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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 vespoid 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 morpho-

logical 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 Moritz 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 vespine 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 vespidae 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 vespidae 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-

1. <i>Nasonia vitripennis</i>	CGCGATG- GCCGGGA- --- COTC- --- GGTTCCGT- GTTAC
2. <i>Apis mellifera</i>	CGCGATGCTCCGGATGAATCCTCGTGGTTTCATCGGCGAGGGCAC
3. <i>Apis dorsata</i>	CGCGATGCTCCGGATGAATCCTCGTGGTTTCATCGGCGAGGGCAC
4. <i>Vespa crabro</i>	AGCGATGTTGCCGAC- --- TACGGT- TGGCGACA- - CGTT
5. <i>Provespa nocturna</i>	AGCGATGTTGCCGAC- --- TACGGTATCGGACA- - CGTT
6. <i>Vespula germanica</i>	AACGATGTTACGGAC- --- TACGGTATCGGACA- - CGTT
7. <i>Polistes dominulus</i>	TGCGTCGTCTCGGAC- --- TTCCGTCTTGGGACT- - CGTA
8. <i>Polistes sagittarius</i>	TGCGTCGTCTCGGAT- --- TTCCGTCTTGGGACG- - CGTA
9. <i>Belonogaster petiolata</i>	TGCGTCGTTTTCGGAC- --- TTCCGTCTTGGGACG- GCGTA
10. <i>Eumenes spec</i>	TGCGGTGTCTGAGG- --- GATACCTTAACGACATTTCCTT
11. <i>Ancistrocerus oviventris</i>	AACGATGTTCCGGAC- --- TACGGTATCGGACA- - CGTT
12. <i>Ancistrocerus nigricornis</i>	GACGATGTTCCGGAC- --- TACGGTATCGGACA- - CGTT
13. <i>Parischnogaster mellyi</i>	CGCGATG- CCCATAA- --- TATTATAAGGT- ATTATGA- GGTAC
14. <i>Liostenogaster vechti</i>	CGCGATG- CCCAAAA- --- TCTT- --- TGC- GTTTTGA- GGTAC
15. <i>Eustenogaster fraterna</i>	CGCGATA- CCTTAAA- --- COTT- --- TGC- GTTTTGA- GGTAC

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

INFORM. 369 :	62002201560121500001002331006452432151051
MAXSTEP 600 :	9300431279144280000210454222958474527216:
MINSTEP 231 :	3100211123132130000110221122313231312111:
WTS 331 / 331 :	111
ACTIV J0 : [331 :	Y Y
ADDIT -331 : +0 :	-----
	€31 €41 €45 €51 €61
1. <i>Nasonia vitripennis</i>	CGCGATG- GCCGGGA- --- COTC- --- GGTTCCGT- G
2. <i>Apis mellifera</i>	CGCGATGCTCCGGATGAATCCTCGTGGTTTCATCGGCGAGG
3. <i>Apis dorsata</i>	CGCGATGCTCCGGATGAATCCTCGTGGTTTCATCGGCGAGG
4. <i>Vespa crabro</i>	AGCGATGTTGCCGAC- --- TACGGT- TGGCGACA- - C
5. <i>Provespa nocturna</i>	AGCGATGTTGCCGAC- --- TACGGTATCGGACA- - C
6. <i>Vespula germanica</i>	AACGATGTTACGGAC- --- TACGGTATCGGACA- - C
7. <i>Polistes dominulus</i>	TGCGTCGTCTCGGAC- --- TTCCGTCTTGGGACT- - C
8. <i>Polistes sagittarius</i>	TGCGTCGTCTCGGAT- --- TTCCGTCTTGGGACG- - C
9. <i>Belonogaster petiolata</i>	TGCGTCGTTTTCGGAC- --- TTCCGTCTTGGGACG- GC
10. <i>Eumenes spec</i>	TGCGGTGTCTGAGG- --- GATACCTTAACGACATTTC
11. <i>Ancistrocerus oviventris</i>	AACGATGTTCCGGAC- --- TACGGTATCGGACA- - C
12. <i>Ancistrocerus nigricornis</i>	GACGATGTTCCGGAC- --- TACGGTATCGGACA- - C
13. <i>Parischnogaster mellyi</i>	CGCGATG- CCCATAA- --- TATTATAAGGT- ATTATGA- G
14. <i>Liostenogaster vechti</i>	CGCGATG- CCCAAAA- --- TCTT- --- TGC- GTTTTGA- G
15. <i>Eustenogaster fraterna</i>	CGCGATA- CCTTAAA- --- COTT- --- TGC- GTTTTGA- G

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

INFORM. 365 :	2015601212000000000233100
MAXSTEP 594 :	31279144240000100454222
MINSTEP 229 :	11123132120000100221122
WTS 397 / 397:	111111111111111111111111
ACTIV]0 : [397:	Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y
ADDIT -397 : +0:	-----
	€36 €46 €51 €56
[1.Nasonia vitripennis	TG- GCCGGGA- - - - CCTC- - - -
[2.Apis mellifera	TGCTCCGGATGAATCCTCGTGG
[3.Apis dorsata	TGCTCCGGATGAATCCTCGTGG
[4.Vespa crabro	TGTTGCCGA- - - - - C TACGG
[5.Provespa nocturna	TGTTGCCGA- - - - - C TACGG
[6.Vespula germanica	TGTTACGGA- - - - - C TACGG
[7.Polistes dominulus	CGTCTCGGA- - - - - C TTCGG
[8.Polistes sagittarius	CGTCTCGGAT- - - - - TTCGG
[9.Belonogaster petiolata	CGTTTCGGA- - - - - C TTCGG
[10.Eumenes spec	TGTCGTGAGG- - - - - GATAC
[11.Ancistrocerus oviventris	TGTCGCGGA- - - - - C TACGG
[12.Ancistrocerus nigricornis	TGTCGCGGA- - - - - C TACGG
[13.Parischnogaster mellyi	TG- CCCATAA- - - TA- TTATAA
[14.Liostenogaster vechti	TG- CCCAAAA- - - T- CTT- - - -
[15.Eustenogaster fraterna	TA- CCCTAAA- - - - CCTT- - - -

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 var-

iables 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; matrilineal 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.

Cotesia glomerata 000000000000000100000000000000
0000000000000000000000000?00-0000001000000

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

Realigned 16S Data

<i>Cotesia glomerata</i>	ATAATAATTTGTTTTTTAATTGAAAAC TAGAATGAATGATTAAATGAAATATAA
<i>Apis mellifera</i>	ATAATAAATTGCTCTTTTAATTGAAGAATTGTATGAAAGAATTAATGAAATAATA
<i>Apis dorsata</i>	ATAATAAATTGCTCTTTTAATTGAAGAATTGTATGAACGAATTAATGAAATAATA
<i>Eumenes coarctatus</i>	AAAATCAATTGTTTTTTAATTGAAAAC TGAATGAAAGAATTAATGAAATAAAA
<i>Ancistrocerus oviventris</i>	ATAATCAATTGTTTTTTAATTGAAAAC TGTATGAAAGAATTGATGAGATAAAA
<i>Ancistrocerus nigricornis</i>	ATAATCAATTGTTTTTTAATTGAAAAC TGTATGAAAGAATTGATGAGATAGAA
<i>Liostenogaster vechti</i>	ATAATAAATTGTTCTTTAATTGAGTTCTGGAATGAAAGGAATAATGAAATAAAA
<i>Eustenogaster fraterna</i>	ATAATAAATTGTTCTTTAATTAGATTCTAGGATGAATGGGATAATGAGATAAAA
<i>Parischnogaster mellyi</i>	ATAATAAATTGATTTTTTAATTAATACTAGGATGAAAGGAGTAATGAAATAAAT
<i>Vespa crabro</i>	AAAATCATTTGTTTTTTAATTGAAAAC TGAATGAAAGAGTGCATGAAATATAA
<i>Provespa nocturna</i>	ATAATCATTTGTTTTTTAATTGAAAAC TGAATGAAAGAGTTAATGAAATATAA
<i>Vespula germanica</i>	AAAATCAATAGTTTTTTAATTGGAAAAC TGAATGAAAGAGTGAATGAAATATAA
<i>Polistes dominulus</i>	AAAATCAATAGTTTTTTAATTGAAAAC TGAATGAAAGAATTGATAAAATATAA
<i>Polistes saggitarius</i>	AAAATCAATAGTTTTTTAATTGAAAAC TGAATGAAAGAATTGATAAAATATAA
<i>Belonogaster petiolata</i>	AAAATCAATAGTTTTTTAATTGAAAAC TAGAATGAAAGAATTAATAAAATATAA
<i>Cotesia glomerata</i>	ACTGTATCAA-ATTTAA-AAAAT-AAATTTATTATTTAAATAAAAAAATTTAAA
<i>Apis mellifera</i>	ACTGTCCTCTAAA-TTATTA-AAT-GAATTTAAATTTTTAGTTAAAATTTCTAAAA
<i>Apis dorsata</i>	ACTGTCCTCTGAA-TTATTA-AAT-GAATTTAAATTTTTAGTTAAAATTTCTAAAA
<i>Eumenes coarctatus</i>	ATTGTCCTCAAT-TTTAA-ATA-TTGAATTTAAATTTTAAGTTAAAATACTTAAA
<i>Ancistrocerus oviventris</i>	ATTGTTTCAATAGTTAA-ATA-T-GAATTTAAATTTTAAGTTAAAATGCTTAAA
<i>Ancistrocerus nigricornis</i>	ATTGTTTCGATAGTTAA-AAA-T-GAATTTAAATTTTAAGTTAAAATGCTTAAA
<i>Liostenogaster vechti</i>	TCTTTATTAA-ATTAA-TA-AATAAAAATTGATTATTAATAAAAAAGGTTTAAA
<i>Eustenogaster fraterna</i>	TCTTTATT-TT-TTAAATA-AATTGAAATTAATTATTAATAAAAAAGGTTTAAA
<i>Parischnogaster mellyi</i>	ACTTTATT-AT-TTAAATA-AATAAAAATTAATTATTAAGTAAAAAGGCTTAAA
<i>Vespa crabro</i>	GTTGTCCTCAATA-TAA-T-TAATTGAATTTAAATTTAAAGTTAAAATGCTTTTA
<i>Provespa nocturna</i>	ATTGTCCTCAAAA-TAA-TATA-TTGAATTTAAATTTAAAGTTAAAATGCTTTTA
<i>Vespula germanica</i>	ATTGTCCTCAAGA-T-GACA-AATTGAATTTAAATTTAAAGTTAAAATGCTTTTG
<i>Polistes dominulus</i>	ACTGTCCTCAA-ATTAAATTTATTTTAATTTAAATAAAAAGTTAAAATGCTTTTA
<i>Polistes saggitarius</i>	ACTGTCCTCAA-ATTAA-TTAAATTTAAATTTAAATTTAAAGTTAAAATGCTTTTA
<i>Belonogaster petiolata</i>	ATTGTCCTCAA-ATTAA-TTAAATTTAAATTTAAATAAAAAGTTAAAATGCTTTTA
<i>Cotesia glomerata</i>	TAATTTTTTAAAGACGAGAAGACCCTAAAGAATTT--T--AATAAT-AAATT-AT
<i>Apis mellifera</i>	TTTAATTATGGGACGATAAGACCCTATAGAATTTAATTTAATTTTTAAATTTAT
<i>Apis dorsata</i>	TTTAATTATGGGACGATAAGACCCTATAGAATTTTATTTAATATTTTA-TTTAT
<i>Eumenes coarctatus</i>	TTTTTTTTATTAGACGAGAAGACCCTATAGAATTTTATAAAATTTATATTAAAGA
<i>Ancistrocerus oviventris</i>	TATTTTTTATTAGACGAAAAGACCCTATAGAATTTTATAAAATTAAGGT-AAAGT
<i>Ancistrocerus nigricornis</i>	TTTATTTTATTAGACGAAAAGACCCTATAGAATTTTATAAAATTAATTT-AAAGT
<i>Liostenogaster vechti</i>	TTTATTTTTAAGACGAGAAGACCCTAGAGAATTTAATA-A-T-AA-TTTTTAAT
<i>Eustenogaster fraterna</i>	TATTTTTTAAAGACGAAAAGACCCTAAAGAATTTAA-ATATA-AATATAATTAA
<i>Parischnogaster mellyi</i>	TGTTATTTTTAGACAAGAAGACCCTAGAGAATTTAATAAATT-AATTTATTTTA
<i>Vespa crabro</i>	TATATTTATAAGACGATAAGACCCTATAGAATTTAATAAAATTTATTTTTTAAA
<i>Provespa nocturna</i>	TTAAATTTATAAGACGATAAGACCCTATAGAATTTAATAAGTTTATTTTTTGT-A
<i>Vespula germanica</i>	TTTAATTATTAGACGAGAAGACCCTATAGAATTTAATAAAATTTATTTTTT-AA
<i>Polistes dominulus</i>	TAAATTTATTAGACGATAAGACCCTA-AGAATTTGAAAATTTAATTTTATTAA
<i>Polistes saggitarius</i>	TTATTTTATTAGACGATAAGACCCTA-AGAATTTAAAGATTTTAATTTA-TTAA
<i>Belonogaster petiolata</i>	TTATTTAATTAGACGAGAAGACCCTA-AGAATTTTATAAA-TTAATA-ATTAAA

APPENDIX 2
(Continued)

Realigned 16S Data (continued)

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-TAAATTGTAAATTAATAATTTTCATATAATTATATTTTATTGGG-AGGAT
Ancistrocerus oviventris	AAATAAAATGTAAATTA-TTTATTTTATTTTTTATATTTAGTTGGG-AGGAT
Ancistrocerus nigricornis	AAATTCAATATAAGATTAA-TTATTTTATTTTATATATTTAGTTGGG-AGGAT
Liostenogaster vechti	AATATAATT-TAAG-T--A-T-ATTA-ATTATTATTATTTAATTGGGGA-GAT
Eustenogaster fraterna	ATTTTTTAA-AT-AAATTT-TTTATTGTATAATT-ATATTTTAAATTGGGGA-GAT
Parischnogaster mellyi	TTATAAATT-TT-AATA--ATTAATAAA-A--TTTATTTTAGTTGGGGC-GAT
Vespa crabro	ATTAATATT-TTTAATAAAATTTAATAAATAA-TTATATTTTATTGGG-AGGAT
Provespa nocturna	ATTATT-TTATTTAATAAAATTTATATAAATAAATTATATTTTATTGGG-AGGAT
Vespula germanica	ATA-T-ATTATGTTATAATTATTTATTAATATTTATATTTTATTGGG-AGGAT
Polistes dominulus	ATGGAATTTATGATATATAAATTAATATTTTAATATTTTATTGGG-AGGGT
Polistes saggitarius	ATAAATTTTATGA-ATTTTAATATTTAAATTATAATTTTATTGGG-AGGGT
Belonogaster petiolata	ATAATTATTAT-TAATAGTATTTTATTAAAT-TTATTTTATTGGG-AGGAT
Cotesia glomerata	TTTAAATTTAAAAATTTTAAATTAA-A--AAAACATT-AATTT--TTGAATA
Apis mellifera	TGGTAAATTTAATAAACTTT-ATTTAAGATTTAA-TTTTGA-TAT-AAAGA-TA
Apis dorsata	TGATAAATTAATAAACTTT-ATTTAATATTTAA-TTTTGA-T-TTAAAGA-TA
Eumenes coarctatus	AATTAATTTAAAAACTTT-AATTT-TATTAATACATAGA-T-T-AATGAATG
Ancistrocerus oviventris	TATAAAATTTAATTAACCTTTTATTTTAAAAACATTGA-TA--AATGAAT
Ancistrocerus nigricornis	TATAAAATTTAATAAACTTTTATTTTATATAGACATTGA-T-T-AATGAA--
Liostenogaster vechti	TATTAATTTAATAAACTTTAA-TATAAATA--AACATA-AATA--AATGA-TT
Eustenogaster fraterna	TATTAATTTAATGAACCTTAA-TAT-TATTAAT-CAAT-AAT-T-ATTGAATT
Parischnogaster mellyi	TAATAAATTTAATAAACTTTAA-T-TATATAAAT-CAAT-AATTT--TTGAGT-
Vespa crabro	AGTTAAATTAATTTAACTTTAA-TA-ATTAGTTAACATAGA-T-T-AATGAATT
Provespa nocturna	AATTAATAAGTTAACTTTAA-TA-AAATATTAACTATTGA-T--GAATGAA-T
Vespula germanica	ATTTAAATTTATTTAACTTTAA-T-TAAA-ATAAACATT-AAT-T-AATGAAA-
Polistes dominulus	AATTAATTTT-TAAACTTTAATTA-AATAAAACATTGATAATTGAATGGATT
Polistes saggitarius	AATTAATTTT-TTAACTTTAA-TAT-TATTATAACACAGATAAAT--GAATT
Belonogaster petiolata	AGTTAAATTTTAACTTTAA-T-TATTTAAAAACATTGATAATT--GAATT
Cotesia glomerata	--T--TT-AAT-ATTTTTTA-AAAAATAAATT-AATTACCTTA
Apis mellifera	ATTTTTTGAATTATAAATGTAAATTAAGAATAAATTACCTTA
Apis dorsata	GAT-ATTGAATTATAATTATAATTAAATAGAGTAAATTACCTTA
Eumenes coarctatus	AATGAGAG-ATTATTA-TTATAATTAAATAGAAAAAATTACCTTA
Ancistrocerus oviventris	-ATTAA-G-ATTATTA-TTATAATTAAAGAAAAAATTACCTTA
Ancistrocerus nigricornis	AATATG-G-ATTATTA-TTATAATTAAAGAGAAAAAATTACCTTA
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-AAATTT-TTTATAATTAAGAGAATTAATTACCTTA
Belonogaster petiolata	TA-AAATG-ATAATT-TTTATAATTATAAGTAATAATTACCTTA

APPENDIX 2
(Continued)

Realigned 28S Data

<i>Nasonia vitripennis</i>	ATCGTCAGCGACGTTGGCTTCCGTGT-GGATCGCGATGG-CCGG--G-A-CCTC
<i>Apis mellifera</i>	ATCGGCAACGGTGCTGGC-TCC-CGTTGGTGCGCGATGCTCCGGATGAATCCTC
<i>Apis dorsata</i>	ATCGGCAACGGTGCTGGC-TCC-CGTTGGTGCGCGATGCTCCGGATGAATCCTC
<i>Eumenes spec</i>	AGCGTCAGCGGCGCTGGC-TCGACATAG-TATGCGGTG-TCGTGAGGGATAC--
<i>Ancistrocerus oviventris</i>	AGCGTCAGCGGCGTTGGC-TCGGCGAAG-TGAACGATG-TCGCG--G-A--CT-
<i>Ancistrocerus nigricornis</i>	AGCGTCAGCGGCGTTGGC-TCGGCGAAG-TGGACGATG-TCGCG--G-A--CT-
<i>Liostenogaster vechti</i>	ATCGTCAGCGACGCTGGCCTCG-CGATGGTGCGCGATG--CCC-AA-AATCTTT
<i>Eustenogaster fraterna</i>	ATCGTCAGCGACGCTGGCCTCG-CGGTGGTGCGCGATG--ACCC-TA-AACCTTT
<i>Parischnogaster mellyi</i>	ATCGTCAGCGACGCTGGCTTCG-CTGTGGTGCGCGATG--CCCATATAT-TAT
<i>Vespa crabro</i>	AGCGTCAGCGGCGCTGGC-TCGGCCGAG-TGAGCGATGTTGCC---G-A-C--T
<i>Provespa nocturna</i>	AGCGTCAGCGGCGCTGGCC-CGGCCGAG-TGAGCGATGTT-GCG--G-A-C--T
<i>Vespula germanica</i>	AGCGTCAGCGGCGCTGGC-TCGGCCGAG-TGAACGATGTTA-CG--G-A-C--T
<i>Polistes dominulus</i>	AGCGTCAGCGGCGTTAAC-TCGGCGGA-TCGTGCG-TCGTCTCG--G-A-C-TT
<i>Polistes saggitarius</i>	AGCGTCAGCGGCGTTAAC-TCGGCGGA-TCGTGCG-TCGTCTCG--G-A--TTT
<i>Belonogaster petiolata</i>	AGCGTCAGCGGCGTTGGC-TCGGCGGA-TAGTGCG-TCGTTTCG--G-A-C-TT
<i>Nasonia vitripennis</i>	--GGT---TC--CG-TGTCACGCGTCC-GCTGC-GGTATGT-CCGACATCGTCG
<i>Apis mellifera</i>	GTGGTTTCATCGGCGAGGGCACACCACCTTCGGCTCGAATGTTCCGGCATCGTAG
<i>Apis dorsata</i>	GTGGTTTCATCGGCGAGGGCACACCACCTTCGGCTTGAACGTTCCGGCATCGTAG
<i>Eumenes spec</i>	-C--T-TAACGAC---ATTCTTATAGCTATGCC-GC--GT-CCGGTGTCGTCG
<i>Ancistrocerus oviventris</i>	ACGGTATCGCGAC---ACG--TTATCACTATGCC-GC--GT-CCGGCGTCGTCG
<i>Ancistrocerus nigricornis</i>	ACGGTATCGCGAC---ACG--TTATCACTATGCC-GCA--T-CCGGCGTCGTCG
<i>Liostenogaster vechti</i>	GC-GT-TTT-GA-GGTACGCCATCG-TCGGTG---TCACGT-CCGGTGTCGTCG
<i>Eustenogaster fraterna</i>	GC-GT-TT-CGA-GGTACGCCATCGC-CGGTG--TT-ACGT-CCGGTGTCGTCG
<i>Parischnogaster mellyi</i>	AAGGTATTATGA-GGTACGCCATCGC-CGGTG--TT-ATGT-CCGGTGTCGTCG
<i>Vespa crabro</i>	ACGGT-TGGCGAC---ACGTTATCACTC-ATGCCCTT---GT-CCGGTGTCGTCG
<i>Provespa nocturna</i>	ACGGTATCGCGAC---ACGTTATCATTC-ATGCCCTT---GT-CCGGTGTCGTCG
<i>Vespula germanica</i>	ACGGTATCGCGAC---ACGTTATCACTC-ATGCCCTT---GT-CCGGCGTCGTCG
<i>Polistes dominulus</i>	-CGGTCCTGGGAC--T-CGTAATCTTTCCATGCCTT---GTTT-GGCGTCGTCG
<i>Polistes saggitarius</i>	-CGGTCCTGGGACG--CGTAATCTTTCCATGCCTT---GTTT-GGCGTCGTCG
<i>Belonogaster petiolata</i>	-CGGTCCTGAGACGG--CGTAATCTTTCCATGCCTT---GTT-CGGCGTCGTCG
<i>Nasonia vitripennis</i>	GCGTGCACTTCTCCTCTAGTAGGACGTCGCGACCCGTTGGGTGCCGGCTACGG
<i>Apis mellifera</i>	TCGTGCACTTCTCCCCTAGTAGAACGTCGCGACCCGTTGGTGTCGGTCTACGG
<i>Apis dorsata</i>	TCGTGCACTTCTCCCCTAGTAGAACGTCGCGACCCGTTGGTGTCGGTCTACGG
<i>Eumenes spec</i>	TCGTGCACTTCTCCTCTAGTAGAACGTCGCGACCCGTTGGGTGCCGGTCTACGG
<i>Ancistrocerus oviventris</i>	TCGTGCACTTCTCCCCTAGTAGAACGTCGCGACCCGTTGGGTGCCGGTCTACGG
<i>Ancistrocerus nigricornis</i>	TCGTGCACTTCTCCCCTAGTAGAACGTCGCGACCCGTTGGGTGCCGGTCTACGG
<i>Liostenogaster vechti</i>	TCGTGCACTTCTCCCCTAGTAGAACGTCGCGACCCGTTGGGTGCCGGTCTAAAA
<i>Eustenogaster fraterna</i>	TCGTGCACTTCTCCCCTAGTAGAACGTCGCGACCCGTTGGGTGCCGGTCTAAGA
<i>Parischnogaster mellyi</i>	TCGTGCACTTCTCCTCTAGTAGAACGTCGCGACCCGTTGGGTGCCGGTCTAAGA
<i>Vespa crabro</i>	TCGTGCACTTCTCCTCTAGTAGAACGTCGCGACCCGTTGGGTGCCGGTCTACGG
<i>Provespa nocturna</i>	TCGTGCACTTCTCCTCTAGTAGAACGTCGCGACCCGTTGGGTGCCGGTCTACGG
<i>Vespula germanica</i>	TCGTGCACTTCTCCTCTAGTAGAACGTCGCGACCCGTTGGGTGCCGGTCTACGG
<i>Polistes dominulus</i>	TCGTGCACTTCTTCCCTAGTAGAACGTCGCGACCCGTTGGGTGTCGGTCTACGG
<i>Polistes saggitarius</i>	TCGTGCACTTCTTCCCTAGTAGAACGTCGCGACCCGTTGGGTGTCGGTCTACGG
<i>Belonogaster petiolata</i>	TCGTGCACTTCTTCCCTAGTAGAACGTCGCGACCCGTTGGGTGCCGGTCTACGG

APPENDIX 2
(Continued)

Realigned 28S Data (continued)

<i>Nasonia vitripennis</i>	CCCG-GTTGGTCTGACTGTCTCGCTCGCGTTTACGCGTGCACGCGG-CAGACCACC
<i>Apis mellifera</i>	CCCAGAG-GGGCGCCTGTCTCGCTCGC-TT--CGCGCGCACGCGG-CAGACCCTC
<i>Apis dorsata</i>	CCCAGAG-GGGCGCCTGTCTCGCTCGC-TT--CGCGCGCACGCGG-CAGACCCTC
<i>Eumenes spec</i>	CCCAGGTGCG-GACTGTCTCGCTCGC-TT--CG-GCGTATGCG-TCAGACCCTC
<i>Ancistrocerus oviventris</i>	CCCAGGTGTAAGACTGTCTCGCTCGC-TT--CG-GCGCACGAGG-CAGACCCTC
<i>Ancistrocerus nigricornis</i>	CCCAGGTGTAAGACTGTCTCGCTCGC-TT--CG-GCGCACGCGG-CAGACCCTC
<i>Liostenogaster vechti</i>	CACGGAAGGTAGACTGTCTCGCTCGCGTAAAACGCGTATGCGGTCTAGACCACC
<i>Eustenogaster fraterna</i>	CACGGAAGGTAGACTGTCTCGCTCGCGTAAAACGCGTATGCGGTCTAGACCCTC
<i>Parischnogaster mellyi</i>	CACGGAAGGTAGACTGTCTCGCTCGCTGTAAAAGGCGTATGCGGTCTAGACCCTC
<i>Vespa crabro</i>	TCCGAG-CGGTAGACTGTCTCGCTCGC-TT--CG-GCGCACGCG-TCAGACCCTC
<i>Provespa nocturna</i>	TCCGAG-CGGTAGACTGTCTCGCTCGC-TT--CG-GCGCACGCG-TCAGACCCTC
<i>Vespula germanica</i>	TCCGAG-CGGTAGACTGTCTCGCTCGC-TT--CG-GCGCACGCG-TCAGACCCTC
<i>Polistes dominulus</i>	TCCGAG-CGGTAGACTGTCTCGCTCGC-TT--CG-GCGCACGCGG-CAGACCCTC
<i>Polistes saggitarius</i>	TCCGAG-CGGTAGACTGTCTCGCTCGC-TT--CG-GCGCACGCGG-CAGACCCTC
<i>Belonogaster petiolata</i>	TCCGAG-TGGTAGACTGTCTCGCTCGC-TT--CG-GCGCACGCGA-CAGACCCTC

<i>Nasonia vitripennis</i>	GGTTGCCCCGGCCGGCTGCCCGGCGGTATAGATCG--C--TAT-AAAACGGTATT
<i>Apis mellifera</i>	GGTCGCCTGGCCGGCTGCACGACGGT--A-CTCGCACGGTATCGGGCCGCAACC
<i>Apis dorsata</i>	GGTCGCCTGGCCGGCTGCACGACGGT--A-CTCGCACGGTATCGGGCCGCAACC
<i>Eumenes spec</i>	GGTTGCCTGGCCGGCTGCTCGGCGGTAGAA--CGCACGGTATCTGGCCGC----
<i>Ancistrocerus oviventris</i>	GGTTGCCCCGGCCGGCTGCCCGGCGGTATAA--CGCACGGTATCTGGCCGC----
<i>Ancistrocerus nigricornis</i>	GGTTTCCCCGGCCGGCTGCCCGGCGGTATAA--CGCACGGTATCTGGCCGC----
<i>Liostenogaster vechti</i>	GAACGTTCCGCCGGCTGCCCGGCGGT--A-CTCGCACGGTATTTGGCCGC----
<i>Eustenogaster fraterna</i>	GAACGTTCCGCCGGCTGCCCGGCGGT--A-CTCGCACGGTATTTGGCCGC----
<i>Parischnogaster mellyi</i>	GATCGTTCCGCCGGCTGCTCGGCGGT--A-CTCGCACGGTATTTGGCCGC----
<i>Vespa crabro</i>	GGTCGCCCCGGCCGGCTGCCCGGCGGTGCAACTTATAAGGTATCTTGCCGC----
<i>Provespa nocturna</i>	GGTCGCCCCGGCCGGCTGCCCGGCGGTGCAACTTATAAGGTATCTTGCCGC----
<i>Vespula germanica</i>	GGTCGCCCCGGCCGGCTGCCCGGCGGTGCAACTTATAAGGTATCTTGCCGC----
<i>Polistes dominulus</i>	GGTCACCTGGCCGGCTGCCCGGCGGTGCAACTTATAAGGTATCTTGCCGC----
<i>Polistes saggitarius</i>	GGTCACCCGGCCGGCTGCCCGGCGGTGCAACTTATAAGGTATCTTGCCGC----
<i>Belonogaster petiolata</i>	GGTCACCCGGCCGGCTGCCCGGCGGTGCAACTTATAAGGTATCTTGCCGC----

<i>Nasonia vitripennis</i>	GGGCC--GGATT--AA-GTGCCTTC-T--GGCTCGTCG--GC-AGGCTC-GT-CTCGCTC
<i>Apis mellifera</i>	AATCCATTTTTCGAATGTGTGTGCGTCAGGCCCGCCGCAAGCTCGG-TCAGTTCTTACCC
<i>Apis dorsata</i>	AATCCATTTTTCGAATGTGTGTGCGTCAGGCCCGCCGCAAGCTCGG-TCAGTTCTTACCC
<i>Eumenes spec</i>	AAT---T---TTCGAAA-----TGCGTCGGGTCCACCGCAAGCGAGA-TCAGTG--T-CCC
<i>Ancistrocerus oviventris</i>	AAT---TT--TT-GA-T---C-TGCGTCGGGCCCCG-CGCAAGCGAGGC-CAGTG--T-CCC
<i>Ancistrocerus nigricornis</i>	AAT---TT--TT-GAA-----C-TGCGTCGGGCCCCG-CGCAAGCGAGGC-CAGTA--T-CCC
<i>Liostenogaster vechti</i>	-AT-----TCGAA--T--GTGCGTCGGGCCCCGCGCAAGCGCGGTTCAGCG--TATCC
<i>Eustenogaster fraterna</i>	-AT-----TT-GAAT-T--TGCGTCGGGCCCCGCGCAAGCTCGGTTTCAGCG--TATCC
<i>Parischnogaster mellyi</i>	-AT-----TCGAA--T--GTGCGTCGGGCCCCGCGCAAGCGTGGTTTCAGGG--TATCC
<i>Vespa crabro</i>	AAT---T--A-TCGAA-----C-TGCGTCGGGCCCCGCGCAAGCGCGG-TCAGTGTC--T--
<i>Provespa nocturna</i>	GAT---T--A-TCGAA-----C-TGCGTCGGGCCCCGTCGCAAGCGCGG-TCAGTGTC--T--
<i>Vespula germanica</i>	AAT---T-G--T-AAAA-----C-TGCGTCGGGCCCCGCGCAAGCGCGG-TCAGTGTC--T-C
<i>Polistes dominulus</i>	AAT---TT--TT-GAA-----C-TGCGTCGGGCCCCGTCGCAAGCGAGGC-CAGTGTC-TTA-
<i>Polistes saggitarius</i>	AAT---T-A-TCGAA-----C-TGCGTCGGGCCCCGTCGCAAGCGAGGC-CAGTGTC-TTAC
<i>Belonogaster petiolata</i>	AATC-AT---TT-GAA-----C-TGCGTCGGGCCCCGTCGCAAGCGCGG-CAGTGTCCTCTC