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On ''Molecular Phylogeny of Vespidae (Hymenoptera) and the Evolution of Sociality in Wasps''

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ABSTRACT

The alignment of molecular sequence data published by Schmitz and Moritz (1998. Molecular phylogeny of Vespidae (Hymenoptera) and the evolution of sociality in wasps, Molecular Phylogenetics and Evolution 9: 183–191) supported closer phylogenetic relationship of Eumeninae to Polistinae + Vespinae than Stenogastrinae, from which they concluded that social behavior has independently evolved twice in the wasp family Vespidae. However, their analyses also showed the Vespidae as paraphyletic in terms of the bee family Apidae. Simultaneous analysis of these molecular data with published morphological and behavioral characters is presented. The resulting cladograms support monophyly of Vespidae, as well as monophyly of social wasps, with the primitively social Stenogastrinae being more closely related to the highly social Polistinae + Vespinae than the solitary Eumeninae. A realignment of the sequence data is also presented, which is more parsimonious than that published by Schmitz and Moritz. Analysis of the realigned sequences also supports monophyly of Vespidae, as well as monophyly of social wasps, with the Stenogastrinae being more closely related to Polistinae + Vespinae than are Eumeninae.

INTRODUCTION

Schmitz and Moritz (1998: 183) published alignments and analyses of two molecular datasets which, they claimed, ''provide strong evidence that sociality has independently evolved twice in the Vespidae.''

The data consisted of sequences from the 16S mt-rDNA and 28S rDNA loci for the following sample of wasps of the family

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Vespidae: three Vespinae (*Vespa crabro*, *Provespa nocturna*, and *Vespula germanica*), three Polistinae (*Belonogaster petiolata* and two species of *Polistes*), three Stenogastrinae (*Liostenogaster vechti*, *Eustenogaster fraterna*, and *Parischnogaster mellyi*), four Eumeninae (two species of *Ancistrocerus* and a different species of the genus *Eumenes* for each sequence dataset). Vespinae and Polistinae are all highly social species, while species of Stenogastrinae are primitively social and those of Eumeninae are mostly solitary (for a review of behavior, see Ross and Matthews, 1991). The datasets also included two bees of the family Apidae (both of them species of *Apis*) and a different parasitoid outgroup for each sequence dataset (one species of Pteromalidae and one of Braconidae).

For the 16S dataset, there were 169 informative characters out of 314 aligned base pairs (Schmitz and Moritz [1998: 187] excluded 62 base pairs from their analyses because of ''poor alignments''); for the 28S dataset, 125 out of 331 aligned base pairs were informative.

Schmitz and Moritz's (1998) analyses included the usual (misguided) distance and likelihood procedures commonly used in molecular systematics, as well as invalid statistics. I will not repeat criticisms of such methods, which have been abundantly detailed in the cladistic literature (Farris, 1981, 1983, 1985, 1999; Farris *in* Werdelin, 1989; Carpenter, 1990, 1992a, 1992b, 1996; Kluge and Wolf, 1993; Farris et al., 1996; Siddall, 1998; Siddall and Whiting, 1999). However, their parsimony cladograms indeed supported a closer relationship of the eumenine sample to the polistines + vespines than the stenogastrines, thus diphyly of social wasps, as figures 1 and 2 show, and this was also supported by analysis of the two alignments combined. This result is in conflict with the cladistic analyses of vespid subfamilies by Carpenter (1981, 1988) and Carpenter and Rasnitsyn (1990), which supported Stenogastrinae as the sister group of Polistinae + Vespinae, with Eumeninae in turn the sister group to that clade, and thus monophyly of social wasps. This latter arrangement is also in line with traditional taxonomic treatment of the group (Richards, 1962). The disparity in results is obviously significant for the evolutionary study of social behavior in wasps: Whether social behavior evolved once or twice independently is crucial for the interpretation of studies of behavioral features in primitively social versus highly social wasps.

Schmitz and Moritz (1998: 183) cited Carpenter (1981) to the effect that the morphological characters grouping Stenogastrinae with Polistinae + Vespinae ''may be prone to homoplasy'', and indeed I have pointed out that the morphological evidence is not abundant and that the behavioral evidence most convincingly supports the grouping (see Carpenter, 1988). The behavioral characters relate mostly to social behavior, but of this Schmitz and Moritz (1998: 184) stated:

> the use of social behavior in cladistic studies may be difficult, since it is well known that sociality evolved independently at least eight times among bees . . . . There seems to be a reasonable risk that one is trapped by homoplasy if social behavior is used as aphylogenetic [sic] character in cladistic analysis of distantly related groups. Furthermore, behavioral traits may show great plasticity, rendering them less informative for phylogenetic studies.

The claim of greater plasticity rendering behavioral characters less informative is actually without foundation (Wenzel, 1992), and behavioral characters do not show elevated levels of homoplasy relative to morphological characters (De Queiroz and Wimberger, 1993). The critical test of whether the social behavior characters in these wasps are informative or not would be to include them in a simultaneous analysis together with the morphological and molecular characters (Nixon and Carpenter, 1996). A related fallacy in the quoted statement is the equation of homoplasy and lack of phylogenetic informativeness. But homoplasy in and of itself does not prevent a character from being informative—a homoplastic character may still be informative in a particular clade, a fact which is part of the foundation of numerical cladistics (the ''Wagner method'' of Farris [1970], which permits evolutionary reversibility) and phylogenetic analysis itself (see Farris, 1983). As Wenzel (1997: 31) put it, ''Eliminating characters because they are expected to show high homoplasy is an unac-

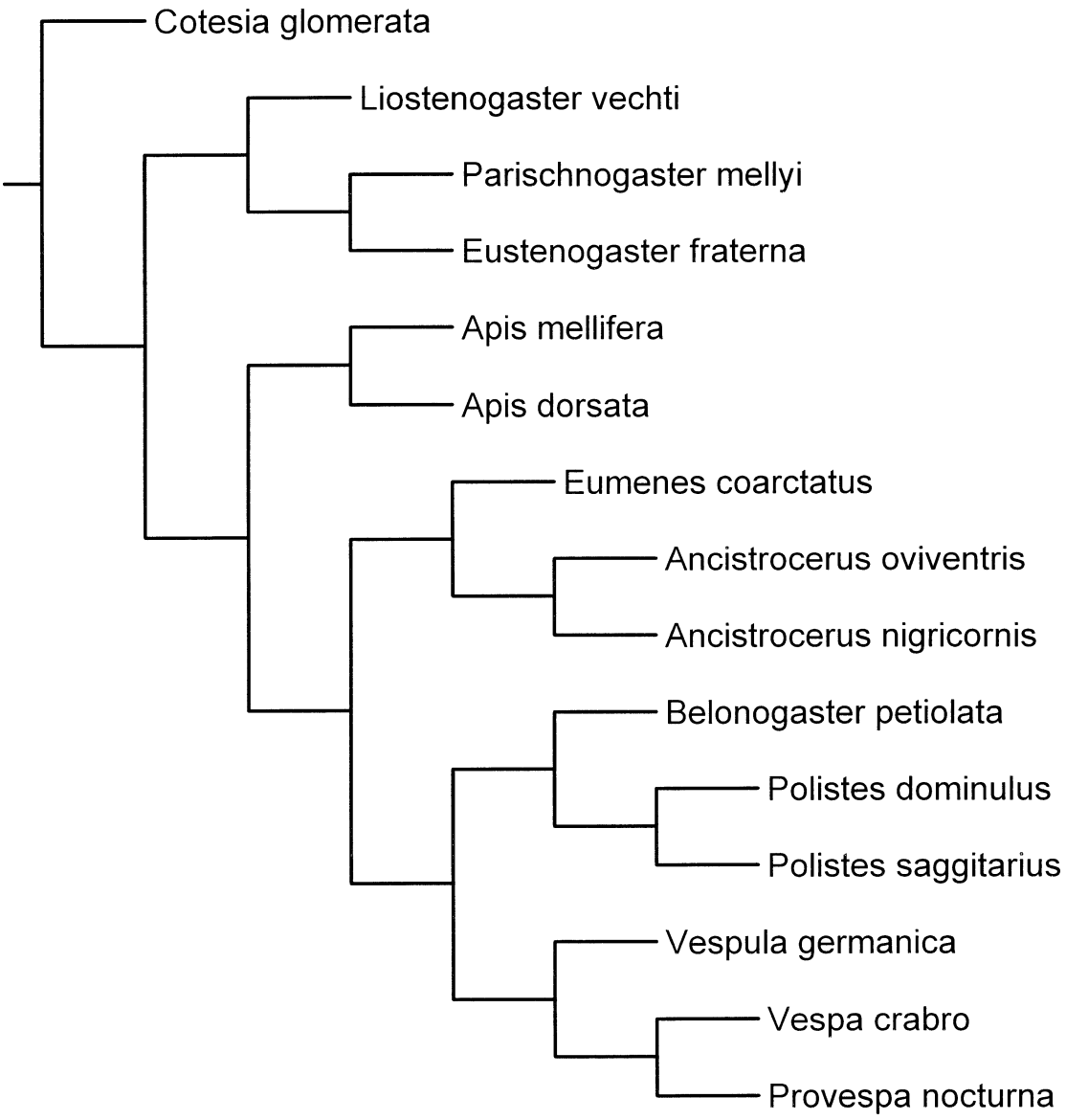

Fig. 1. Cladogram for the 16S mt-rDNA alignment of Schmitz and Moritz (1998). The length is 512 steps; consistency index = 0.52 and retention index = 0.54.

ceptable *ad hoc* protection of an hypothesis from a legitimate test.''

Figures 1 and 2 also show the family Vespidae as paraphyletic, in terms of the bee genus *Apis*. Vespids and apids are placed in different superfamilies and are not at all closely related (see Brothers and Carpenter, 1993; Brothers, 1999). The separation of these two families is supported by abundant morphological data. Schmitz and Moritz (1998: 189) termed their arrangement ''unusual'', and stated, ''To clarify the exact position of the Stenogastrinae among the aculeate hymenoptera, a more extensive study, including a range of additional vespid and nonvespid members of the Vespoidea, is required.'' Nevertheless, Schmitz and Moritz (1998: 190) concluded that their data provide

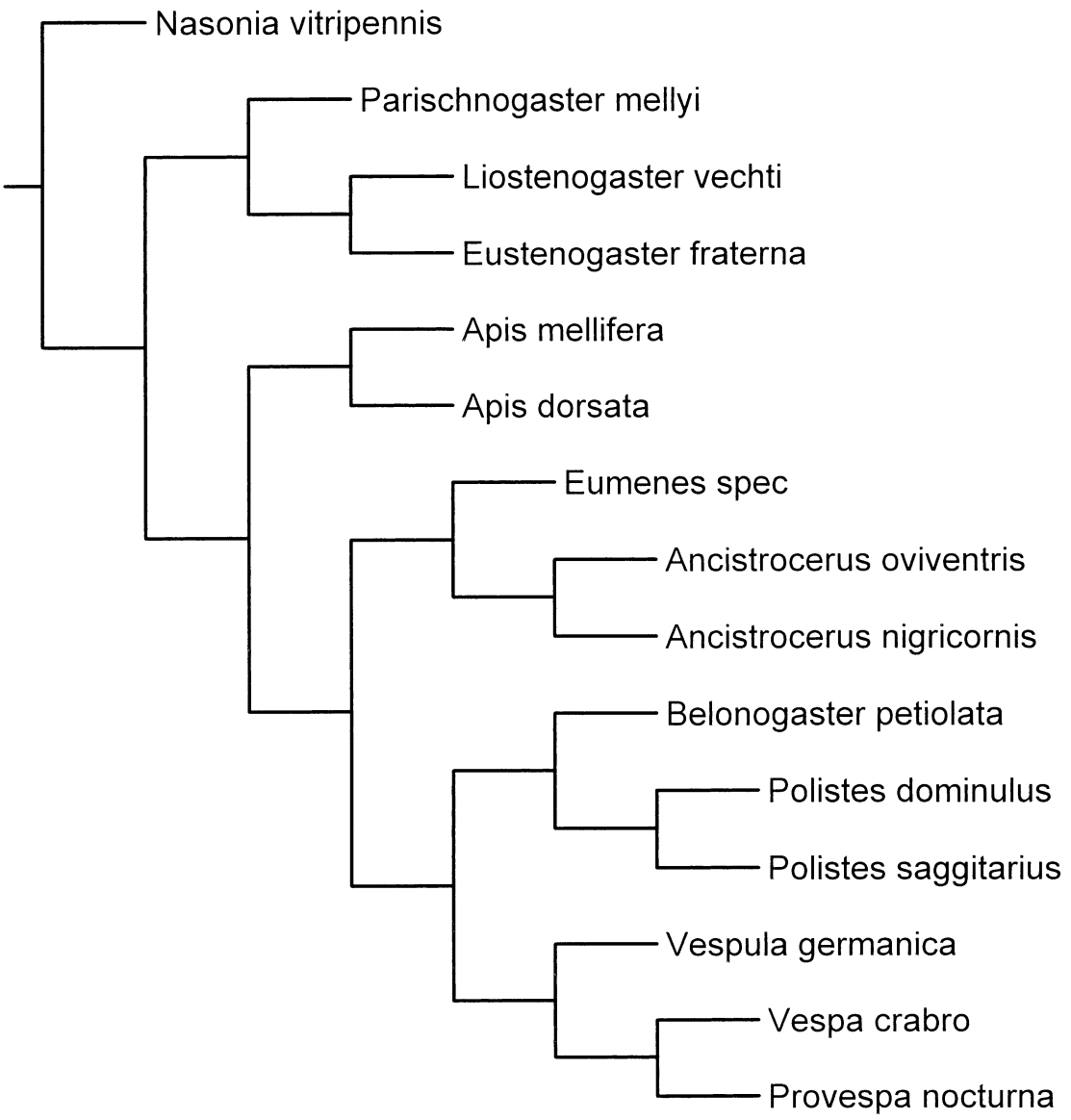

Fig. 2. Cladogram for the 28S rDNA alignment of Schmitz and Moritz (1998). The length is 302 steps; consistency index = 0.76 and retention index = 0.80.

''strong evidence for the sister group relationship of Eumeninae to Polistinae + Vespinae.''

SIMULTANEOUS ANALYSIS

Whether the evidence of Schmitz and Moritiz is ''strong'' should really be assessed through simultaneous analysis of the molecular data combined with morphological and behavioral characters (see Nixon and Carpenter, 1996, for review). I now present such an analysis. I adduced the relevant morphological and behavioral data from Carpenter (1981, on vespid subfamilies; 1987, on vespine genera; 1988, on stenogastrine genera; 1989b, on social behavior; 1991, on polistine genera), Brothers and Carpenter (1993, for aculeate family relationships), Ronquist et al. (1999, for superfamily relationships), and

Hunt (1999, on vespid subfamilies), for a total of 125 additional variables. These variables are listed in appendix 1 and in a matrix scored for the sequenced taxa at the end of that appendix.

The matrix was produced by: (1) taking each cited character matrix, reducing each to the pertinent family, subfamily, or generic vectors using the program Winclada (Nixon, 2002) through deletion of irrelevant terminals, (2) flagging the uninformative characters by use of the ''mop uninformative chars'' command of Winclada, and then deleting those characters; and (3) combining the pertinent scores as summary scores with the sequenced exemplars by merging the matrices (see Nixon and Carpenter [1996] on mechanics and terminology). None of the 16S data were excluded.

Analysis with the program Nona (Goloboff, 1999) results in a single cladogram for the 16S alignment combined with morphology and behavior (fig. 3) and two cladograms for the 28S alignment combined with morphology and behavior (fig. 4 is the consensus tree); the minimum-length tree was found by most searches. The results of the simultaneous analysis do not indicate a reclassification of Aculeata, nor reinterpretation of vespid phylogenetic relationships. Vespidae are supported as a monophyletic family, and social wasps are supported as monophyletic; that is, Stenogastrinae are the sister group of Polistinae + Vespinae. Simultaneous analysis of both alignments combined with the morphology and behavior leads to the same result (fig. 5). This is in accord with the results published by Carpenter (1981, 1988), and it is thus seen that the data of Schmitz and Moritz (1998) are scarcely ''strong''. When other characters are considered, the sequence alignments are overruled.

In support of their conclusions, Schmitz and Moritz (1998: 189) observed that rearranging their tree for both alignments to support monophyly of Vespidae required 31 additional steps. Rearranging the cladogram of figure 5 to support paraphyly of Vespidae, with Stenogastrinae excluded, requires 49 additional steps. Schmitz and Moritz also cited bootstrap values as confirming their tree, 100% for the Eumeninae + (Polistinae + Vespinae) and placement of Apidae as sister group to this clade in the tree for both alignments (the values are, respectively, 98% and 58% if 16S sites are not excluded). Use of bootstrap values for assessing confidence is misplaced (see Farris *in* Werdelin, 1989; Carpenter, 1992a, 1996; Kluge and Wolf, 1993), but in the present context it is perhaps worth pointing out that the monophyly of Vespidae is likewise supported by 100% bootstrap values (1000 replicates) for each combination of alignment with morphological and behavioral characters (figs. 3–5). Bremer support values, on the other hand, show a discrepancy: as calculated with Nona, for the combination of the two alignments, paraphyly of Vespidae is supported by just 1 step, while for the combination of molecular with morphological and behavioral characters, monophyly of Vespidae is supported by 36 steps.

REALIGNMENT

The lack of stability of the results supported by the sequence alignments could be due to a number of factors, but one obvious possibility is the alignments themselves. Schmitz and Moritz (1998: 186) produced their alignments ''by using the CLUSTAL V program . . . which were improved by comparison of the secondary structure of the rRNAs.'' Unfortunately, criticism of any alignment raises a difficult issue: in general, there is no optimality criterion for alignment. Thus, the common practice of inputting sequences into the Clustal program, obtaining an output alignment, and then changing that alignment by the user is acceptable, despite the fact that the changes are made on an arbitrary basis. Of course, authors may state that they are taking into account secondary structure or applying a weighting scheme for multiple substitution, and so on, but replicability of such procedures is, to state the obvious, not straightforward. To this is added the common practice of discarding data on the grounds that they are ''difficult to align''. Even if a program is employed, with defined cost parameters that the user does not override ad libitum, those parameters are purely arbitrary. This has been cogently discussed by Wheeler (1995), who proposed to deal with the problem by means of sensitivity analysis, wherein the cost parameters are var-

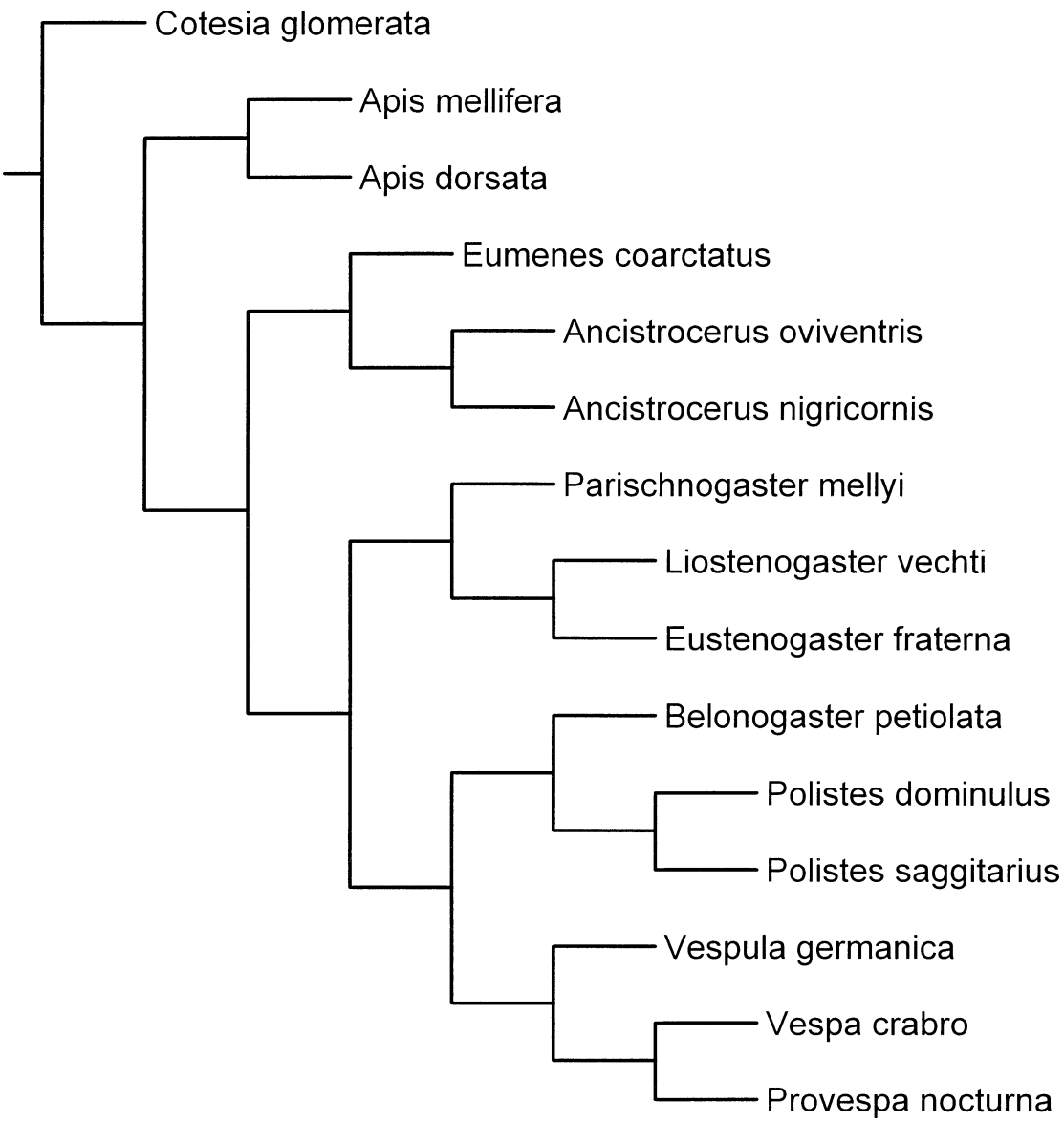

Fig. 3. Cladogram for the combined 16S data and the morphological and behavioral characters (see appendix 1). The length is 652 steps; consistency index = 0.64 and retention index = 0.69.

ied and multiple reanalyses undertaken in order to assess how sensitive the output alignment is to particular parameters. He also proposed to select among parameter schemes by means of congruence, with the costs chosen based on minimization of incongruence among datasets. Thus he appealed to an optimality criterion external to the alignment procedure to select the alignment.

Application of an alignment optimality criterion would obviate the necessity for sensitivity analysis, and in the realignment that follows I propose to use parsimony, specifically in the sense of preferring an alignment that implies fewer steps than another. As with the use of parsimony in phylogenetic analysis (Farris, 1983), parsimonious alignment would maximize similarities accounted for

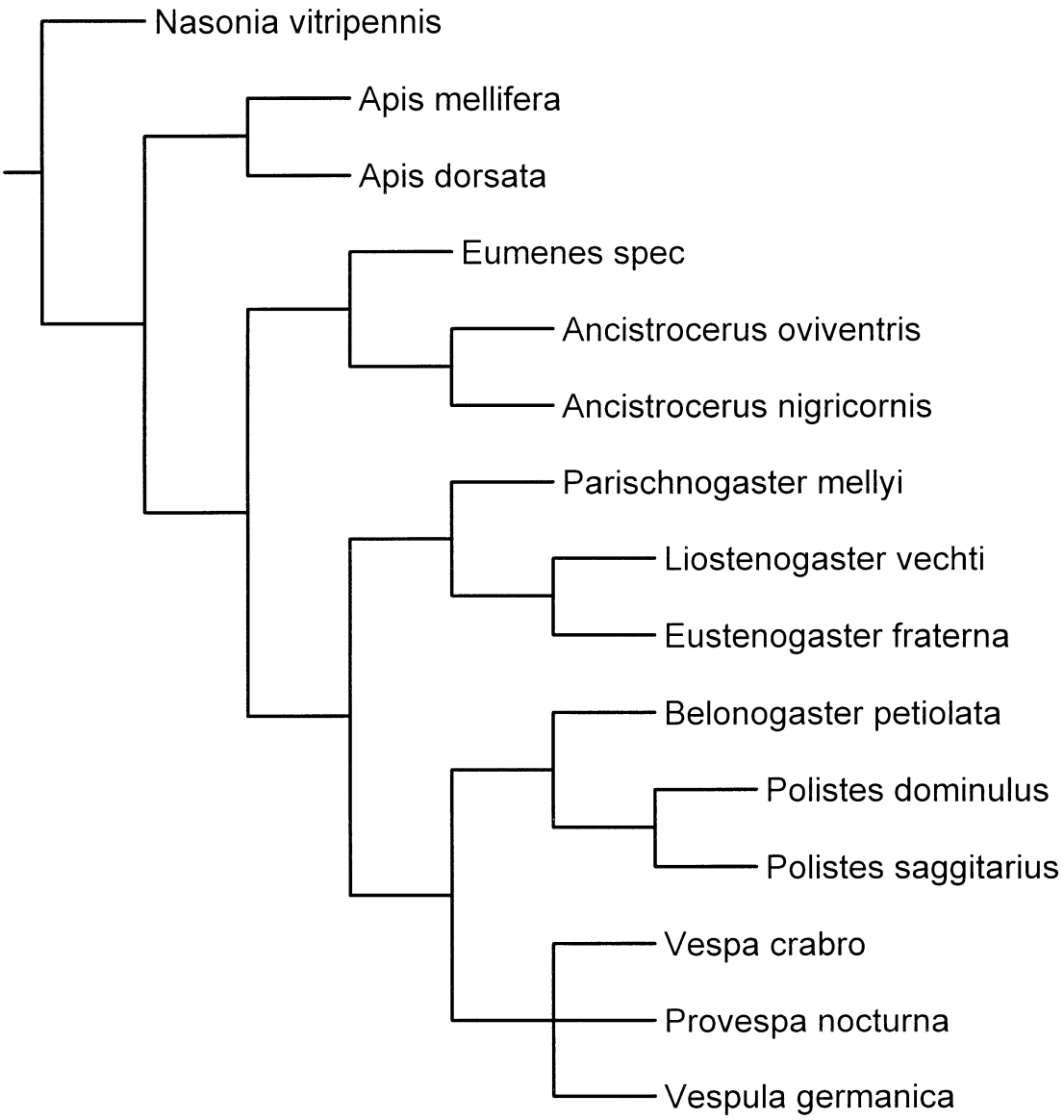

Fig. 4. Consensus tree for the combined 28S data and the morphological and behavioral characters (see appendix 1). The length is of the two underlying cladograms is 458 steps; consistency index = 0.81 and retention index = 0.85.

by ancestry (inheritance) by minimizing separate origins of features (= nucleic acid residues). Obviously, for this criterion, gaps must be factored so as to avoid trivial invariant alignments, as could be obtained by treating gaps as ''missing'' (i.e., no cost) and merely inserting them so that the alignment implied no steps. Treatment of gaps as a ''fifth state'' would obviate that problem, and then a bound could be set on changes. For example, if the most parsimonious alignment is the one that has the fewest changes, set as lower bound the length of the longest sequence (= maximum number of steps on a bush). Then the difference between maximum steps minus the longest sequence could be minimized.

However, Schmitz and Moritz (1998)

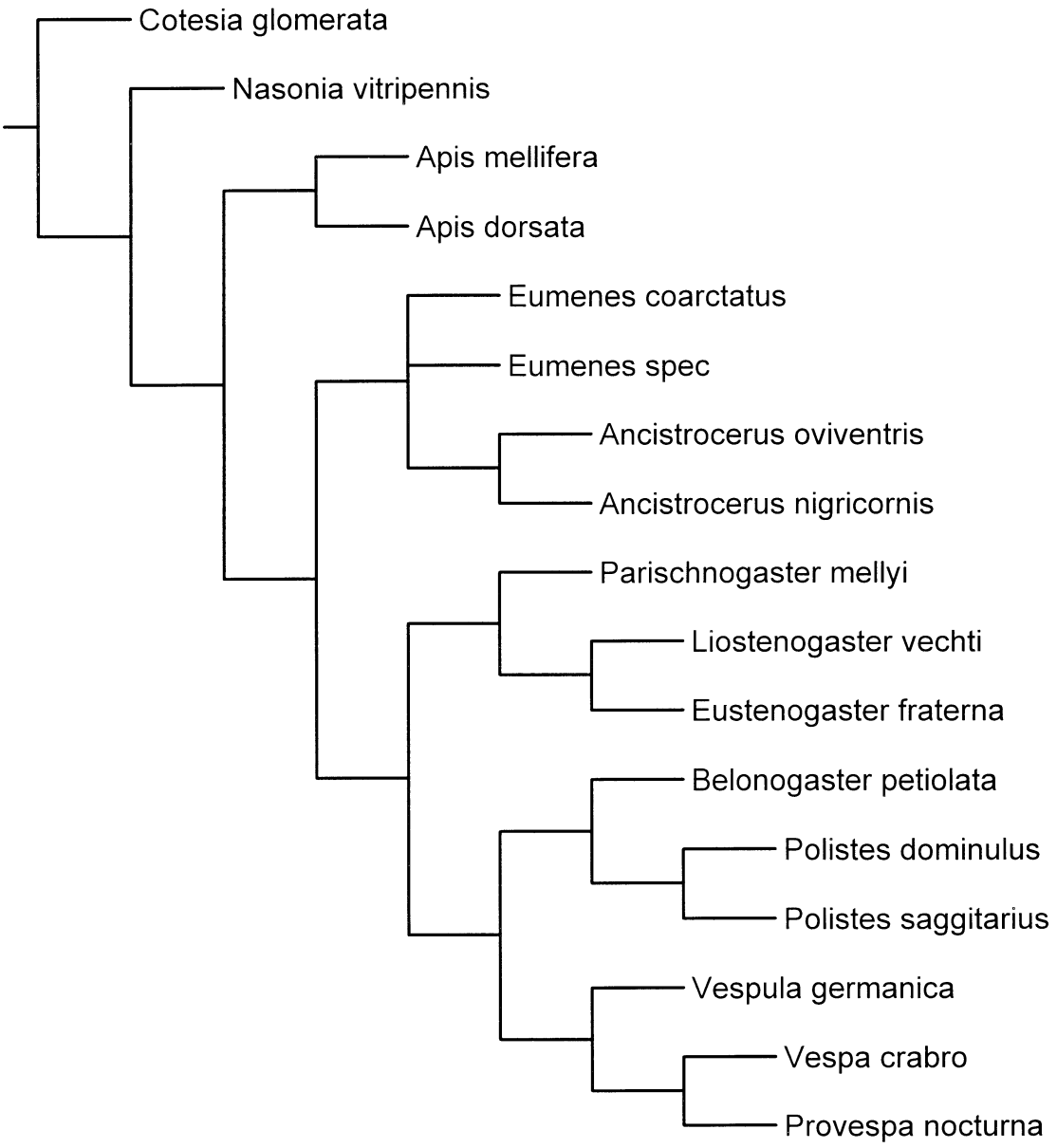

Fig. 5. Cladogram for the combined sequence datasets and the morphological and behavioral characters. The length is 907 steps; consistency index = 0.68 and retention index = 0.75.

treated gaps as missing, and so for this realignment will I. Rather than beginning with the raw sequences, I began with their published alignment, which can be ''improved'' if the gaps can be rearranged so that the same number of aligned variables is maintained, but fewer steps are implied. This can be readily accomplished. Schmitz and Moritz's alignment for 16S of 314 variables implies a maximum number of steps of 802 and a minimum of 267, with the difference being 535; their 28S alignment of 331 variables implies a maximum of 600 steps and a minimum of 231, with a difference of 369. These numbers were improved using Winclada, using the Alignment Insert/Delete function. The pro-

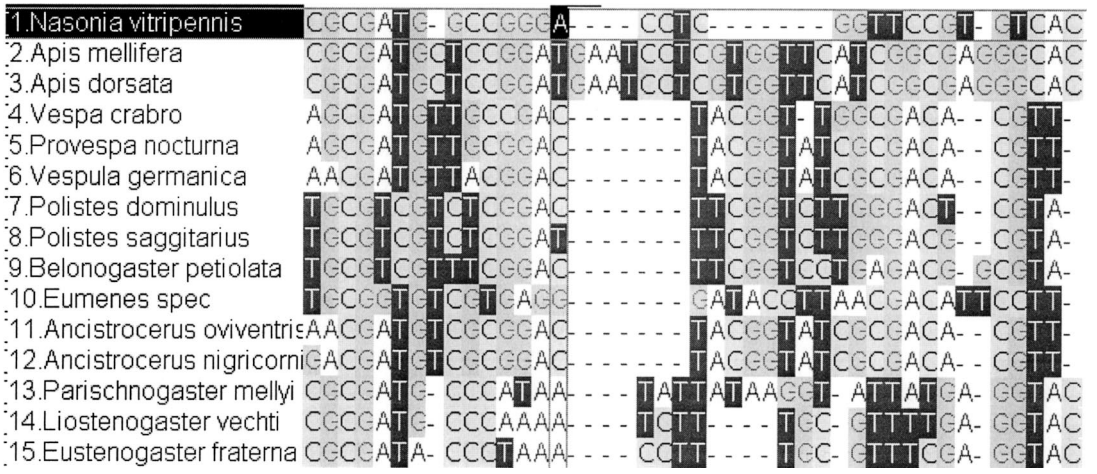

Fig. 6. Portion of 28S alignment of Schmitz and Moritz (1998) as displayed on the screen by Winclada.

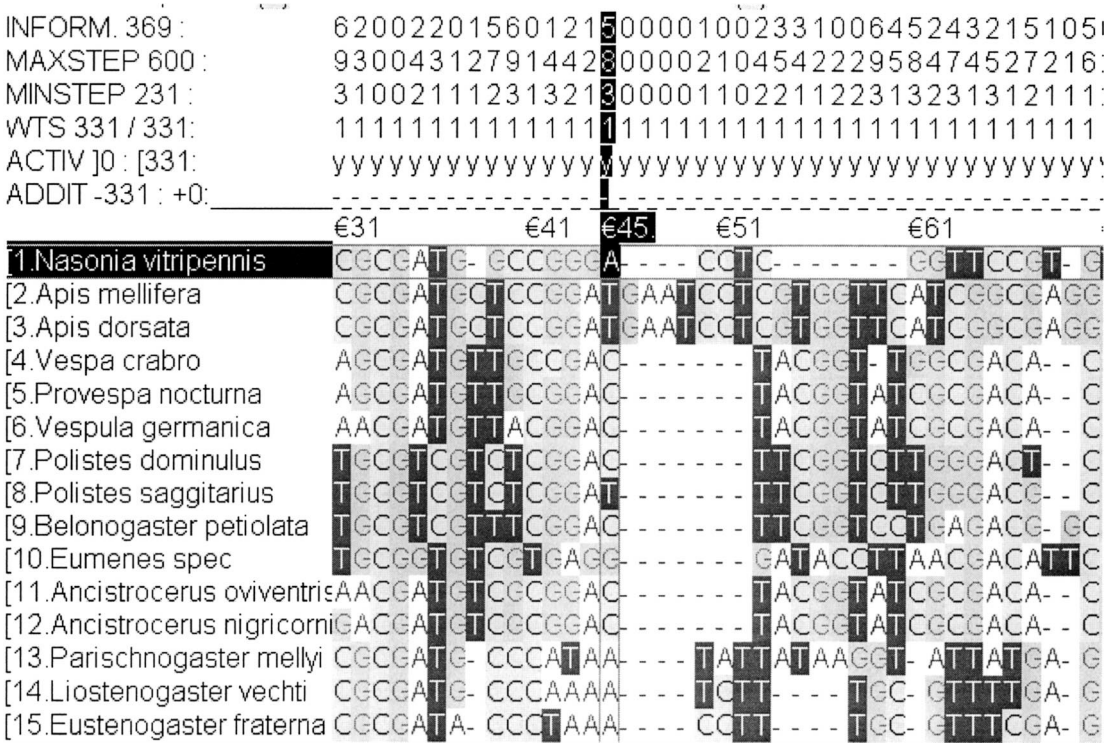

Fig. 7. Screen display of same portion of the 28S alignment as in figure 6, with the show character statistics toggle of Winclada set to on. See text for explanation of the numbers displayed.

cedure is illustrated in figures 6–9. Figure 6 shows a screen display of a portion of the 28S alignment published by Schmitz and Moritz. In Figure 7, the display of character statistics for this alignment has been toggled on, which shows at the left top, in order: the total information content (= difference between maximum and minimum steps), the

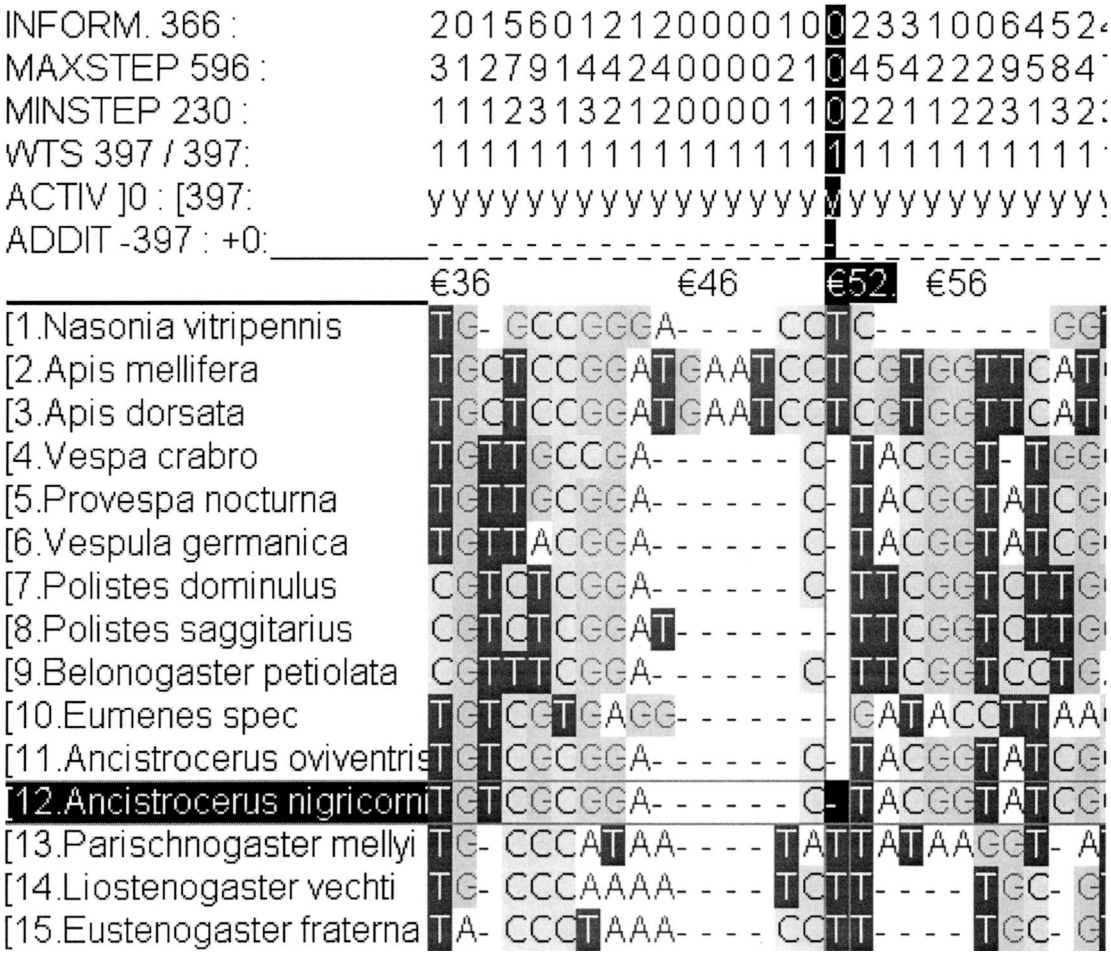

Fig. 8. Screen display of first step in realignment. See text for explanation of gap insertion.

maximum number of steps, the minimum number of steps, the character weights (all = 1), the character activities (all active), and character additivites (all nonadditive). The same numbers are shown for each character above that character's column. I merely inserted or deleted gaps, checking the step totals each time and saving changes when these numbers went down. This is shown for one of the characters in figure 8: gaps have been inserted in front of the cytosines in character 45, moving them to character 51. This results in a reduction in maximum number of steps to 596, an improvement of 4, with decreases as well in other step counts (note that the values for weights and additivities now appear higher; this is because Winclada adds gaps at the end of the matrix when the Alignment Insert/Delete function is toggled on; at the end of the procedure the trailing gaps are stripped). Figure 9 shows the subsequent step, where gaps were inserted in the place of the adenine in character 51, moving it to character 50, and deleted in front of the thymines in character 50, moving them to character 49. This reduces the maximum number of steps to 594.

Improvement of the alignments following this procedure was rapid in each case, up to a reduction of around 100 maximum steps, after which it became more difficult, and so was halted. This indicates a potential limitation of the method, similar to the well-known problem with local optima during tree

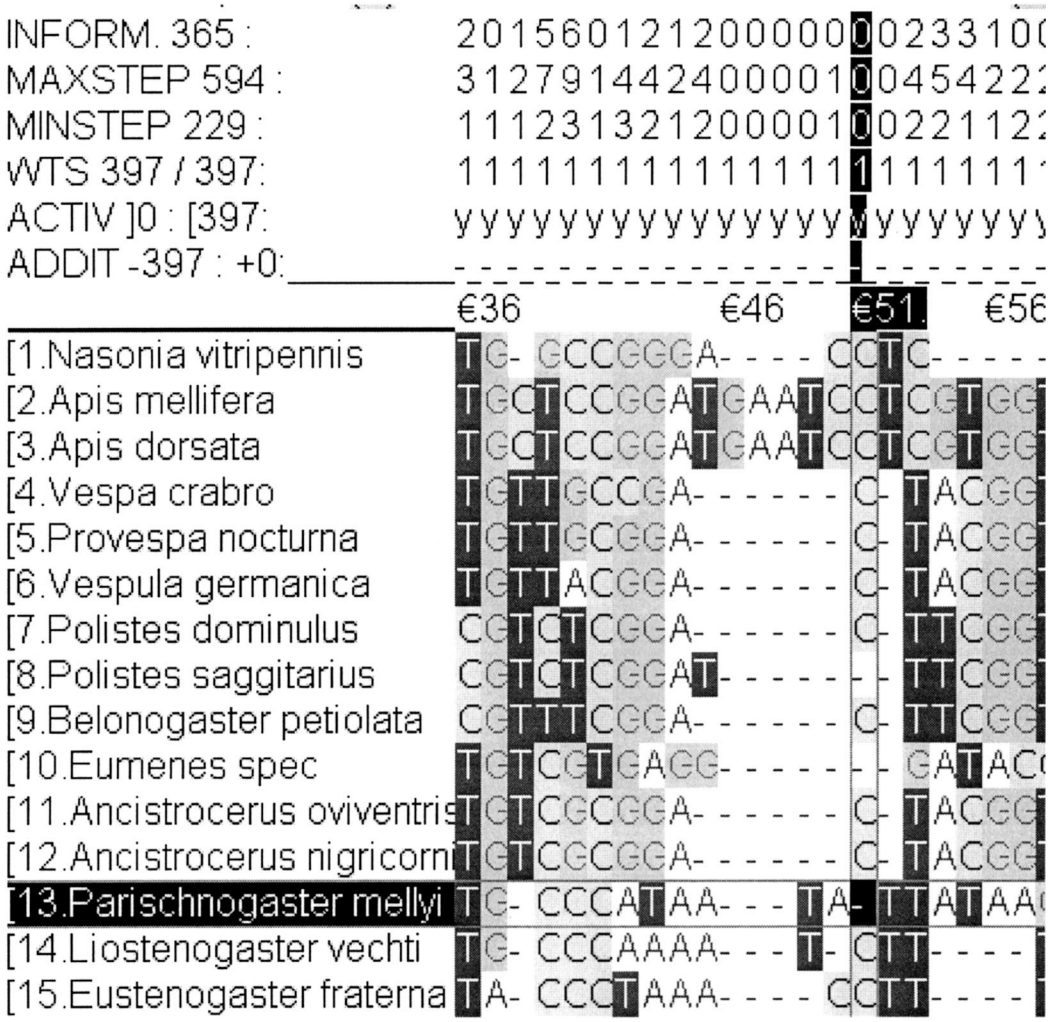

Fig. 9. Screen display of second step in realignment. See text for explanation of gap insertion.

searching: beginning the realignment at particular regions may limit the ability to attain a global optimum. As the procedure is manual, there is no guarantee that a global optimum has in fact been attained (but note that if there is a sufficient number of terminals that a global optimum cannot be guaranteed with multiple alignment programs). However, it is clear that the realignments produced are a substantial improvement over those published by Schmitz and Moritz, and they are sufficient to illustrate a critical point. The realignments are given in appendix 2. For the 16S data, the resulting alignment of 314 variables implies a maximum number of steps of 713 and a minimum of 256, with the difference being 457. Analysis of the alignment results in a single cladogram, with topology identical to that of figure 3 and length of 463, consistency index of 0.55, and retention index of 0.54. For the 28S data, the resulting alignment of 331 variables implies a maximum of 499 steps and a minimum of 209, with a difference of 290. Analysis of the alignment results in a single cladogram, with topology identical to that of figure 4, except that relationships among Vespinae are resolved as *Vespula* + (*Provespa* + *Vespa*)

with a length of 260, consistency index of 0.80, and retention index of 0.82. Combination of the two realignments then results in a single cladogram, identical to that of figure 5, with a length of 723, consistency index of 0.64, and retention index of 0.65.

The sequence alignments of Schmitz and Moritz (1998) are thus not robust, and improved alignments support Vespidae as a monophyletic family and social wasps as monophyletic, that is, Stenogastrinae are the sister group of Polistinae + Vespinae.

CONCLUSIONS

Note that the results shown in figures 3–5 are not completely in accord with other studies even now; specifically, generic relationships depicted within Stenogastrinae and Vespinae do not match those in Carpenter (1987, 1988) and Carpenter and Starr (2000). That deserves reexamination, but rather than undertake that with these data, I note that Schmitz and Moritz (2000) have more recently published a paper alluding to sequence data for some additional vespid taxa, including two species of the subfamily Masarinae. Through a more judicious selection of outgroups (e.g., using just *Apis*), some of their analyses no longer resulted in vespid paraphyly (a result that however resurfaced when the parasitic wasps were included as outgroups), but still showed Eumeninae as more closely related to Polistinae + Vespinae than Stenogastrinae (which in yet another highly novel arrangement on one of their trees are less closely related than the Masarinae, which are sister group to Eumeninae). I do not anticipate that these results would withstand simultaneous analysis any better, or realignment. However, I cannot investigate this at present. Despite Schmitz and Moritz's citation of Genbank accession numbers for these sequences, not all of them are in fact in that database, including both Masarinae.

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APPENDIX 1

MORPHOLOGICAL AND BEHAVIORAL CHARACTERS EXTRACTED FROM THE LITERATURE AS CITED BELOW

The character matrix is given after the list. Characters are treated as additive unless specified otherwise.

Characters from Carpenter (1981). For character matrices see Carpenter and Rasnitsyn (1990); Carpenter (1993). Numbers in parentheses correspond to the numbering in the text of Carpenter (1981). The polarity of certain of the characters has been modified as discussed in Carpenter (1988, 1989a).

Plaiting (1): absent = 0; present = 1.
Second submarginal cell shape: quadrilateral = 0; square = 1.
Marginal cell (7): truncate = 0; narrowed = 1; pointed = 2.
Hindwing cells (9): three = 0; two = 1.
Jugal lobe (10): long = 0; short = 1; absent = 2. (nonadditive)
Ligula (15): short = 0; attenuate = 1.
Acroglossals (16): absent = 0; present = 1.
Posterior lingual plate (17): narrow = 0; broad = 1; absent = 2. (nonadditive)
Occipital carinae (18): two = 0; one = 1; incomplete = 2; complete to hypostoma = 3.
Clypeus (21): dorsally straight, ventrally emarginate = 0; dorsally bisinuate = 1; ventrally pointed = 2; ventrally pointed, laterally angulate = 3. (nonadditive)
Pronotal lobe (23): near tegula = 0; separated = 1.
Secondary spiracular entrance (24): absent = 0; present = 1.
Mesoscutal lamella (25, 26): present adjoining tegula = 0; reduced = 1; parategula = 2. (nonadditive)
Propodeal orifice (28): dorsally broad = 0; dorsally acute = 1.
Hindcoxa (32): smooth = 0; carinate = 1.
Claws (33): simple = 0; toothed = 1; bifid = 2.
Parameral spines (37): sharp = 0; blunt = 1.
Volsellar apodeme (40): absent = 0; present = 1.
Aedeagus (41): terminally broad = 0; narrow and attenuate = 1.
Van der Vecht's gland (42): absent = 0; present = 1.
Larval labrum (45): wide = 0; narrow = 1.
Larval clypeus (46): ventral to mandibular bases = 0; at level of or dorsal to mandibular bases = 1.
Larval pronotum (47): bare = 0; with long bristles = 1.
Larval spiracle collar processes (48): absent = 0; present and simple = 1; branched = 2.
Larval provisions (49 modified): arthropods = 0; pollen = 1; masticated paste = 2; carrion = 3. (nonadditive)

Characters for social behavior from the discussion by Carpenter (1988) and the optimization by Carpenter (1989b); see Carpenter (1992c).

Division of labor: solitary = 0; temporary eusociality = 1; dominance hierarchies = 2; permanent sterility = 3.
Number of queens: absent = 0; short-term monogyny = 1; matrifilial monogyny = 2; polygyny = 3. (nonadditive)
Progressive provisioning: absent = 0; present = 1.
Extended brood care: absent = 0; present = 1.
Nest sharing: absent = 0; present = 1.
Overlap of adult generations: absent = 0; present = 1.
Cell reuse: absent = 0; present = 1.
Adult–adult trophallaxis: absent = 0; present = 1.
Free nests: absent = 0; present = 1; enclosed = 2.

Characters from Carpenter (1988: table 2). Numbers in parentheses correspond to the numbering in that paper. The occipital carina character (no. 3 in Carpenter, 1988) is consolidated into the occipital carina character scored from Carpenter (1981). The propodeal sculpture character (no. 16) has been deleted (it is now known to be polymorphic within genera; see Carpenter and Starr, 2000).

Pronotal fovea: absent = 0; present = 1.
Male clypeus (4): emarginate or truncate = 0; pointed = 1; slightly rounded = 2.
Male mandibular teeth (5): four = 0; three = 1; two = 2; one = 3. (nonadditive)

Characters from Carpenter (1991). Numbers in parentheses correspond to the numbering in that paper. The antennal articles character (no. 1), pronotal fovea (no. 12), scutal lamella (no. 19), and larval mandible (no. 28) have been consolidated into the appropriate characters scored from Carpenter (1981). The dorsal groove character (no. 16) has been scored for stenogastrines and vespines, the scrobal sulcus (no. 17) scored for stenogastrines, the implied polarity of the epicnemial carina (no. 18) reversed to take other vespids into account, and the thyridium (no. 25) scored for vespines.

Pronotal carina (13): present = 0; absent = 1.
Dorsal groove (16): present = 0; absent = 1.
Epicnemium (18): carina absent = 0; present = 1.
Thyridium (25): absent = 0; linear = 1; not transverse or basal = 2.

Characters from Carpenter (1987: tables 1

and 2). Numbers in parentheses correspond to the numbering in that paper. The occipital carina character (no. 6), scutal lamella (no. 16), hindcoxa carina (no. 18), larval spiracle collar processes (no. 32), and aerial nest (no. 33) have been consolidated into the appropriate characters scored from Carpenter (1981). The implied polarity of the aedeagus (no. 24) has been reversed, as has that for the paramere process (nos. 26 and 27, which have been combined), to take other vespids into account.

Base of second submarginal cell (3): basally acute = 0; basally truncate = 1.
Hamuli placement (5): basad of fork of R1 and RS = 0; at fork = 1.
Labial palp segment 3 (11): with large, recurved seta = 0; without large, recurved seta = 1; with 2 setae = 2. (nonadditive)
Pretegular carina (14): present = 0; absent = 1.
Aedeagus apex (24): rods fused apically = 0; rods separated apically = 1.
Paramere dorsal process (26, 27): absent = 0; present = 1; fingerlike = 2.
Envelope: absent = 0; present = 1.
Suspensoria (37): absent = 0; pillarlike = 1.
Royal court (44): absent = 0; present = 1.
Hunger signal (35): absent = 0; consistent scraping = 1; low frequency = 2.
Queen cells: absent = 0; present = 1.

Characters from the optimization by Hunt (1999). Excluded because of duplication are progressive provisioning, nest sharing, adult–adult trophallaxis, permanent sterility, and cell dimorphism.

Prey capture: with sting = 0; with mandibles = 1.
Malaxation of prey: absent = 0; present = 1.
Larval diapause: present = 0; absent = 1.
Adult emergence: protandry = 0; protogyny = 1.
Open brood cells: none = 0; single = 1; multiple = 2.
Larval–adult trophallaxis: absent = 0; licking of secretion = 1; direct = 2.
Nest material: soil = 0; wax = 1; mixed = 2; paper = 3. (nonadditive)
Antivertebrate venom: absent = 0; present = 1.

Characters from Brothers and Carpenter (1993). Numbers in parentheses correspond to the numbering in Brothers (1975), including the use of asterisks.

Pubescence (4): simple = 0; plumose = 1.
Clypeus (5.2): moderate = 0; dorsally produced = 1.
Eye form (7.2): oval = 0; reniform = 1.
Labio-maxillary complex (15.1): short = 0; prementum and stipes long = 1.
Labio-maxillary complex (15.2): short = 0; glossa and paraglossa produced = 1.
Pronotal hind margin (18.1)*: straight = 0; V-shaped = 1.
Pronotal articulation: free = 0; fused to prepectus = 1.
Pronotal posterolateral angle (21.1)*: rounded = 0; truncate = 1; notched = 2; acute above = 3.
Pronotal posterolateral angle (21.2)*: rounded = 0; lobe = 1.
Pronotal ventral angle (23.1+)*: rounded = 0; narrowly rounded = 1; acute = 2.
Pronotal ventral angle (23.2+)*: rounded = 0; produced = 1; almost contact = 2.
Propleural separation (24.1)*: diverging = 0; contiguous = 1.
Prosternum (25)*: plane = 0; sunken = 1; not visible = 2.
Prepectus (29.1)*: contiguous = 0; separated = 1; separated and short = 2; narrow and short = 3.
Prepectus (29.2)*: contiguous = 0; fused ventrally = 1.
Mesepimeron (30.2)*: sulcus distinct = 0; reduced sclerite = 1.
Mesosternum (31.2)*: posteriorly truncate = 0; mesally produced, with coxae = 1; mesally produced, without coxae = 2. (nonadditive)
Mesocoxal contiguity (32.1): separated = 0; contiguous = 1.
Metapostnotum (35.1)*: groove = 0; shortened = 1; invaginated = 2.
Metapostnotum (35.3)*: groove = 0; triangular area = 1.
Metapleuron: straight = 0; ventrally concave = 1.
Metapleuron: straight = 0; dorsally angulate = 1.
Metasternum (38.1): depressed anteriorly = 0; entirely depressed = 1.
Metasternal differentiation (39): present = 0; absent = 1.
Metathoracic-propodeal pleural suture (42): distinct = 0; partly obliterated = 1.
Propodeal length (43): moderate = 0; shortened = 1.
Forewing venation extent (45)*: reaching apical margin = 0; retracted = 1.
Pterostigmal sclerotization (48): heavy = 0; intermediate = 1; reduced = 2.
Basal hamuli (54.1): dispersed = 0; basal cluster = 1.
Basal hamuli (54.2): dispersed = 0; absent = 1.
Plical lobe (55): moderate incision = 0; shallow notch = 1.
Claws (59): toothed = 0; simple = 1.
Midtibial spur number (63.1): two = 0; one = 1.
Mid- and hindtibial spur form (64.1): circular = 0; flattened dorsally = 1; serrate margins = 2.
Hindtibial calcar (68.2±)*: absent = 0; carina = 1.
Sternum I differentiation (76±): absent = 0; present = 1.

Female tergum VII (78)*: exposed = 0; hidden = 1; membranous = 2.
Female gonapophysis IX (81.2): arcuate = 0; straight = 1.
Male sternum VII (82): well-developed = 0; reduced = 1; hidden = 2.
Male hypopygium concealment (84.2): absent = 0; concealed = 1.
Larval mandibular teeth (87.2): four = 0; two = 1.
Provision type (92): arthropods = 0; vegetable matter = 1.
Third phragma: flange = 0; even = 1; reduced or thin = 2.
Third phragma: reduced or thin = 0; weakly expanded = 1; greatly expanded = 2; plates = 3. (nonadditive)
Second phragma: oblique = 0; muscles posterior = 1.
Hypopharynx pubescence: present = 0; reduced = 1.
Sternum I and tergum II: not articulated = 0; articulated = 1; hinged = 2.
Trochantellus: present = 0; reduced = 1; absent = 2.
Sternum II: curved = 0; notches = 1; desclerotized areas = 2; median notch = 3. (nonadditive)

Characters from Ronquist et al. (1999).

Articles of female antenna: more than 16 = 0; 16 = 1; 15 = 2; 14 = 3; 13 = 4; 12 = 5.
Flagellomere sex dimorphism: absent = 0; present = 1.
Pronotum length: long dorsomedially = 0; short dorsomedially = 1.
Pronotal-mesepisternal attachment: loose = 0; rigid = 1.
Mesocoxal base: wide = 0; narrow = 1.
Crossvein 2r-m: tubular = 0; nebulous = 1; absent = 2.
2m-cu: tubular = 0; nebulous to absent = 1.
Hindwing jugal lobe: separate = 0; incorporated into vannus = 1.
Direction of 1r-m: subvertical = 0; reclivous = 1.
Female sternum VII: flat = 0; conical = 1.
Cercus of female: present = 0; reduced = 1; absent = 2.
Apex of female metasoma: open at rest = 0; closed = 1.
Second valvifer structure: entire = 0; divided by postarticular incision = 1.
Valvilli: absent = 0; two, attached separately = 1; two, attached singly = 2.
Third valvula structure: unisegmented = 0; two-segmented = 1.
Furcula: absent = 0; free = 1.

Morphological and Behavioral Character Matrix

Cotesia glomerata 000000000000000010000000000000
000 000 000 000 000 000 000 000 0?0 0–00 000 001 000 000
000 000 000 000 000 000 000 000 000 000 000 000 001 112
1110000100

Nasonia vitripennis 000 000 000 000 000 100 000 000 000
000000000000000000000000000?00–000 000 000 000
000 000 000 000 000 000 000 000 000 000 000 004
000121110000000

Apis mellifera 000100000000000100000000132111111
200000000000000000101–112211100100001020101120
011 101 101 000 001 200 212 111 101 002 351 000 000 012
11211

Apis dorsata 000100000000000100000000132111111
2000000000000000101–11221110010000102010112 00
111 011 010 000 012 002 121 111 010 023 510 000 000 121
1211

Vespa crabro 102020111000001011011002232111111
110001010000111111111112231011011130201230011
20001001021111001110100023012005111000–012110
11

Provespa nocturna 102 020 111 000 000 011 011 001 232
1111111100110111011011 1?111112231011011130201
230011200010010211110011101000230120051110 00–
01211011

Vespula germanica 102 020 111 000 101 011 011 001 333
111111210011011111021102111112231011011130201
230011200010010211110011101000230120051110 00–
01211011

Polistes dominulus 102 010 111 301 010 011 011 100 231
111 111 110 0001 100 000 000 000 111 122 310 110 111 302
012300112000100102111100111010002301200511100
0001211011

Polistes saggitarius 102010111301010011011100 2311
111 111 1000 0110 0000 000 000 111 1223 1011 0111 3020
1230011 2000 100 102 111 100 111 010 002 301 200 511 100
0001211011

Belonogaster petiolata 102 010 111 301 000 011 011 100
231111111100011020020000000011112231011 01113
020 123 001 120 001 001 021 111 001 110 100 023 012 00511
1000001211011

Eumenes coarctatus 10 101 010 100 020 120 000 000 000
000000001000010000000000000000000100001101113020
12300112000100102111100111010002301200511100 0
001211011

Eumenes spec 101010101000201200000000000000000
0100001000000000000000001000011011130201230011
20001001021111001110100023012005111000012110
11

Ancistrocerus oviventris 101010101000201200000000
000 000 000 010 000 100 000 000 000 000 001 000 011 0111
302 012 3001 120 001 001 021 111 001 110 100 023 012 005
111000001211011

Ancistrocerus nigricornis 101010101000201200000000
000000000010000100000000000000000010000110111 30
20123001120001001021111001110100023012005111 0
00001211011

Parischnogaster mellyi 012 111 023 210 000 100 101 010
2211111111011100000010000000111121200110111 30
20123001120001001021111001110100023012005111 0
00001211011

Liostenogaster vechti 012 111 022 210 000 10 01 010 102
211111111011100000010000000111121200110111302
01230011200010010211110011101000230120051110
00001211011

Eustenogaster fraterna 01211102321000010010101021
111111110231000000100000001111212001101113020
12300112000100102111100111010002301200511100 0
001211011

APPENDIX 2
REALIGNMENTS OF THE SEQUENCE DATA OF SCHMITZ AND MORITZ (1998)

Realigned 16S Data

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Cotesia glomerata              ATAATAATTTGTTTTTTAATTGAAAACTAGAATGAATGATTAAATGAAATATAA
Apis mellifera                 ATAATAAATTGTCTTTTAATTGAAGAATTGTATGAAAGAATTAATGAAATAATA
Apis dorsata                   ATAATAAATTGTCTTTTAATTGAAGAATTGTATGAACGAATTAATGAAATAATA
Eumenes coarctatus             AAAATCAATTGTTTTTTAATTGAAAACTGGAATGAAAGAATTAATGAAATAAAA
Ancistrocerus oviventris       ATAATCAATTGTTTTTTAATTGAAAACTTGTATGAAAGAATTGATGAGATAAAA
Ancistrocerus nigricornis      ATAATCAATTGTTTTTTAATTGAAAACTTGTATGAAAGAATTGATGAGATAGAA
Liostenogaster vechti          ATAATAAATTGTTCTTTAATTGAGTTCTGGAATGAAAGGAATAATGAAATAAAA
Eustenogaster fraterna         ATAATAAATTGTTCTTTAATTAGATTCTAGGATGAATGGGATAATGAGATAAAA
Parischnogaster mellyi         ATAATAAATTGATTTTTAATTAAAATCTAGGATGAAAGGAGTAATGAAATAAAT
Vespa crabro                   AAAATCATTTGTTTTTTAATTGAAAACTGGAATGAAAGAGTGCATGAAATATAA
Provespa nocturna              ATAATCATTTGTTTTTTAATTGAAAACTGGAATGAAAGAGTTAATGAAATATAA
Vespula germanica              AAAATCAATAGTTTTTTAATTGGAAACTGGAATGAAAGAGTGAATGAAATATAA
Polistes dominulus             AAAATCAATAGTTTTTTAATTGAAAACTGGAATGAAAGAATTGATAAAATATAA
Polistes saggitarius           AAAATCAATAGTTTTTTAATTGAAAACTTGAATGAAAGAATTGATAAAATATAA
Belonogaster petiolata         AAAATCAATAGTTTTTTAATTGAAAACTAGAATGAAAGAATTAATAAAATATAA

Cotesia glomerata              ACTGTATCAA-ATTTAA-AAAAT-AAATTTATTATTTAAATAAAAAAATTTAAA
Apis mellifera                 ACTGTCTCTAAA-TTATTA-AAT-GAATTTAAATTTTTAGTTAAAATTCTAAAA
Apis dorsata                   ACTGTCTCTGAA-TTATTA-AAT-GAATTTAAATTTTTAGTTAAAATTCTAAAA
Eumenes coarctatus             ATTGTCTCAAT-TTTAA-ATA-TTGAATTTAAATTTTAAGTTAAAATACTTAAA
Ancistrocerus oviventris       ATTGTTTCAATAGTTAA-ATA-T-GAATTTAAATTTTAGGTTAAAATGCTTAAA
Ancistrocerus nigricornis      ATTGTTTCGATAGTTAA-AAA-T-GAATTTAAATTTTAAGTTAAAATGCTTAAA
Liostenogaster vechti          TCTTTATTAA-ATTAA-TA-AATAAAAATTGATTATTAAATAAAAAGGTTTAAA
Eustenogaster fraterna         TCTTTATT-TT-TTAAATA-AATTGAAATTAATTATTAAATAAAAAGGTTTAAA
Parischnogaster mellyi         ACTTTATT-AT-TTAAATA-AATAAAATTTAATTATTAAGTAAAAAGGCTTAAA
Vespa crabro                   GTTGTCTCAATA-TAA-T-TAATTGAATTTAAATTAAAAGTTAAAATGCTTTTA
Provespa nocturna              ATTGTCTCAAAA-TAA-TATA-TTGAATTTAAATTAAAAGTTAAAATGCTTTTA
Vespula germanica              ATTGTCTCAAGA-T-GACA-AATTGAATTTAAATTAAAAGTAAAAATGCTTTTG
Polistes dominulus             ACTGTCTCAA-ATTAAATTTATTTTAATTTAAATAAAAAGTTAAAATGCTTTTA
Polistes saggitarius           ACTGTCTCAA-ATTAA-TTAAATTGAATTTAAATTAAAAGTTAAAATGCTTTTA
Belonogaster petiolata         ATTGTCTCAA-ATTAA-TTAAATTAAATTTAAATAAAAAGTTAAAATGCTTTTA

Cotesia glomerata              TAATTTTTAAAGACGAGAAGACCCTAAAGAATTT--T--AATAAT-AAATT-AT
Apis mellifera                 TTTAATTATGGGACGATAAGACCCTATAGAATTTAATTTAATTTTTAAATTTAT
Apis dorsata                   TTTAATTATGGGACGATAAGACCCTATAGAATTTTATTTAATATTTTA-TTTAT
Eumenes coarctatus             TTTTTTTATTAGACGAGAAGACCCTATAGAATTTTATAAAATTTATATTAAAGA
Ancistrocerus oviventris       TATTTTTATTAGACGAAAAGACCCTATAGAATTTTATAAAATTAAGGT-AAAGT
Ancistrocerus nigricornis      TTTATTTATTAGACGAAAAGACCCTATAGAATTTTATAAAATTAAATT-AAAGT
Liostenogaster vechti          TTTATTTTTAAGACGAGAAGACCCTAGAGAATTTAATA-A-T-AA-TTTTTAAT
Eustenogaster fraterna         TATTTTTTAAAGACGAAAAGACCCTAAAGAATTTAA-ATATA-AATATAATTAA
Parischnogaster mellyi         TGTTATTTTTAGACAAGAAGACCCTAGAGAATTTAATAAATT-AATTTATTTTA
Vespa crabro                   TATATTTATAAGACGATAAGACCCTATAGAATTTAATAAAATTTATTTTTTAAA
Provespa nocturna              TTAAATTATAAGACGATAAGACCCTATAGAATTTAATAAGTTTTATTTTTGT-A
Vespula germanica              TTTAATTATTAGACGAGAAGACCCTATAGAATTTAATAAAATTTATTTTTT-AA
Polistes dominulus             TAAATTTATTAGACGATAAGACCCTA-AGAATTTGAAAATTTTAATTTTATTAA
Polistes saggitarius           TTATTTTATTAGACGATAAGACCCTA-AGAATTTAAAGATTTTAATTTA-TTAA
Belonogaster petiolata         TTATTTAATTAGACGAGAAGACCCTA-AGAATTTTATAAA-TTAATA-ATTAAA
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APPENDIX 2
(Continued)

Realigned 16S Data (continued)

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Cotesia glomerata              T-A-TAATTATT--T--ATA-ATTT-AT-T-A-T---ATT-AATTGGGGAA-AT
Apis mellifera                 TTA-TAATT-TTAATAAATATATTTAAGAT---TAA-ATTTGATTGGG-AGGAT
Apis dorsata                   TAAATAATTATTAATTTGTAAATAAGATGT---TAA-ATTTGATTGGG-AGGAT
Eumenes coarctatus             GT-TAAATTGTAAAATTAAATAATTTCATATAATTATATTTTATTGGG-AGGAT
Ancistrocerus oviventris       AAATAAAATGTAAAATTA-TTTATTTTATTTTTTTATATTTAGTTGGG-AGGAT
Ancistrocerus nigricornis      AAATTCAATATAAGATTAA-TTATTTTATTTTATTATATTTAGTTGGG-AGGAT
Liostenogaster vechti          AATATAATT-TAAG-T--A-T-ATTA-ATTATTATTATTTTAATTGGGGA-GAT
Eustenogaster fraterna         ATTTTTAA-AT-AAATTT-TTTATTGTATAATT-ATATTTTAATTGGGGA-GAT
Parischnogaster mellyi         TTATAAATT-TT-AATA--ATTAAATAAA-A--TTTATTTTAGTTGGGGC-GAT
Vespa crabro                   ATTAATATT-TTTAATAAAATTTAATAAATAA-TTATATTTTATTGGG-AGGAT
Provespa nocturna              ATTATT-TTATTTAATAAAATTATATAAATAAATTATATTTTATTGGG-AGGAT
Vespula germanica              ATA-T-ATTATGTTATAATTATTTATTAAATATTTATATTTTATTGGG-AGGAT
Polistes dominulus             ATGGAATTTATGATATATAAATTAATATTTTAATATTTTTTTATTGGG-AGGGT
Polistes saggitarius           ATAAATTTTATGA-ATTTTAATATTTAAATTATAATTTTTTTATTGGG-AGGGT
Belonogaster petiolata         ATAATTATTAT-TAATAGTATTTTTTATTAAAAT-TTATTTTATTGGG-AGGAT

Cotesia glomerata              TTTAAAATTTAAAAAATTTTTAATTAA-A--AAAACATT-AATTT--TTGAATA
Apis mellifera                 TGGTAAATTTAATAAACTTT-ATTTAAGATTTAA-TTTTGA-TAT-AAAGA-TA
Apis dorsata                   TGATAAATTAATTAAACTTT-ATTTAATATTTAA-TTTTGA-T-TTAAAGA-TA
Eumenes coarctatus             AATTAAATTTAAAAAACTTT-AATTT-TATTAATACATAGA-T-T-AATGAATG
Ancistrocerus oviventris       TATAAAATTTAATTAACTTTTAATTTATTTAAAAACATTGA-TA--AATGAAT-
Ancistrocerus nigricornis      TATAAAATTTAATAAACTTTTATTTTTTATATAGACATTGA-T-T-AATGAA--
Liostenogaster vechti          TATTAAATTTAATAAACTTTAA-TATAAATA--AACATA-AATA--AATGA-TT
Eustenogaster fraterna         TATTAAATTTAATGAACTTTA--TAT-TATTAAT-CAAT-AAT-T-ATTGAATT
Parischnogaster mellyi         TAATAAATTTAATAAACTTTAA-T-TATATAAAT-CAAT-AATTT--TTGAGT-
Vespa crabro                   AGTTAAATTAATTAAACTTTAA-TA-ATTAGTTAACATAGA-T-T-AATGAATT
Provespa nocturna              AATTAAATAAGTTAAACTTTAA-TA-AAATATTAACATTGA-T--GAATGAA-T
Vespula germanica              ATTTAAATTTATTTAACTTTAA-T-TAAA-ATAAACATT-AAT-T-AATGAAA-
Polistes dominulus             AATTAAATTT-TAAAACTTTAATTA-AATAAAAACATTGATAATTGAATGGATT
Polistes saggitarius           AATTAAATTT-TTTAACTTTAA-TAT-TATTATAACACAGATAAAT---GAATT
Belonogaster petiolata         AGTTAAATTTTTTAAACTTTAA-T-TATTTAAAAACATTGATAATT---GAATT

Cotesia glomerata              --T--TT-AAT-ATTTTTTA-AAAAAATAAATT-AATTACCTTA
Apis mellifera                 ATTTTTTGAATTATAAATTGTAATTAAAAGAATAAATTACCTTA
Apis dorsata                   GAT-ATTGAATTATAATTTATAATTAATAGAGTAAATTACCTTA
Eumenes coarctatus             AATGAGAG-ATTATTA-TTATAATTAATAGAAAAAATTACCTTA
Ancistrocerus oviventris       -ATTAA-G-ATTATTA-TTATAATTAAAAGAAAAAATTACCTTA
Ancistrocerus nigricornis      AATATG-G-ATTATTA-TTATAATTAAAAGAGAAAATTACCTTA
Liostenogaster vechti          -A--AATG-ATAA----TTA-AATAAATAGAA-AAATTACCTTA
Eustenogaster fraterna         T-T---TG-ATAAG----T-TAATAAATAGAA-AAATTACCTTA
Parischnogaster mellyi         -AT-AA-G-ATAA----TTA-AATATTAAGAA-AAATTACCTTA
Vespa crabro                   -ATT--TG-ATTATTA-TTATAATTATTAGAATAAATTACCTTA
Provespa nocturna              -ATTT-TG-ATTATTA-TTACAATTATTAGAATAAATTACCTTA
Vespula germanica              GTTAT-TG-ATTATTA-TTATAATTATTAGAATAAATTACCTTA
Polistes dominulus             AATTTATG-ATAATTA-TTATAATTATGAGAATAAATTACCTTA
Polistes saggitarius           T-T-AATG-AAAATT-TTTATAATTAAGAGAATTAATTACCTTA
Belonogaster petiolata         TA-AAATG-ATAATT-TTTATAATTATAAGTAATAATTACCTTA
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APPENDIX 2
(Continued)

Realigned 28S Data

```
Nasonia vitripennis            ATCGTCAGCGACGTTGGCTTCCGTGT-GGATCGCGATGG-CCGG--G-A-CCTC
Apis mellifera                 ATCGGCAACGGTGCTGGC-TCC-CGTTGGTGCGCGATGCTCCGGATGAATCCTC
Apis dorsata                   ATCGGCAACGGTGCTGGC-TCC-CGTTGGTGCGCGATGCTCCGGATGAATCCTC
Eumenes spec                   AGCGTCAGCGGCGCTGGC-TCGACATAG-TATGCGGTG-TCGTGAGGGATAC--
Ancistrocerus oviventris       AGCGTCAGCGGCGTTGGC-TCGGCGAAG-TGAACGATG-TCGCG--G-A--CT-
Ancistrocerus nigricornis      AGCGTCAGCGGCGTTGGC-TCGGCGAAG-TGGACGATG-TCGCG--G-A--CT-
Liostenogaster vechti          ATCGTCAGCGACGCTGGCCTCG-CGATGGTGCGCGATG--CCC-AA-AATCTTT
Eustenogaster fraterna         ATCGTCAGCGACGCTGGCCTCG-CGGTGGTGCGCGAT--ACCC-TA-AACCTTT
Parischnogaster mellyi         ATCGTCAGCGACGCTGGCTTCG-CTGTGGTGCGCGATG--CCCATAATAT-TAT
Vespa crabro                   AGCGTCAGCGGCGCTGGC-TCGGCCGAG-TGAGCGATGTTGCC---G-A-C--T
Provespa nocturna              AGCGTCAGCGGCGCTGGCC-CGGCCGAG-TGAGCGATGTT-GCG--G-A-C--T
Vespula germanica              AGCGTCAGCGGCGCTGGC-TCGGCCGAG-TGAACGATGTTA-CG--G-A-C--T
Polistes dominulus             AGCGTCAGCGGCGTTAAC-TCGGCGGA-TCGTGCG-TCGTCTCG--G-A-C-TT
Polistes saggitarius           AGCGTCAGCGGCGTTAAC-TCGGCGGA-TCGTGCG-TCGTCTCG--G-A--TTT
Belonogaster petiolata         AGCGTCAGCGGCGTTGGC-TCGGCGGA-TAGTGCG-TCGTTTCG--G-A-C-TT

Nasonia vitripennis            --GGT---TC--CG-TGTCACGCGTCC-GCTGC-GGTATGT-CCGACATCGTCG
Apis mellifera                 GTGGTTCATCGGCGAGGGCACACCACCTTCGGCTCGAATGTTCCGGCATCGTAG
Apis dorsata                   GTGGTTCATCGGCGAGGGCACACCACCTTCGGCTTGAACGTTCCGGCATCGTAG
Eumenes spec                   -C--T-TAACGAC---ATTCCTTATAGCTATGCC-GC--GT-CCGGTGTCGTCG
Ancistrocerus oviventris       ACGGTATCGCGAC---ACG--TTATCACTATGCC-GC--GT-CCGGCGTCGTCG
Ancistrocerus nigricornis      ACGGTATCGCGAC---ACG--TTATCACTATGCC-GCA--T-CCGGCGTCGTCG
Liostenogaster vechti          GC-GT-TTT-GA-GGTACGCCATCG-TCGGTG---TCACGT-CCGGTGTCGTCG
Eustenogaster fraterna         GC-GT-TT-CGA-GGTACGCCATCGC-CGGTG--TT-ACGT-CCGGTGTCGTCG
Parischnogaster mellyi         AAGGTATTATGA-GGTACGCCATCGC-CGGTG--TT-ATGT-CCGGTGTCGTCG
Vespa crabro                   ACGGT-TGGCGAC---ACGTTATCACTC-ATGCCTT---GT-CCGGTGTCGTCG
Provespa nocturna              ACGGTATCGCGAC---ACGTTATCATTC-ATGCCTT---GT-CCGGTGTCGTCG
Vespula germanica              ACGGTATCGCGAC---ACGTTATCACTC-ATGCCTT---GT-CCGGCGTCGTCG
Polistes dominulus             -CGGTCTTGGGAC--T-CGTAATCTTTCCATGCCTT---GTTT-GGCGTCGTCG
Polistes saggitarius           -CGGTCTTGGGACG---CGTAATCTTTCCATGCCTT---GTTT-GGCGTCGTCG
Belonogaster petiolata         -CGGTCCTGAGACGG--CGTAATCTTTCCATGCCTT---GTT-CGGCGTCGTCG

Nasonia vitripennis            GCGTGCACTTCTCCTCTAGTAGGACGTCGCGACCCGTTGGGTGCCGGCCTACGG
Apis mellifera                 TCGTGCACTTCTCCCCTAGTAGAACGTCGCGACCCGTTGTGTGTCGGTCTACGG
Apis dorsata                   TCGTGCACTTCTCCCCTAGTAGAACGTCGCGACCCGTTGTGTGTCGGTCTACGG
Eumenes spec                   TCGTGCACTTCTCCTCTAGTAGAACGTCGCGACCCGTTGGGTGCCGGTCTACGG
Ancistrocerus oviventris       TCGTGCACTTCTCCCCTAGTAGAACGTCGCGACCCGTTGGGTGCCGGTCTACGG
Ancistrocerus nigricornis      TCGTGCACTTCTCCCCTAGTAGAACGTCGCGACCCGTTGGGTGCCGGTCTACGG
Liostenogaster vechti          TCGTGCACTTCTCCCCTAGTAGAACGTCGCGACCCGTTGGGTGCCGGTCTAAAA
Eustenogaster fraterna         TCGTGCACTTCTCCCCTAGTAGAACGTCGCGACCCGTTGGGTGCCGGTCTAAGA
Parischnogaster mellyi         TCGTGCACTTCTCCCCTAGTAGAACGTCGCGACCCGTTGGGTGCCGGTCTAAGA
Vespa crabro                   TCGTGCACTTCTCCTCTAGTAGAACGTCGCGACCCGTTGGGTGCCGGTCTACGG
Provespa nocturna              TCGTGCACTTCTCCTCTAGTAGAACGTCGCGACCCGTTGGGTGCCGGTCTACGG
Vespula germanica              TCGTGCACTTCTCCTCTAGTAGAACGTCGCGACCCGTTGGGTGCCGGTCTACGG
Polistes dominulus             TCGTGCACTTCTTCCCTAGTAGAACGTCGCGACCCGTTGGGTGTCGGTCTACGG
Polistes saggitarius           TCGTGCACTTCTTCCCTAGTAGAACGTCGCGACCCGTTGGGTGCTGGTCTACGG
Belonogaster petiolata         TCGTGCACTTCTTCCCTAGTAGAACGTCGCGACCCGTTGGGTGCCGGTCTACGG
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APPENDIX 2
(Continued)

Realigned 28S Data (continued)

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Nasonia vitripennis          CCCG-GTTGGTCGACTGTCGCGTCGCGTTTACGCGTGCACGCGG-CAGACCACC
Apis mellifera               CCCGAGA-GGGCGCCTGTCGCGTCGC-TT--CGCGCGCACGCGG-CAGACCCTC
Apis dorsata                 CCCGAGA-GGGCGCCTGTCGCGTCGC-TT--CGCGCGCACGCGG-CAGACCCTC
Eumenes spec                 CCCGAGGTGCG-GACTGTCACGTCGC-TT--CG-GCGTATGCG-TCAGACCCTC
Ancistrocerus oviventris     CCCGAGGTGTAAGACTGTCGCGTCGC-TT--CG-GCGCACGAGG-CAGACCCTC
Ancistrocerus nigricornis    CCCGAGGTGTAAGACTGTCGCGTCGC-TT--CG-GCGCACGCGG-CAGACCCTC
Liostenogaster vechti        CACGGAAAGGTAGACTGTCGCGTCGCGGTAAAACGCGTATGCGGTCAGACCACC
Eustenogaster fraterna       CACGGAAAGGTAGACTGTCGCGTCGCGGTAAAACGTGTACGCGGTCAGACCTCC
Parischnogaster mellyi       CACGGAAAGGTAGACTGTCGCGTCGCTGTAAAAGGCGTATGCGGTCAGACCTCC
Vespa crabro                 TCCGAG-CGGTAGACTGTCGCGTCGC-TT--CG-GCGCACGCG-TCAGACCCTC
Provespa nocturna            TCCGAG-CGGTAGACTGTCGCGTCGC-TT--CG-GCGCACGCG-TCAGACCCTC
Vespula germanica            TCCGAG-CGGTAGACTGTCGCGTCGC-TT--CG-GCGCACGCG-TCAGACCCTC
Polistes dominulus           TCCGAG-CGGTAGACTGTCGCGTCGC-TT--CG-GCGCACGCGG-CAGACCCTC
Polistes saggitarius         TCCGAG-CGGTAGACTGTCGCGTCGC-TT--CG-GCGCACGCGG-CAGACCCTC
Belonogaster petiolata       TCCGAG-TGGTAGACTGTCGCGTCGC-TT--CG-GCGCACGCGA-CAGACCCTC

Nasonia vitripennis          GGTTGCCCGGCCGGCTGCCCGGCGGTATAGATCG--C--TAT-AAAACGGTATT
Apis mellifera               GGTCGCCTGGCCGGCTGCACGACGGT--A-CTCGCACGGTATCGGGCCGCAACC
Apis dorsata                 GGTCGCCTGGCCGGCTGCACGACGGT--A-CTCGCACGGTATCGGGCCGCAACC
Eumenes spec                 GGTTGCCTGGCCGGCTGCTCGGCGGTAGAA--CGCACGGTATCTGGCCGC----
Ancistrocerus oviventris     GGTTGCCCGGCCGGCTGCCCGGCGGTATAA--CGCACGGTATCTGGCCGC----
Ancistrocerus nigricornis    GGTTTCCCGGCCGGCTGCCCGGCGGTATAA--CGCACGGTATCTGGCCGC----
Liostenogaster vechti        GAACGTTCGGCCGGCTGCCCGGCGGT--A-CTCGCACGGTATTTGGCCGC----
Eustenogaster fraterna       GAACGTTCGGCCGGCTGCCCGGCGGT--A-CTCGCACGGTATTTGGCCGC----
Parischnogaster mellyi       GATCGTTCGGCCGGCTGCTCGGCGGT--A-CTCGCACGGTATTTGGCCGC----
Vespa crabro                 GGTCGCCCGGCCGGCTGCCCGGCGGTCGAACTTATAAGGTATCTTGCCGC----
Provespa nocturna            GGTCGCCCGGCCGGCTGCCCGGCGGTCGAACTTATAAGGTATCTTGCCGC----
Vespula germanica            GGTCGCCCGGCCGGCTGCCCGGCGGTCGAACTTATAAGGTATCTTGCCGC----
Polistes dominulus           GGTCACCTGGCCGGCTGCCCGGCGGTCGAACTTATAAGGTATCTTGCCGC----
Polistes saggitarius         GGTCACCCGGCCGGCTGCCCGGCGGTCGAACTTATAAGGTATCTTGCCGC----
Belonogaster petiolata       GGTCACCCGGCCGGCTGCCCGGCGGTCGAACTTATAAGGTATCTTGCCGC----

Nasonia vitripennis          GGGCC--GGATT--AA-GTGCGTTC-T--GGCTCGTCG---GC-AGGCTC-GT-CTCGCTC
Apis mellifera               AATCCATTTTTTCGAATGTGTGTGCGTCAGGCCCGCCGCAAGCTCGG-TCAGTTCTTACCC
Apis dorsata                 AATCCATTTTTTCGAATGTGTGTGCGTCAGGCCCGCCGCAAGCTCGG-TCAGTTCTTACCC
Eumenes spec                 AAT---T---TTCGAAA-----TGCGTCGGGTCCACCGCAAGCGAGA-TCAGTG--T-CCC
Ancistrocerus oviventris     AAT---TT--TT-GA-T---C-TGCGTCGGGCCCG-CGCAAGCGAGGC-CAGTG--T-CCC
Ancistrocerus nigricornis    AAT---TT--TT-GAA----C-TGCGTCGGGCCCG-CGCAAGCGAGGC-CAGTA--T-CCC
Liostenogaster vechti        -AT--------TCGAA--T--GTGCGTCGGGCCCGCCGCAAGCGCGGTTCAGCG--TATCC
Eustenogaster fraterna       -AT-------TT-GAAT-T---TGCGTCGGGCCCGCCGCAAGCTCGGTTCAGCG--TATCC
Parischnogaster mellyi       -AT--------TCGAA--T--GTGCGTCGGGCCCGCCGCAAGCGTGGTTCAGGG--TATCC
Vespa crabro                 AAT---T--A-TCGAA----C-TGCGTCGGGCCCGCCGCAAGCGCGG-TCAGTGTC--T--
Provespa nocturna            GAT---T--A-TCGAA----C-TGCGTCGGGCCCGTCGCAAGCGCGG-TCAGTGTC--T--
Vespula germanica            AAT---T-G--T-AAAA---C-TGCGTCGGGCCCGCCGCAAGCGCGG-TCAGTGTC--T-C
Polistes dominulus           AAT---TT--TT-GAA----C-TGCGTCGGGCCCGTCGCAAGCGAGGC-CAGTGTC-TTA-
Polistes saggitarius         AAT----T-A-TCGAA----C-TGCGTCGGGCCCGTCGCAAGCGAGGC-CAGTGTC-TTAC
Belonogaster petiolata       AATC-AT---TT-GAA----C-TGCGTCGGGCCCGTCGCAAGCGCGGC-CAGTGTCCTCTC
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