Direct optimization, sensitivity analysis, and the evolution of the hymenopteran superfamilies

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ABSTRACT

Even as recent studies have focused on the construction of larger and more diverse datasets, the proper placement of the hymenopteran superfamilies remains controversial. In order to explore the implications of these new data, we here present the first direct optimization-sensitivity analysis of hymenopteran superfamilial relationships, based on a recently published total evidence dataset. Our maximum parsimony analyses of 111 terminal taxa, four genetic markers (18S, 28S, COI, EF-1α), and 392 morphological/behavioral characters reveal areas of clade stability and volatility with respect to variation in four transformation cost parameters. While most parasitican superfamilies remain robust to parameter change, the monophyly of Proctotrupoidea sensu stricto is less stable; no set of cost parameters yields a monophyletic Diaprioidea. While Apoidea is monophyletic under eight of the nine parameter regimes, no set of cost parameters returns a monophyletic Vespoidea or a monophyletic Chrysidoidea. The relationships of the hymenopteran superfamilies to one another demonstrate marked instability across parameter regimes. The preferred tree (i.e., the one that minimizes character incongruence among data partitions) includes a paraphyletic Apocrita, with (Orussoidea + Stephanoidea) sister to all other apocritans, and a monophyletic Aculeata. "Parasitica" is rendered paraphyletic by the aculeate clade, with Aculeata sister to (Trigonaloidea + Megalyroidea).

INTRODUCTION

Despite being one of the most diverse, well-studied, and economically important groups of insects (Goulet and Huber, 1993; Grimaldi and Engel, 2005), the Hymenoptera (ants, bees, wasps, and sawflies) still present major problems for phylogenetic systematists. While certain aspects of the group's phylogeny—the paraphyly of the "Symphyta," the monophyly of the aculeate wasps, and the rise of the Aculeata from within a paraphyletic "Parasitica"—are relatively uncontroversial, the proper placement of the order's 22 extant superfamilies (Sharkey, 2007) remains elusive.

While recent efforts associated with the Hymenoptera Tree of Life project (HymAToL; e.g., Vilhelmsen et al., 2010; Heraty et al., 2011; Sharkey et al., 2012; Klopfstein et al., 2013) represent major advances in taxon sampling and character scoring, the results of those studies still point to a deep instability among higher-order hymenopteran relationships. In an effort to further explore the implications of these new data, and to more precisely define regions of topological instability, we here present the first direct optimization-sensitivity analysis of hymenopteran superfamilial relationships, based on a reanalysis of the most recently published total evidence dataset (Sharkey et al., 2012).

BACKGROUND I:
GENERAL OUTLINE OF HYMENOPTERAN PHYLOGENY

A long list of synapomorphies—including a unique hamulus-based wing-joining mechanism, protibial antennal cleaners, and a haplodiploid sex determination system (among others, see Sharkey, 2007)—clearly unites the hyperdiverse membership of the Hymenoptera as a natural group (Goulet and Huber, 1993; Grimaldi and Engel, 2005). In addition, the general outline of the order's higher-level relationships are more or less clear: a basal grade, the "Symphyta," comprising no more than 5% of hymenopteran diversity leads to an extremely diverse suborder, the Apocrita, united by the evolution of the wasp waist (Vilhelmsen et al., 2010) and a series of highly successful developments in the parasitic lifestyle.

The Apocrita, or true wasps, are further subdivided into two groups, the "Parasitica" (12 superfamilies) and the Aculeata (three superfamilies), the latter defined by an unambiguous synapomorphy in the form of a complex ovipositor based sting apparatus. No readily apparent morphological character unites the extremely diverse parasitican superfamilies (Sharkey et al., 2012), and the results of many phylogenetic studies have pointed to an aculeate origin from within the group (Rasnitsyn, 1988; Dowton and Austin, 1994; Downton et al., 1997; Carpenter and Wheeler, 1999; Davis et al., 2010; Vilhelmsen et al., 2010; Peters et al., 2011; Heraty et al., 2011; Sharkey et al., 2012; but see Ronquist et al., 1999; Dowton and Austin, 2001).

While this basic outline (fig. 1) is relatively uncontroversial (Sharkey, 2007), the details of the superfamilial relationships, and especially of the exact position of the aculeate clade within "Parasitica," are far from settled. Among the more acute problems facing the higher-order hymenopteran systematist are: (1) establishing the monophyly of each of the 22 superfamilies proposed by Sharkey (2007); (2) establishing the basal most lineage within the order: either

FIG. 1. Schematic representation of hymenopteran relationships. Box size proportional to estimated species diversity, based on conservative estimates in Goulet and Huber (1993): "Symphyta": ~ 15,000 sp.; Aculeata: ~ 92,000 sp.; "Parasitica": 200,000 sp. Bars represent key synapomorphies: the wasp-waist (a) and the defensive sting apparatus (b). Some estimates of species diversity within "Parasitica" are much higher; see, for instance, the 375,000 to 500,000 chalcidoids predicted by Heraty and Darling (2009).
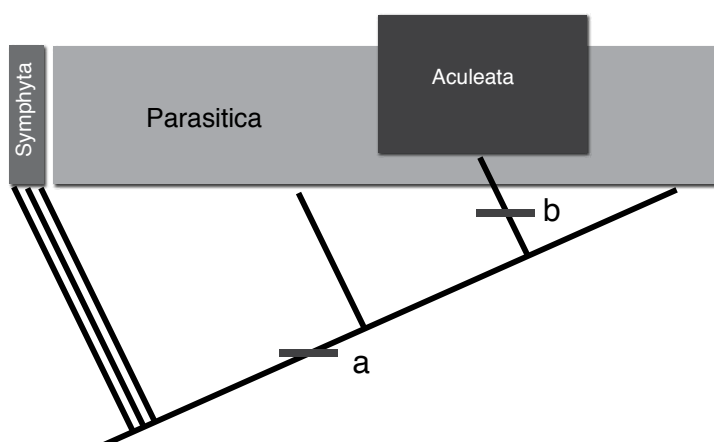
Xyeloidea or some combination of Xyeloidea + other symphytan clades; (3) resolving the phylogenetic structure of the Vespina (Orussoidea + Apocrita) and determining whether the symphytan Orussoidea renders Apocrita paraphyletic (as suggested by Heraty et al., 2011); and, finally, (4) inferring the position of the Aculeata among the parasitican lineages and establishing the identity of the group's sister taxon.

In order to contribute to these efforts, we bring a powerful set of analytical tools—direct optimization and sensitivity analysis—to bear on a slightly expanded version of a recently published total-evidence dataset. Sharkey et al. (2012) examined 111 taxa, including three outgroups and 84 generic exemplars within Apocrita, using 392 morphological and behavioral characters, along with eye-aligned sequence data from four genes. While this represents the most extensive phylogenetic study of the Hymenoptera to date, their total-evidence analysis returned only weak support for a number of important clades and did not address issues of parametric contingency in parsimony analysis (Wheeler, 1995; Giribet, 2003). The current study was designed to expand upon the previous work's findings, and to further explore the implications of the newly available HymaToL data.

BACKGROUND II:
DIRECT OPTIMIZATION

When analyzing molecular-sequence characters, conventional phylogenetic methods require two separate and sequential optimization procedures: an initial multiple-sequence alignment (MSA), followed by some form of character optimization and tree search. Sequence alignment is a necessary first step given that variations in sequence length, which presumably reflect long series of historical insertion and deletion events, are a pervasive feature of comparative molecular datasets.

MSAs are methods for "correcting" this length heterogeneity through the insertion of gaps, placeholders that stand in for absent homologous nucleotides. In doing so, they establish putative homologies among nucleotide base positions across terminal taxa, and at the same time present a visible manifestation of that homology in the form of neat columns of molecular

characters. Once these putative homologies are established, researchers can treat sequence-based datasets just as they would any other set of phylogenetically informative static characters (Wheeler, 2001). Firmly embedded in a static matrix, aligned nucleotide characters can reveal the patterns of state change and stasis that form the backbone of phylogenetic analysis.

There are, however, problems associated with this separate and sequential approach. Given that true multiple-sequence alignment is computationally prohibitive for all but the most trivial of datasets (Schulmeister et al., 2002), all of the currently implemented MSA optimization methods rely on some form of heuristic search: most often a "binary 'guide' tree" that points the way, via a series of simpler pairwise alignments, toward an approximation of the global optimum (Wheeler, 2001). Unfortunately, different guide trees can produce vastly different optimum alignments, which in turn may result in vastly different phylogenetic outcomes. In a worst-case, but probably common scenario, the optimum cladogram for a given alignment will not represent the lowest-cost cladogram that could have been generated from the same sequence data given a different static alignment.

Wheeler's (1996) optimization alignment (i.e., direct optimization) algorithm solves this problem by combining the sequence alignment and character optimization/tree search steps. Putative homologies are no longer determined a priori via a separate and prior MSA, but rather with reference to each unique cladogram encountered during a given tree search. Homologies are thus "dynamically determined and uniquely tailored to each topology" (Wheeler, 2001: S5), with direct optimization based cladograms routinely obtaining lower costs than cladograms derived from conventional analyses (Wheeler, 2001). For an introduction to the mechanics of the optimization alignment algorithm, see Wheeler (1996); for an extended discussion of its advantages in total evidence analysis, see Schulmeister et al. (2002).

BACKGROUND III:
SENSITIVITY ANALYSIS

At its most basic, the phylogenetic implementation of maximum parsimony is a method for determining the minimum amount of character change demanded by: (1) a given dataset, (2) the assumption of common descent, and (3) Hennig's auxiliary principle (Hennig, 1966). As a test of the null hypothesis that putative homology reflects final homology, it does nothing more than minimize the number of ad hoc hypotheses of evolutionary convergence required to explain patterns present within the data.

Despite this logical simplicity, parsimony methods cannot escape the need to assign a priori costs to the various character transformations we seek to optimize (Wheeler, 1995; Donoghue and Ackerly, 1996). While changes in the relative magnitudes of these costs can have a dramatic effect on the outcome of phylogenetic analyses, no empirical, extraphylogenetic methods exist for determining "realistic" cost assignments.

Sensitivity analysis (sensu Wheeler, 1995) allows for a liberal exploration of the effects of varying cost parameters on the outcome of parsimony analyses. By choosing an expanded set of transformation cost regimes and using them as the basis for multiple parallel analyses of the same character data, we can explore the sensitivity of a given clade or clades to changes in those

cost parameters (Wheeler, 1995; Schulmeister et al., 2002). Clades that hold together regardless of changes in the relative costs of transitions, transversions, insertion/deletion events, or morphological changes may be considered more stable or more "robust" than those that exist only under one or a few cost regimes (Giribet, 2003); such robustness may justify more confidence in the clade, and thus function as a form of clade support (Schulmeister et al., 2002).

Of course, the many trees produced by even a small-scale sensitivity analysis still leave us with the dilemma of choosing a "best" tree from among the phylogenetic hypotheses derived from competing cost regimes. Wheeler (1995) suggested using one of two measures of congruence, either taxonomic (based on topological agreement) or character based (a measure of character conflict among constituent datasets, e.g., the incongruence length difference of Mickevich and Farris, 1981). Whichever set of cost parameters minimizes the chosen incongruence measure yields the preferred phylogenetic hypothesis.

MATERIALS AND METHODS

Taxa and Characters: Our dataset was nearly identical to the one analyzed by Sharkey et al. (2012); it contained the same 111 genus-level terminals (108 ingroup, 3 outgroup), the same genetic markers (18S, 28S, COI, EF-1α), and the same 392 morphological/behavioral characters. However, ours also included fragments of 23 additional sequences downloaded from GenBank and used to fill in gaps in the molecular data matrix (accession numbers in table 1). In some cases, these sequences provided molecular characters for genera (*Orgilus, Plumarius, Spalangia,* and *Urocerus*) that were previously represented by morphology alone (Sharkey et al., 2012). All other sequence, morphological, and behavioral data were obtained directly from one of the previous study's authors (J.M.C.).

Sequences were initially aligned by eye using Geneious Pro version 5.5 (Drummond et al., 2010). This temporary alignment facilitated the identification of nonoverlapping sequence regions (e.g., leading and trailing gaps), and allowed for the partitioning of sequences into shorter homologous fragments (14 subfragments in 18S; 21 in 28S; 6 in COI; 10 in EF1-α). All gaps were removed prior to the direct-optimization phylogenetic analyses described below.

Phylogenetic Analyses: Nine total-evidence maximum parsimony analyses were performed simultaneously using POY version 4.1.2.1 (Varón et al., 2010). These nine analyses differed only in terms of the costs assigned to four classes of character transformations: insertion/deletion events, transversion substitutions, transition substitutions, and morphological/behavioral changes (table 2). "Neuroptera" was designated as the outgroup for all analyses.

Each analysis began with a 15 hour tree search using POY's default search command on four processors:

search(max_time:00:15:00)

The trees produced by these nine simultaneous searches were concatenated into a single file that served as the input tree file for the next round of heuristic search. Subsequent tree-search iterations each performed 1000 rounds of tree fusing followed by swapping on unique trees:

fuse(iterations:1000) select() swap(trees:10) select()

The best trees from all nine analyses were again concatenated and used as input for subsequent rounds of fusing and swapping; this procedure continued iteratively until the costs of all nine output tree sets equaled the costs of all nine input tree sets for three consecutive rounds (in this case, after four rounds of tree fusing and swapping).

In addition to the total-evidence analyses, all four individual gene partitions and the morphological/behavioral dataset were analyzed separately, using the same iterative procedure described above. Analyses were terminated once the input and output tree costs were the same for at least two consecutive rounds of tree search (five rounds each for the 18S, 28S, COI, and EF1-α partitions; three rounds for the morphology/behavior partition).

The preferred tree was chosen after calculating the incongruence length difference (ILD; Mickevich and Farris, 1981; Wheeler, 1995; Schulmeister et al., 2002) for each total-evidence tree and selecting the parameter set that minimized the statistic. The ILD here represents a measure of character incongruence, i.e., the character conflict created by the combination of multiple data partitions.

Clade sensitivities for groups within the preferred tree were calculated and visualized using Cladescan version 1.0 (Sanders, 2010). Bremer supports were calculated using POY version 5.0.1 alpha (Varón et al., 2011) and based on exhaustive enumeration of the TBR neighborhood of the preferred tree:

swap(tbr,all,visited:"bremertrees.tre") report(graphsupports:bremer:"bremertrees.tre")

RESULTS

Each of the nine total evidence analyses returned a set of one or more most parsimonious trees (fig. 2); of these, the fully resolved tree generated by the 2:2:1:1 parameter set (indels equal to transversions, twice transitions and morphological/behavioral changes) resulted in the lowest ILD score and was thus chosen as the preferred phylogenetic hypothesis (table 3). Details of this minimum ILD (mILD) tree, including Bremer supports and major clade sensitivities, are shown in figures 3 and 4, respectively.

The mILD tree differed in a number of respects from the equal weights parsimony (EWP) consensus tree; figure 5 shows a side-by-side comparison of these trees. A simplified version of the EWP tree, with sensitivity plots for major clades superimposed, is shown in figure 6.

DISCUSSION

The results of this study reveal and formalize deep instabilities among higher order hymenopteran phylogenetic relationships, at least with respect to variation in four key transformation cost parameters. Such instability is consistent with a history of competing, mutually incompatible phylogenetic hypotheses (reviewed in Sharkey, 2007; see also Vilhelmsen et al., 2010; Heraty et al., 2011; Sharkey

et al., 2012), and serves as a reminder of the difficulties facing hymenopteran systematists. What follows are notes on some of the major implications of our results:

The mILD Tree vs. the EWP Tree

Two of our final consensus trees, the mILD (2:2:1:1) and EWP (1:1:1:1) cladograms, deserve special attention: the former, because it maximizes an objective optimality criterion (in this case the minimization of the ILD statistic), and the latter because it is the tree most consistent with an agnostic, equal-weights approach to parsimony that also minimizes the overall number of transformations.

Of the two, the mILD tree deviates the most from a traditional and intuitive classification of the Hymenoptera. The most dramatic of these deviations is almost certainly a polyphyletic Ichneumonoidea, with its closely related families Braconidae and Ichneumonidae placed far apart on the tree (fig. 5); given the long list of synapomorphies uniting these families (Goulet and Huber, 1993; references therein), this result seems unlikely to reflect actual phylogenetic relationships. In addition, the mILD tree also renders Apocrita paraphyletic with respect to Orussoidea, as discussed below.

These features contrast with the more traditional scheme found in the EWP tree, which also returns the largest proportion of extant superfamilies as monophyletic groups. The two cladograms also differ on many of the details of apocritan relationships.

While we designate the mILD tree as the "preferred" phylogenetic hypothesis, we recognize the value of the EWP tree as an alternate hypothesis and discuss the results of both analyses below.

The Monophyly of the Hymenopteran Superfamilies

Of the 22 superfamilies evaluated here, 14 (Xyeloidea [S], Tenthredinoidea [S], Pamphili-oidea [S], Cephoidea [S], Xiphydroidea [S], Stephanoidea [P], Evanioidea [P], Trigonaloidea [P], Megalyroidea [P], Ceraphronoidea [P], Mymarommatoidea [P], Platygastroidea [P], Cyni-poidea [P], and Chalcidoidea [P]; S = "Symphyta," P = "Parasitica," and A = Aculeata) were stable across all nine transformation cost parameter sets. Three more (Orussoidea [S], Ichneu-monoidea [P], and Apoidea [A]) were monophyletic in eight out of nine analyses. To the extent that a clade's robustness to parametric change may function as a form of clade support (Giribet, 2003), we consider these groups well supported by the sensitivity analysis.

Siricoidea, composed of the symphytan families Anxyelidae and Siricidae, was a monophy-letic group in six of nine analyses, while Proctotrupoidea sensu stricto [P] (i.e., sensu Sharkey, 2007: Austroniidae + Heloridae + Pelecinidae + Peradeniidae + Proctotrupidae + Proctorenyxi-dae + Roproniidae + Vanhorniidae) was monophyletic only in four. The remaining three super-families: the parasitican Diaprioidea (again sensu Sharkey, 2007: Diapriidae + Monomachidae + Maamingidae) and the aculeate Chrysidoidea and Vespoidea did not appear as natural groups under any of the cost regimes.

Diaprioidea is a relatively new concept (Sharkey, 2007), and while the group appeared in both the total-evidence parsimony tree of Sharkey et al. (2012) and in the maximum likelihood,
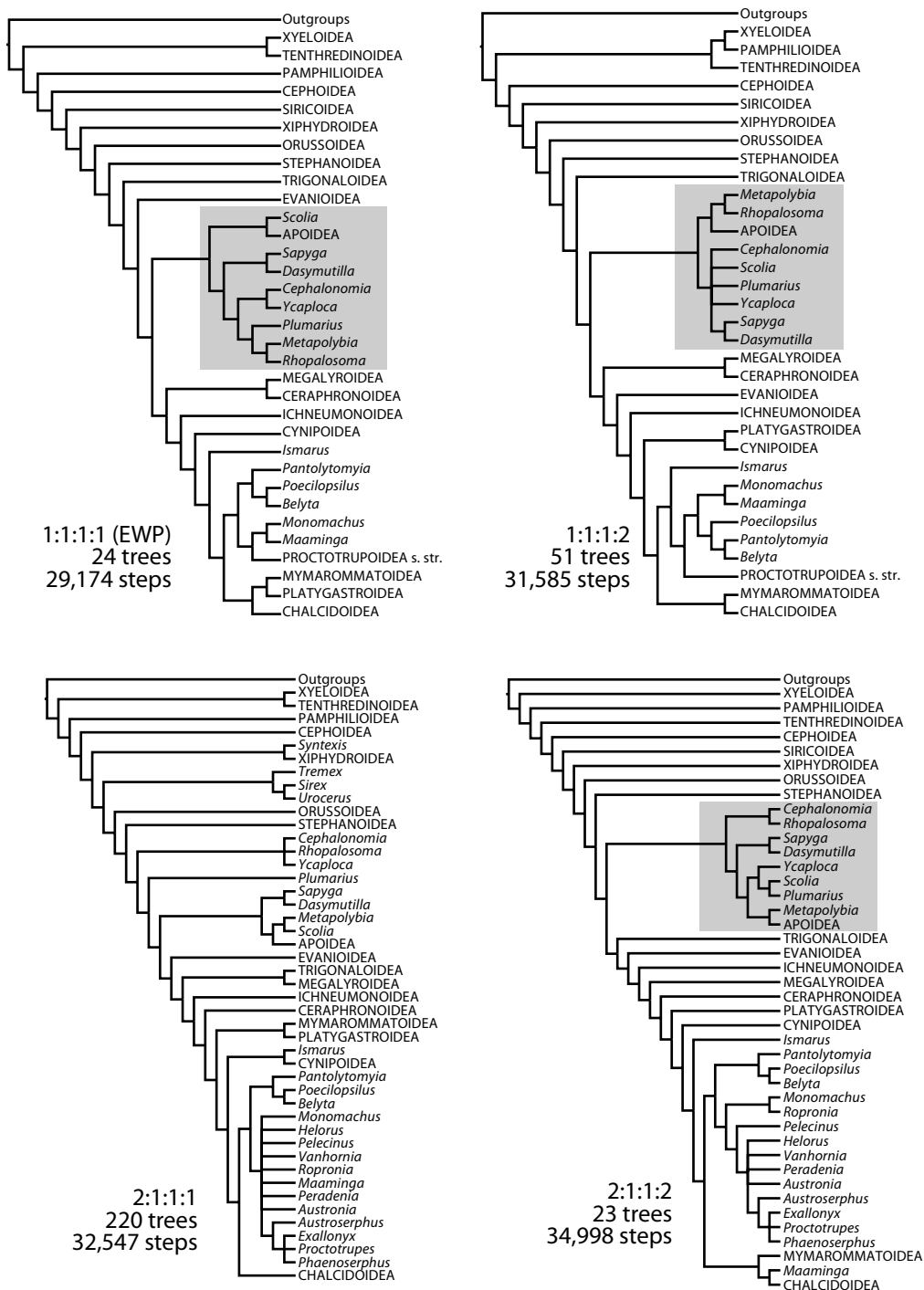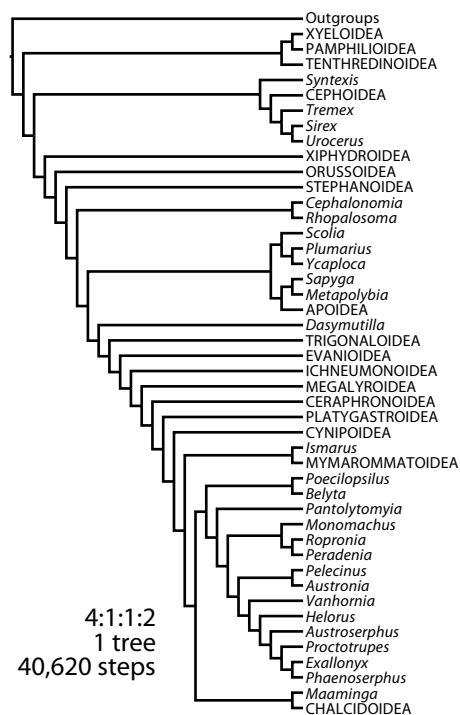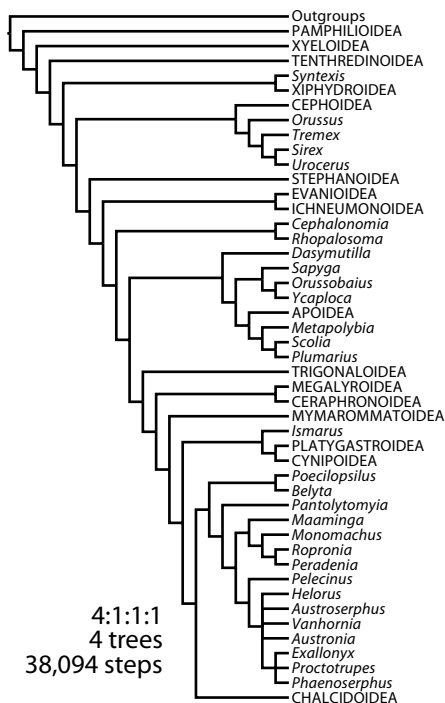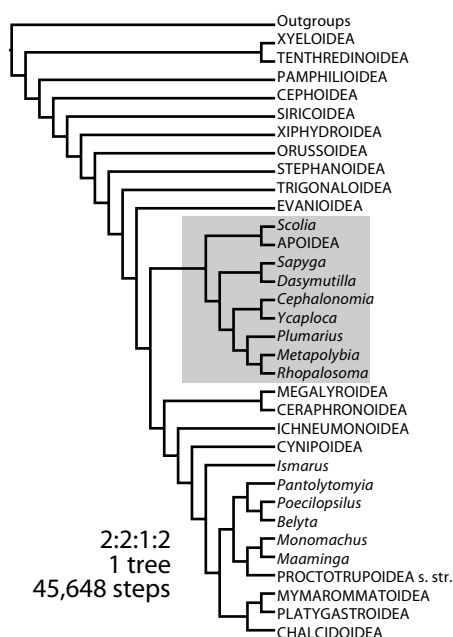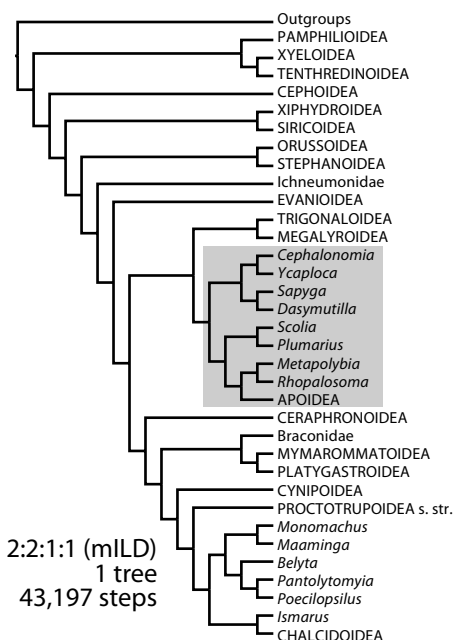
FIG. 2. A–C. Strict consensus trees (on this and following pages) produced by all nine transformation cost parameter sets and simplified, when possible, to the superfamilial level; total tree length, as well as the number of most parsimonious trees, are shown at the bottom left of each tree. Monophyletic Aculeata highlighted.
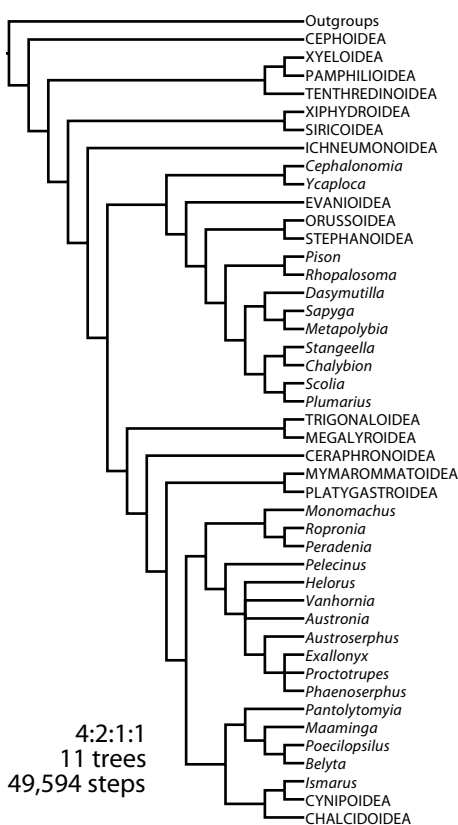
```
                    ┌─── Outgroups
                    ├─── CEPHOIDEA
                    ├─── XYELOIDEA
                    ├─── PAMPHILIOIDEA
                    ├─── TENTHREDINOIDEA
                    ├─── XIPHYDROIDEA
                    ├─── SIRICOIDEA
                    ├─── ICHNEUMONOIDEA
                    ├─── Cephalonomia
                    ├─── Ycaploca
                    ├─── EVANIOIDEA
                    ├─── ORUSSOIDEA
                    ├─── STEPHANOIDEA
                    ├─── Pison
                    ├─── Rhopalosoma
                    ├─── Dasymutilla
                    ├─── Sapyga
                    ├─── Metapolybia
                    ├─── Stangeella
                    ├─── Chalybion
                    ├─── Scolia
                    ├─── Plumarius
                    ├─── TRIGONALOIDEA
                    ├─── MEGALYROIDEA
                    ├─── CERAPHRONOIDEA
                    ├─── MYMAROMMATOIDEA
                    ├─── PLATYGASTROIDEA
                    ├─── Monomachus
                    ├─── Ropronia
                    ├─── Peradenia
                    ├─── Pelecinus
                    ├─── Helorus
                    ├─── Vanhornia
                    ├─── Austronia
                    ├─── Austroserphus
                    ├─── Exallonyx
                    ├─── Proctotrupes
                    ├─── Phaenoserphus
                    ├─── Pantolytomyia
                    ├─── Maaminga
                    ├─── Poecilopsilus
                    ├─── Belyta
                    ├─── Ismarus
                    ├─── CYNIPOIDEA
                    └─── CHALCIDOIDEA

          4:2:1:1
          11 trees
          49,594 steps
```

FIG. 2. *(continued).*

Bayesian, and eye-aligned parsimony trees of Heraty et al. (2011), it was not necessarily well supported (MP tree: bootstrap < 50% [Heraty et al., 2011]; MP tree: symmetric resampling frequency difference = 0 [Sharkey et al., 2012]). In the current study, the group breaks up in a variety of ways depending on the cost parameters investigated; however, one consistent feature is the excision of the diapriid genus *Ismarus* from the rest of Diapriidae and its relocation elsewhere within Proctotrupomorpha. In the EWP tree, *Ismarus* is sister to a clade composed of ((Chalcidoidea + (Mymarommatoidea + Platygastroidea)) + (Diapriidae + (Proctotrupoidea sensu stricto + (Maamingidae + Monomachidae)))). In the mILD tree, the topology is ((Chalcidoidea + *Ismarus*) + the remaining Diaprioidea). This wayward *Ismarus* and its relationship to the rest of the Diapriidae were anticipated in part by Sharkey (2007), who doubted the latter's monophyly; Vilhelmsen et al. (2010) reached a similar conclusion based on morphological data alone, while Sharkey et al. (2012) raised the Ismarinae to family status, Ismaridae.

Serious doubts about the monophyly of Vespoidea have been building for some time (Sharkey, 2007; Pilgrim et al., 2008; Heraty et al., 2011; Debevec et al., 2012; Sharkey et al. 2012; Wilson et al., 2013) and the current study supports that notion. At the moment, the more interesting question is not whether Vespoidea constitutes a paraphyletic group, but rather how, exactly, other aculeates render that paraphyly. In the current study, we a see a variety of vespoid deconstructions: In the EWP tree, *Scolia* is sister to Apoidea, while a polyphyletic Chrysidoidea shows up twice among the remaining Vespoidea. In the mILD tree, Apoidea is sister to *Metapolybia* + *Rhopalosoma,* while *Scolia* is sister to the chrysidoid genus *Plumarius*, and *Sapyga* + *Dasymutilla* is sister to the rest of the chrysidoids. No clear picture of aculeate relationships emerges, with the possible exception of a clade composed of Sapygidae + Mutillidae (in six out of nine analyses), and, of course, the monophyly of Apoidea.

The meaning of a paraphyletic or polyphyletic Chrysidoidea is much harder to gauge. The group has traditionally been considered to be a well-established clade, united by a number of key synapomorphies (enlarged female femora, reduction of the Cu_2 vein of the forewing, etc. [Grimaldi and Engel, 2005]). Among the recent HymAToL studies, Vilhelmsen et al. (2010) and Heraty et al. (2011) both recovered a nonmonophyletic Chrysidoidea, while Sharkey et al. (2012) united their three chrysidoid genera (*Plumarius* [Plumariidae], *Cephalonomia* [Bethylidae], and *Ycaploca* [Scolebythidae]; the same taxa used in the current study). The true nature of chrysidoid relationships, both within the group and with the other aculeates, is thus unclear.

The Basalmost Lineage of the Hymenoptera

That the "Symphyta" form a paraphyletic grade at the base of the hymenopteran tree has never really been in doubt (Schulmeister et al., 2002); instead, debate has centered on the precise nature of the relationships of the symphytan superfamilies (Xyeloidea, Pamphilidoidea, Tenthredinoidea, Siricoidea, Cephoidea, Xyphidroidea, and Orussoidea) to one other (reviewed in Schulmeister et al., 2002) and to the considerably more speciose and economically important Apocrita.

Most recent analyses (Vilhelmsen, 2001; Schulmeister et al., 2002; Schulmeister, 2003; Sharkey et al., 2012,) place Xyeloidea, with its single small and geographically restricted family, in the basalmost position within Hymenoptera, a placement bolstered in part by the group's ancient fossil record (Goulet and Huber, 1993; Grimaldi and Engel, 2005). That said, Heraty et al. (2011) united Xyeloidea with Tenthredinoidea as the basal lineage of the order, an arrangement found in three of our nine analyses, including the EWP tree. Four of the nine analyses, including the preferred mILD tree, produced a basal lineage composed of Pamphilioidea + (Xyeloidea + Tenthredinoidea); only two of our cladograms place Xyeloidea alone as the basal lineage.

Of these hypotheses, the last is the most intuitive. The remaining Hymenoptera (the so-called Neohymenoptera; Grimaldi and Engel, 2005) share a number of putative morphological synapomorphies including certain details of wing venation and postspiracular mesothoracic sclerites, among others. Unfortunately, our analyses do little to resolve this debate, except to confirm a place for Xyelidae within the basal lineage; whether or not that relict family is joined by Tenthredinoidea and Pamphilioidea is unclear.

The Phylogenetic Structure of Vespina

While some authors have challenged apocritan monophyly through the unification of Orussoidea and Stephanoidea (reviewed in Schulmeister et al., 2002; Heraty et al., 2011), support for this clade has never been particularly strong, and in fact requires the reversal of the wasp waist constriction on the lineage leading to modern orussids. (The close relationship of Orussoidea to Apocrita, of course, has never been in doubt.)

The traditional and intuitive arrangement of Vespina (= Euhymenoptera of Grimaldi and Engel, 2005) is Orussoidea + (Stephanoidea + all other apocritans); this topology allows for a single origin of parasitoid behavior on the branch leading to Vespina, followed by a single, unreversed origin of the wasp waist constriction in the branch leading to Stephanoidea + the other apocritans. In fact, this arrangement is present here in six out of nine analyses, including the EWP tree; only the mILD tree has the Orussoidea + Stephanoidea clade as sister to the rest of Apocrita.

The Position of Aculeata within "Parasitica"

Deciphering the precise relationships among Aculeata and the other apocritan lineages is probably the most challenging issue facing hymenopteran systematists. From the mostly unresolved tree of Königsmann (1978, in Whitfield, 1992) to the more or less resolved, but poorly
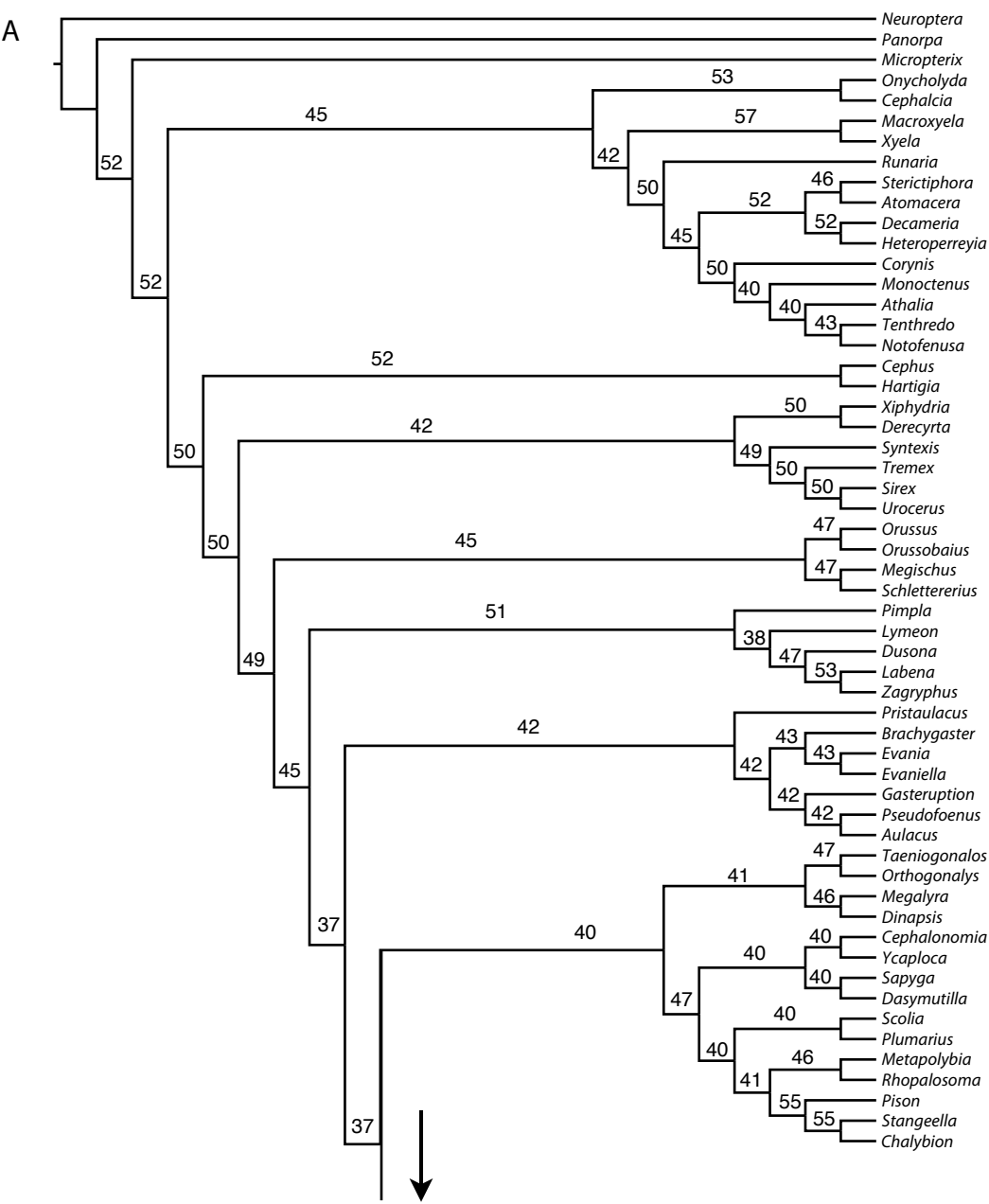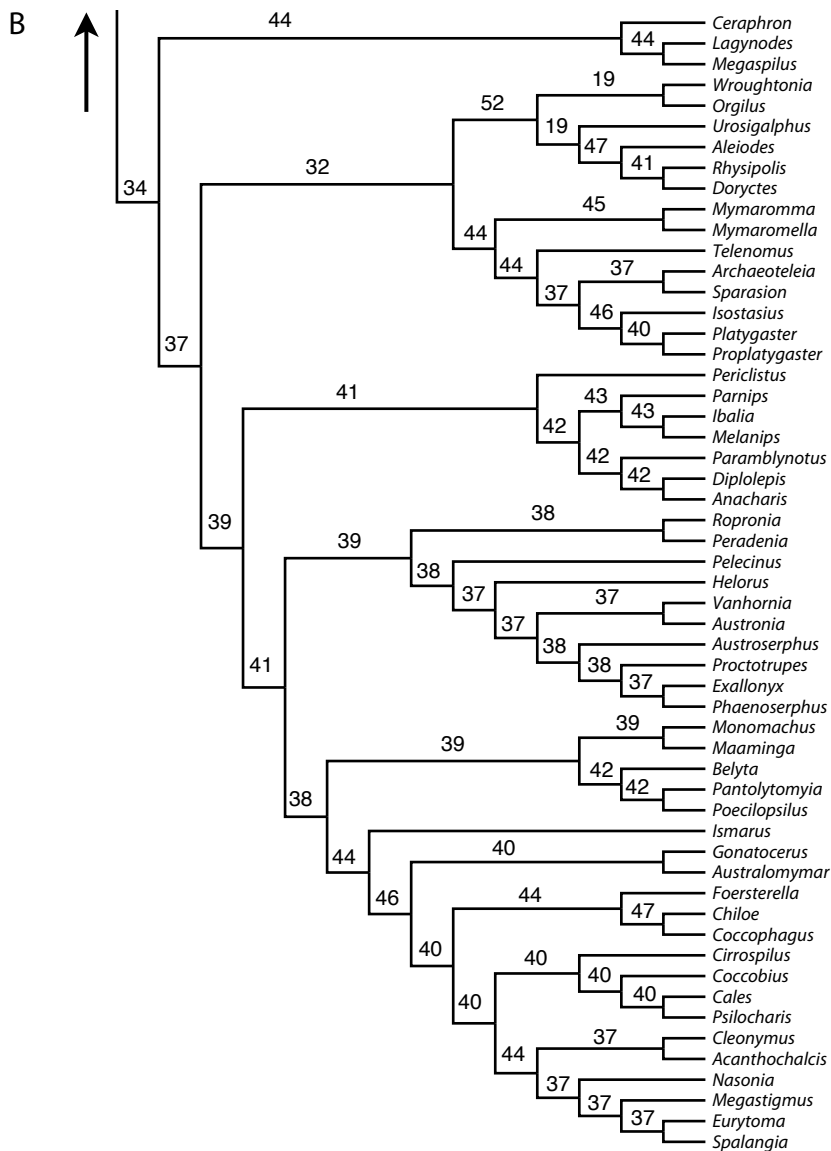
FIG. 3A–B. Fully resolved genus-level cladogram produced by the 2:2:1:1 (mILD) transformation cost parameter set, with Bremer supports.

supported, total-evidence cladogram of Sharkey et al. (2012), a variety of aculeate sister-group hypotheses have been proposed including, but not limited to:

- Aculeata sister to Ichneumonoidea (= Ichneumonomorpha; Rasnitsyn, 1988; Dowton and Austin, 1994; Dowton et al., 1997; Sharkey, 2007; Vilhelmsen et al., 2010)
- Aculeata sister to a monophyletic Parasitica (Ronquist et al., 1999; Dowton and Austin, 2001)
- Aculeata sister to Evanioidea (Peters et al., 2011; Sharkey et al., 2012)
- Aculeata sister to Trigonaloidea or Trigonaloidea + Megalyroidea (Heraty et al., 2011; Klopfstein et al., 2013)
- Aculeata sister all apocritans except Stephanoidea (Vilhelmsen et al., 2010)
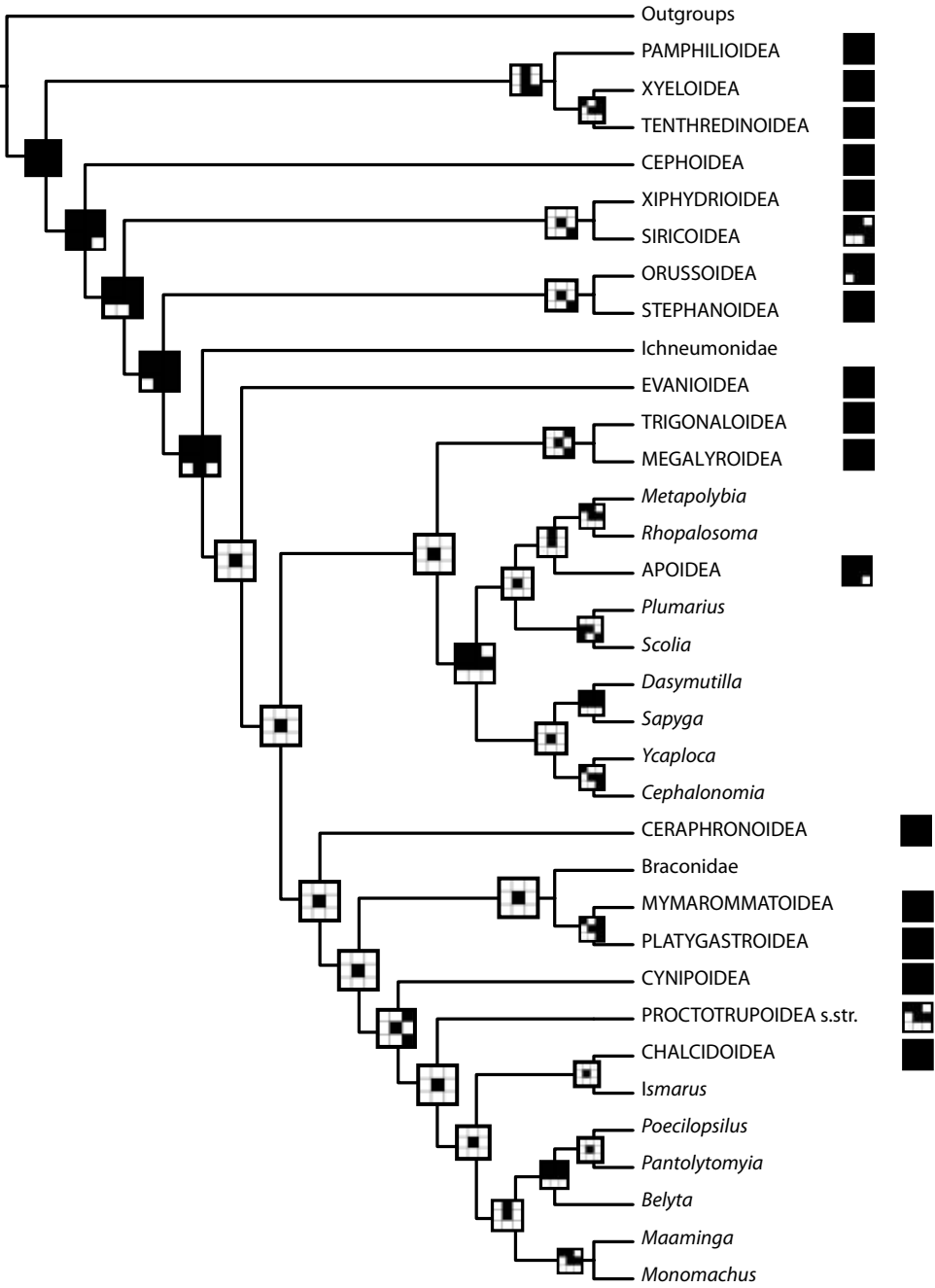
FIG. 4. Simplified 2:2:1:1 (mILD) tree with sensitivity plots for each node. Superfamily sensitivity plots are shown to the right of each superfamilial terminal.
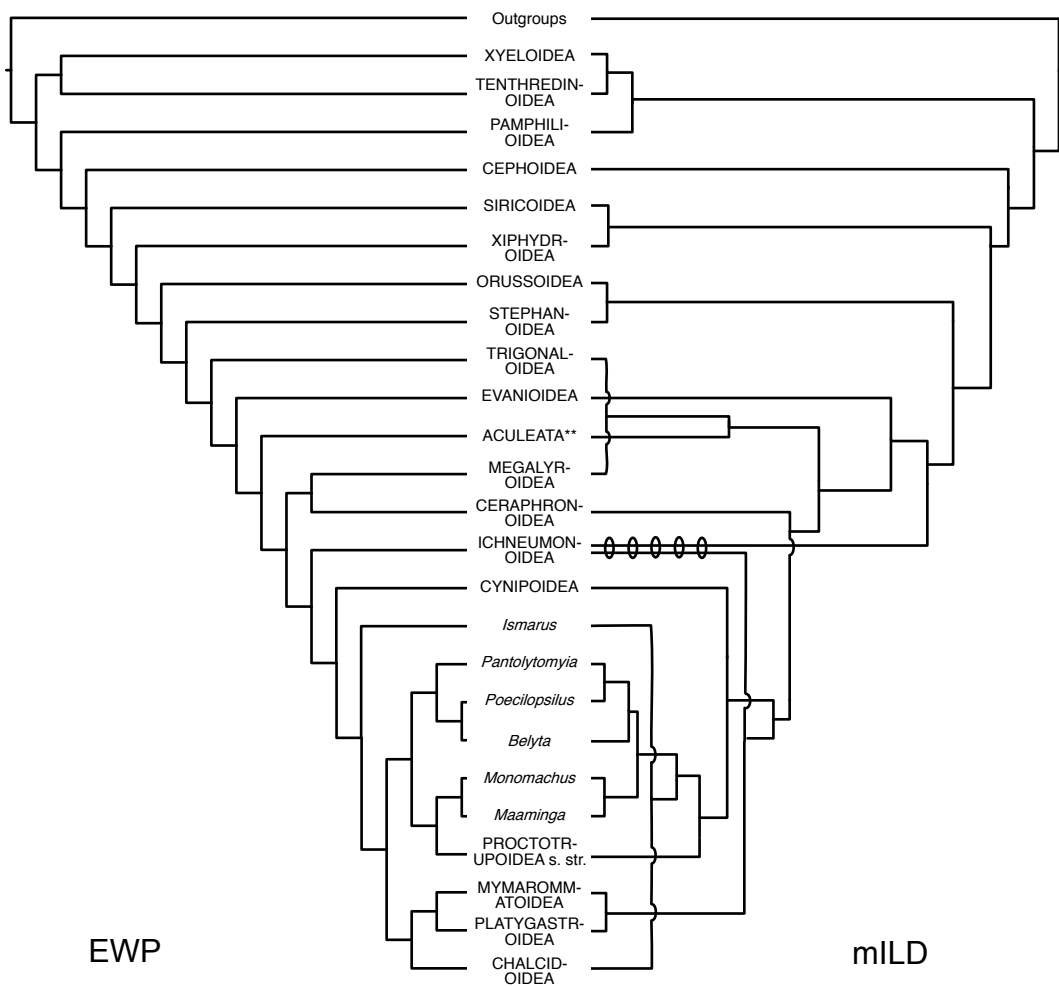
FIG. 5. Direct comparison of the 2:2:1:1 (mILD) and 1:1:1:1 (EWP) topologies (simplified to superfamilial level). Note the polyphyletic Ichneumonoidea in the mILD tree.

Our trees present a wide range of possible sister groups (fig. 2), underlining the topological instabilities inherent in apocritan relationships, at least given the current state of taxon and character sampling. In our mILD tree, Aculeata is sister to Trigonaloidea + Megalyroidea, while the EWP tree has Aculeata sister to all other apocritans excluding Stephanoidea, Trigonaloidea, and Evanioidea. The important point is that these relationships demonstrate too much instability to allow for confident statements regarding final relationships within the true wasps.

THE DEEP STRUCTURE OF HYMENOPTERAN PHYLOGENY

Besides the monophyly of Hymenoptera itself, none of the order's deepest and oldest relationships were unanimously supported across all parameter sets; nevertheless, two important clades were present in eight out of nine analyses: Unicalcarida (all Hymenoptera with the

FIG. 6. Simplified consensus of 24 trees produced by the 1:1:1:1 (EWP) cost parameter set, with sensitivity plots for each node. Superfamily sensitivity plots are shown to the right of each superfamilial terminal.

exception of Xyeloidea, Tenthredinoidea, and Pamphilioidea) and Proctotrupomorpha (Platygastroidea + Cynipoidea + Proctotrupoidea sensu stricto + Diaprioidea + Mymmaromatoidea + Chalcidoidea). The symphytan lineages as a whole clearly form a basal grade relative to Apocrita, which may or may not include the orussids as sister to Stephanoidea.

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