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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 (Orussoidea + Apocrita). Within Vespina, our results indicate a sister-group relationship between Ichneumonoidea and Proctotrupomorpha, while the stinging wasps (Aculeata) are monophyletic and nested inside Evaniomorpha. 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, Orussidae 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.

1. Introduction

The Hymenoptera represent one of the most successful Mesozoic 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 (Orussidae + 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 (Eggleton 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, Tenthredinoidea, Pamphilioidea, Cephoidea, Siricoidea (Anaxyelidae + Siricidae), Xiphydrioidea and Orussoidea leading to Apocrita (Sharkey, 2007; Vilhelmsen, 2006; Vilhelmsen

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et al., 2010). Three clades are considered particularly well supported: Unicalcarida (all Hymenoptera except Xyelidae, Tenthredinoidea and Pamphilioidea), Vespina (Orussidae + Apocrita) and Apocrita (Rasnitsyn and Zhang, 2010; Ronquist et al., 1999; Schulmeister, 2003a,b; Vilhelmsen, 2006; Vilhelmsen et al., 2010). Evidence for this scenario is largely derived from morphological analyses (Rasnitsyn and Zhang, 2010; Schulmeister, 2003a,b; Vilhelmsen, 1997, 2001, 2006; Vilhelmsen 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 Cephoidea, 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 (Stephanoidea), Ceraphronomorpha (Ceraphronoidea, Megalyroidea and Trigonaloidea), 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 Evaniomorpha (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 Evaniomorpha, 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 Evaniomorpha *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 Diaprioidea. The second is within Evaniomorpha *s.l.* and consists of Trigonaloidea and Megalyroidea (Dowton and Austin, 2001; Dowton et al., 1997). Within Proctotrupomorpha, either Platygastroidea or Diaprioidea (*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 Mymarommatoidea (Gibson, 1986, 1999), a group not sequenced prior to our study. Two traditional superfamilies, Evanioidea and Proctotrupoidea *sensu stricto* (without Diaprioidea), 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. Mymarommatoidea 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

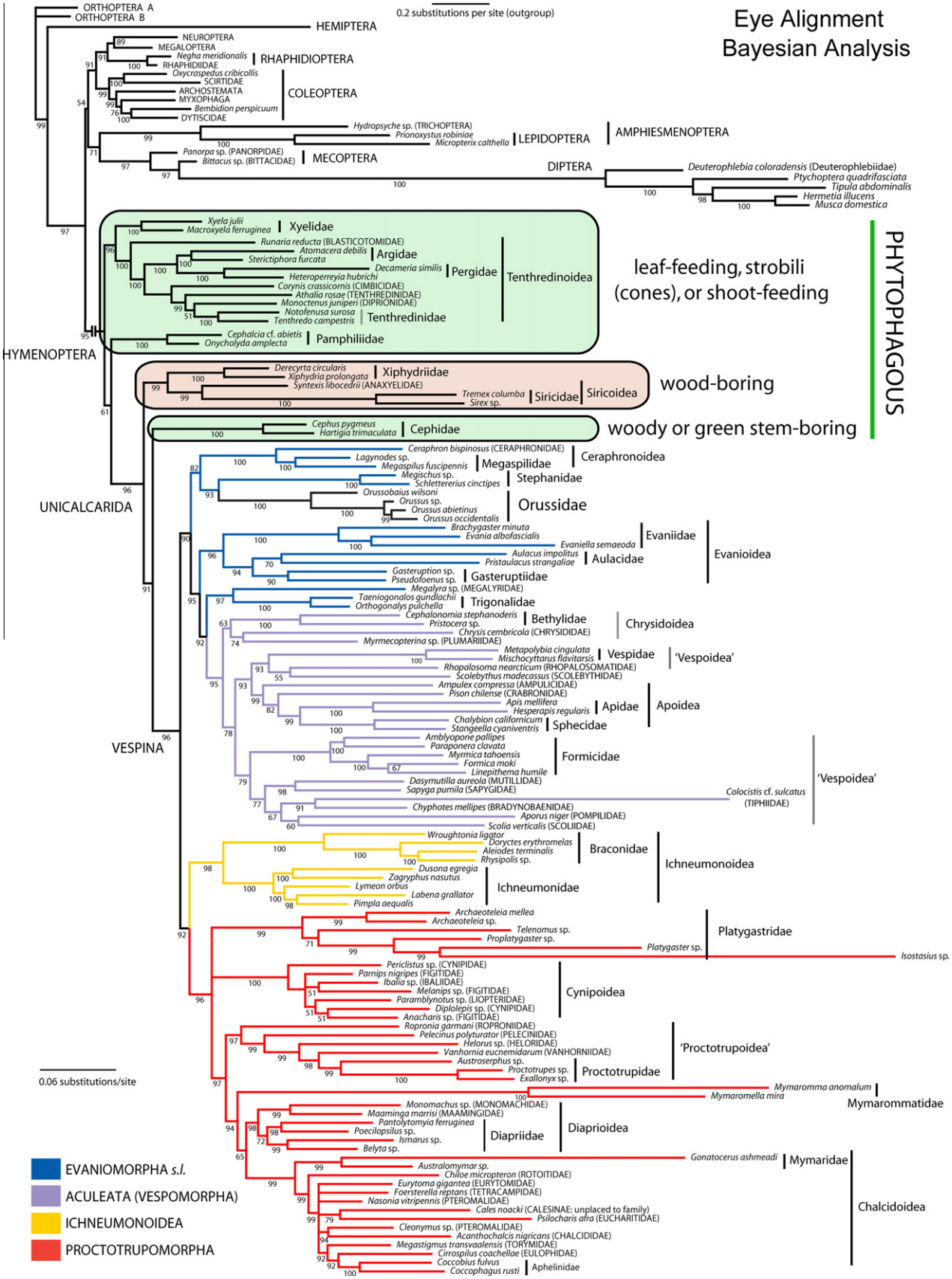

Fig. 1. Hymenopteran relationships based on Bayesian inference (MrBayes, four runs of eight chains each, 100 M gen.) of combined 18S, 28S, EF-1α and CO1 data with nt3 included (7190 bp). Ribosomal sequences aligned by-eye with hypervariable regions excluded. Posterior probability (PP) indicated on branches (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.

Taxa		GenBank Accession Numbers			
		18S	28S	COI	EF1-alpha (F2)
Orthoptera A[a]	Composite taxon	AY859547[1]	AY859546[1]	DQ230733[2]	AY181377[3]
Orthoptera B	Composite taxon	AY121145[4]	AY125285[4]	EF030116[5]	DQ531738[6]
Hemiptera	Composite taxon	LHU06476[7]	DQ133584[8]	AY253038[9] AY744838[10]	DQ194507[11]
Neuroptera	Composite taxon	AF423790[12]	AY521794[12]	AY743812[13]	N/A
Megaloptera	Composite taxon	AY521864[14]	AY521793[14]	AY750519[15]	AY620201[16]
Rhaphidioptera					
Inocellidae	*Negha meridionalis* U. Aspöck	AY521865	AY521795	N/A	N/A
Rhaphidiidae	Rhaphidiidae sp.	GU169690	GU169693	GU169696	N/A
Mecoptera					
Panorpidae	*Panorpa* sp.	GU169691	GU169694	GU169697	N/A
Bittacidae	*Bittacus* sp. (composite)	AF286290[17]	AF423933[17]	EF050551[18]	N/A
Coleoptera					
Belidae	*Oxycraspedus cribricollis* (Blanchard)	FJ867778	FJ867698	FJ867811	FJ867881
Scirtidae	Genus unknown	GU591990	GU591989	N/A	N/A
Dytiscidae	Genus unknown	GU591992	GU591991	N/A	N/A
Carabidae	*Bembidion perspicuum* Leconte	GQ503348	GQ503347	N/A	GQ503346
Myxophaga	Composite taxon	GU591993[19]	GU591994[19]	GQ503342[20]	GQ503345[20]
Archostemata	Composite taxon	EU797411[21]	GU591995[22,23]	EU839762[24]	GQ503344[25]
Lepidoptera					
Cossidae	*Prionoxystus robiniae* (Peck)	AF423783	AY521785	N/A	N/A
Micropterigidae	*Micropterix calthella* L.	GU169692	GU169695	N/A	N/A
Trichoptera					
Hydropsychidae	*Hydropsyche* sp.	AF286291	AF338267	EF513857	N/A
Diptera					
Deuterophlebiidae	*Deuterophlebia coloradensis* Pennak	FJ040539	FJ040539	GQ465781	N/A
Ptychopteridae	*Ptychoptera quadrifasciata* Say	FJ040542	GQ465777	GQ465782	GQ465785
Tipulidae	*Tipula abdominalis* Say (composite)	FJ040553	GQ465778	AY165639	GQ465786
Stratiomyidae	*Hermetia illucens* L.	DQ168754	GQ465779	GQ465783	GQ465787
Muscidae	*Musca domestica* L.	DQ656974	GQ465780	AF104622	DQ657113
Hymenoptera					
Apoidea					
Ampulicidae	*Ampulex compressa* (Fabricius)	GQ410619	GQ374726	GQ374639	GQ410718
Apidae	*Apis mellifera* Linnaeus	AY703484	AY703551	AF250946	AF015267
	Hesperapis regularis (Cresson)	AY995665	–[b]	GQ374630	AY585151
Crabronidae	*Pison chilense* Spinola	GQ410608	GQ374715	GQ374629	GQ410710
Sphecidae	*Chalybion californicum* (Saussure)	GQ410620	GQ374727	N/A	EF013407
	Stangeella cyaniventris (Guérin-Ménevill	GQ410616	GQ374723	GQ374637	GQ410716
Cephoidea					
Cephidae	*Cephus pygmeus* (Linnaeus)	GQ410588	GQ374695	EF032228	GQ410693
	Hartigia trimaculata (Say)	GQ410589	GQ374696	EF032230	GQ410694
Ceraphronoidea					
Ceraphronidae	*Ceraphron bispinosus* (Nees)	GQ410626	GQ374733	GQ374642	GQ410721
Megaspilidae	*Lagynodes* sp.	GQ410624	GQ374731	N/A	GQ410719
	Megaspilus fuscipennis (Ashmead)	GQ410625	GQ374732	N/A	GQ410720
Chalcidoidea					
Aphelinidae	*Coccobius fulvus* (Compere & Annecke)	GQ410673	GQ374780	GQ374675	N/A
	Coccophagus rusti Compere	GQ410674	GQ374781	GQ374676	GQ410755
Calesinae	*Cales noacki* Howard	GQ410670	GQ374777	N/A	GQ410752
Chalcididae	*Acanthochalcis nigricans* Cameron	GQ410679	GQ374786	GQ374680	GQ410759
Eucharitidae	*Psilocharis afra* Heraty	GQ410680	GQ374787	N/A	N/A
Eulophidae	*Cirrospilus coachellae* Gates	GQ410672	GQ374779	GQ374674	GQ410754
Eurytomidae	*Eurytoma gigantea* Walsh	GQ410671	GQ374778	GQ374673	GQ410753
Mymaridae	*Australomymar* sp.	GQ410668	GQ374775	GQ374671	N/A
	Gonatocerus ashmeadi Girault	GQ410667	GQ374774	AY971871	GQ410750
Pteromalidae	*Cleonymus* sp.	GQ410678	GQ374785	GQ374679	GQ410758
	Nasonia vitripennis Walker	GQ410677	GQ374784	GQ374678	GQ410757
Rotoitidae	*Chiloe micropteron* Gibson & Huber	GQ410669	GQ374776	GQ374672	GQ410751
Tetracampidae	*Foersterella reptans* (Nees)	GQ410675	GQ374782	N/A	N/A
Torymidae	*Megastigmus transvaalensis* (Hussey)	GQ410676	GQ374783	GQ374677	GQ410756
Chrysidoidea					
Bethylidae	*Cephalonomia stephanoderis* Betrem	GQ410610	GQ374717	GQ374632	GQ410712
	Pristocera sp.	GQ410622	GQ374729	N/A	EF013494
Chrysididae	*Chrysis cembricola* Krombein	GQ410611	GQ374718	GQ374633	N/A
Plumariidae	*Myrmecopterina* sp.	GQ410618	GQ374725	N/A	N/A
Scolebythidae	*Scolebythus madecassus* Evans	GQ410609	GQ374716	GQ374631	GQ410711

Table 1 (*continued*)

Taxa		GenBank Accession Numbers			
		18S	28S	COI	EF1-alpha (F2)
Cynipoidea					
Cynipidae	*Diplolepis* sp.	GQ410647	GQ374754	GQ374659	GQ410734
	Periclistus sp.	GQ410648	GQ374755	AF395181	GQ410735
Figitidae	*Anacharis* sp.	GQ410651	GQ374758	N/A	GQ410738
	Melanips sp.	GQ410649	GQ374756	GQ374660	GQ410736
	Parnips nigripes (Barbotin)	GQ410650	GQ374757	GQ374661	GQ410737
Ibaliidae	*Ibalia* sp.	GQ410645	GQ374752	GQ374657	GQ410732
Liopteridae	*Paramblynotus* sp.	GQ410646	GQ374753	GQ374658	GQ410733
Diaprioidea					
Diapriidae	*Belyta* sp.	GQ410663	GQ374770	N/A	GQ410748
	Ismarus sp.	GQ410662	GQ374769	GQ374668	N/A
	Pantolytomyia ferruginea Dodd	GQ410660	GQ374767	GQ374666	GQ410746
	Poecilopsilus sp.	GQ410661	GQ374768	GQ374667	GQ410747
Maamingidae	*Maaminga marrisi* Early et al.	GQ410664	GQ374771	GQ374669	GQ410749
Monomachidae	*Monomachus* sp.	GQ410652	GQ374759	GQ374662	GQ410739
Evanioidea					
Aulacidae	*Aulacus impolitus* Smith	GQ410638	GQ374745	GQ374652	N/A
	Pristaulacus strangaliae Rohwer	GQ410635	GQ374742	GQ374649	GQ410728
Evaniidae	*Brachygaster minuta* (Olivier)	GQ410634	GQ374741	AY800156	N/A
	Evania albofacialis Cameron	GQ410632	GQ374739	GQ374647	N/A
	Evaniella semaeoda Bradley	GQ410633	GQ374740	GQ374648	GQ410727
Gasteruptiidae	*Gasteruption* sp.	GQ410636	GQ374743	GQ374650	GQ410729
	Pseudofoenus sp.	GQ410637	GQ374744	GQ374651	GQ410730
Ichneumonoidea					
Braconidae	*Aleiodes terminalis* Cresson	GQ410603	GQ374710	N/A	GQ410707
	Doryctes erythromelas (Brullé)	GQ410602	GQ374709	GQ374627	GQ410706
	Rhysipolis sp.	GQ410601	GQ374708	GQ374626	GQ410705
	Wroughtonia ligator (Say)	GQ410600	GQ374707	GQ374625	GQ410704
Ichneumonidae	*Dusona egregia* (Viereck)	GQ410597	GQ374704	AF146682	GQ410701
	Labena grallator (Say)	GQ410595	GQ374702	GQ374622	GQ410699
	Lymeon orbus (Say)	GQ410599	GQ374706	GQ374624	GQ410703
	Pimpla aequalis Provancher	GQ410598	GQ374705	AF146681	GQ410702
	Zagryphus nasutus (Cresson)	GQ410596	GQ374703	GQ374623	GQ410700
Megalyroidea					
Megalyridae	*Megalyra* sp.	GQ410629	GQ374736	GQ374645	GQ410724
Mymarommatoidea					
Mymarommatidae	*Mymaromella mira* Girault	GQ410666	GQ374773	N/A	N/A
	Mymaromma anomalum (Blood & Kryger)	GQ410665	GQ374772	GQ374670	N/A
Orussoidea					
Orussidae	*Orussobaius wilsoni* Benson	GQ410607	GQ374714	N/A	N/A
	Orussus abietinus (Scopoli)	GQ410604	GQ374711	EF032236	GQ410708
	Orussus occidentalis (Cresson)	GQ410605	GQ374712	GQ374628	GQ410709
	Orussus sp.	GQ410606	GQ374713	N/A	N/A
Pamphilioidea					
Pamphiliidae	*Cephalcia* cf. *abietis* (Linnaeus)	GQ410587	GQ374694	EF032225	GQ410692
	Onycholyda amplecta (Fabricius)	GQ410586	GQ374693	EF032223	GQ410691
Platygastroidea					
Platygastridae	*Archaeoteleia mellea* Masner (Chile)	GQ410639	GQ374746	GQ374653	GQ410731
	Archaeoteleia sp. (Australia)	GQ410640	GQ374747	N/A	N/A
	Isostasius sp.	GQ410644	GQ374751	N/A	N/A
	Platygaster sp.	GQ410641	GQ374748	GQ374654	N/A
	Proplatygaster sp.	GQ410643	GQ374750	GQ374656	N/A
	Telenomus sp.	GQ410642	GQ374749	GQ374655	N/A
Proctotrupoidea					
Heloridae	*Helorus* sp.	GQ410653	GQ374760	GQ374663	GQ410740
Pelecinidae	*Pelecinus polyturator* (Drury)	GQ410655	GQ374762	GQ374664	GQ410742
Proctotrupidae	*Austroserphus* sp.	GQ410654	GQ374761	N/A	GQ410741
	Exallonyx sp.	GQ410656	GQ374763	N/A	GQ410743
	Proctotrupes sp.	GQ410657	GQ374764	N/A	N/A
Roproniidae	*Ropronia garmani* Ashmead	GQ410659	GQ374766	GQ374665	GQ410745
Vanhornidae	*Vanhornia eucnemidarum* Crawford	GQ410658	GQ374765	DQ302100	GQ410744
Siricoidea					
Anaxyelidae	*Syntexis libocedrii* Rohwer	GQ410594	GQ374701	EF032234	GQ410698
Siricidae	*Sirex* sp.	GQ410593	GQ374700	GQ374621	GQ410697
	Tremex columba (Linnaeus)	GQ410592	GQ374699	EF032233	GQ410696
Stephanoidea					
Stephanidae	*Megischus* sp.	GQ410630	GQ374737	GQ374646	GQ410725
	Schlettererius cinctipes (Cresson)	GQ410631	GQ374738	EF032237	GQ410726

(*continued on next page*)

Table 1 (*continued*)

Taxa		GenBank Accession Numbers			
		18S	28S	COI	EF1-alpha (F2)
Tenthredinoidea					
Argidae	*Atomacera debilis* Say	GQ410580	GQ374687	GQ374618	N/A
	Sterictiphora furcata (Villers)	GQ410578	GQ374685	EF032222	GQ410685
Blasticotomidae	*Runaria reducta* Malaise	GQ410581	GQ374688	EF032212	GQ410686
Cimbicidae	*Corynis crassicornis* (Rossi)	GQ410577	GQ374684	EF032220	GQ410684
Diprionidae	*Monoctenus juniperi* (Linnaeus)	GQ410582	GQ374689	EF032278	GQ410687
Pergidae	*Decameria similis* (Enderlein)	GQ410579	GQ374686	GQ374617	N/A
	Heteroperreyia hubrichi Malaise	GQ410585	GQ374692	GQ374620	GQ410690
Tenthredinidae	*Athalia rosae* (Linnaeus)	GQ410576	GQ374683	GQ374616	GQ410683
	Notofenusa surosa (Konow)	GQ410584	GQ374691	N/A	GQ410689
	Tenthredo campestris Linnaeus	GQ410583	GQ374690	GQ374619	GQ410688
Trigonaloidea					
Trigonalidae	*Orthogonalys pulchella* (Cresson)	GQ410628	GQ374735	GQ374644	GQ410723
	Taeniogonalys gundlachii (Cresson)	GQ410627	GQ374734	GQ374643	GQ410722
Vespoidea					
Bradynobaenidae	*Chyphotes mellipes* (Blake)	AY703485	AY703552	N/A	EF013409
Formicidae	*Amblyopone pallipes* (Haldeman)	AY703487	AY703554	DQ353291	EF013381
	Formica moki Wheeler	AY703493	AY703560	AF398151	EF013425
	Linepithema humile (Mayr)	EF012875	EF013003	AY233690	EF013439
	Myrmica tahoensis Weber	AY703495	AY703562	DQ353360	EF013459
	Paraponera clavata (Fabricius)	AY703489	AY703556	GQ374640	GQ422822
Mutillidae	*Dasymutilla aureola* (Cresson)	GQ410621	GQ374728	N/A	EF013414
Pompilidae	*Aporus niger* (Cresson)	GQ410615	GQ374722	GQ374636	GQ410715
Rhopalosomatidae	*Rhopalosoma nearcticum* Brues	GQ410617	GQ374724	GQ374638	GQ410717
Sapygidae	*Sapyga pumila* Cresson	GQ410612	GQ374719	GQ374634	GQ410713
Scoliidae	*Scolia verticalis* Fabricius	EF012932	EF013060	GQ374641	EF013507
Tiphiidae	*Colocistis* (=*Aglyptacros*) cf. *sulcatus* (M.&K.)	GQ410623	GQ374730	N/A	EF013379
Vespidae	*Metapolybia cingulata* (Fabricius)	GQ410613	GQ374720	GQ374635	GQ410714
	Mischocyttarus flavitarsis (Saussure)	GQ410614	GQ374721	N/A	EF013451
Xiphydrioidea					
Xiphidriidae	*Derecyrta circularis* Smith	GQ410591	GQ374698	N/A	N/A
	Xiphydria prolongata (Geoffroy)	GQ410590	GQ374697	EF032235	GQ410695
Xyeloidea					
Xyelidae	*Macroxyela ferruginea* (Say)	GQ410574	GQ374681	EF032211	GQ410681
	Xyela julii (Brebisson)	GQ410575	GQ374682	EF032210	GQ410682

[a] Composite taxa comprised of sequences from more than one taxons follows: Acrididae: [1]Gomphocerinae sp. JM-2004, [2]*Gomphocerippus rufus* (L.); Gryllidae: [3]*Gryllus veletis* (Alexander, R.D. & Bigelow). Stenopelmatidae: [4]*Stenopelmatus fuscus*; [5]*Stenopelmatus 'mahogani'* isolate F613; Hodotermitidae: [6]species JD-709; Miridae: [7]*Lygus hesperus* Knight; Phymatidae: [8]*Phymata* sp. (D1–6); Miridae: [9]*Lygus elisus* (Van Duzee); Cixiidae: [10]*Pintalia alta* Osborn (D7–10); Cicadidae: [11]*Maoricicada campbelli* (Myers); Hemerobiidae: [12]*Hemerobius* sp.; Chrysopidae: [13]*Chrysoperla agilis* Henry et al.; Sialidae: [14]*Sialis* sp.; Corydalidae: [15]*Nigronia fasciatus* (Walker); [16]*Sialis lutaria* (Fabricius); Bittacidae: [17]*Bittacus strigosus* Hagen; [18]*Bittacus* sp.; Lepiceridae: [19]*Lepicerus inaequalis* Motschulsky; Sphaeriusidae: [20]*Sphaerius* sp.; Cupedidae: [21]*Prolixocupes lobiceps* (LeConte) (18S); [22]*P. lobiceps,* D2–D5 (GU591995) and Ommatidae: [23]*Tetraphalerus bruchi* Heller, D1 and D6–D10 (Maddison BToL, not yet deposited), and Cupedidae: [24]*Priacma serrata* LeConte; [25]*Tenomerga* sp.

[b] A combination of AY654456, AY654457, and AY654522.

et al., 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-1α was amplified using primers from Danforth et al. (1999) and four EF-1α primers developed by D.M. (F2F3 5′-GAG CGN GAR CGT GGT ATC AC-3′; F2R2 5′-GCA GCA CCN TTN GGN GGG TTG-3′; F1R3 5′-GCN CCT TTN GGN GGR TGR TCT-3′; For7 5′-GTB GAR ATG CAT CAC GAR GC-3′). EF-1α amplicons were generated with a semi-nested or nested approach, using HaF2For1 and Cho10(mod) primers (Danforth et al., 1999) for the first PCR round and a combination of other primers, depending on the taxa, for the second round. Gene homology for the F2 copy was determined by two methods. First, the F2 copy is distinguished from the F1 copy by highly conserved intron patterns. Following Danforth and Ji (1998), the two F2 intron positions are located at positions 753/754 and 1029/1030, while the one F1 intron, when present, is found at 823/824. Second, gene homology was determined by phylogenetic analysis. An EF-1α data set was built using the 90 putative EF-1α F2 sequences and 45 putative F1 sequences generated from exemplars across Hymenoptera, treating all sequences as unique exemplars, and parsimony analysis was used to confirm that the gene tree could be rooted between the F1 and F2 copies. A similar analysis was performed for the outgroup taxa. Because of difficulties in amplifying the F1 copy, the F1 data set was so incomplete that we decided not to include it in the final analyses. Introns were removed from the F2 sequences for a final sequence length of 1095 bp (Table 2).

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-1α:1095).

Secondary Structure Alignment (SS) – Ribosomal RNA sequences of 18S and 28S were aligned manually by A.P.D. using secondary structure. Notations follow Kjer et al. (1994) and Kjer (1995), with modifications by Gillespie et al. (2004). For 18S, the alignment

Table 2
Gene and alignment summary for Hymenoptera and outgroups. Hypervariable regions and introns removed (see text). Base frequencies are uncorrected values from PAUP* (Swofford, 2002). Partition models calculated using the Bayesian Inference Criterion (BIC) as calculated in jModelTest 0.1.1 (Posada, 2008).

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	
EA without nt3	6563	24.5	23.5	23.3	28.7	36.3	
Secondary Structure with nt3	6993	25.1	24.2	23.1	27.6	40.8	
SS without nt3	6366	24.7	23.3	23.2	28.8	35.2	
28S (eye)	3405	23.2	21.0	24.6	31.2	41.8	GTR + 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	SYM + I + G
18S (SS)	1860	25.3	24.7	22.8	27.2	30.5	SYM + I + G
COI	786	31.5	40.0	14.6	13.9	65.6	TVM + 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 + 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	TrN + I + G
EF1 (nt3)	365	17.3	26.8	34.1	21.8	95.3	TPM1uf + I + G
COI + EF1 (nt3 only)	627	30.1	21.5	13.2	35.2	97.3	GTR + I + G

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), Evaniidae (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-1α:1095).

The base composition, percentage of informative sites for the complete alignment and each gene region, and estimated models for each gene partition as calculated in jModeltest 1.01 (Posada, 2008) are reported in Table 2. The EA and SS alignments, with and without nt3, are deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S10858). The SS alignment and structural mask are deposited at jrna (http://hymenoptera.tamu.edu/rna/models.php). Analyses were run on both the EA and SS alignments and with nt3 of the coding genes (COI and EF-1α) either included or excluded (cf. Table 3). Gene regions were analyzed both independently and combined, but only the combined results are reported.

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, CO1 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 contrasting 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 obtained 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 substitution rates. Parameter estimation and bootstrapping were carried 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 bootstrap search algorithm (RBS) (Stamatakis et al., 2008), in which bootstrap analyses are conducted first with 500 repetitions, followed by fast and then slow searches on the sampled trees to find the BKL tree.

Parsimony 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. Virtually all of the heterogeneity among MCMC runs in the SS analyses concerned the position of Orussoidea, Stephanoidea, and the resolution of Evaniomorpha and Aculeata. 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 Evaniomorpha *s.l.* and Aculeata, were consistently resolved and the variation among runs in estimated posterior probabilities (PP) of clades was negligible.

There were distinct differences between the EA and SS analyses. Orussoidea were placed as the sister group of Stephanoidea inside the Evaniomorpha *s.l.* grade in the EA analyses (Fig. 1), but as the sister group to the Apocrita, including Stephanoidea, in the SS analyses (Table 3). However, both signals were present with intermediate 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) support 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), Vespina (PP 96–100; PMO 22 or 30), Evanioidea (PP 89–100), Ichneumonoidea (PP 98–100; PMO 65 or 190), Proctotrupomorpha (PP 92–100; PMO 11 or 27), core Proctotrupomorpha (Proctotrupoidea including Mymarommatoidea, Diaprioidea and Chalcidoidea) (PP 92–100; PMO 12 or 31), and Chalcidoidea (PP 99–100) (Tables 3 and 4). Diapriidae (PP 72–77) and Diaprioidea (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 Xyeloidea and Tenthredinoidea (EA; PP 96; Fig. 1) or Xyeloidea, Tenthredinoidea and Pamphilioidea (SS; PP 92) as a monophyletic sister group to the Unicalcarida. However, the EAnt12 and SSnt12 analyses both rooted the hymenopteran tree between Xyeloidea and other Hymenoptera (PP 100). In the likelihood (RAxML), parsimony (nt12) analyses, Xyeloidea were always sister to the remaining Hymenoptera (Figs. 2 and 3; Table 3), which is similar to morphology-based hypotheses that treat Xyeloidea as a monophyletic or paraphyletic sister group to the remaining Hymenoptera. Xyelidae were monophyletic in most of the single gene analyses but sister to the remaining Hymenoptera only with COI (STable 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 Hymenoptera (Fig. 1).

More unexpectedly, the woodwasps – siricids, anaxyelids and xiphydriids but not orussids – emerged as monophyletic (PP 83–99; PMO 110 or 120). Proctotrupoidea *sensu stricto* is also monophyletic (PP 97–100; but excluding the basal Roproniidae in the SS analysis). Trigonaloidea always grouped strongly with Megalyroidea (PP 97–100). Lastly, Ichneumonoidea were consistently placed as sistergroup to a monophyletic Proctotrupomorpha (PP 85–92).

Analyses of the separate genes showed that much of the signal resolving higher-level relationships emerged only after gene regions were combined (STable A1). Of the four markers studied, 28S provided the best resolution of basal nodes on its own. It strongly supported monophyly of Unicalcarida (PP 93–100) and Vespina (PP 93–100). In the eye alignments, there was additional evidence for Proctotrupomorpha (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), Vespina (PP 89–93) and core Proctotrupomorpha (PP 94–97). On their own, the EF-1α 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 signal, for example the grouping of Stephanidae with *Apis mellifera* in the EF-1α analyses (PP 93–96). More interesting signal concerning higher relationships included support for Diaprioidea (PP 94–96 in CO1 analyses), Evaniomorpha + Aculeata (PP 79–91 in EF-1α analyses) and core Prototrupomorpha, including Chalcidoidea (PP 99 in 28S and 83 in COI analyses) (STable 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.

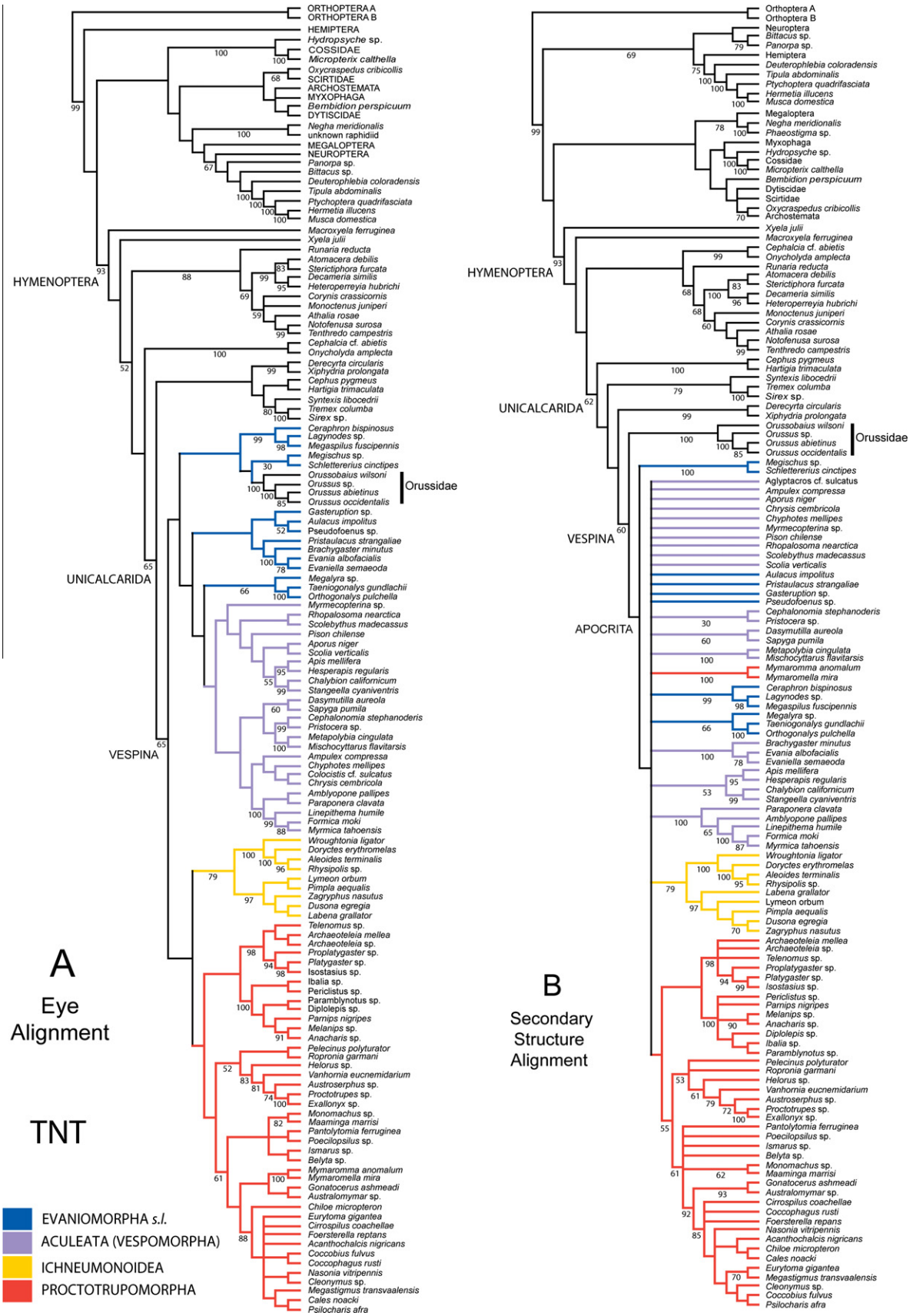

Fig. 3. Parsimony analysis (TNT, New Technology Search) of combined 18S, 28S, EF-1α and CO1 data with nt3 excluded for coding genes: A, eye alignment (6563 bp; 22201 steps, consensus of 16 trees, r.i. 0.46); B, secondary structure alignment (6366 bp; 20277 steps, consensus of 172 trees, r.i. 0.47). Bootstrap 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). Xyelidae 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 Macroxyelinae + remaining Hymenoptera (Table 3). Pamphilioidea consistently appeared as sister to Unicalcarida, although with weak support (BS <50–77). Orussoidea 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 + Proctotrupomorpha were monophyletic in all results, but without bootstrap support. Mymarommatoidea were sister to Chalcidoidea only in the EAnt12 analysis (no BS); otherwise a paraphyletic Diaprioidea 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 (STable A1). Contrary to the combined results, 28S alone provided weak support for a monophyletic Xyeloidea + 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 Proctotrupoidea, 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), Proctotrupoidea (BS 57–71) and Chalcidoidea (BS 88) (Table 3). Considerably more structure was obtained from the nt12 analyses for both alignments (Fig. 3), although the SS analyses were generally unresolved for Apocrita and did not include Mymarommatoidea within Proctotrupomorpha (Fig. 3B). Results for the EAnt12 analysis (Fig. 3A) were generally concordant with the likelihood results for the same dataset. Xyeloidea were paraphyletic, and the remaining Hymenoptera monophyletic. Unicalcarida were monophyletic, but with Cephoidea included within the woodwasp clade. Orussoidea 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 sister to a monophyletic Proctotrupomorpha, which also included the 'core Proctotrupomorpha' clade. Diaprioidea were monophyletic only in the EA results. Mymarommatoidea were sister to Mymaridae, 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 support for some groups (STable A1), including core Proctotrupoidea with Chalcidoidea (clade present in the 28S and COI nt1–3 analyses).

4. Discussion

Generally speaking, the combined analyses based on eye alignments were more resolved and agreed better with previous morphology-based hypotheses of relationships than those based on secondary-structure alignments, except for placement of Orussoidea. This could potentially be due to observer bias towards expected relationships because eye alignments of this size necessarily 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 increases 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 alignment of these regions is less objective and we chose to exclude them. Fewer sites were excluded in the EA as hypervariable; however 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 major impact on the parsimony analyses, resulting in both greater resolution 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. Apparently, 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 attraction 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 morphology-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 higher-level taxa, and recovered many of the higher-level groups previously 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 Apocrita, with either inclusion or exclusion of Orussoidea 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 + Neuropterida (Megaloptera, Neuroptera, Raphidioptera) + Amphiesmenoptera (Lepidoptera + Trichoptera) + Antliophora (Mecoptera + Diptera) as sister group to Hymenoptera. These results conflict with the earlier hypothesis of a sister-group relationship between Hymenoptera and Mecopterida (Kristensen, 1999), but they are congruent with recent molecular (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 Xyelidae (and hence 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, STable 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 Xyelidae appears to be impacted by the exclusion of nt3 for the coding regions, with exclusion favoring diphyly 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 Xyelidae.

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 and Aculeata and cannot separate the impact of molecular or morphological data. Our results provide the first robust molecular support for Unicalcarida and Vespina, while they are less clear on the monophyly of the Apocrita (Figs. 1–3).

Morphological analyses suggest that Cephoidea is the sister group of the remaining Unicalcarida, and woodwasps (Anaxyelidae, Siricidae, and Xiphydrioidea) form a paraphyletic grade, with xiphydriids most closely related to Vespina (Davis et al., 2010; Rasnitsyn and Zhang, 2010; Schulmeister, 2003b; Vilhelmsen, 1997, 2001; Vilhelmsen et al., 2010). Previous studies using only molecular data failed to resolve these relationships (Dowton and Austin, 1994; Schulmeister, 2003b). In our study, only the parsimony analysis of the SS dataset with nt12 provided a similar hierarchy (Fig. 3B), but without any BS support. Otherwise, Cephoidea 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 Xiphydrioidea + 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 Xiphydrioidea + 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 Xiphydrioidea share at least one potential morphological synapomorphy: an invagination on the distal labial palp segment with specialized rodlike sensilla. In Xiphydrioidea 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.* (Fig. 1). 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 (Gibson, 1985), the ocellar corona, and a specialized labrum (Vilhelmsen, 1996), characters usually assumed to be convergences or shared plesiomorphies lost in apocritans other than stephanids. However, we are skeptical of this hypothesis because of the lack of more substantial morphological evidence for this placement of orussids and because of the difficulty of reliably placing orussids and stephanids both in our and previous molecular analyses (Dowton and Austin, 1994; Schulmeister et al., 2002).

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 (Evaniomorpha *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 Stephanidae, 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

Table 3
Table of support values for selected clades across all analyses. Datasets are based on Eye (EA) and Secondary Structure (SS) alignments, and inclusion (EA, SS) or exclusion of the 3rd base position of CO1 and EF-1α (EAnt12, SSnt12). Support values are reported as posterior probability percentage above 50 for Bayesian results, and bootstrap support percentage above 50 for RAxML and TNT analyses. Abbreviations: AN, Anaxyelidae; CH, Chalcidoidea; DI, Diaprioidea; MM, Mymarommatoidea; MY, Mymaridae; OR, Orussidae; SI, Siricidae; ST, Stephanidae; XI, Xiphydriidae; y = yes, group recovered but without bootstrap support; –, not monophyletic; *p*, paraphyletic. 'Diapriidae' or 'Diaprioidea' refers to paraphyletic group. Italic values, taxa strongly supported as monophyletic across most analyses.

Clade	Bayesian analyses				Likelihood (RAxML) analyses				Parsimony (TNT) analyses			
	EA	EAnt12	SS	SSnt12	EA	EAnt12	SS	SSnt12	EA	EAnt12	SS	SSnt12
Hymenoptera	*95*	*100*	*96*	*100*	*100*	*78*	*99*	*87*	*91*	*93*	*87*	*93*
Xyelidae	*100*	–	*99*	–	*93*	*p*	*97*	*70*	*99*	*p*	*98*	*p*
Hymenoptera excluding Xyelidae	–	100	–	100	57	66	55	80	–	52	–	y
Xyelidae + Tenthredinoidea	96	–	–	–	–	–	–	–	–	–	–	–
Xyelidae + Tenthredinoidea + Pamphilioidea	–	–	92	–	–	–	–	–	–	–	70	–
Pamphilioidea + Unicalcarida	61	100	–	99	53	77	y	63	–	y	–	–
Unicalcarida: (Siricoidea + XI + CE + Vespina)	*96*	*100*	*97*	*100*	*100*	*91*	*99*	*92*	*52*	*65*	*y*	*62*
Siricoidea *sensu* Rasnitsyn (AN + XI + SI)	99	83	99	–	92	y	94	–	y	–	–	–
Cephidae + Vespina	91	76	93	73	y	y	52	y	–	–	–	–
Vespina	*96*	*100*	*97*	*100*	*100*	*94*	*98*	*94*	*y*	*65*	–	*60*
Apocrita (Vespina excluding Orussidae)	–	–	58	–	–	–	y	–	–	–	–	y
Orussidae + Stephanidae	93	66	–	–	y	y	–	–	y	y	–	–
Ceraphronoidea+(OR + ST)	82	–	–	–	–	–	–	–	–	y	–	–
Evanioidea	96	100	89	100	55	79	y	y	–	y	–	–
Megalyridae + Trigonalidae	*100*	*100*	*97*	*100*	*61*	*84*	–	*82*	–	*66*	–	*66*
Aculeata (=Vespomorpha)	95	82	–	95	y	61	y	53	–	y	–	–
Apoidea	99	100	–	–	y	y	–	–	–	–	–	–
Ichneumonoidea	*98*	*100*	*99*	*100*	*93*	*100*	*99*	*99*	*y*	*79*	–	*79*
Ichneumonoidea + Proctotrupomorpha	92	85	91	92	y	y	y	y	–	y	–	–
Proctotrupomorpha	*96*	*100*	*92*	*100*	*99*	*88*	*72*	*88*	–	*y*	–	–
Proctotrupoidea (*sensu stricto*)	*97*	*99*	–	*100*	*69*	*86*	*57*	*67*	*71*	*52*	*57*	*53*
Mymarommatoidea + Chalcidoidea	–	–	–	–	–	y	–	–	–	y	–	–
Mymarommatoidea+(DI + CH)	94	100	–	99	y	–	y	65	–	–	–	–
Proctotrupoidea + (MM + DI + CH) ['core clade']	*97*	*100*	*92*[a]	*100*	*67*	*86*	*61*	*81*	–	*y*	–	*55*
Diaprioidea	98	–	98	–	–	*p*	–	–	–	y	–	–
Diaprioidea + Chalcidoidea	65	94	97	99	y	–	68	69	y	–	57	61
Diapriidae	72	–	77	–	–	y	–	–	–	–	–	–
'Diapriidae' + Chalcidoidea	–	96	–	100	y	–	–	57	–	–	–	–
Chalcidoidea	*99*	*100*	*99*	*100*	*100*	*100*	*100*	*100*	*88*	*92*[b]	*88*	*92*
Chalcidoidea excluding Mymaridae	*99*	*100*	*100*	*100*	*70*	*68*	*98*	*97*	*y*	*88*	*74*	*85*
Chalcidoidea excluding MY and Rotoitidae	99	100	–	–	53	55	y	y	–	y	–	–

[a] Monophyletic, but Roproniidae placed outside of Proctotrupoidea.
[b] Monophyletic in bootstrap analysis but not parsimony (Fig. 3A).

Table 4
Estimated posterior model odds for selected clades of interest. The odds for a clade is the ratio of the posterior probabilities of trees with and without the clade, with the prior putting equal probability on all fully resolved trees. For abbreviations of taxa, see Table 3.

Clade	Posterior model odds	
	EA	SS
Siricoidea *sensu* Rasnitsyn (AN + XI + SI)	110	120
Cephidae + Vespina (including Orussidae)	9.8	13
Siricoidea *sensu* Rasnitsyn + Vespina	<0.0001	<0.0001
Vespina	22	30
Apocrita (excluding Orussidae)	<0.0001	1.4
Aculeata	19	0.95
Ichneumonoidea	65	190
Evaniomorpha *s.l.*	<0.0001	<0.0001
Proctotrupomorpha	27	11
Ichneumonoidea + Aculeata	<0.0001	<0.0001
Evaniomorpha *s. l.* + Aculeata	<0.0001	1.2
Evaniomorpha *s. l.* + Orussidae + Aculeata	9.0	0.61
Proctotrupomorpha + Aculeata	<0.0001	<0.0001
Megalyridae + Trigonalidae + Aculeata	11	0.5
Trigonalidae + Evaniidae	<0.0001	<0.0001
Core Proctotrupomorpha (including CH)	31	12

model-based analyses of the by-eye alignments (Fig. 1; Table 3). Chrysidoidea, except for the scolebythid, formed a monophyletic group that was sister to the remaining Aculeata in most analyses. In the combined results of the EAnt12 ML analysis, Scolebythidae grouped with a paraphyletic Chrysidoidea (sister to Chrysididae) as the sister group of a monophyletic Apoidea + Vespoidea. 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 Chrysidoidea in the EA and SS analyses. Apoidea, with Ampulicidae as sister to the remaining taxa (Fig. 1), were monophyletic and placed within a paraphyletic Vespoidea 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 Vespoidea that included Scoliidae (Fig. 1) to Scoliidae alone (RAxML: EA and SS).

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 Proctotrupomorpha. 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 Aculeata + Ichneumonoidea is doubtful.

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

There is no consensus among previous analyses concerning relationships within the Proctotrupomorpha. On the basis of morphological and fossil evidence, Rasnitsyn (1980, 1988) suggested that the clade falls into two lineages: (1) Chalcidoidea + Platygastroidea, probably also including the Mymarommatoidea, 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 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 Proctotrupomorpha, except for Diapriidae forming a monophyletic lineage with Monomachidae + 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 Diaprioidea, and a sister-group relationship with Chalcidoidea (Fig. 1). However, when third codon positions were excluded, Diaprioidea instead appeared as a grade leading to Chalcidoidea. The ML and parsimony analyses treated them as paraphyletic or monophyletic, with the diapriid genus *Ismarus* as sister to Chalcidoidea in the ML analyses (Fig. 2). No putative morphological synapomorphies are currently known for Diaprioidea 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 diaprioid 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 Diaprioidea + Chalcidoidea instead. Based on morphological evidence, Gibson (1985, 1986) argued convincingly for a sister-group relationship between Chalcidoidea and Mymarommatoidea, 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 Mymarommatoidea. 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 Mymarommatoidea as the sister group of Diaprioidea + Chalcidoidea (Figs. 1 and 2).

Previous molecular analyses confirmed hypotheses based on morphological evidence that Proctotrupoidea in the traditional sense are polyphyletic (Castro and Dowton, 2006; Dowton and Austin, 1994, 2001; Dowton et al., 1997). However, apart from the diaprioid lineages, analyses have disagreed widely on relationships. Our results are the first to suggest that Proctotrupoidea, exclusive of Diaprioidea, are monophyletic (Figs. 1 and 2). The position of Roproniidae is still somewhat uncertain; they are sister to the remaining proctotrupoid clade in almost all of the results, statistical or parsimony (Figs. 1–3), although the Bayesian SS tree had them unplaced within Proctotrupomorpha (complete alignment) or sister to Pelecinidae (nt12). An unexpected result of our analyses was strong support for a core clade of Proctotrupomorpha that includes Prototrupoidea (*sensu stricto*), Diaprioidea, Mymarommatoidea and Chalcidoidea (Figs. 1–3, Table 4). This novel assemblage of "core Proctotrupomorpha" families has never before been proposed as a monophyletic lineage. The sister group of core proctotrupomorphs 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 diapriid lineages formed the sister lineage of Cynipoidea but our analyses place Diaprioidea firmly within the core Proctotrupomorpha.

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 Aculeata, followed by impressive independent shifts to eusociality in the Vespoidea 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 Vespoidea and

Apoidea, 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 Evaniomorpha *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 undoubtably 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

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2011.04.003.

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