitsyn’s evidence in terms of quantitative characters and subjected them to parsimony analysis. While Ichneumonoidea and Aculeata were recovered as monophyletic, Proctotrupomorpha and Evaniomorpha were not. A subsequent study with modified wing characters showed even less resolution (Sharkey and Roy, 2002). Recently, Rasnitsyn and Zhang (2010) proposed that Evaniomorpha (sensu lato) were not monophyletic and divided them into three distinct lineages, Stephanomorpha (Stephanoidae), Ceraphronomorpha (Ceraphronoidea, Megaloroidae and Trigonaloidae), and a reduced Evaniomorpha (sensu stricto) that includes just Evanioidea.

Molecular analyses have provided some insight into apocritan relationships, but also contradictory results (Castro and Dowton, 2006; Dowton and Austin, 1994, 2001; Dowton et al., 1997; Sharanowski et al., 2010). An early study supported monophyly of Evaniomorpha s.l. (Dowton and Austin, 1997), but later analyses based on broader taxon sampling and more sequence data suggested that they formed a grade with respect to Aculeata (Castro and Dowton, 2006; Dowton and Austin, 2001). The trend was the opposite for Proctotrupomorpha, in which later and more comprehensive analyses (Castro and Dowton, 2006; Dowton and Austin, 2001) supported its monophyly, despite early indications to the contrary (Dowton and Austin, 2001; Dowton et al., 1997; see also Sharanowski et al., 2010). Similarly, a sister–group relationship between Ichneumonoidea and Aculeata was supported in earlier analyses (Dowton and Austin, 1994; Dowton et al., 1997), but later with Aculeata usually nested within Evanionioidea (Castro and Dowton, 2006; Dowton and Austin, 2001). None of the molecular analyses that included at least one other symphytan outgroup ever supported a sister–group relationship between Orussidae and Apocrita (Dowton and Austin, 1994, 2001; Schulmeister, 2003b); nor did they ever include a broad sampling of both Symphyta and Apocrita in the same analysis.

Relationships among Evanionioidea, Proctotrupomorpha, Ichneumonoidea and Aculeata have been equivocal, with no emerging consensus between morphological and molecular datasets (Rasnitsyn and Zhang, 2010). The same is true for relationships within Evanionioidea s.l. and Proctotrupomorpha. However, two new clades appeared consistently in the molecular analyses. The first falls within Proctotrupomorpha and consists of the Diapriidae, Monomachidae and Maamingidae (Castro and Dowton, 2006; Dowton and Austin, 2001), a clade that Sharkey (2007) proposed as Diaprioidae. The second is within Evanionioidea s.l. and consists of Trigonaloidae and Megaloroidae (Dowton and Austin, 2001; Dowton et al., 1997). Within Proctotrupomorpha, either Platygastroidea or Diaprioidae (sensu Sharkey, 2007) appeared as the sister–group of Chalcidoidea (Castro and Dowton, 2006; Dowton and Austin, 1994; Dowton et al., 1997). Based on EST analyses, Sharanowski et al. (2010) proposed a very different hypothesis, in which Chalcidoidea were excluded from Proctotrupomorpha, but the taxon sampling was minimal (10 Hymenoptera) and the results varied depending on method of analysis. Importantly, morphological studies indicate that the chalcidoid sister group is Mymarommatoidae (Gibson, 1986, 1999), a group not sequenced prior to our study. Two traditional superfamilies, Evaniioidea and Proctotrupoidae sensu stricto (without Diaprioidae), were not recovered as monophyletic in any of these earlier molecular analyses. Monophyly of Aculeata was always demonstrated; however, too few taxa were included to test superfamily relationships within Aculeata.

Previous molecular studies of hymenopteran phylogeny used mitochondrial 16S and COI and small fragments of ribosomal 18S and 28S (D2–D3), or more recently, EST data. Generally, these studies focused on either Symphyta (Schulmeister, 2003b; Schulmeister et al., 2002) or Apocrita (Castro and Dowton, 2006; Dowton and Austin, 1994, 2001; Dowton et al., 1997). Here, we attempt to increase our understanding of the relationships of Hymenoptera using a more complete analysis spanning the entire order and including all superfamilies. Mymarommatoidae are included for the first time, and we employ extensive outgroup sampling outside of Hymenoptera. We combined approximately 7 kb of sequence data from four gene regions that include nearly complete 18S, 28S, EF-1α and COI. The analysis represents part of the Hymenoptera Assembling the Tree of Life effort, and will be complemented by more detailed studies of target subgroups.

2. Materials and methods

Taxonomic Sampling – A total of 116 species of Hymenoptera were sampled, representing 65 families and all 22 superfamilies (sensu Sharkey, 2007). Twenty-four families were not sampled; either because they are extremely rare (Austrocynipidae, Austroniidae, Embolemidae, Peradeniidae and Sclerogibbidae) or they were closely related to taxa already sampled (several families of Chalcidoidea and Apoidea). Taxa were chosen to represent the breadth of taxonomic and biological diversity across Hymenoptera. Twenty-three outgroup taxa were selected. Composite outgroup taxa, as indicated in Table 1, were developed by concatenating sequences from different taxa either from our own sequences or those deposited in Genbank. Outgroup taxa covered a diversity of taxa both closely and distantly related to Hymenoptera. Voucher specimens are deposited at the American Museum of Natural History (AMNH), University of California, Riverside (UCR), Swedish Museum of Natural History (NHRS) or the University of Kentucky (UKY).

Molecular data – Data for four gene regions were gathered using previously published primers for 28S (Belshaw and Quicke, 2002; Campbell et al., 1993, 2000; Gillespie et al., 2005b; Harry et al., 1996; Kim, 2003; Nunn et al., 1996; Schulmeister, 2003b; Wiegmann et al., 2000), 18S and COI (Schulmeister, 2003b). Amplification and sequencing followed established protocols at UCRC (Heraty et al., 2004), AMNH (Schulmeister, 2003b), UKY (Sharkey...
Fig. 1. Hymenopteran relationships based on Bayesian inference (MrBayes, four runs of eight chains each, 100 M gen.) of combined 18S, 28S, EF-1a and CO1 data with nt3 included (7190 bp). Ribosomal sequences aligned by-eye with hypervariable regions excluded. Posterior probability (PP) indicated on branches (in percent); branches with PP below 50% collapsed. Scale is different for outgroup and ingroup parts of tree.
Table 1
List of taxa and gene regions sampled for Hymenoptera and outgroups as discussed in text.

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Tenthredinoida

Argidae

Argusiger (Cresson) 18S-441 F 5'-TGG TGA GGT TTC CCG TGT T-3' 18S-1299 R 5'-GCA GCA TAT TAC TTT TTA GCC-3'.

Blastocotomidae

Blastocotoma sp.; F2R2 5'-AAA TTA CCC ACT CCC GGC A-3'.

Cimbicidae

Corynis cristisonis (Rossi) 18S-441 F 5'-AAG CGN GAR CGT GGT ATC AC-3' 18S-1299 R 5'-GCA GCA TAT TAC TTT TTA GCC-3'.

Diprionidae

Monocotenus juniperi (Linnaeus) 18S-441 F 5'-AAA TTA CCC ACT CCC GGC A-3'.

Pergidae

Decameria similis (Enderlein) 18S-441 F 5'-AAA TTA CCC ACT CCC GGC A-3'.

Tenthredinidae

Athalia rosea (Linnaeus) 18S-441 F 5'-AAA TTA CCC ACT CCC GGC A-3'.

Trigonolidae

Trigonolus pulchella (Cresson) 18S-441 F 5'-AAA TTA CCC ACT CCC GGC A-3'.

Vespidae

Bradybomyidae

Chyphotes mellite (Blake) 18S-441 F 5'-AAA TTA CCC ACT CCC GGC A-3'.

Formicidae

Ambylopone pallipes (Haldeman) 18S-441 F 5'-AAA TTA CCC ACT CCC GGC A-3'.

Formenta moki Wheeler 18S-441 F 5'-AAA TTA CCC ACT CCC GGC A-3'.

Lyccophila haeohens Weber 18S-441 F 5'-AAA TTA CCC ACT CCC GGC A-3'.

Myrmica tahoensis Ward, Brady 18S-441 F 5'-AAA TTA CCC ACT CCC GGC A-3'.

Paraparacera clavata (Fabricius) 18S-441 F 5'-AAA TTA CCC ACT CCC GGC A-3'.

Mutilillidae

Dasyinyxia aurea (Cresson) 18S-441 F 5'-AAA TTA CCC ACT CCC GGC A-3'.

Pompilidae

Aporus niger (Cresson) 18S-441 F 5'-AAA TTA CCC ACT CCC GGC A-3'.

Rhopalosomatidae

Rhopalosoma nearticicum Brues 18S-441 F 5'-AAA TTA CCC ACT CCC GGC A-3'.

Sappidae

Sappya pupila Cresson 18S-441 F 5'-AAA TTA CCC ACT CCC GGC A-3'.

Scolidae

Scolia verticalis Fabricius 18S-441 F 5'-AAA TTA CCC ACT CCC GGC A-3'.

Tiphiidae

Colocistis (=aglyptacros) cf. sulcatus (M.B.K.) 18S-441 F 5'-AAA TTA CCC ACT CCC GGC A-3'.

Vespidae

Metopopolyta cingulata (Fabricius) 18S-441 F 5'-AAA TTA CCC ACT CCC GGC A-3'.

Mycolyca flavitarsis (Saussure) 18S-441 F 5'-AAA TTA CCC ACT CCC GGC A-3'.

Xiphidioidae

Xiphidius sp.; F2R2 5'-AAA TTA CCC ACT CCC GGC A-3'.

Xyelidae

Xyelus sp.; F2R2 5'-AAA TTA CCC ACT CCC GGC A-3'.

a Composite taxa comprised of sequences from more than one taxons follows: Acrididae: 18S:1904, 28S:3405, COI:786, EF-1-alpha (F2)

b A Combination of IY654546, IY654457, and IY654522.

e t., 2006) and FSU. New 18S primers were developed by D.H. (18S-441 F 5'-AAA TTA CCC ACT CCC GGC A-3'; 18S-1299 R 5'-TGG TGA GGT TTC CCG TGT T-3').

The F2 copy of EF-1 alpha was amplified using primers from Danforth et al. (1999) and four EF-1 alpha primers developed by D.M. (2002). The F2 copy of EF-1 alpha was amplified using primers from Danforth et al. (1999) and four EF-1 alpha primers developed by D.M. (2002). The F2 copy of EF-1 alpha was amplified using primers from Danforth et al. (1999) and four EF-1 alpha primers developed by D.M. (2002). The F2 copy of EF-1 alpha was amplified using primers from Danforth et al. (1999) and four EF-1 alpha primers developed by D.M. (2002).

Outgroup sequences were generated from available sequences on GenBank or supplied by Tree-of-Life collaborators in the Diptera (Wiegmann), Formicidae (Ward, Brady), and Coleoptera (Farrell, Maddison) projects (Table 1).

2.1. Alignment

Eye Alignment (EA) – Ribosomal DNA was aligned manually by D.H. for a total of 6695 bp (18S:2014, 28S:4681). A total of 1326 bp in 19 variable regions in which alignment of ribosomal sequence was extremely difficult were excluded. An additional 46 bases were excluded from the outgroup alignment because these were missing for all Hymenoptera. The final by-eye alignment was 8576 bp without exclusions, and 7190 bp with data exclusions (18S:1904, 28S:3405, COI:786, EF-1x:1095).

initially followed the secondary structure model of Arthropoda by Gillespie et al. (2005a) with refinements based on an ichneumonid model (Gillespie et al., 2005c). For 28S, the secondary structure model was derived from Ichneumonoidea (Gillespie et al., 2005c), Chalcidoidea (Gillespie et al., 2005b), Evanidae (Deans et al., 2006), and the honeybee (Gillespie et al., 2006). All regions exhibiting variability in sequence length (Kolaczkowski and Thornton, 2007) and base composition (e.g. hairpin-stem loops) were evaluated in the program Mfold (version 3.1; http://mfold.bioinfo.rpi.edu/cgi-bin/dna-form1.cgi), which folds RNA based on free energy minimizations (Mathews et al., 1999; Zuker et al., 1999). Potential helices were confirmed by the presence of compensatory base changes across taxa included in the matrix. A total of 50 regions of ambiguous alignment, representing highly variable loop regions and 1370 bp, typically in highly variable loop regions, were excluded from final analyses. The structural alignment for analysis was 6993 bp (18S:1860, 28S:3252, COI:786, EF-1 (nt3) 365 17.3 26.8 34.1 21.8 95.3 TPM1uf + I + G
EF1 (nt1 & 2) 730 29.6 22.0 27.6 20.8 15.8 TrN + I + G
COI (nt1 & 2) 524 24.7 18.6 19.3 37.4 48.5 TVM + I + G
COI (nt3) 262 45.2 45.1 6.6 3.1 100 GTR + I + G
EF1-alpha (F2 copy) 1095 25.5 22.8 26.1 25.6 42.3 SYM + I + G
EF1 (nt1 & 2) 730 29.6 22.0 27.6 20.8 15.8 TVM + I + G
EF1 (nt3) 365 17.3 26.8 34.1 21.8 95.3 TPM1uf + I + G
COI + EF1 (nt3 only) 627 36.1 21.5 13.2 35.2 97.3 GTR + I + G

Table 2
Gene partition or combination Aligned base pairs A (%) T (%) C (%) G (%) % Parsimony informative bp BIC model
Eye Alignment (EA) with nt3 7190 24.9 24.4 23.2 27.5 41.6 GTR + I + G
EA without nt3 6563 24.5 23.5 23.3 28.7 36.3 SYM + I + G
Secondary Structure with nt3 6993 25.1 24.2 23.1 27.6 40.8 GTR + I + G
SS without nt3 6366 24.7 23.3 23.2 28.8 35.2 SYM + I + G
28S (eye) 3405 23.2 21.0 24.6 31.2 41.8 SYM + I + G
28S (SS) 3252 23.5 20.7 24.4 31.4 40.1 GTR + I + G
18S (eye) 1904 25.1 25.0 22.8 27.1 31.1 GTR + I + G
18S (SS) 1860 25.3 24.7 22.8 27.2 30.5 Sym + I + G
COI (nt1 & 2) 524 24.7 18.6 19.3 37.4 48.5 TVM + I + G
COI (nt3) 262 45.2 45.1 6.6 3.1 100 GTR + I + G
EF1-alpha (F2 copy) 1095 25.5 22.8 26.1 25.6 42.3 SYM + I + G
EF1 (nt1 & 2) 730 29.6 22.0 27.6 20.8 15.8 TVM + I + G
EF1 (nt3) 365 17.3 26.8 34.1 21.8 95.3 TPM1uf + I + G
COI + EF1 (nt3 only) 627 36.1 21.5 13.2 35.2 97.3 GTR + I + G

2.2. Phylogenetic analyses

We explored parsimony, Maximum Likelihood (ML) and Bayesian approaches to the analysis of our data set both because a wide range of opinions on the merit of these approaches exist among Hymenopterists and because we believe that the methods differ in their strengths and weaknesses, such that a combined approach gives a better chance to evaluate the phylogenetic signal in the data. For instance, the Bayesian approach tends to be more robust to modest over-parameterization (Huelsenbeck and Rannala, 2004) while the maximum likelihood approach may be less sensitive to long-branch attraction.

Bayesian analyses – Bayesian analyses were performed using MrBayes 3.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Except as noted, we used the default settings. The combined data set was analyzed using a GTR + I + G model with all substitution model parameters unlinked across the four gene partitions (18S, 28S, COI and EF-1α). Compared to the models suggested by the model-testing procedure (Table 2), GTR + I + G was the closest, more parameter-rich model implemented in MrBayes for all partitions except 18S and complete EF-1α, where the slightly simpler SYM + I + G model that constrains all stationary state frequencies to be equal was implied. Because Bayesian inference is known to be robust to modest over-parameterization (Huelsenbeck and Rannala, 2004), and we expect this to be true in particular of the state frequency parameter, we also analyzed these partitions using the GTR + I + G model. We also noted that model testing suggested that stationary state frequencies should not be assumed to be equal for the EF-1α nt12 or 28S partitions, which should evolve in a similar fashion to the EF-1α and 18S partitions. The nt3 of both COI and EF-1α is highly saturated, and especially for COI has an extremely high AT bias (Table 2), which might warrant exclusion of nt3 (Castro and Dowton, 2006; Dowton and Austin, 2001) even though the gamma model of rate variation across sites should largely accommodate rate differences among codon positions. Each gene, with nt3, was also analyzed separately under an unpartitioned GTR + I + G model. The temperature coefficient was set to 0.1 to increase the acceptance rate of swaps between Metropolis-coupled chains. We used a relative burn-in of 25% and ran four independent analyses with eight chains each in increments of 10 M generations until the tree samples reached a standard deviation of split frequencies (ASDSF) of 0.01, or until the analyses hit 100 M generations if that target was not reached. For the separate gene analyses, we used an ASDSF of 0.02.

Relative support for selected groups based on the EA and SS alignments was estimated using the posterior model odds (PMO). Bayesian model comparison is more typically based on evaluation of the Bayes factor (BF), which is the same as the PMO when the prior model odds are 1:1 (Gelman et al., 2004). However, the PMO has some distinct advantages in this context. First, calculating BF for monophyly hypotheses is demanding, requiring at least one full MCMC analysis for each BF unless the taxon set is small. Second, BF can be misleading in some cases because of the dependency between different parts of the tree.

Assume, for instance, that we were interested in testing the null hypothesis of monophyly of Ichneumonoidea + Aculeata, an unlikely group according to our results. If we assume equal prior probability of all trees, a naive BF test is unlikely to provide evidence against the null hypothesis. This is simply because there are so few trees that are consistent with the hypothesis and many orders of magnitude more trees that conflict with it. If we were to take into account in the prior that some other groups are likely to be supported – like Unicalcarida, Ichneumonoidea, and Aculeata – then the prior odds would shift dramatically and the BF is more likely to provide evidence against the null hypothesis. One can
argue that the PMO provides a more balanced view of the contrast-
ing hypotheses and it is also much easier to calculate, simply being
the ratio of the number of trees in the MCMC sample supporting
the hypothesis divided by the number of trees conflicting with it.
When one group was not represented in the MCMC sample, we ob-
tained a conservative estimate of the PMO by simply adding one
sample to the missing group, based on the notion that the next
MCMC sample could go against the signal seen in all the previous
ones.

RAxML analyses – A different likelihood approach was taken for
the combined (EA and SS, and with or without nt3) and single gene
data sets using RAxML v.7.0.0 (Stamatakis et al., 2007, 2008). Gene
regions were partitioned for separate optimization of per-site sub-
stitution rates. Parameter estimation and bootstrapping were car-
cied out locally on a 2 node, 8 processor Power Mac G5 Quad
Beowulf-like mini-cluster. Ten randomized starting trees were
generated to determine the initial rearrangement setting (-i) and
number of distinct rate categories (-c). Independent searches of
1000 repetitions were used to find the best-known likelihood
(BKL) tree and bootstrap searches using the “rapid hill climbing
algorithm” (Stamatakis et al., 2007). Additional analyses, including
single gene searches, were conducted using the CIPRES portal
(http://www.phylo.org/sub_sections/portal/) and the rapid boot-
strap search algorithm (RBS) (Stamatakis et al., 2008), in which
bootstrap analyses are conducted first with 500 repetitions, fol-
lowed by fast and then slow searches on the sampled trees to find
the BKL tree.

Parasimony analyses – Heuristic tree searches were conducted in
TNT version 1.1 (Goloboff et al., 2008) using the New Technology
Search with default settings, except for using a sectorial search,
ratchet weighting probability of 5% with 200 iterations, tree-
drifting of 50 cycles, tree-fusing of 5 rounds, and best score hit of
25 times, followed by swapping to completion on all trees found.
Analyses were conducted on the EA and SS alignments both with
and without nt3 for coding regions. Support was calculated using
non-parametric bootstrapping with 1000 replicates.

3. Results

3.1. Bayesian results

The Bayesian trees were highly resolved for most relationships
across Hymenoptera, with the greatest discrepancy in placement
of Xyelidae at the base of Hymenoptera (Fig. 1; Table 3). The
ASDSF fell below 0.01 in 50 M generations or less for all combined
data sets except SS, for which the ASDSF was still 0.020 after
100 M generations, at which point we stopped the analysis. Virtu-
ally all of the heterogeneity among MCMC runs in the SS analyses
concerned the position of Orussoidea, Stephanoidea, and the res-
olution of Eevaniomorpha and Acaleuta. The ambiguous clades
were poorly supported in all runs and the discordance among
runs only concerned their exact posterior probabilities, including
which clades climbed above the 50% mark. Other parts of the tree,
including the internal relationships of Eevaniomorpha s.l. and
Acaleuta, were consistently resolved and the variation among
runs in estimated posterior probabilities (PP) of clades was negli-
gible.

There were distinct differences between the EA and SS analyses.
Orussoidea were placed as the sister group of Stephanoidea inside
the Eevaniomorpha s.l grade in the EA analyses (Fig. 1), but as the
sister group to the Apocrita, including Stephanoidea, in the SS anal-
yses (Table 3). However, both signals were present with intermedi-
ate levels of support in the tree sets produced from each analysis.
With two exceptions noted below, the EAnt12 and SSnt12 results
were quite similar to the EA and SS results, respectively, although
posterior probabilities were lower for some clades and higher for
others (Table 3). There was weak Posterior Model Odds (PMO) sup-
port for Apocrita (excluding Orussidae) in the SS results, but not in
the EA (Table 4).

Nine clades were consistently recovered with high PP. They
included Hymenoptera (PP 95–100 percent), Unicalcarida (PP
96–100), Vespana (PP 96–100; PMO 22 or 30), Eanjoidea (PP 89–
100), Ichneumonoidea (PP 98–100; PMO 65 or 190), Proctotrupa-
morpha (PP 92–100; PMO 11 or 27), core Proctotrumpomorpha
(Proctotruidaeco including Myrmamorrtu, Diaprioidea and
Chalciidoidea) (PP 92–100; PMO 12 or 31), and Chalciidoidea
(PP 99–100) (Tables 3 and 4). Diapriidae (PP 77–77) and Diapriidea
(PP 98) were each monophyletic in analyses that included nt3
(EA and SS), but with exclusion of nt3 (EAnt12 and SSnt12) causing
both groups to be paraphyletic.

Bayesian analyses of the complete data sets placed either
Xyelidea and Tenthredinoida (EA; PP 96; Fig. 1) or Xyelidea,
Tenthredinoida and Pamphilioida (SS; PP 92) as a monophyletic
sister group to the Unicalcarida. However, the EAnt12 and SSnt12
analyses both rooted the hymenopteran tree between Xyelidea
and other Hymenoptera (PP 100). In the likelihood (RAxML), parsi-
mony (nt12) analyses, Xyelidea were always sister to the remain-
ing Hymenoptera (Figs. 2 and 3; Table 3), which is similar to
morphology-based hypotheses that treat Xyelidea as a monophy-
letic or paraphyletic sister group to the remaining Hymenoptera.
Xyelidea were monophyletic in most of the single gene analyses
but sister to the remaining Hymenoptera only with COI (Table
A1). Clearly, the unusual rooting of the EA and SS trees depends
critically on signal in third codon position sites. The outgroup
Holometabola were always monophyletic and sister to the Hyme-
noptera (Fig. 1).

More unexpectedly, the woodwasps – sricids, anayzelds and
xiphidiids but not orussids – emerged as monophyletic (PP
83–99; PMO 110 or 120). Proctotruidaeco sensu stricto is also mon-
ophyletic (PP 97–100; but excluding the basal Roproniidae in the
SS analysis). Trigonalidea always grouped strongly with Megaly-
rdoida (PP 97–100). Lastly, Ichneumonoidea were consistently
placed as sistergroup to a monophyletic Proctotrumpomorphapa (PP
85–92).

Analyses of the separate genes showed that much of the signal
resolving higher-level relationships emerged only after gene re-
ions were combined (Table A1). Of the four markers studied,
2PS provided the best resolution of basic nodes on its own. It
strongly supported monophyly of Unicalcarida (PP 93–100) and
Vespana (PP 93–100). In the eye alignments, there was additional
evidence for Proctotrumpomorphapa (PP 81). Additionally, there
was also some apparently spurious signal, such as the grouping of
Stephanidae with Ichneumonoidea (PP 93–96) in the secondary
structure alignments. Results of the 18S data analyses were much
less resolved but did support Unicalcarida (PP 77–91), Vespana
(PP 89–93) and core Proctotrumpomorphapa (PP 94–97). On their
own, the EF-1a and CO1 sequences provided little signal concerning
basal hymenopteran relationships. Of the few interesting higher clades
that were supported, some clearly appeared due to misleading sig-
ual, for example the grouping of Stephanidae with Apis mellifera
in the EF-1a analyses (PP 93–96). More interesting signal concerning
higher relationships included support for Diaprioidea (PP 94–96 in
CO1 analyses), Eevaniomorpa + Acaleuta (PP 79–91 in EF-1a anal-
yses) and core Proctotrumpomorphapa, including Chalciidoidea (PP 99
2PS and 83 in CO1 analyses) (Table A1).

3.2. Likelihood results

For the combined results, the ML trees were highly concordant
with the Bayesian results, both in terms of groups supported and in
disagreement between the eye and secondary structure alignments
Fig. 2. Maximum likelihood analysis (RAxML, RBS gamma search tree and 500 standard bootstrap replicates) of combined 18S, 28S, EF-1α and CO1 data with nt3 included (6993 bp). Ribosomal sequences aligned based on secondary structure information with regions of ambiguous alignment deleted. Single resulting tree with bootstrap proportions above 50% indicated on branches.
Fig. 3. Parsimony analysis (TNT, New Technology Search) of combined 18S, 28S, EF-1α and C01 data with nt3 excluded for coding genes: A, eye alignment (6563 bp; 22201 steps, consensus of 16 trees, r.i. 0.46); B, secondary structure alignment (6366 bp; 20277 steps, consensus of 172 trees, r.i. 0.47). Bootstrap proportions above 50% indicated on branches.
(Fig. 2; Table 3). The ML results supported the traditional sister-
group relationship between Xyeloidea and the remaining
Hymenoptera across both datasets although with poor support
(BS 55–57). Xyeloidea were monophyletic in the EA, SS and SSnt12
analyses (BS 70–97), and paraphyletic in the EAnt12 analysis, but
with no BS (clade present but with <50% support) for Macroxyel-
inae + remaining Hymenoptera (Table 3). Pamphilioidea consis-
tently appeared as sister to Unicalcarida, although with weak
support (BS <50–77). Orussoidae was sister to Apocrita only in
the SS results (no BS), and sister to Stephanoidea within Apocrita
in both EA analyses (Table 3). Aculeata is monophyletic across all
datasets although with weak support. Ichneumonoidea + Procto-
trupomorpha were monophyletic in all results, but without boot-
strap support. Mymaromatoidea were sister to Chalcidoidea
only in the EAnt12 analysis (no BS); otherwise a paraphyletic Dia-
prioidea were the sister group of Chalcidoidea, but with only weak
bootstrap support obtained in the SS analyses (Table 3).

Single gene analyses were nearly identical to those from the
Bayesian results (Table A1). Contrary to the combined results,
28S alone provided weak support for a monophyletic Xyelo-
da + Tenthredinoidea for both the EA and SS alignments (BS 50
[SS] to 65 [EA]). No resolution of basal taxa was obtained from
18S alone. COI provided strong support (BS 88–89) for Xyeloidea
as sister to the remaining Hymenoptera; EF-1α grouped Xyeloidea
with Pamphilioidea, but otherwise the early branching events
within Hymenoptera were poorly resolved. A core Proctotrupedia,
including Chalcidoidea, was weakly supported in each of the 18S,
28S EA and COI analyses.

3.3. Parsimony analyses

The strict consensus trees from the TNT analyses based on the
complete EA and SS datasets were very poorly resolved other than
supporting a few stable groups including Hymenoptera (BS 87–91),
Unicalcarida (BS <50–52), Proctotrupedia (BS 57–71) and Chalci-
doidea (BS 88) (Table 3). Considerably more structure was ob-
tained from the nt12 analyses for both alignments (Fig. 3),
although the SS analyses were generally unresolved for Apocrita
and did not include Mymaromatoidea within Proctotrupomor-
pha (Fig. 3B). Results for the EAnt12 analysis (Fig. 3A) were gener-
ally concordant with the likelihood results for the same dataset.
Xyeloidea were paraphyletic, and the remaining Hymenoptera
monophyletic. Unicalcarida were monophyletic, but with
Cephioidea included within the woodwasp clade. Orussoidae were
sister to Stephanoidea in the EA analysis (Fig. 3A), but to Apocrita
in the SS analysis (Fig. 3B); neither hypothesis garnered BS support.
Aculeata were monophyletic (no BS), but Chrysidoidea were
scattered throughout the clade, and Ampulicidae were not placed
with Apoidea. Ichneumonoidea were monophyletic (BS 79) and sis-
ter to a monophyletic Proctotrupomorpha, which also included the
‘core Proctotrupomorpha’ clade. Diaprioidea were monophyletic
only in the EA results. Mymaromatoidea were sister to Mymari-
dae, rendering Chalcidoidea non-monophyletic in the parsimony
EAnt12 results, although Chalcidoidea were monophyletic (BS 92)
in the bootstrap analysis of the same dataset (Fig. 3A; Table 3).
Single gene analyses were largely unresolved but showed weak sup-
port for some groups (Table A1), including core Proctotrupedia with
Chalcidoidea (clade present in the 28S and COI nt1–3 analyses).

4. Discussion

Generally speaking, the combined analyses based on eye align-
ments were more resolved and agreed better with previous mor-
phology-based hypotheses of relationships than those based on
secondary-structure alignments, except for placement of
Orussoidae. This could potentially be due to observer bias towards
expected relationships because eye alignments of this size neces-
sarily use some grouping information to facilitate comparison
across sequences. However, the eye alignments were capturing all
of the stem region information as well as additional alignments
from within regions of ambiguous alignment. This latter fact in-
creases credibility in the eye alignment and the extra resolution;
however, we stress only those results that were robust to both
alignment protocols.

The secondary-structure (SS) alignment excluded regions of
ambiguous alignment (slip-strand compensation, expansion and
contraction, and loop regions) (Gillespie et al., 2004, 2005b,c),
which if included, may have added resolution. However the align-
ment of these regions is less objective and we chose to exclude
them. Fewer sites were included in the EA as hypervariable; how-
ever these regions generally corresponded with the SS exclusions.
The EA was also made longer by spreading the alignment of stem
regions to reduce homoplasy, which can be forced in a model-
based approach. This apparently resulted in a qualitatively better
signal best demonstrated in Aculeata, which are more resolved
and produce expected relationships.

Deletion of the third base position for COI and EF-1α had a ma-
jor impact on the parsimony analyses, resulting in both greater res-
olution and more comparable results to the other analyses. There
was little impact of this deletion on either the Bayesian or RAxML
analyses, which might be expected given that third codon positions
tend to be downweighted by the gamma model of rate variation
across sites because of their fast evolutionary rate. However, it is
interesting that third-codon positions nevertheless affected the
rooting of the hymenopteran tree in the Bayesian analyses. Appar-
ently, this is caused by spurious attraction in third-codon positions
among the long basal hymenopteran branches, possibly worsened
by non-stationary base frequencies in this part of the tree. Bayesian
inference is expected to be more sensitive to long-branch attrac-
tion than maximum likelihood because of the influence of branch-length priors (Kolaczkowski and Thornton, 2007). Thus,
we see no reason given these results to question the morphol-
ogy-based consensus view on the first branching events in the
Hymenoptera.

The results presented in Figs. 1–3 provide a summary of the
well-supported clades across the majority of results (Table 3). All
analyses were almost identical in their support for various high-
level taxa, and recovered many of the higher-level groups previ-
ously hypothesized by morphological evidence. Except for the
anomalous rooting of the Hymenoptera in some of the Bayesian
analyses, the primary differences were in the monophyly of Apo-
crita, with either inclusion or exclusion of Orussoidae in Apocrita.

4.1. Rooting and basal relationships of the Hymenoptera

Even given the poor taxon sampling, higher-level relationships
within Holometabola were well resolved in our analyses, with a
monophyletic Coleoptera + Neuropteraida (Megaloptera, Neuro-
ptera, Raphidioptera) + Amphiphiemoptera (Lepidoptera + Trichop-
tera) + Antiophora (Mecoptera + Diptera) as sister group to
Hymenoptera. These results conflict with the earlier hypothesis of
a sister-group relationship between Hymenoptera and Mecopte-
rida (Kristensen, 1999), but they are congruent with recent molec-
ular (McKenna and Farrell, 2010; Misof et al., 2007; Savard et al.,
2006; Schulmeister, 2003b; Wiegmann et al., 2009), as well as
morphological studies (Kukalová-Peck and Lawrence, 2004; Rasnitsyn,
1980, 2002a; Rohdendorf and Rasnitsyn, 1980). Thus, our results contribute to an emerging consensus with respect to
holometabolan ordinal relationships, and we expect our outgroups
to provide a reasonable signal for rooting the hymenopteran tree.
Recent morphological and molecular analyses suggest that early branching events in the Hymenoptera follow the pattern (Xyeloidea (Tenthredinoidea (Pamphilioidea, Unicalcarida))) (Rasnitsyn, 2002b; Schulmeister, 2003a,b; Schulmeister et al., 2002; Vilhelmsen, 1997, 2001; Vilhelmsen et al., 2010), but the evidence has been weak and there has been some uncertainty regarding the monophyly of the Xyeloidea. Except for the anomalous rooting in the Bayesian analyses of the complete alignments mentioned above (see also Fig. 1), our analyses (Bayesian nt12, ML, parsimony nt12, and single gene analyses of 28S, EF-1α and COI) uniformly support the pattern suggested by morphology, with Xyeloidea as either a mono- or paraphyletic sister group to Hymenoptera (Table 3, Table A1). The support is fairly strong in the Bayesian nt12 analyses, but poor in the ML and parsimony analyses (Fig. 2, Table 3). Monophyly of Xyeloidea appears to be impacted by the exclusion of nt3 for the coding regions, with exclusion favoring diphyle over monophyly (Fig. 3, Table 3). It is difficult to determine which one of these results is more reliable, leaving uncertainty regarding the status of the Xyeloidea.

Morphologically, the best-supported basal hymenopteran clades are the Unicalcarida, Vespina and Apocrita (Vespina excluding Orussoidea) (Rasnitsyn and Zhang, 2010; Schulmeister, 2003a,b; Schulmeister et al., 2002; Vilhelmsen, 2001). There has not been strong support for any of these groups in previous molecular analyses. In a recent matrix-based supertree approach, Davis et al. (2010) expressed similar results, but their results are hardly comparable as their analyses constrained the monophyly of Apocrita (Figs. 1–3). For the most part, higher relationships within this clade were placed as either sister to Vespina in the Bayesian and ML analyses (Figs. 1 and 2; PMO 9.8 or 13, Table 4), or within the woodwasp lineage in the parsimony (nt12) analysis (Fig. 3A). Excluding Cephoidea, our statistical results suggest that instead of being paraphyletic, the woodwasps may form a monophyletic group (Figs. 1 and 2). This result was consistent across most alignment and analytical methods (Tables 3 and 4), even though this same relationship was not supported in any of the single gene analyses except EF-1α.

Up to 16 morphological synapomorphies have been proposed for Xiphydrioidae + Vespina (Gibson, 1985; Rasnitsyn and Zhang, 2010; Vilhelmsen, 2001; Vilhelmsen et al., 2010), the majority of which are related to radical changes in the mesothoracic flight mechanism. The states in other woodwasps have usually been interpreted as more primitive stages in the transition to the Xiphydrioidae + Vespina flight mechanism, but might instead represent alternative directions in the early evolution of the new flight mechanism, which would be consistent with woodwasp monophyly. Recent morphological analyses included no characters that can be readily interpreted as woodwasp synapomorphies (Rasnitsyn and Zhang, 2010; Schulmeister, 2003a; Vilhelmsen, 1997, 2001), but this does not mean they do not exist. For instance, most Siricidae and the genus Xiphydria (unknown for other xiphydriid genera and Anaxyelidae) live in a symbiotic relationship with a fungus for which the females have pockets called mycangia (Kajimura, 2000). Such mycangia for symbiotic fungi are unknown in any other hymenopterans and may be a woodwasp synapomorphy. Cephoidea, Siricoidea and Xiphydrioidae share at least one potential morphological synapomorphy: an invagination on the distal labial palp segment with specialized rodlike sensilla. In Xiphydrioidae and Siricoidea, it becomes a deeply invaginated pocket, while it is absent from all Vespina (Vilhelmsen, 1996). Our results may contradict data based on thoracic features (Vilhelmsen et al., 2010), but may be supported by other character systems that need to be explored.

4.2. Relationships of Vespina (Orussoidea + Apocrita)

In contrast with all previous molecular analyses, our results support a single origin of parasitism (Vespina) across all analyses (Tables 3 and 4). This is highly satisfactory since monophyly of Vespina is probably the strongest result emerging from morphology-based analyses (Gibson, 1985; Rasnitsyn and Zhang, 2010; Schulmeister, 2003a,b; Schulmeister et al., 2002; Vilhelmsen, 1997, 2001, 2007; Vilhelmsen et al., 2010). Within Vespina, it is widely assumed that Orussoidea are the sister group of the remaining taxa, the Apocrita (Rasnitsyn and Zhang, 2010). This was the result we obtained from analyses of almost all of the secondary structure alignments (Figs. 2 and 3B; Tables 3 and 4). However, in the eye alignment, orussids grouped with stephanids inside Evaniomorpha s.l. Grouping orussids with stephanids would imply that orussids are derived apocritans that secondarily lost the wasp-waist. This is not unprecedented, as it is known to have occurred in other apocritan lineages (Gibson et al., 1999), although some critical features of the propodeal fusion, such as fusion of the metapleuron with the propodeum in Apocrita, are absent in the Orussidae and make hypotheses of secondary loss of the wasp-waist unlikely (Rasnitsyn and Zhang, 2010). On the other hand, there are unique morphological similarities shared between orussids and stephanids that suggest an orussid + stephanid clade might be correct. Similarities include the elongate basalare in Xiphydrioidae + Vespina flight mechanism, but may be supported by other character systems that need to be explored.

Orussids disregarded, the Evaniomorpha s.l. + Aculeata are monophyletic in almost all of our statistical results, with a monophyletic Aculeata nested in a paraphyletic Evaniomorpha s.l. (Figs. 1 and 2). For the most part, higher relationships within this clade were poorly supported, but two robustly supported clades are worth noting, i.e., Evanioidea and Trigonaloidea + Megalyroidea. Monophyly of Evanioidea (Evanioidea s.s.) has been widely doubted because of the lack of morphological synapomorphies, apart from the high attachment point of the metasoma, grouping these rather heterogeneous lineages. The Trigonaloidea + Megalyroidea clade was proposed in earlier molecular analyses (Castro and Dowton, 2006; Dowton and Austin, 2001; Dowton et al., 1997), although Rasnitsyn (1988) considered them to form part of an evaniomorph lineage that also included Stepehanidae, and more recently he placed the Trigonaloidea, Megalyroidea and Ceraphronoidea in the Ceraphronomorpha (Rasnitsyn and Zhang, 2010). Notably, none of our results supported a monophyletic Ceraphronomorpha.

Aculeata were generally recovered as monophyletic, as expected, but resolution within the clade was poor, especially in the secondary structure analyses. The greatest congruence with traditional morphological groupings was obtained with the
Scolebythids were sister to Bethylidae (another chrysidoid) in both the SS and EA analyses of 28S alone, and grouped with Chrysis in the 18S results. However, Scolebythidae had scattered groupings in the COI and EF-1α results, which may be responsible for its novel, and likely incorrect grouping outside of Chrysidioidea in the EA and SS analyses. Apoidea, with Ampulicidae as sister to the remaining taxa (Fig. 1), were monophyletic and placed within a paraphyletic Vespoidae in most statistical analyses, which is in agreement with molecular and morphological studies by Pilgrim et al. (2008) and Vilhelmsen et al. (2010), but contrary to the supertree results of Davis et al. (2010). Formicidae were monophyletic across all analyses, but with variable sister-group relationships in the statistical analyses that ranged from a monophyletic section of Vespoidae that included Scoliidae (Fig. 1) to Scoliidae alone (RAxML: EA SS EAnt12 SSnt12).

In a comparative morphological study of the ovipositor, Oeser (1961) showed that ichneumonoids and aculeates share a valve-like mechanism for pushing venom into the ovipositor canal (sting). Aculeates and ichneumonoids also share a similar configuration of the waist, including a distinct articulation involving a pair of projecting lateral condyles (Rasnitsyn, 1988; Rasnitsyn and Zhang, 2010), but which are known to occur in a variety of other apocritans (Vilhelmsen et al., 2010). Early molecular analyses of 16S rDNA data supported the Aculeata + Ichneumonoidea as monophyletic (Dowton and Austin, 1994; Dowton et al., 1997), but later analyses placed Aculeata inside Evaniomorpha (Castro and Dowton, 2006; Dowton and Austin, 2001), with Ichneumonoidea grouping either with Proctotrupomorpha (Dowton and Austin,
2001) or more basally within Apocrita (Castro and Dowton, 2006). Our Bayesian results provide fairly strong and consistent signal grouping Ichneumonoidea with Proctotrumpomorpha. The same results were obtained from the ML and Parsimony (EAnt12) analyses, but without strong support. Our results suggest that the valvilli of the sting/egg canal and the lateral condyles of the metasomal foramen may be plesiomorphic or independently derived in the two groups, and the hypothesis of a monophyletic Auculeata + Ichneumonoidea is doubtful.

Rasnitsyn (1988, 2010) listed several putative morphological and biological apomorphies supporting monophyly of Proctotrumpomorpha, but none of these characters is unambiguous. Early molecular analyses generally supported Proctotrumpomorphomorpha even though single taxon often fell outside, such as Cynipoidea (Dowton et al., 1997) or Heloridae (Dowton and Austin, 2001). Proctotrumpomorpha was strongly supported (PP 98) by Castro and Dowton (2006). Sharanski et al. (2010) provided a novel hypothesis that Chalcidoidea were always well supported as being sister that placed Mymarommatidae elsewhere within the Apocrita (Tables 3 and 4), except in some parsimony analyses (Fig. 1-3, Table 4) and within the single gene analyses for 18S, 28S and COI. The relationships proposed by Sharanski et al. (2010), with Chalcidoidea excluded from Proctotrumpomorpha and the latter group sister to Auculeata, was not obtained in any of our results.

There is no consensus among previous analyses concerning relationships within the Proctotrumpomorpha. On the basis of morphological and fossil evidence, Rasnitsyn (1980, 1988) suggested that the clad falls into two lineages: (1) Chalcidoidea + Platygastroidea, probably also including the Mymarommatidae, with Pelecinidae and Proctotrupidae appearing more basally; and (2) Cynipoidea + Diapriidae, with Monomachidae, Austroniidae, Roproniidae and Heloridae appearing more basally. Gibson (1986) made a strong case, based on morphological evidence, that the Mymarommatidae form the sister group of Chalcidoidea. He also pointed out a number of putative morphological synapomorphies grouping Platygastroidea with Proctotrupidae and Pelecinidae rather than with Chalcidoidea (Gibson, 1985, 1999). This grouping of Platygastroidea was supported in a recent analysis of 173 morphological characters of the mesosoma across Apocrita, but not a sister-group relationship between Mymarommatidae and Chalcidoidea (Vilhelmsen et al., 2010).

There was little consensus in previous molecular analyses concerning relationships within Proctotrumpomorpha, except for Diapriidae forming a monophyletic lineage with Monomachiidae + Maamingidae (Castro and Dowton, 2006; Dowton and Austin, 2001). Sharkey (2007) proposed that the three families be grouped together in the Diaprioidea. Our Bayesian results of the complete alignments supported the monophyly of Diapriidea, and a sister-group relationship with Chalcidoidea (Fig. 1). However, when third codon positions were excluded, Diapriidea instead appeared as a grade leading to Chalcidoidea. The ML and parsimony analyses treated them as paraplythetic or monophyletic, with the diaprid genus Ismarus as sister to Chalcidoidea in the ML analyses (Fig. 2). No putative morphological synapomorphies are currently known for Diapriidea but they may well share a significant biological apomorphy, namely endoparasitism of dipteran larvae. The Diapriidae are predominantly, and apparently also primitively, dipteran parasitoids, which also appears to be the case for Monomachidae (Musetti and Johnson, 2004). Unfortunately, the hosts of Maamingidae remain unknown. Rasnitsyn (1988, 2002b) suggested that the diaprid group may include the Austroniidae, a rare Australian taxon that has never been sequenced and whose biology is unknown.

Early molecular analyses tended to support a sister-group relationship between Chalcidoidea and Platygastroidea (Dowton and Austin, 1994, 2001; Dowton et al., 1997). However, in a more recent analysis, Castro and Dowton (2006) favored Diapriidea + Chalcidoidea instead. Based on morphological evidence, Gibson (1985, 1986) argued convincingly for a sister-group relationship between Chalcidoidea and Mymarommatidae, a morphologically isolated apocritan lineage not sequenced prior to our study. The supertree approach of Davis et al. (2010) placed mymarommatids within Chalcidoidea, and further suggested the non-monophyly of Chalcidoidea, but both of these results are considered an artifact of the method and are not based on any new data. Our results support the monophyly of each of Chalcidoidea (except parsimony EAnt12, Fig. 3, but which is supported in the bootstrap analysis, Table 3) and Mymarommatidae. However, a sister-group relationship between these two taxa was supported only in the RAxML-EAnt12 analysis. Instead, most of the model-based analyses instead supported a clade consisting of Mymarommatidae as the sister group of Diapriidea + Chalcidoidea (Figs. 1 and 2).

Previous molecular analyses confirmed hypotheses based on morphological evidence that Proctotrumpomorpha in the traditional sense are polyphyletic (Castro and Dowton, 2006; Dowton and Austin, 1994, 2001; Dowton et al., 1997). However, apart from the diapriid lineages, analyses have disagreed widely on relationships. Our results are the first to suggest that Proctotrumpomopha, exclusive of Diapriidea, are monophyletic (Figs. 1 and 2). The position of Roproniidae is still somewhat uncertain: they are sister to the remaining proctotrupid clade in almost all of the results, statistically or parsimony (Figs. 1–3), although the Bayesian SS tree had them unplaced within Proctotrumpomorpha (complete alignment) or sister to Pelocinidae (nt12). An unexpected result of our analyses was strong support for a core clade of Proctotrumpomorpha that includes Prototrupoidea (sensu stricto), Diapriidea, Mymarommatoida and Chalcidoidea (Figs. 1–3, Table 4). This novel assemblage of “core Proctotrumpomorpha” families has never before been proposed as a monophyletic lineage. The sister group of core proctotrumpomorphs is uncertain: Bayesian (EA and SS) and parsimony (EAnt12 and SSnt12) analyses favor a monophyletic Platygastroidea + Cynipoidea as their sister group, whereas likelihood favors Cynipoidea alone as their sister. Neither of these hypotheses garner strong support. Rasnitsyn (1988, 2002b) suggested that the diaprid lineages formed the sister lineage of Cynipoidea but our analyses place Diapriidea firmly within the core Proctotrumpomorpha.

Among the megadiverse insect orders, Hymenoptera demonstrate a past history of punctuated events that have led to one of the most impressive animal radiations on our planet. A succession of early life history shifts from leaf-feeding through wood-boring and stem mining are summarized as a grade of phytophagous lineages leading to the single evolution of parasitism in the Vespina. In no other insect group, has parasitism resulted in such a single explosive radiation (Davis et al., 2010; Whitfield, 2003; Wiegmann et al., 1993), with an extraordinary subsequent radiation in the Ichneumonoidea and Chalcidoidea (Heraty, 2009). Interestingly, Davis et al. (2010) propose that the evolution of “special” parasitism in the Apocrita is the important diversification shift with Hymenoptera; however, they mistakenly do not make a sister-group comparison that considers the Vespina (Orussidae + Apocrita). We would argue that all of our results support the thesis that the “discovery” of parasitism in the ancestor of the Vespina is the single most important shift in Hymenoptera. Within the Vespina, provisioning developed only within the Anculaeata, followed by impressive independent shifts to eusociality in the Vespoidae and Apoidea (Pilgrim et al., 2008). Neither of these events are considered as important shifts in the diversification analyses of Davis et al. (2010); however, this may be based on faulty assumptions such as a sister-group relationship between Vespoidae and
Apoida, which is not supported in any of our analyses or other recent analyses (Pilgrim et al., 2008; Vilhelmsen et al., 2010). Despite these discrepancies, we agree with other authors that, except for a major successful shift back to phytophagy through nectar and pollen-feeding in the bees and gall-making in some isolated lineages, parasitism is the major and most successful trait of the vast majority of Hymenoptera (Grissell, 1999; Whitfield, 2003).

While our study resolves some of the phylogenetic relationships across Hymenoptera, many questions remain. In particular, there is still considerable uncertainty regarding the relationships of Evanio- morpha s.l., the position of stephanids, and the most basal splits in Apocrita. A fair amount of signal in our study comes from 28S rDNA data, and this is also likely to be true for previous analyses of the Apocrita (Castro and Dowton, 2006; Dowton and Austin, 2001). Additional nuclear protein-coding genes are a potential source of information that could test and extend the current results. Extensive genomic sampling has recently suggested novel hypotheses (Sharanowski et al., 2010) that will require greater taxonomic sampling for verification. Some of the controversy in the molecular relationships will undoubtedly be resolved through combined analyses with morphological data. However we must continue to address the independent results of each data source to understand the causes of any conflicting signal and refine our understanding of the evolution of Hymenoptera.

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Appendix A. Supplementary material


References


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