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Simultaneous Analysis and the Origin of Eusociality in the Vespidae (Insecta: Hymenoptera)

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> Abstract

A review of the literature concerning the phylogenetics of the Vespidae is presented. We also present a new phylogenetic analysis of the Vespidae based on what is by far the largest taxon sample to include molecular data, and the largest phenotypic character dataset ever compiled. Relationships among the subfamilies are Euparagiinae + (Masarinae + (Eumeninae + (Stenogastrinae + (Polistinae + Vespinae)))), with all the subfamilies monophyletic. A single origin of eusociality is thus supported. Our results empirically supersede all previous treatments and should be the preferred scaffold of the family for studies of social behavior.

> Key words

Polistes, *Polybia*, Eumeninae, solitary wasps, social wasps, congruence, direct optimization.

1. Introduction

Wasps in the family Vespidae have played a central role in the understanding and development of the evolution of social habits. For this reason, and because of their often conspicuous and beautiful nests – and most certainly because of their pugnacious defense of those nests – social wasps are the most well known members of the family. But of the more than 5,000 species in the family (see Tab. 1), social wasps comprise only a fifth. Attention to the entire family, however, permits elucidation of the transition from solitary to social habits, as the majority of vespids are solitary. While many scientific articles begin by drawing attention to the potential for studying this transition in the Vespidae, few investigators actually do investigate it. Our treatment is phylogenetic, so it will reveal phylogenetic transitions in social habit. Our treatment is also broader by far in its taxon sampling than any previous work to include molecular data. Furthermore, as taxonomists, we present this work not only for what it says about behavior, but also for what it tells the community about the taxonomy of the group, following cladistic principles of monophyly and synapomorphy. In order to present context for this last point, we begin with the taxonomic history of the group. We then proceed to review recent phylogenetic analyses of the group, which have come to quite different conclusions, in particular regarding the origin of eusociality. Carpenter (1981, 2003) supported monophyly of the social subfamilies (see Tab. 1), hence a single origin of eusociality. Schmitz & Moritz (1998) and Hines et al. (2007) argued for no close relationship of Stenogastrinae to other social wasps, hence a diphyletic origin of eusociality. We will discuss these studies in detail before presenting our new data.

2. Taxonomic history

As the higher-level classification of the Vespidae gradually took shape over the first half of the nineteenth century, three major groups came to be generally recognized. In the comprehensive revision by DE SAUSSURE (1852–1858) these were treated as tribes: Masariens, Euméniens and Vespiens. The former two comprise the solitary vespids, and the latter the social wasps. De Saussure was emphatic that the masarines and eumenines were distinct groups, both distinct from the social wasps, but there were a number of taxa whose placement in these three groups fluctuated over the next century. De Saussure subdivided the eumenines into two sections based on differences in wing venation, Anomaloptères and Euptères, and placed only the genera *Raphiglossa*, *Stenoglossa* (= *Psiliglossa*) and *Gayella* in Anomaloptères. He later separated *Zethus*, *Calligaster* and *Discoelius* from the rest of his Section Euptères as the group "Zethites," distinguished by short, truncate mandibles from the remaining eumenines, the "Euménites." He placed the genus *Ischnogaster* (= *Stenogaster*) in the vespines, but indicated that it was entirely intermediate in characters between the Euméniens and Vespiens. All these genera were poorly known at the time, and some came to be transferred repeatedly as classification changed.

Whereas de Saussure treated Vespidae as a single family with three major divisions, ASHMEAD (1902a–c) exemplified a trend to treat these major groups as separate families: Masaridae, Eumenidae and Vespidae. Ashmead divided these families into subfamilies or tribes. For the Vespidae, these were Vespinae and Polistinae. He placed *Ischnogaster* in one of four subfamilies of the Eumenidae, Ischnogasterinae, the others being Discoelinae (= Zethinae), Eumeninae and Raphiglossinae (for *Raphiglossa*, *Stenoglossa* and *Gayella*). Masaridae were divided into Masarini and Euparagiini, the latter including *Euparagia* (described subsequent to DE SAUSSURE 1852–1858) and *Paramasaris*.

BEQUAERT (1918) recognized just a single family Vespidae, but with 10 subfamilies. He divided masarines into the Masarinae and Euparagiinae, following Ashmead but also including *Paragia* in Euparagiinae along with *Euparagia* and *Paramasaris*. Eumenines were divided into three subfamilies, Raphiglossinae, Zethinae and Eumeninae; Bequaert continued to include *Gayella* in the former, but stated that it might have to be removed from this subfamily due to the fact that the "longitudinal plaiting of the front wings is very obsolete." Social wasps comprised the Stenogastrinae, Epiponinae, Ropalidiinae, Polistinae and Vespinae. BRADLEY (1922) restricted Euparagiinae to *Euparagia* and *Paramasaris*, and created a subfamily for *Gayella*, Gayellinae, based on wing venation. BEQUAERT (1928) transferred *Paramasaris* to the Gayellinae on the basis of hindwing venation.

RICHARDS (1962) returned to a system with three families, Masaridae, Eumenidae and Vespidae, each composed of three subfamilies. He included the Gayellinae and Euparagiinae in the Masaridae. He placed the Stenogastrinae in the Vespidae, and within Polistinae treated Bequaert's subfamilies Epiponinae, Ropalidiinae and Polistinae as tribes. Eumenidae included Raphiglossinae, Discoeliinae and Eumeninae.

In the first application of cladistic methods to phylogenetic relationships in Vespidae, CARPENTER (1981) showed that Masaridae in Richards' sense is paraphyletic, with *Euparagia* the sister-group of all other Vespidae, and reclassified the group again into a single family, with six subfamilies. These were Euparagiinae, Masarinae (including Gayellini and Masarini as tribes), Eumeninae (including raphiglossines, zethines and eumenines but not recognizing any of these as formal taxa because of probable paraphyly of some of these groups), Stenogastrinae, Polistinae and Vespinae. The latter three were supported by Carpenter's analysis as a monophyletic lineage, which accorded with the views of previous authors such as DE SAUSSURE (1852–1858) and RICHARDS (1962). Carpenter's nomenclature quickly superseded the three-family classification of RICHARDS (1962), and Carpenter's work went unchallenged for more than a decade and a half.

3. Monophyly of social wasps

3.1. SCHMITZ & MORITZ (1998)

SCHMITZ & MORITZ (1998) challenged the view of social wasp monophyly, presenting analyses which, they claimed, "provide strong evidence that sociality has independently evolved twice in the Vespidae" (p. 183). Their data consisted of molecular sequences from the 16S mt-rDNA and 28S rDNA loci for the following 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). The outgroup included two Apidae (species of *Apis*) and a different parasitoid for each sequence (one species of Pteromalidae and one of Braconidae).

Tab. 1. Subfamilies of Vespidae, number of taxa and distribution. The subfamilies are listed in the order of branching in the cladogram of Carpenter (1981). The first three are solitary and the last three are eusocial.

Subfamily	Numbers of genera (and species)	Distribution
Euparagiinae	1 (10)	Southwestern U.S.A. and Mexico
Masarinae	14 (344)	Neotropical and southwestern Nearctic, western Palearctic, southern Africa, Australia
Eumeninae	210 (3579)	Cosmopolitan
Stenogastrinae	7 (58)	Oriental Region
Polistinae	26 (958)	Cosmopolitan
Vespinae	4 (69)	Holarctic, Oriental Region; adventive elsewhere

For 16S there were 314 aligned base pairs (169 informative characters); for 28S there were 331 aligned base pairs (125 informative). Analyses of these data with the usual gamut of techniques resulted in trees that showed a closer relationship of the eumenines to the polistines + vespines than the stenogastrines, thus diphyly of social wasps.

However, their trees also showed the family Vespidae as paraphyletic, in terms of the bee genus *Apis*. This is an absurd result – vespids and apids are traditionally placed in different superfamilies, and are not at all closely related, with their separation supported both by abundant morphological data (Brothers 1975; Brothers & Carpenter 1993; Brothers 1999) and molecular data (Pilgrim et al. 2008). Schmitz & Moritz (1998: 189) attempted to downplay this aspect of their results, terming it "unusual", and stating, "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." Their promotion of the diphyly of social wasps is thus a kind of double-talk, all too common in molecular systematics, with a preferred part of results played up as "strong" and embarrassing parts brushed aside. Equally common for molecular-only studies, all the results were based on poor taxon sampling (for a family consisting of nearly 5,000 described species) and little data.

Carpenter (2003) reanalyzed the data of Schmitz & Moritz (1998), as follows. First, he scored 125 (published) morphological and behavioral characters for the taxa used by Schmitz & Moritz (1998), and combined these characters with their alignment. Simultaneous analysis supported (1) monophyly of Vespidae, and (2) monophyly of social wasps, with the Stenogastrinae being more closely related to the Polistinae + Vespinae than the Eumeninae. Carpenter (2003) also realigned Schmitz & Moritz's (1998) sequence data, producing an alignment that is more parsimonious (implying fewer steps). Analysis of the realigned sequences, alone or in combination with the morphological characters, also supported monophyly of Vespidae, and monophyly of social wasps. Thus the data in Schmitz & Moritz (1998) do not actually support a reclassification of Aculeata, nor reinterpretation of vespid relationships.

3.2. Hunt & Amdam (2005)

More recently, Hunt & Amdam (2005) implied a new phylogenetic arrangement by which the social wasps would be rendered non-monophyletic, and this view was proposed as a necessary component of a new hypothesis regarding the evolution of sociality in the behaviorally important model genus *Polistes*. The idea proposed by Hunt & Amdam (2005) is that the dichotomy of workers and queens in social wasps is derived from ancestral regulatory circuitry of bivoltinism present in a solitary "eumenine-like" ancestor. In other words, Hunt & Amdam's view asserts that the most recent common ancestor of *Polistes* – a completely social genus – was solitary, although the authors presented no evidence whatsoever that suggested this. In fact, Hunt & Amdam's view is predicated on many critical assumptions that are at odds with all available phylogenetic, biogeographic, and ecological data for the wasps of interest. All published phylogenetic analyses that treat *Polistes* – whether based on morphology (Carpenter 1991), behavior (Wenzel 1993), morphology and behavior (Wenzel & Carpenter 1994), molecules (Schmitz & Moritz 1998; Carpenter 2003) or the simultaneous analysis of morphology, molecules and behavior (Carpenter 2003; Arévalo et al. 2004; Pickett & Wenzel 2004; Pickett et al. 2006) – give no harbor to Hunt & Amdam's critical assumption that the ancestor of *Polistes* was a eumenine-like, solitary wasp. Rather, all of these studies show a monophyletic *Polistes*; as all *Polistes* are social, so the ancestor of *Polistes*, logically, was social. All relevant phylogenetic studies (Carpenter 1991; Wenzel 1993; Wenzel & Carpen-

TER 1994; SCHMITZ & MORITZ 1998; CARPENTER 2003; PICKETT & WENZEL 2004; PICKETT et al. 2006) agree that *Polistes* plus its closest relatives (the Polistinae, all of which are social) are the sister-group to the all-social Vespinae. None of these studies show *Polistes* closely related to the Eumeninae or any other solitary group. Even Hunt's own strictly molecular work (in HINES et al. 2007; see below) does not show *Polistes* as closely related to the eumenines (something HINES et al. 2007 neglected to mention).

In elaboration of their claims, HUNT & AMDAM (2005) pointed out that the Stenogastrinae are "facultatively eusocial," and "originated in a nonseasonal environment," which they claimed is unlike *Polistes*. Both components of this conjecture, however, are also without empirical support. All three subfamilies of social wasps occur together only in monsoonal (not non-seasonal, *contra* HUNT & AMDAM 2005) Southeast Asia, where Stenogastrinae are endemic, which has given rise to the inveterate view (VAN DER VECHT 1965; RICHARDS 1971) that social wasps arose in the Southeast Asian tropics. *Polistes*, therefore, cannot be shown to differ from the Stenogastrinae in this respect. Further, and of particular note, recent work by SAITO et al. (2006, 2009) discovered stenogastrine bivoltinism. This finding is fundamentally incompatible with the bivoltine framework of HUNT & AMDAM (2005), which contrasts the evolution of the Stenogastrinae and *Polistes*.

As we have already addressed, the general claim that the most recent common ancestor of *Polistes* was "eumenine-like" is unsupported phylogenetically, and this error is due to a misrepresentation of the nature of *Polistes*, and the phylogeny of the Vespidae. But even if the ancestor of *Polistes* were "eumenine-like," the bivoltine ancestor assertion would still be untenable, as it is based on an unsupported assumption about the nature of the Eumeninae. HUNT & AMDAM (2005) cited SEGER (1983) in support of their claim that "Bivoltinism occurs commonly in solitary eumenines …," a necessary correlate of their notion that the regulatory circuitry of the "eumenine-like" bivoltine ancestor of *Polistes* evolved into caste circuitry. However SEGER (1983) provides no such support. Seger studied six bivoltine populations of four species of the 3578 nominal Eumeninae species (excluding subspecies). No statement regarding commonality of bivoltinism was offered by SEGER (1983), nor can one be determined from such a small sample. Currently, the frequency of bivoltinism in eumenines is unknown. Therefore, even if *Polistes* were derived from within the Eumeninae (which is clearly not the case), the bivoltine groundplan suggested by HUNT & AMDAM (2005) does not necessarily follow. In addition to this, HUNT & AMDAM stated that their hypothesis "... shifts emphasis away from altruism, away from costs and benefits, and away from conflict and cooperation." However, SEGER (1983) explained bivoltine-based eusociality in terms of the evolution of altruism, specifically discussing costs and benefits in light of kin selection theory.

3.3. HUNT (2006)

HUNT (2006) amplified the views presented in HUNT & AMDAM (2005) regarding novel relationships of eumenines. Like HUNT & AMDAM (2005), HUNT (2006) was not an empirical contribution, and so we treat only the more significant claims here. HUNT (2006) asserted:

"Diverse species of Eumeninae have seemingly informative behavioral and life history traits that have drawn the attention of numerous naturalists attempting to understand the evolution of vespid sociality. ... However, these investigations cannot reveal ancestral states of sociality in the social subfamilies if Eumeninae is monophyletic. Faced with this conundrum, I reject monophyly of Eumeninae, and therefore the six subfamily phylogeny, as implausible."

Taken at face value, this statement asserts that ancestral character optimization is only possible if the Eumeninae are paraphyletic. But characters can be optimized on any tree of any shape, and even Hunt himself has engaged in such optimizations that seek to reconstruct the evolution of sociality in the Vespidae even when Eumeninae are monophyletic (HUNT 1999). HUNT (2006: 418) continued:

"A strong test of whether sociality evolved in a matrifilial or semisocial context could be a phylogenetic test – whether shared nesting or solitary nesting characterizes the non-social sister group to Polistinae + Vespinae. Such a test presupposes, however, that Eumeninae as currently recognized is paraphyletic with regard to the social subfamilies, and that a sister taxon to Polistinae + Vespinae will be identified among the living wasps, which can only be within Eumeninae."

Hunt thus repeated his untenable view that cladistics can only inform social behavior in the Vespidae if the eumenines are not monophyletic, but this quotation reveals much more. Here Hunt asserted that a phylogenetic test must presuppose that the result Hunt prefers is correct. This is not a test at all, but evidence of *a priori* bias. In other words, if the purpose of the phylogeny is to test the phylogenetic relationships, then assuming the patterns *a priori* is clearly unacceptable. But Hunt did not stop there:

"New molecular data are needed … and these data should be analyzed separately from existing data. Separate analysis of new molecular data will place the six subfamily hypothesis at risk and so would constitute

a strong test of the six subfamily hypothesis." (HUNT 2006: 418).

That more molecular data are needed is not in dispute (see below), but the notion that existing data – gathered across centuries of careful study – should be jettisoned is completely unjustified.

3.4. HUNT (2007)

In the book, "The Evolution of Social Wasps," HUNT (2007) discussed at length his doubts that the social wasps are monophyletic. *Inter alia*, Hunt stated: "Others before me have argued that sociality in Stenogastrinae is separate from that of Polistinae, and they based their arguments on the same reasons that initiated my thinking – that there are numerous and often dramatic trait differences between stenogastrines and other vespids" (p. 67).

The differences between the Stenogastrinae and the Polistinae + Vespinae (that is, the autapomorphies of the former, and the synapomorphies of the latter) do not inform the relationship of the two. Just as the possession of feathers in birds does not inform the relationship of birds to non-feathered vertebrates, so unique characters of lineages do not inform their relationship to other lineages. Similarly, humans have many traits unique to them that the other apes lack, but this does not mean we are not apes. Only traits that are shared by lineages provide such information (HENNIG 1966). HUNT (2007) attempted to evade the irrelevance of autapomorphies by first, admitting as much ("Carpenter is correct on this point of phylogenetic interpretation" [that autapomorphies are irrelevant to determining relationship]), but continues in the very next sentence: "I would point out that there are numerous and distinctive autapomorphies of Polistinae + Vespinae as well as of Stenogastrinae." But of course, autapomorphies of any particular taxon are irrelevant to its relationships to other taxa. Extending the comparison, the unique traits of humans tell us nothing about our status as sister to chimpanzees, and neither do the unique traits of chimpanzees. We are apes, no matter how much evolution happened on our lineage *subsequent* to our split from our common ancestor with chimps. But Hunt continued:

"The many autapomorphies of Stenogastrinae and those of Polistinae + Vespinae as well as other life history differences between them (table 4.2) include major aspects of morphology, development and life cycle. The number and importance of the differences so greatly exceed the number and importance of the synapomorphies (table 4.1) that reexamination of the argument for recent common ancestry seems called for."

The lists HUNT (2007) references are quite truncated, and Hunt went on to suggest that CARPENTER's (1981) work showing monophyletic social wasps is based on only three characters, but this is not the case. The characters that were optimized by CARPENTER (1981) might have been arranged in some other way, had another arrangement been more optimal. Hunt presented the picture that, if only these characters were allowed to tell their story, the results would change. But those characters were allowed to tell their story: CARPENTER treated them, without chauvinism of some character types over others, and the results optimized 15 autapomorphies for the Stenogastrinae, 6 synapomorphies of the Polistinae and Vespinae, 6 autapomorphies for the Polistinae alone, and 8 autapomorphies for the Vespinae. All of those characters, optimized as they were, might have told a different story, but they did not. While true that in the end only 3 characters directly subtend the clade of social wasps, the other characters HUNT (2007) was convinced would overturn the monophyly of social wasps did not do so, even though they were permitted the chance. Further, the Eumeninae are supported as monophyletic by 11 synapomorphies, the social wasps are nested in a clade showing the Eumeninae as their sister, and that clade is supported by 13 synapomorphies (and all of this must be false under Hunt's preferred scenario, with a paraphyletic Eumeninae, Stenogastrinae sister to the remaining Vespidae, and Eumeninae sister to the Polistinae + Vespinae; see HINES et al. 2007, below). In other words, Hunt's characterization that only three characters support the monophyly of social wasps – and that if autapomorphies were considered matters would change – is itself without support.

Hunt continued his attempt to purge from consideration all characters that suggest the unity of the social wasps by claiming that sociality itself is no indication of monophyly. Taking his argument directly from long-refuted objections to the use of behavioral characters in phylogeny, Hunt claimed, "To use [social behaviors] as evidence of common ancestry for taxa categorized as "eusocial" constitutes a fallacy of affirming the consequent." Would Hunt agree that, "To use the vertebral column as evidence of common ancestry for taxa categorized as 'vertebrates' constitutes a fallacy of affirming the consequent?" Are molecular phylogenetic studies circular if they employ molecular data? These exact arguments have been dissected, refuted and rejected long ago. We feel little need to treat their underpinnings much more than to cite well known literature (WENZEL 1992; DELEPORTE 1993). But briefly, to say that similarity is not potential evidence of shared ancestry is to deny evolution. If Hunt, for example, denies that all social wasps reuse cells, then he can present evidence that they do not. Or, perhaps Hunt does not believe this putative homology is herit-

able. That evidence too he can present. But to claim that data, behavioral or otherwise, that suggest common ancestry are suspect because the investigator knows better is not an empirical argument.

3.5. HINES et al. (2007)

In the fourth of four contributions presented by J.H. Hunt relating to the topic at hand, HINES et al. (2007) produced a new hypothesis of the phylogeny of the Vespidae. Their analysis contains only 27 of the more than 5,000 nominal species, and only molecular data were considered. This is similar to SCHMITZ & MORITZ (1998) in its breadth, although having more terminals. These approaches contrast, for example, with the work of CARPENTER (1981) in which 506 species across 136 genera and subgenera of Vespidae – plus 45 species of scoliid outgroup taxa in both subfamilies and all three tribes – were examined. HINES et al. (2007) came up with results that are quite at variance with the much more thorough work of Carpenter. Below we discuss the results of HINES et al. (2007), which they characterize as "a firm foundation" for the phylogeny of the Vespidae. We show that their analysis is deficient in many respects, does not unambiguously lead to the results they present, and, when their molecular data are combined with approximately one-tenth as many morphological and behavioral characters, the results provide no support whatsoever for their novel claims.

To begin, we note one interesting aspect of the results of HINES et al. (2007) – the presented tree exhibits all of the precise details predicted by HUNT (2006, 2007; it is noteworthy however that the results contradict the predictions of HUNT & AMDAM 2005, as discussed above): diphyly of the social wasps, and paraphyly of the eumenines in terms of the Polistinae + Vespinae. Below we do what any good scientist would do in light of very different results than the status quo: we dissect their contribution. This is in keeping with science: search and research, especially when new assertions contradict decades of study. The results we present show that the contribution of HINES et al. is rife with misrepresentations, omissions, errors and fallacies. HINES et al. excluded all previously published data from their phylogenetic treatment, choosing instead to discuss the "evolution" of the characters *post hoc*, absent any phylogenetic optimization of the characters being discussed. HINES et al. excluded portions of their own data (by treating gaps as missing data during phylogenetic analyses). HINES et al. used inconsistent character weighting strategies with no justification (treating gaps as extremely expensive in the "alignment stage" and as worthless in the "phylogenetic stage"). The veracity of support indices was exaggerated, important phylogenetic details (concerning the relationships deriving from individual gene partitions) were misrepresented, and the canon of vespid literature was misconstrued. Important details permitting a thorough re-evaluation of their contribution were not provided (including details of phylogenetic search methodology and optimality scores of the results), but we present a reanalysis below nonetheless.

3.5.1. Repeatability

One of the many striking features of HINES et al. (2007) is the way in which critical details of the analysis are opaque. Among the omissions are a lack of standard details of the phylogenetic methodologies and alignment procedures employed. Most egregious, the optimality scores for the presented trees themselves are not reported. It is, in fact, impossible for an outside investigator to repeat the procedure HINES et al. followed to test if even they obtained the correct answer given their own methods.

We disagree with HUNT (2006: 417) that "reanalysis of the existing data is a waste of time." Hunt and his colleagues chose to ignore any data that are known not to fit their preferred hypotheses (namely, morphology); this contrasts sharply with a cornerstone of the hypothetico-deductive reasoning process: the most rigorous tests are those based on the most data (e.g., WILEY 1975), and this requires considering previously published data, including the data of HINES et al. (2007). HUNT (2006: 416) said previous data used are "incorrect and inappropriate," but neither description is justified. No errors were detailed by Hunt in character delimitation. Criteria that might lead to a character's being "inappropriate" for phylogenetic analysis include that the trait is not heritable, or that the trait varies within species, for example. Ultimately, HINES et al.'s exclusion of previous data serves only to protect their new data from potential falsification. HINES et al. do "reanalyze" existing data, but they do so in such a way that it cannot disconfirm their preferred hypotheses, as we show below.

Here we reanalyze all available data – that presented by HINES et al. and previously published data. After showing that the HINES et al. data, even when treated by themselves, do not support the assertions of HINES et al., we also include newly described characters. All characters presented here were subjected to phylogenetic analysis. All character states treated here either survived the phylogenetic test of congruence – and so remain putative homologies – or they failed that test, and so are rendered homoplasies. Phylogenetics and the test of congruence, after all, are the only scientific methods that permit such deter-

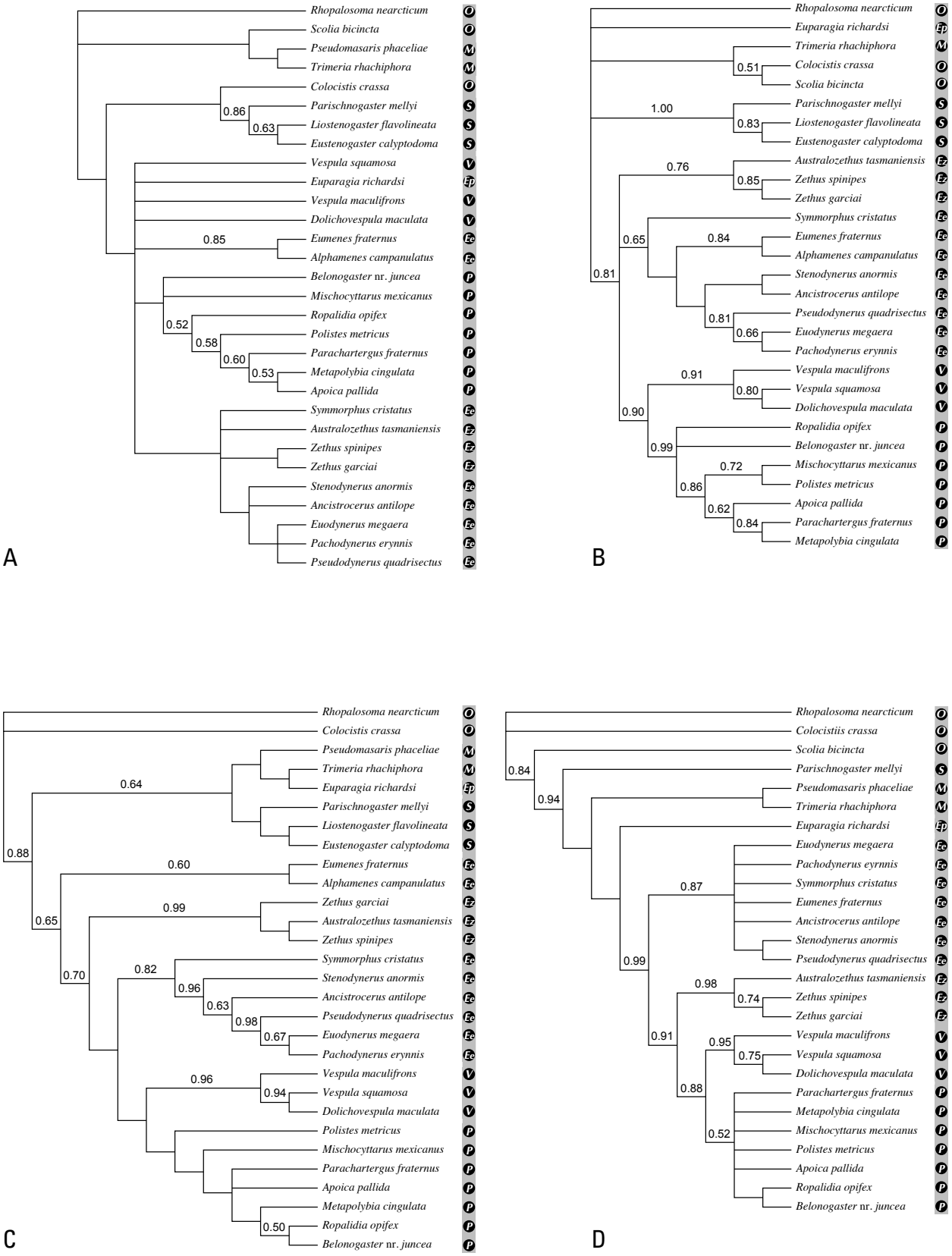


Fig. 1. Phylogenies obtained by analysis of individual gene fragments from Hines et al. (2007). **A**: 18S; **B**: 28S; **C**: RNA-polII; **D**: Abd-A. Bootstrap values indicated upon branches if ≥0.5. Taxon labels inside circles represent subfamilies as follows: O = Outgroup taxa; Ep = Euparagiinae; M = Masarinae; Ee = Eumeninae s.str.; Ez = zethine Eumeninae; S = Stenogastrinae; V = Vespinae; and P = Polistinae.

minations. In line with our desire to reanalyze all existing data, we requested that the authors of HINES et al. (2007) provide us with the unaligned, un-manipulated sequence data (for example, the fasta formatted data before they were read into Clustal). Despite no fewer than three requests, the authors never provided those data. Ultimately, the authors provided only the 'un-gapped' aligned data. This is all we have to work with, and it is unfortunate. Primary among our reasons for wanting the un-manipulated, raw sequence data is that it is well established that manual manipulation and re-alignment of sequence data permits for the alteration of the primary data, including inadvertent deletion of nucleotides (GIRIBET et al. 2002). Nevertheless, our reanalysis of the data made available to us follows.

3.5.2. Robustness: individual gene fragment partitions

HINES et al. (2007) asserted: "analyses of individual gene fragments resulted in highly resolved and well supported phylogenies with similar patterns of relationship." This is simply not true. Our reanalysis of their data (aligned as HINES et al. aligned it, gaps treated as they did) shows a very different story (see Fig. 1). Their 18S data give rise to 16 trees (L = 148); in the strict consensus (Fig. 1A), Vespidae itself is paraphyletic, the Vespinae is not a group, the zethines are not a group, but the zethines do group with all but two eumenines. The 28S data yield 17 trees (L = 800); again, in the consensus (Fig. 1B) Vespidae is paraphyletic, and in the underlying trees, the zethines are never sister to the Vespinae + Polistinae (a critical feature of HINES et al.'s preferred pattern). The RNA polymerase II data (Fig. 1C) yield two trees (L = 1133); Masarinae is paraphyletic, and again the zethines are not close to Vespinae + Polistinae. Only the Abd-A data (giving 12 trees, L = 688) recovers a tree (Fig. 1D) that contains all of the novel elements of the paper: diphyly of the social wasps, and zethines sister to a clade of polistines and vespines; but even here, neither the eumenines nor the Polistinae are resolved internally. So, in other words, the analyses of individual gene fragments resulted in quite different patterns of relationship – patterns that are often quite poorly supported (see bootstraps in Fig. 1) – refuting their own claim that the underlying concordance of the loci lends strong credence to their phylogeny. Of course, we do not much care if the individual fragments show what HINES et al. (2007) claimed they show or not, except insofar as HINES et al. showcased this attribute as indicative of "robustness" of their result. For us, only the tree of combined evidence matters in the end.

3.5.3. Robustness: bootstraps

HINES et al. (2007) also claim that their results are "strongly supported." In fact, the words "strong support" (or variants thereof) appear five times in the article (with multiple other instances of the self-description "robust"). Despite these claims, the support (in the resampling sense) for the clades they report is not as high as they claim, and many of their most unexpected bifurcations are quite poorly supported. Here we only consider HINES et al.'s bootstrap analysis; we do not address the even higher Bayesian clade support values that they present, as these are very well-known to be extremely inflated relative to properly conducted bootstrap and jackknife analyses, even for the very same data (RANNALA & YANG 1996; LEACHÉ & REEDER 2002; WHITTINGHAM et al. 2002; SUZUKI et al. 2002; CUMMINGS et al. 2003; DOUADY et al. 2003; ERIXON et al. 2003; SIMMONS et al. 2004; SVENBLATT et al. 2006).

One reason for the inflated support reported by HINES et al. is that they conducted a very superficial bootstrap analysis. Their bootstrap frequencies are based on only 400 replicates. This number is too low to achieve stability of the result, as has long been known (e.g. HEDGES 1992; MORT et al. 2000; SALAMIN et al. 2003; FREUDENSTEIN et al. 2004). Even one thousand pseudoreplicates would very likely give different results than a more rigorous analysis, especially for clades supported by less than 70% (see HEDGES 1992). A more appropriate analysis would employ 10,000 replicates, one random addition sequence per replicate, one tree held per replicate, and TBR swapping on each replicate; this strategy has been shown to be sufficient to reach an asymptotic result (see FREUDENSTEIN et al. 2004; all bootstrap analyses conducted herein use this methodology, implemented in TNT [GOLOBOFF et al. 2008] or POY4 [VARÓN et al. 2010]). Fig. 2 shows the results of that analysis applied to the HINES et al. data. With this more appropriate bootstrap analysis, several elements of the unusual findings of HINES et al. begin to show as weak. First, the sister relationship between the Polistinae + Vespinae and the zethines is supported by 68%, considerably lower than the 98–75% values reported by HINES et al. Second, the unprecedented finding of the stenogastrines as the sister to the remaining Vespidae is not supported (vs. the 55–62% reported by HINES et al.). By simply doing a more rigorous bootstrap analysis, we show the reported "robustness" of the results to be tenuous, and these results alone open the door for one component of the classical view: *Euparagia* and the Masarinae being the basal-most lineages; and this, from their own data, organized (i.e., aligned) in the way they organized the data, with gaps treated as missing data. In the next section, however, we show that this organization is suboptimal in the extreme.

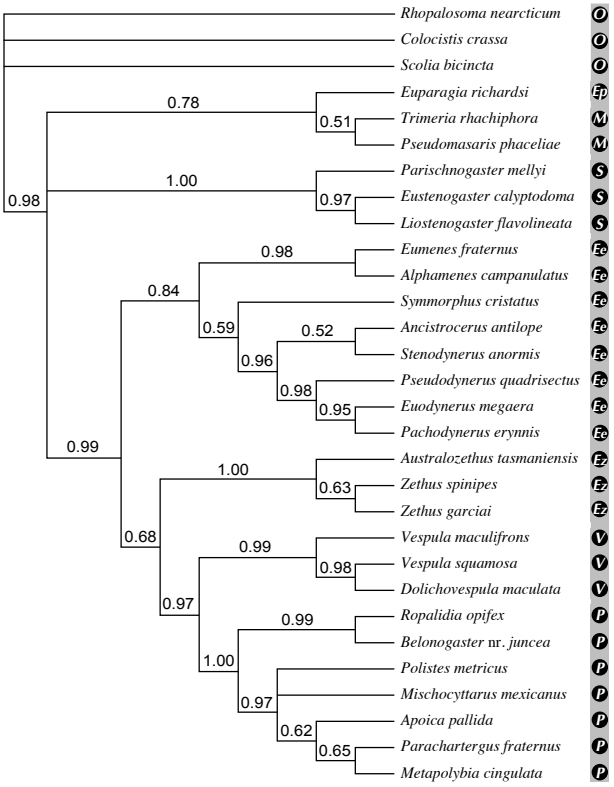


Fig. 2. The bootstrap frequency tree (10,000 replicates; see text), deriving from reanalysis of the entire Hines et al. (2007) molecular dataset. Taxon labels inside circles represent subfamilies as follows: O = Outgroup taxa; Ep = Euparagiinae; M = Masarinae; Ee = Eumeninae s.str.; Ez = zethine Eumeninae; S = Stenogastrinae; V = Vespinae; and P = Polistinae.

3.5.4. Robustness: alignments and optimization

Phylogenetic analysis of molecular data typically proceeds by a two-step process. First, investigators try to organize the data in a static, multiple sequence alignment, and second, that static alignment is used as the basis for selecting the optimal tree (that is, the topology that best fits that static alignment). But, as has long been known, this is not an optimal procedure. Multiple sequence alignment is a heuristic approach that exists as an alternative to directly optimizing characters on a given tree (Sankoff 1973; Sankoff & Rousseau 1975; Sankoff et al. 1975; Sankoff & Cedergren 1983). Sankoff and colleagues suggested multiple sequence alignment be a temporary measure in lieu of the ideal approach pioneered by Needleman & Wunsch (1970) and Sellers (1974), as this approach was far too computationally complex for the computers of the time.

But matters have changed in the last 40 years, and techniques that optimize raw sequence data directly onto trees have been available for more than 10 years (see Wheeler 1996). Whether under the parsimony criterion (Ogden & Whiting 2003; Wheeler 2003b; Whiting et al. 2006), or when using explicit models of maximum likelihood (Whiting et al. 2006; see Wheeler 2006), multiple sequence alignments and manipulations thereof have been consistently shown to produce sub-optimal tree scores when compared to Direct Optimization (Wheeler 1996) of the same data. Indeed, it is often the case that differences in primary data organization (i.e., varying multiple alignments) are more responsible for differences in results than differences in optimality criteria (see Whiting et al. 2006), and global phylogenetic results can be profoundly influenced by alignment choices early on (Wheeler 1994).

As is typical, although suboptimal, Hines et al. conducted a 'two-step' analysis, including an alignment and then a phylogenetic tree search using that static alignment. As we show, *seriatim* below, there are many problems implicit in their approach, analysis, and results.

One of the problems implicit in Hines et al. (2007) is that they did not provide any precise description of their alignment procedure. All that is said of their methodology is that they employed "default parameters of Clustal W in Bioedit", "further refined the alignment … using protein translation," and "aligned 28S and 18S rDNA sequences to secondary structure." No scientist can repeat this procedure using this description. The initial default cost parameters in Clustal W are well-known (if not justified; see below); they are gap opening penalty = 15 (on a range of 0 to 100); transition = 0.5 (on a range of 0 to 1); gap extension penalty = 0.44 times the gap opening penalty. Hines et al. offered no rationale for this combination of parameters, and differences in these values greatly influence results. At a minimum, the authors should have provided some justification for their choices, but at least this component of their alignment procedure is repeatable. However, the second component, refinement "using protein translation" is indeterminable. What criteria did the authors employ that allowed them to alter the alignment such that they knew the alteration was an improvement? Such *ad hoc* manipulation of the data opens the door for a host of concerns.

Ultimately, an optimality criterion is the only basis for selecting among tree topologies, given data. As Hines et al. open the door to *post hoc* 'hand' or 'eye' manipulation of the data, with no optimality criterion, we pursue this a bit further. Here, using the procedure outlined by Carpenter (2003), we show that their alignment is extremely suboptimal. We do not believe that such hand manipulation of the data is the best approach, even when employing an optimality criterion as we do here (see Carpenter 2003). However, we do this here simply to show that the alignment of Hines et al. is so poor that it can be improved, even without the aid of a computer-run heuristic algorithm.

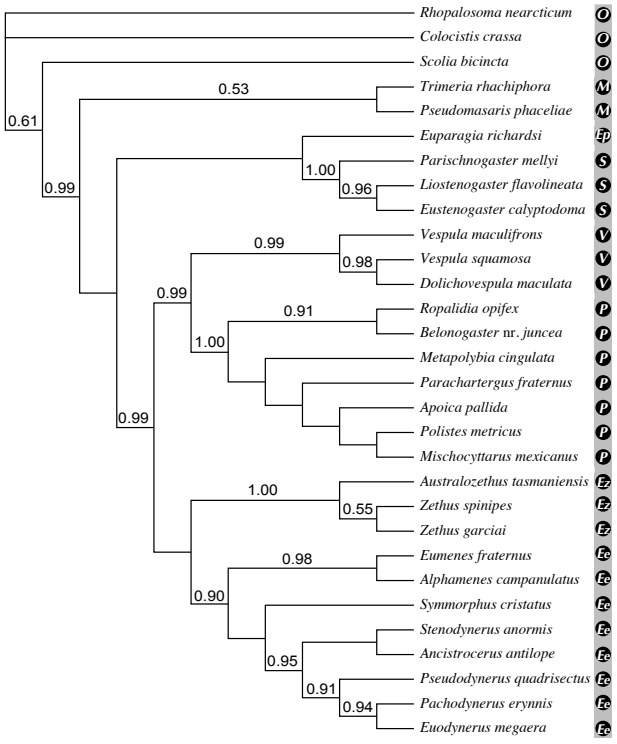


Fig. 3. The single most parsimonious (L = 2338) tree that resulted from analysis of the realigned HINES et al. (2007) molecular data as discussed in the text (parsimony analysis conducted in TNT: 1000 random additions, each with 40 rounds of the parsimony ratchet, 30 rounds of tree fusing, and 20 rounds of tree drifting). Bootstrap values indicated upon branches if ≥0.5. Taxon labels inside circles represent subfamilies as follows: O = Outgroup taxa; Ep = Euparagiinae; M = Masarinae; Ee = Eumeninae s.str.; Ez = zethine Eumeninae; S = Stenogastrinae; V = Vespinae; and P = Polistinae.

3.5.5. Realignment, with justification (parsimony of alignment length)

As indicated above, HINES et al. (2007) did not report their tree scores for any of their analyses. We obtained the scores by optimization of the data on their trees. The parsimony length for the HINES et al. tree, treating gaps as missing data as they did, is 2573. Realignment of the data given to us by the authors (that is, beginning with their alignment), invoking parsimony of the alignment length (as discussed in CARPENTER 2003) yielded a new alignment with 1212 maximum steps (maxsteps) vs. the 1402 maxsteps of the HINES et al. alignment. Parsimony analysis of the shorter alignment, treating gaps as missing as HINES et al. did, yields a single most parsimonious tree of length 2338, over two hundred steps and almost ten percent more optimal than the 2573 of HINES et al.

Our alignment and resulting tree are also superior under a homogeneous Markov process (i.e., typical) likelihood criterion. We obtained the ML score of the topology they presented via optimization of their data on the given topology in PAUP; again, this was necessary as HINES et al. did not report the optimality scores of their trees. The –ln ML score for the HINES et al. topology under their alignment and their stipulated model and fixed parameters was 16071.15611. Estimating the substitution rate matrix and all free parameters except base frequencies, for which so-called 'empirical' frequencies were stipulated (as in HINES et al.), the –ln ML score was 16074.849359 (conducted in raxML, as this analysis was much more computationally intensive, and raxML is vastly more efficient than PAUP). For our realigned data, under the model of HINES et al., the –ln ML score was 15160.77963 (estimated in PAUP, as above); estimating the rate matrix and all free parameters (as above) on the given tree in raxML gave the improved score of 15158.011363. Both of these scores are much better than the score of 16071.15611 for the HINES et al. tree and their alignment, and so even under a homogeneous ML criterion, our realignment is vastly superior.

But not only are the alignment length, parsimony tree score, and likelihood tree scores more optimal. The trees derived from our more optimal alignments differ sharply from that of HINES et al. The parsimony tree resulting from the realigned data is presented in Fig. 3. Two significant differences from the HINES et al. tree are immediately clear. First, although the stenogastrines do not form a group with the other social wasps, their placement is now closer to them (that is, no longer sister to all other vespids). Second – and perhaps most devastating to the thesis of HINES et al. – is the monophyly of the Eumeninae. No longer are the zethines sister to the Polistinae + Vespinae as proposed by HINES et al. HINES et al. discussed at length the importance of the paraphyly of the eumenines, the grouping of the zethines with the Polistinae + Vespinae, and erected an incorrect scheme for the evolution of sociality based on this finding. In fact, many of the assertions that support that scheme are based on misreadings of the available literature (see Appendix 1). Now given a phylogenetic reanalysis, their scheme is meaningless, as that more rigorous treatment of their data shows the necessary "intermediate" Zethinae is in fact not phylogenetically intermediate at all.

3.5.6. Robustness: data exclusion

3.5.6.1. The exclusion of data: morphology and behavior. HINES et al. (2007) chose to analyze their new molecular data without any phenotypic data. They did not do this because they denied the importance of these characters. On the contrary, a large portion of the article is dedicated to evolutionary interpretations of those morphological and behavioral characters. The problem is that HINES et al. simply talked about those characters in light of their molecule-only phylogeny. A select mi-

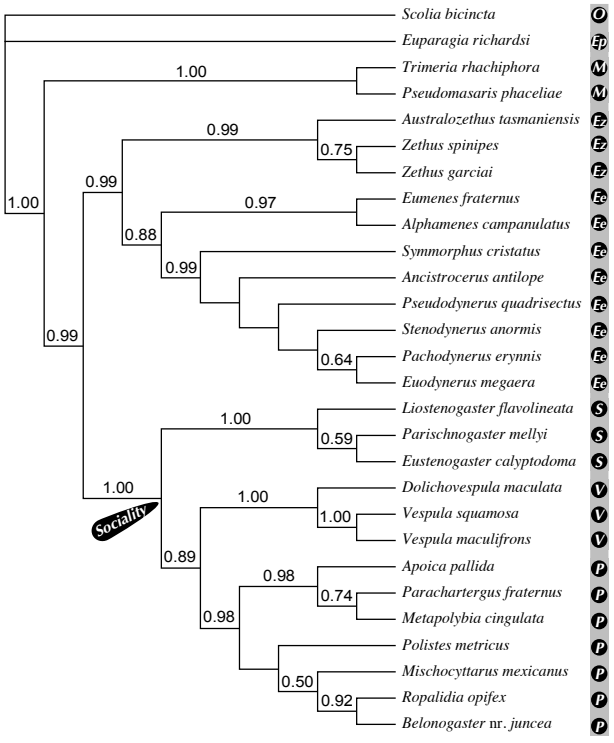


Fig. 4. The single most parsimonious tree (L=689) from analysis of the morphological and behavioral characters (see Appendix 2) alone (parsimony analysis conducted in TNT: 1000 random additions, each with 40 rounds of the parsimony ratchet, 30 rounds of tree fusing, and 20 rounds of tree drifting). Taxon labels inside circles represent subfamilies as follows: O = Outgroup taxa; Ep = Euparagiinae; M = Masarinae; Ee = Eumeninae s.str.; Ez = zethine Eumeninae; S = Stenogastrinae; V = Vespinae; and P = Polistinae.

nority of morphological and behavioral attributes was highlighted in a *post hoc* fashion, but these characters were never subjected to the test of congruence with the molecules, nor are the conclusions from the molecules tested in this way either. Ultimately, the authors protected the conclusions drawn from their molecular data from refutation by additional data. This kind of data chauvinism is not in keeping with empiricism; it amounts to nothing more than the assertion that some types of data are better than others. But as the authors show by their discussion, it is the phenotypic data, and the evolutionary story they tell, that is most interesting.

We have already shown that the alignment presented by Hines et al. is extremely suboptimal, and when a more optimal alignment of only their data is treated, critical details of their new findings are not supported. However, even with this realignment, certain details of the phylogeny are still unexpected, namely the polyphyly of social wasps. Therefore, this unusual finding must be subjected to more test. In phylogenetics, the way one tests homologies is to subject them to potentially disconfirming data. Here we do this, by adding to Hines et al.'s molecular data published and new morphological and behavioral data. If the pattern survives additional, potentially refuting data, only then can the result possibly be described as robust.

It is still common for phylogeneticists to fear that molecular data will swamp out the signal of phenotypic characters such as morphology and behavior. Here we add to the Hines et al. data approximately one-tenth as many phenotypic characters. With no *a priori* preference for the signal deriving from either partition, 'swamping' of one preferred signal by another need not be feared. We are interested in the most supported hypothesis, and so we simply combine all available data. But before we combine the data here, first we present the new phenotypic data alone.

The new phenotypic data include 267 morphological characters and 66 behavioral characters, for a total of 333 phenotypic characters. Some of these characters are extracted directly from the literature, unaltered. Others are new character descriptions (see Appendix 2 for a description of the morphological and behavioral data treated here). These phenotypic characters were coded for the Hines et al. taxa and subjected to phylogenetic analysis in TNT (Goloboff et al. 2008; *Rhopalosoma nearcticum* and *Colocistis crassa* were not coded). The following search parameters were employed: 1000 random taxon addition sequences (RAS), with 40 rounds of the parsimony ratchet, 30 rounds of tree fusing, and 20 rounds of tree drifting per RAS. Each RAS was refined with TBR swapping. The resulting tree is shown in Fig. 4 (L=689). The tree is unsurprisingly consistent in every detail with the standard view of the phylogeny of the Vespidae: *Euparagia* is sister to the remaining vespids and does not form a clade with the masarines (*Trimeria* and *Pseudomasaris*); the eumenines are monophyletic; and the social wasps are monophyletic. Bootstrap support for each of these traditionally recognized clades is strong (≥99).

Adding this comparably paltry amount of morphological character data to the "robust" molecular data of Hines et al., aligned the way they aligned their data (and treating gaps the way they do, as missing), results in two equally parsimonious trees (L=3344). The strict consensus of those trees is shown in Fig. 5. While a few relationships within groups are altered (rearrangements within the Eumeninae, Polistinae, and Vespinae), the relationships of the six subfamilies are identical to the traditional view of Carpenter (1981).

When the phenotypic data are added to the better organized, realigned data of Hines et al. (see above), the resulting three equally parsimonious trees have a length of 3085 steps (see Fig. 6A). This score is 259 steps (~8%) more optimal than the tree deriving from combination of the phenotypic data and the Hines et al. data organized their way (L=3344), and the bootstrap scores are much improved for many nodes. The topological results from this analysis still give the

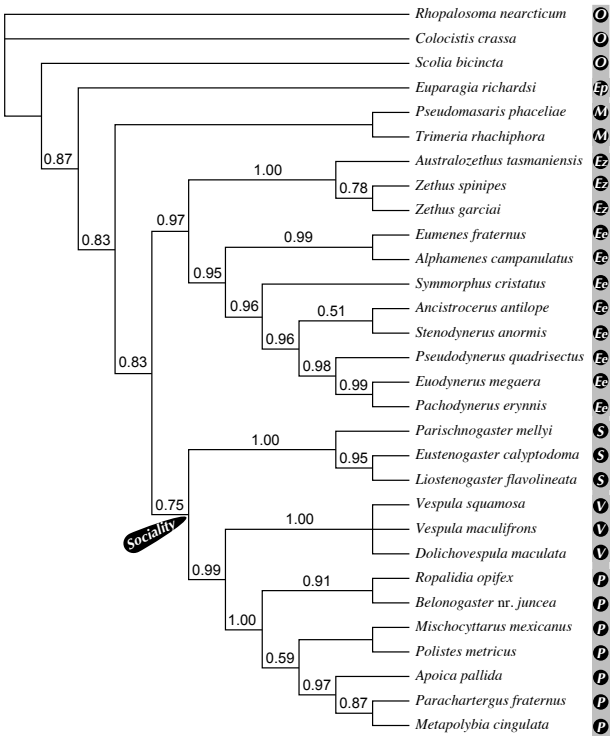


Fig. 5. Strict consensus of two equally parsimonious trees (L = 3344) deriving from analysis of the HINES et al. (2007) molecular data, using their alignment and with gaps as missing data, plus morphology and behavior (see Appendix 2) (parsimony analysis conducted in TNT: 1000 random additions, each with 40 rounds of the parsimony ratchet, 30 rounds of tree fusing, and 20 rounds of tree drifting). Bootstrap values indicated upon branches if ≥0.5. Taxon labels inside circles represent subfamilies as follows: O = Outgroup taxa; Ep = Euparagiinae; M = Masarinae; Ee = Eumeninae s.str.; Ez = zethine Eumeninae; S = Stenogastrinae; V = Vespinae; and P = Polistinae.

traditional view of the subfamilies, monophyly of the Eumeninae, and monophyly of the social wasps. Even when the behavioral data are excluded entirely from the analysis, the topology is unchanged in these aspects (see Fig. 6B).

3.5.6.2. HINES et al.'s exclusion of their own data: indel events. We have thus far treated all indel events as HINES et al. did: as missing data for phylogenetic construction (cf., during alignment; see below). We have done this only for consistency of comparison with HINES et al.'s own work, to show that our realignment is more optimal under their chosen cost regime. However, it has long been appreciated that treating indels as missing amounts to nothing more than unjustified data exclusion (see GIRIBET & WHEELER 1999). As such, HINES et al. excluded many informative evolutionary events from their analysis. As we state above, our realignment of their data was an exercise merely to show that their alignment was severely suboptimal, and the conclusions are thus suspect. Below, we treat indel events, and all other transformations, in a cohesive analysis implemented under Direct Optimization (WHEELER 1996).

3.5.6.3. Direct Optimization of HINES et al.'s molecular data. While organizing and analyzing their data, HINES et al. treated indels in profoundly different ways at different times, leading to their presented tree. As is common, HINES et al. used various methods to generate a static multiple sequence alignment. These included common use of Clustal, "refine[ment] ... using protein translation", and alignment "to secondary structure." As indicated above, it is unclear from this exactly what was done, and thus repeatability is not possible. However, it is clear that one guiding principle was the minimization of indels, or at least the consolidation of indels into a minimal number of opening events. This, for example, is often what is meant when investigators say they "refined" computer-generated alignments to protein translation. This practice, if roughly translated into an explicit cost statement, would amount to inducing high cost on gap opening vs. substitution (minimizing gaps), followed by an affine cost that ensured that gaps cluster together. The simplest way to accomplish this is simply to make substitutions cheap and gaps very expensive overall (and, perhaps with extension gaps less expensive than opening gaps but more expensive than [or =] substitutions). So, in this "alignment stage," gaps were very costly, and thus very important in the computation. However, HINES et al. then made an about face and treated all gaps as valueless (i.e., cost = 0) in the so-called "phylogenetic stage."

HINES et al. provided no justification for why they treated indels so differently in different parts of their analysis. Indels are either evolutionary events or they are not; they cannot be both extremely important and simultaneously worthless.

Their shifting computational methods – treating gaps as extremely expensive relative to other evolutionary events in one part of their analysis, and treating them as free, or worthless relative to other events in another part of their analysis – surely had a significant impact on their results. Absent justification for these actions, their results are in doubt. Here we present a reanalysis of the data of HINES et al. (2007), treating indels and substitutions the same, throughout the analysis. As discussed above, the so-called "alignment" and "phylogenetic" stages of analysis are artificial subsets of what is optimally a single analytical process (SANKOFF 1975; FELSENSTEIN 1988; WHEELER 1996). For philosophical reasons (e.g., GRANT & KLUGE 2005), we treat all evolutionary events as equal in cost in all Direct Optimization analyses presented hereafter. Other cost schemes might be justified or tested (GIRIBET & WHEELER 2007), but the important point is to use whichever cost scheme is used consistently, throughout the entire analysis. Shifting cost schemes

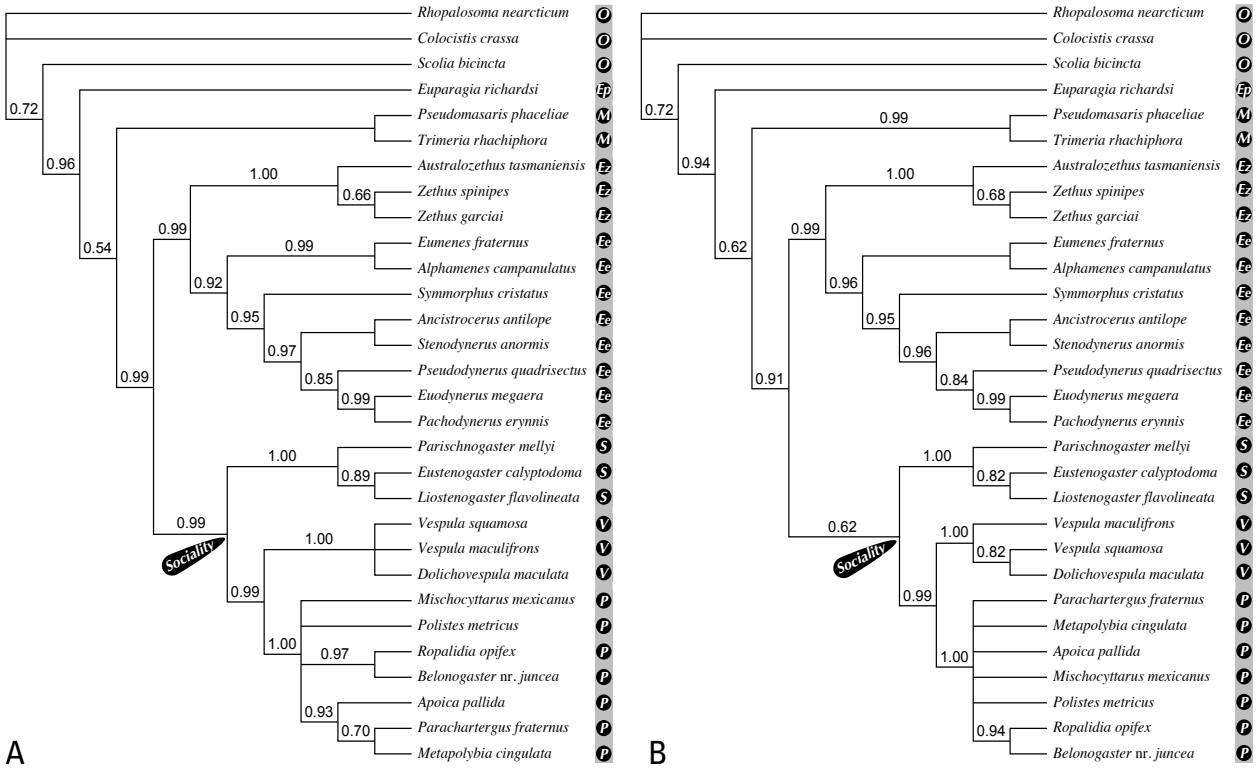


Fig. 6. Results of combined analysis of realignment of Hines et al. (2007) data (gaps treated as missing data) and phenotypic characters (see Appendix 2). **A**: With behavioral characters (strict consensus of three equally parsimonious trees of L=3085). **B**: Without behavioral characters (strict consensus of three equally parsimonious trees of L=2982). Parsimony analysis conducted in TNT: 1000 random additions, each with 40 rounds of the parsimony ratchet, 30 rounds of tree fusing, and 20 rounds of tree drifting. Bootstrap values indicated upon branches if ≥0.5. Taxon labels inside circles represent subfamilies as follows: O = Outgroup taxa; Ep = Euparagiinae; M = Masarinae; Ee = Eumeninae s.str.; Ez = zethine Eumeninae; S = Stenogastrinae; V = Vespinae; and P = Polistinae.

mid-analysis is not justifiable. We carried out our reanalysis via Direct Optimization (Wheeler 1996) as implemented in POY4 (Varón et al. 2010).

For this Direct Optimization analysis, an optimal tree was selected from 20 initial Wagner builds (Farris 1970). This tree was used solely to divide algorithmically the data at highly conserved regions ("auto_sequence_partition" command). The same initial tree was used to implement the implied alignment search algorithm ("auto_static_approx" command; see Wheeler 2003b). Next, we issued the timed "search()" command in POY4. Given the time allotted to the "search" command, POY4 implements standard Direct Optimization (Wheeler 1996) via a variety of tree search methods, including multiple random addition sequence builds, plus multiple rounds of tree swapping, ratcheting (Nixon 1999), tree fusing and drifting (Goloboff 1999). Four 24-hour rounds of "search()" were followed by 24 hours of exhaustive Direct Optimization ("set(exhaustive_do)" command) and two 24-hour rounds of iterative pass optimization searching ("set(iterative:exact)" command; Wheeler 2003a). This entire week-long search strategy was repeated iteratively, starting with the most optimal trees from the previous week's search – until a superior tree could not be found (driven search: stopping point at >100 consecutive trees of same score). At the end of this proceedure, the unpartitioned data (that is, not treated by "auto_sequence_partition") were re-optimized onto the resulting topologies for final score calculations. All commands were executed in parallel via LAM Message Passing Interface across 64 hyperthreaded 2.8 GHz Pentium-class, Myrnet-linked Linux PC nodes maintained at the American Museum of Natural History.

The strict consensus of the three equally parsimonious trees resulting from the POY4 analysis of the Hines et al. data is shown in Fig. 7. The length of those trees was 2796, which is much more optimal than the length of 2869 resulting from the Hines et al. alignment analyzed under the same cost scheme (i.e., gaps as a fifth state, with cost equal to substitutions). Although in the strict consensus shown (Fig. 7) the zethines, remaining eumenines and Vespinae + Polistinae form a trichotomy, in none of the three equally optimal Direct Optimization trees are the zethines sister to the social wasps. It is also noteworthy that in one of the equally optimal solutions, the Eumeninae (including the zethines) are monophyletic. Again, the sister relationship of the Zethinae to the Polistinae + Vespinae

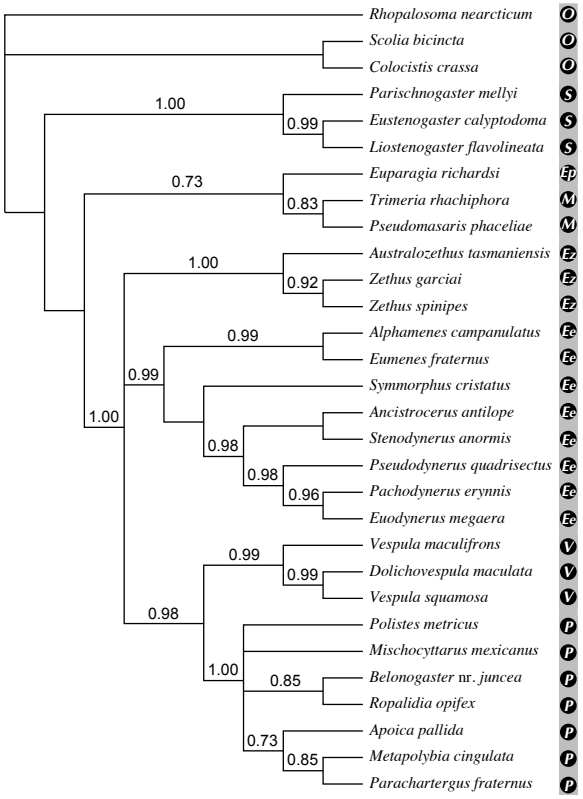


Fig. 7. Direct Optimization of the HINES et al. (2007) molecular data, implemented in POY4 (see text for search strategy and commands). Taxon labels inside circles represent subfamilies as follows: O = Outgroup taxa; Ep = Euparagiinae; M = Masarinae; Ee = Eumeninae s.str.; Ez = zethine Eumeninae; S = Stenogastrinae; V = Vespinae; and P = Polistinae.

Fig. 8. Direct Optimization of the HINES et al. (2007) molecular data, plus morphology and behavior (see Appendix 2), implemented in POY4 (bootstraps implemented in POY4 during optimization, according to same parameters as described in FREUDENSTEIN et al. 2004; see text for search strategy and commands). Taxon labels inside circles represent subfamilies as follows: O = Outgroup taxa; Ep = Euparagiinae; M = Masarinae; Ee = Eumeninae s.str.; Ez = zethine Eumeninae; S = Stenogastrinae; V = Vespinae; and P = Polistinae.

reported by HINES et al. is crucial to their hypothesis regarding the evolution of social behavior in vespids. That result is not supported here by our more optimal solution of HINES et al.'s own data.

Although the HINES et al. data analyzed under Direct Optimization do continue to show polyphyletic social wasps, the addition of the phenotypic data (see Appendix 2) —analyzed via the same search strategy as for the Hines et al. (2007) data treated alone— results in the traditional view. The single most parsimonious tree (L = 3576; see Fig. 8) resulting from the combined analysis of all data (molecular and phenotypic) under Direct Optimization shows the Euparagiinae sister to the remaining Vespidae, a monophyletic Eumeninae (with bootstrap = 100%), and a monophyletic social wasp clade (with bootstrap = 94%), The bootstrap values reported for this Direct Optimization analysis of both molecular and phenotypic data are much higher (with 23 of 27 nodes ≥94%) than those reported by HINES et al., refuting sharply any suggestion that the data partitions are somehow combating each other. On the contrary, the molecular data, the morphology and the behavioral characters act in concert to produce a much better supported phylogeny than any of these datasets do when treated in isolation, as is common in social wasp studies (ARÉVALO et al. 2004; PICKETT & WENZEL 2004; PICKETT et al. 2006).

3.6. Summary of review of prior work and its reanalysis

Here we have detailed the problems inherent to recent vespid studies that purport to show diphyly of sociality and paraphyly of the Eumeninae. In so doing we have revealed two general patterns. First, most of the molecular studies have used extremely small sample sizes; second, these studies employed suboptimal methods of data organization (i.e., alignment). When realigned using an optimality criterion to guide multiple sequence alignment, the findings of SCHMITZ &

Moritz (1998) – namely, paraphyly of Vespoidea and diphyly of sociality – are refuted. The predictions of Hunt & Amdam (2005) – that *Polistes* has a solitary ancestor and renders the Eumeninae paraphyletic – have no empirical support whatsoever; indeed, subsequent work by one of these authors (Hines et al. 2007) fails to support Hunt & Amdam (2005). The assertions of Hunt (2006, 2007) are based on no empirical evidence and misreading of natural history literature. Finally, the findings of Hines et al. (2007) – paraphyly of the Eumeninae with zethines as sister to Polistinae + Vespinae and the diphyly of the social wasps – are shown to be either unsupported by their own data or unsupported when challenged by other data in a simultaneous analysis. Specifically, the claim that each individual gene partition supports the same topology as the combined data is shown to be false (Fig. 1). The data of Hines et al. (2007), when analyzed under their alignment and cost structure, fail to support any of their unusual findings when analyzed with ~10% more morphological and behavioral data (Fig. 5). When realigned with an explicit alignment criterion, the newly aligned data are more optimal in alignment length and tree cost, and they show a monophyletic Eumeninae (Fig. 3). When phenotypic data are added to the more optimal alignment, none of Hines et al.'s findings survive (Fig. 6A), even when the behavioral data are excluded (Fig. 6B). When the data from Hines et al. are treated via Direct Optimization (Fig. 7), they fail to show a sister relationship between the Zethinae and the Polistinae + Vespinae – a finding that is critical to their notions about the origins of sociality in the group. And finally, when the Hines et al. data are organized via Direct Optimization and combined with phenotypic characters (Fig. 8), none of their unexpected findings remain; instead, a tree perfectly consistent with Carpenter (1981) is recovered.

In short, when subjected to a variety of tests, analyzed under a variety of methods employed by researchers today, the findings of Hines et al. (2007) are found to be wanting, and under any scheme of molecular data organization (including their own), their findings are completely lacking when a comparatively paltry amount of phenotypic data are added to the analysis.

We do not claim to have solved all matters Vespidae. The numbers of taxa employed in the molecular analyses conducted so far are such small samples of the available vespid species diversity that it would be premature to make strident claims about the veracity of these findings. Thus, below we present a new phylogenetic analysis, with new molecular data for 130 vespid taxa (plus outgroup taxa) – the largest vespid phylogeny to include molecular data presented to date.

4. New data and analyses

4.1. Dataset

The analysis presented below is based on multiple nuclear and mitochondrial loci – including ~1,078 unaligned sites of COI, ~1,000 unaligned sites of 28S, ~350 unaligned sites of 12S, and ~515 unaligned sites of 16S (GenBank accession GU596504-GU596949; for *Polybioides melainus*, a small discontinuous fragment of 28S is unavailable via GenBank) as well as 267 morphological characters and 66 behavioral characters (Appendix 2). The ingroup consists of 130 vespid species, and two scoliid outgroup taxa are included. This taxon sampling is more than four times that of Hines et al. (2007). Also, Hines et al. analyzed ~2,780 unaligned characters; we include here ~2,943 unaligned sites plus 333 phenotypic characters (minus missing data). We include all data here, following the well-established precepts of simultaneous analysis (Kluge 1989; Nixon & Carpenter 1996).

We include here 333 morphological and behavioral characters. Although neither of us believes that the evolutionary character content of a single nucleotide substitution, for example, is comparable to any given morphological character – most of which are the polygenic result of multiple, interacting genes – philosophical consistency dictates that we treat all characters as equal contributors to the topology. Thus, here we are willingly increasing the relative power of single nucleotides or indel events to that of complex morphological characters (no doubt the result of many thousands of nucleotides). So, in effect, we are sharply minimizing the phylogenetic impact of the morphology relative to the molecular characters. As a result, none can argue that we have unfairly stacked the deck against the molecules. Nearly 3,000 unaligned molecular characters can surely assert their signal when faced with what amounts to about a tenth as many phenotypic characters.

4.2. Methods

As stated above, our preferred methodology for phylogenetic analysis of these data is Direct Optimization (Wheeler 1996). To recap, in this context, all molecular characters, including substitutions and inferred indel events, are treated the same throughout the analysis. In common implementations, an 'alignment' is constructed using one suite of substitution and indel cost parameters, and the phylogenetic tree is inferred

using a different, often contradictory, suite of costs (see discussion above). Here we implemented Direct Optimization and related algorithms (see above) as implemented in POY (VARÓN et al. 2010) to accomplish a consistent, unified cost regime approach. In keeping with our philosophical objection to *a priori* differential weighting, the cost regime implemented was substitutions = 1, and indel event (whether opening or extending) = 1 (that is, there are no affine costs). In keeping with simultaneous analysis, the morphology and behavior were analyzed with the molecular data, in POY4, according to the same costs; additivities in phenotypic characters were retained.

As the tree-search space and the tree-alignment space for 132 taxa and this many characters is very large, our search strategy had to be rigorous. We employed POY4 to implement the same strategy discussed above for the reanalysis of the HINES et al. (2007) data, but many more "search()" runs were implemented. As before, in this Direct Optimization analysis an optimal tree was selected from 20 initial Wagner builds, and the best tree was again used to execute automatic sequence partitioning and the implied alignment search algorithms. Multiple loops of the week-long "search()" strategy (see above) were initiated, seeding each new round with the best trees from the previous. Searches were continued until a better answer could not be found by extensive subsequent searching (driven search: >100 consecutive trees of same score), and final scores were calculated by reoptimization of the unpartitioned data, as before. All commands were again executed on 64 nodes of the AMNH cluster (described above). In this case, this ultimately meant weeks of time, and thousands of processor-hours of searching with some of the most sophisticated phylogenetic search algorithms available.

4.3. Results and discussion

The strict consensus of the seven most parsimonious trees found (L=16880) is presented in Fig. 9. Two traditional features that would normally not be noteworthy, but for recent claims, are immediately apparent: (1) the Eumeninae are monophyletic, and (2) the social wasps are also monophyletic. The six subfamilies recognized by CARPENTER (1981) are recovered as monophyletic here, and the relationships of these six are exactly as found in CARPENTER (1981). All genera with multiple representatives are monophyletic save two: *Ceramius*, here paraphyletic in terms of *Ceramiopsis*; and *Polybia*, here paraphyletic in terms of *Epipona* + (*Asteloeca* + *Metapolybia*).

Relationships among the genera within Masarinae (Fig. 9A) differ from the cladogram in CARPENTER (1993) in showing *Ceramius* + *Ceramiopsis* as a clade, rather than *Ceramiopsis* as more closely related to *Jugurtia* + *Trimeria*. This may be an effect of the paraphyly of *Ceramius* shown in Fig. 9. That genus has been subdivided into eight or more species groups (see GESS 1996: tab. 4); our sample represents four of these and one species of uncertain placement. We therefore defer discussion of this genus until a larger taxon sample is investigated.

Moving down the tree (Fig. 9A), the monophyletic Eumeninae are sister to the three subfamilies of social wasps. The relationships among the eumenine genera correspond to a *Eumenes* s.l. clade and an *Odynerus* s.l. clade, both seen in the previous cladistic analyses of genera in this group (CARPENTER & CUMMING 1985; VERNIER 1997). Closer relationship of *Monobia* to *Parancistrocerus* than *Ancistrocerus* differs from CARPENTER & CUMMING (1985), but that study did not include *Ancistroceroides*.

Relationships among stenogastrine genera (Fig. 9A) correspond to those shown by CARPENTER (1988; 2001). *Liostenogaster* and *Anischnogaster* are monophyletic, and *Anischnogaster*'s placement as sister to *Parischnogaster*, with *Liostenogaster* sister to that clade, is consistent with the previous studies. These morphological relationships have never been tested before with molecular data, and so the relationships presented in CARPENTER (1988, 2001) have now survived potential refutation via simultaneous analysis with molecular data.

Relationships among vespine genera (Fig. 9A), with monophyletic hornets on the one hand and yellowjackets on the other, differ from CARPENTER (1987) in which *Provespa* was sister to the yellowjackets. As in the Stenogastrinae, there has been little genetic data brought to bear on generic relationships (but see VARVIO-AHO et al. 1984 for allozyme data, SCHMITZ & MORITZ 1990 for mtDNA restriction fragment size polymorphism, and PANTERA et al. 2003 for amino acid data). That we found monophyly of all four genera (*Vespa*, *Provespa*, *Dolichovespula*, and *Vespula*) and that the yellowjackets (*Dolichovespula* + *Vespula*) form a clade are both consistent with CARPENTER (1987). Monophyly of the hornets is novel, but this is the first study to bring molecular, morphological, and behavioral data to investigate all four vespine genera.

Finally, concerning relationships within Polistinae, resolution of tribal relationships is different from any previous publication, with Ropalidiini (represented by *Polybioides* and *Belonogaster*) sister to Mischocyttarini + (Epiponini + Polistini) (Fig. 9A,B). Previous studies have usually placed Polistini as sister to the other tribes, but have otherwise differed from each other: CARPENTER (1991) and ARÉVALO et al. (2004) had

relationships among the remaining three tribes unresolved, while WENZEL (1993) and WENZEL & CARPENTER (1994) showed Ropalidiini as most closely related to Epiponini.

Within Epiponini (Fig. 9B), the base of the tribe corresponds to the relationships shown in CARPENTER (1991) and WENZEL & CARPENTER (1994), with *Apoica* as sister to all remaining epiponine genera, then *Agelaia*, then *Pseudopolybia* + *Chartergellus*. ARÉVALO et al. (2004) had similar relationships in this part of the tree, except relative placement of *Apoica* and *Agelaia* was unresolved. NOLL et al. (2004) had *Apoica* and *Agelaia* as sisters and did not have *Pseudopolybia* and *Chartergellus* so. Relationships among the other genera differ considerably from previous studies. The one point of similarity to all previous work includes close relationship of *Metapolybia* and *Asteloeca* (not applicable to the taxa in ARÉVALO et al. 2004). Much of this part of the tree was unresolved in CARPENTER (1991), and while *Epipona* was closely related to *Metapolybia* + *Asteloeca*, *Charterginus* was not closely related to *Brachygastra*, *Protonectarina* and *Protopolybia*. WENZEL & CARPENTER's (1994) and NOLL et al.'s (2004) trees were resolved, but there is no other correspondence. ARÉVALO et al. (2004) also had this part of the tree largely unresolved, and also had *Epipona* as closely related to *Metapolybia*, and *Protonectarina* and *Brachygastra* as sisters, and did not resolve *Polybia* as monophyletic.

Three polistine genera are represented by many species: *Polistes*, *Mischocyttarus*, *Polybia*. Only the first two of these are found to be monophyletic. The monophyly of *Polybia* has been weakly supported (see CARPENTER et al. 2000), but is not found here. Because the species samples in these genera are sufficiently large, and because these three genera have historically been subdivided into subgenera, we consider the correspondence of our tree to subgenera below. Some of the findings are at odds with prior work, and although this alone is no reason to doubt the novel findings, there is independent reason to think that nomenclatorial action relating to the subgenera should await further work. First, the morphological and behavioral data included here vary primarily at the genus level and above. While we did code each individual species for each adult morphological character with specimens in hand, and this did result in some within-genus variation, the characters themselves tend to reveal higher-level relationships. As such, some characters that vary only within *Polistes* (for example) were not coded, but these would surely provide much needed clarity to relationships within *Polistes*. Second, the *Polybia* species in our analysis have the most missing molecular fragments of any of the groups examined. We believe that this shortcoming does not necessarily vitiate the analysis, and such individual character partition shortcomings are specifically accommodated by simultaneous analysis, as was recently shown forcefully in GOLOBOFF et al. (2009). Third, and most important, the species sample in each genus is far from comprehensive. Therefore, we will forgo nomenclatorial action and await focused phylogenetic treatments of these genera.

4.3.1. *Polistes*

Four subgenera are currently recognized within *Polistes* (after CARPENTER 1996): the New World *Aphanilopterus*, the East Asian and Indo-Australian *Gyrostoma*, the Austral-Asian and African *Polistella*, and the Eurasian and African *Polistes* s.str. Although RICHARDS (1973, 1978) recognized twelve subgenera, the four recognized by CARPENTER (1996) are the only supported both by broad taxon sampling and cladistic methodology. In the tree in Fig. 9B, three of these subgenera are monophyletic: *Gyrostoma* (*P. tenebricosus* and *P. jokahamae*), *Polistella* (*P. japonicus*, *P. sagittarius*, *P. snelleni*, and *P. stigma bernardii*), and *Polistes* s.str. (*P. biglumis*, *P. dominula*, *P. gallicus*, *P. marginalis*, and *P. nimpha*). The sister relationship between *Polistes* s.str. and the remaining *Polistes* found here is consistent with the findings of ARÉVALO et al. (2004); it differs from CARPENTER (1996) and PICKETT et al. (2006), where it was sister to *Aphanilopterus*. The position of *Polistella* differs from previous studies.

Only the subgenus *Aphanilopterus* is paraphyletic, rendered so by *Gyrostoma*. The sister relationship of *Gyrostoma* to the former *Epicnemius* (the *P. pacificus* to *P. testaceicolor* component in Fig. 9; subgenus of RICHARDS 1973, 1978) is quite unprecedented. Moving down the figure, the clade including *P. major major* to *P. aurifer* is surprising. The close relationship of *P. major major* (former subgenus *Palisotius* of RICHARDS 1973, 1978) to the remaining taxa in the clade is expected, but PICKETT et al. (2006) found *P. major* sister to the former *Epicnemius* (which accords with the fact that all of these taxa have an epicnemial carina). The placement of *P. carnifex carnifex* (former subgenus *Onerarius* of RICHARDS 1973, 1978) within the former subgenus *Fuscopolistes* is unprecedented. At the bottom of the tree (*P. biguttatus* and following), the recovery of the former subgenus *Aphanilopterus* (*sensu* RICHARDS 1973, 1978) is consistent with ARÉVALO et al. (2004) and PICKETT et al. (2006), although species-level relationships are different.

4.3.2. *Mischocyttarus*

Eleven subgenera are currently recognized (SILVEIRA 2008), eight of which are represented here (see Fig. 9A). Of the four represented by more than one ter-

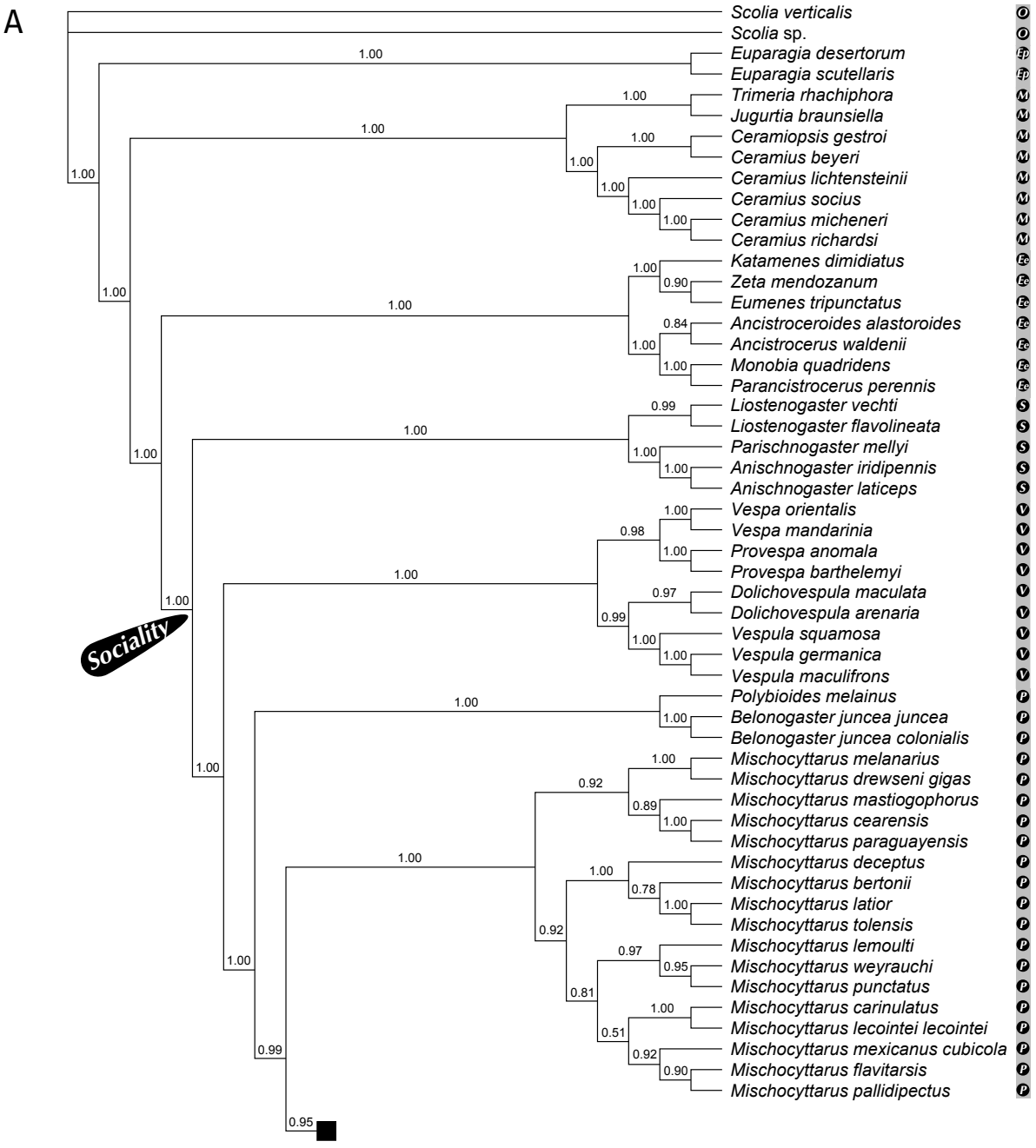
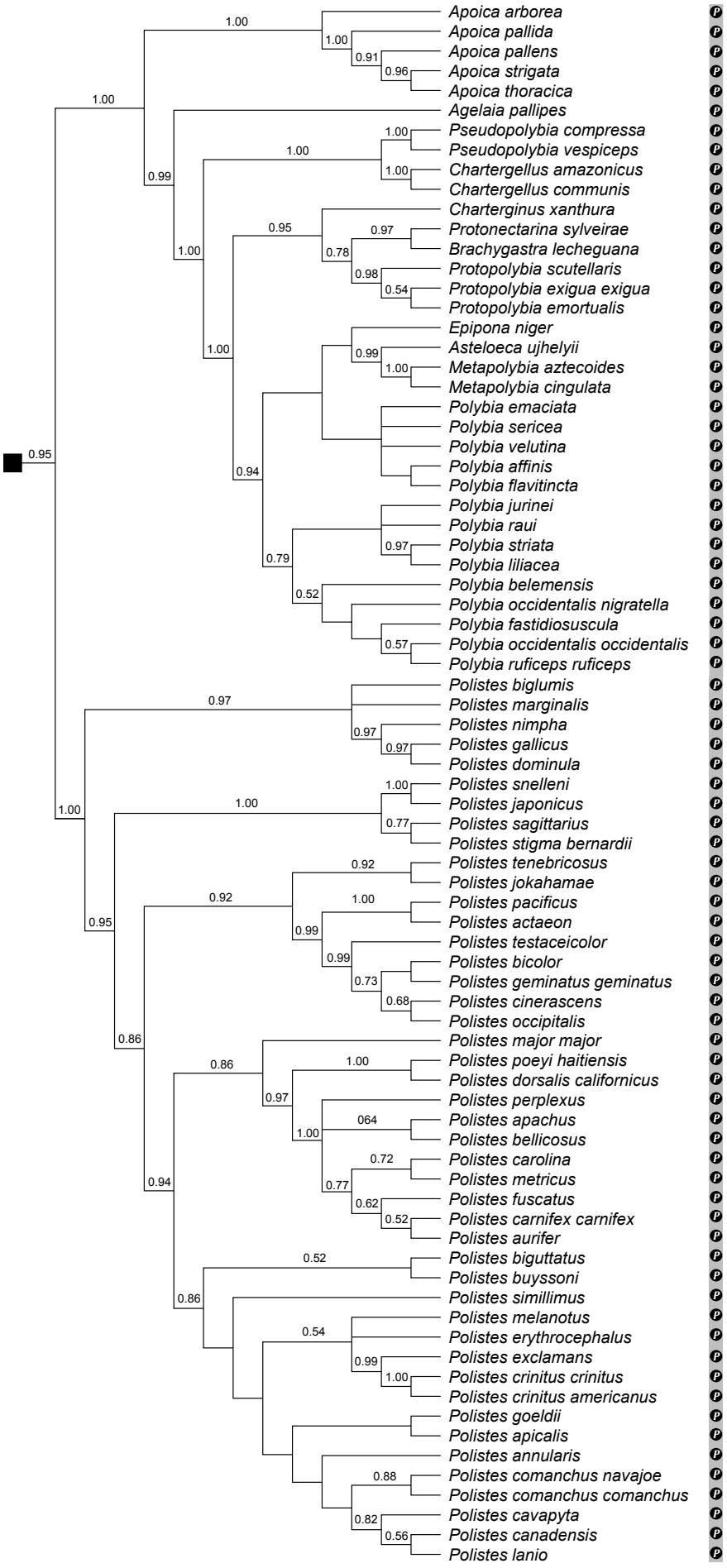


Fig. 9. Direct Optimization of our molecular data (see text sections 4.1. and 4.2.) plus morphology and behavior (see Appendix 2), implemented in POY4 (bootstraps implemented in POY4 during optimization, according to same parameters as described in FREUDENSTEIN et al. 2004; see text for search strategy and commands). Taxon labels inside circles represent subfamilies as follows: O = Outgroup taxa; Ep = Euparagiinae; M = Masarinae; Ee = Eumeninae s.str.; S = Stenogastrinae; V = Vespinae; and P = Polistinae. The cladogram is split into figures 9A and 9B. **A**: The major clades (after scoliid outgroup taxa), from top to bottom, are Euparagiinae, Masarinae, Eumeninae, Stenogastrinae, Vespinae, and the polistine tribes Ropalidiini and Mischocyttarini. The first three clades are solitary vespids, and the single origin of sociality in the Vespidae is indicated. **B**: The first sister relationship separates the polistine tribes Epiponini (top) and Polistini (bottom).

minal, *Kappa* (*M. bertonii*, *M. deceptus*, *M. latior*, *M. tolensis*), *Mischocyttarus* s.str. (*M. drewseni gigas*, *M. melanarius*) and *Monogynoecus* (*M. carinulatus*, *M. lecointei lecointei*) are monophyletic. The subgenus *Phi* is not, with part (*M. paraguayensis* + *M. cearensis*) sister to *Scytokeraia* (*M. mastigophorus*), and part (*M. mexicanus cubicola* + (*M. flavitarsis* + *M. pallidipectus*)) sister to *Monogynoecus*. The subgenera *Artifex* (*M. lemoulti*), *Haplometrobius* (*M. weyrauchi*) and *Omega* (*M. punctatus*) form a clade, which corresponds to SILVEIRA (2008), but none of the other subgeneric relationships do. In view of the much larger taxon sample in SILVEIRA (2008), we defer further discussion of this genus.

B


Apoica arborea
Apoica pallida
Apoica pallens
Apoica strigata
Apoica thoracica
Agelaia pallipes
Pseudopolybia compressa
Pseudopolybia vespiceps
Chartergellus amazonicus
Chartergellus communis
Charterginus xanthura
Protonectarina sylveirae
Brachygastra lecheguana
Protopolybia scutellaris
Protopolybia exigua exigua
Protopolybia emortualis
Epipona niger
Asteloeca ujhelyii
Metapolybia aztecoides
Metapolybia cingulata
Polybia emaciata
Polybia sericea
Polybia velutina
Polybia affinis
Polybia flavitincta
Polybia jurinei
Polybia raui
Polybia striata
Polybia liliacea
Polybia belemensis
Polybia occidentalis nigratella
Polybia fastidiosuscula
Polybia occidentalis occidentalis
Polybia ruficeps ruficeps
Polistes biglumis
Polistes marginalis
Polistes nimpha
Polistes gallicus
Polistes dominula
Polistes snelleni
Polistes japonicus
Polistes sagittarius
Polistes stigma bernardii
Polistes tenebricosus
Polistes jokahamae
Polistes pacificus
Polistes actaeon
Polistes testaceicolor
Polistes bicolor
Polistes geminatus geminatus
Polistes cinerascens
Polistes occipitalis
Polistes major major
Polistes poeyi haitiensis
Polistes dorsalis californicus
Polistes perplexus
Polistes apachus
Polistes bellicosus
Polistes carolina
Polistes metricus
Polistes fuscatus
Polistes carnifex carnifex
Polistes aurifer
Polistes biguttatus
Polistes buyssoni
Polistes simillimus
Polistes melanotus
Polistes erythrocephalus
Polistes exclamans
Polistes crinitus crinitus
Polistes crinitus americanus
Polistes goeldii
Polistes apicalis
Polistes annularis
Polistes comanchus navajoe
Polistes comanchus comanchus
Polistes cavapyta
Polistes canadensis
Polistes lanio

4.3.3. *Polybia*

Paraphyly of *Polybia* in terms of *Epipona* + (*Asteloeca* + *Metapolybia*) is quite novel (Fig. 9B). Eleven subgenera are currently recognized in *Polybia* (CARPENTER et al. 2000), but just five are represented here. Two of the three represented by more than one terminal are monophyletic: *Myrapetra* (*P. belemensis*, *P. fastidiosuscula*, *P. occidentalis nigratella*, *P. occidentalis occidentalis*, *P. ruficeps ruficeps*) and *Polybia* s.str. (*P. liliacea*, *P. striata*). The subgenus *Trichinothorax* is not monophyletic, with *P. raui* more closely related to subgenera *Apopolybia* (*P. jurinei*) and *Polybia* s.str., the remaining *Trichinothorax* (*P. affinis*, *P. flavitincta*, *P. sericea*, and *P. velutina*) in a clade with *Pedothoeca* (*P. emaciata*), and this latter clade closely related to *Epipona* + (*Asteloeca* + *Metapolybia*). Relationships are obviously quite different from those in CARPENTER et al. (2000), but until a study encompassing all the subgenera is undertaken, we defer discussion.

5. Conclusion

Although this is a large-scale study of vespid phylogeny, we do not claim it is definitive. Taxa important for a comprehensive understanding of the group that are missing include the masarine tribe Gayellini and subtribes Paragiina and Priscomasarina, and the former eumenine subfamilies Raphiglossinae and Zethinae. We have acquired fresh specimens of Gayellini, Paragiina and Zethinae in the course of our field work, and are currently undertaking a much larger analysis of the Vespidae, with more than twice as many terminals as included here. That will better approach the sort of sample that a group of 5,000 described species deserves.

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8. Appendix 1: Factual errors in HINES et al. (2007)

The paper by HINES et al. (2007) contains so many errors of simple fact that correcting them compels us to devote a separate section to the task, as this would be extremely distracting as part of the main text. We proceed *seriatim* (citations from HINES et al. 2007 in Italics).

(1) HINES et al. (2007: 3295): "*Additional behavioral characters proposed as shared, derived traits (synapomorphies) for the clade (5, 35) appear to be ambiguously characterized (19, 37)*."

This statement is misrepresentation. First, citations 19 and 37 are self-citations, of HUNT (2006, 2007). HUNT (2006) did not present any argument regarding the behavioral characters adduced by CARPENTER (1991, 2003 [citations 5 and 35]), he simply cited HUNT (2007). HUNT (2007) did not actually show that any of these behavioral characters were "ambiguously characterized." He only mentioned those presented in CARPENTER (1991), dismissing most of them merely by stating (p. 71) "I disagree with Carpenter's interpretation of most of the behavioral traits." Among the numerous other characters presented by CARPENTER (2003) were those extracted from HUNT (1999). This latter paper is cited with evident approval by HINES et al. (2007) and HUNT (2006, 2007).

(2) HINES et al. (2007: 3295), speaking of SCHMITZ & MORITZ (1998): "*an analysis that remains controversial because of the absence of some ingroup sub-*

families and the inappropriate selection of outgroups, which resulted in uncertainties in rooting the phylogeny (31)."

None of the reasons given has anything to do with why that analysis might be "controversial." CARPENTER (2003) presented a better alignment that resulted in a tree in line with CARPENTER (1981), with just the ingroups and outgroups published by SCHMITZ & MORITZ (1998).

(3) HINES et al. (2007: 3296): "*The perspective that eumenines are the nearest relatives of eusocial Vespinae + Polistinae is not new. Earlier observers argued for this relationship on the basis of behavioral and morphological traits (41–45), such as the longitudinal folding of wings, a commonly used diagnostic feature of Vespidae that occurs only in eumenines, polistines, and vespines.*"

First, the morphology mentioned is wrong, as longitudinal folding of wings occurs in Masarinae too (the genera *Celonites* and *Quartinia*). This is a fact discussed in CARPENTER (1981), cited by HINES et al. (2007).

Second, none of the authors cited argued anything of the sort:

Citation 41. DUCKE (1914) argued that social wasps arose out of the eumenines – and included Stenogastrinae (as *Ischnogaster*) in the social wasps. He did not mention masarines or euparagiines.

Citation 42. BEQUAERT (1918) said nothing about relationship of eumenines to polistines or vespines – what he said was "in fact the solitary as well as the social wasps are polyphyletic" (p. 12).

Citations 43–44. ROUBAUD (1911) said nothing about relationships, merely stating that habits in *Synagris* (the only eumenine discussed) are "in full course of evolution toward a higher type, toward the mode of rearing the young so entirely different, which exists among the social wasps" (p. 508). ROUBAUD (1916) likewise said nothing about relationship, but discussed evolution of behavior within eumenines as the genesis of social behavior. Roubaud also never mentioned masarines or euparagiines.

Citation 45. WILLIAMS (1919) placed stenogastrines (as *Stenogaster*) in the social wasps (as Vespinae), while stating "they have some characters of the Eumeninae and some of neither subfamily" (p. 166). Of Eumeninae, he stated "Some of the more highly specialized Eumeninae have habits in common with their social brethren" (p. 150). He also did not mention masarines or euparagiines.

(4) HINES et al. (2007: 3296): "*Our finding that Eumeninae is paraphyletic accords well with the distribution of their trait variation and supports an earlier taxonomic classification (39) of two subfamilies, Zethinae and Eumeninae.*"

This misstates both trait variation (see below) and classification. BEQUAERT (1928) [citation 39] did not divide eumenines into the two subfamilies Zethinae and Eumeninae; he included Raphiglossinae (see also BEQUAERT 1918; CARPENTER 1981), and placed all three as subfamilies within Vespidae.

(5) HINES et al. (2007: 3296): "*Eumenine relationships similar to our results were obtained in morphological analyses of Eumeninae by Vernier (46) and Carpenter (22), both of whom found a zethine taxon to be sister to the remaining Eumeninae.*"

While it is true that CARPENTER & CUMMING (1985) and VERNIER (1997) both had zethines basal within a monophyletic Eumeninae, there is no similarity whatsoever to HINES et al.'s (2007) result of paraphyletic Eumeninae.

(6) HINES et al. (2007: 3296): "*'Zethinae,' the sister group to the eusocial taxa, exhibits traits that may be transitional between those of the ancestral eumenines and the eusocial Vespinae + Polistinae. For instance, rather than the typical eumenine nest construction with mud, the zethine genera* Zethus *and* Calligaster *are known to construct nests of plant material, a behavior that could precede the construction of nests from long-fiber wood pulp in the manner of Vespinae and Polistinae (41, 47, 48).*"

No such interpretation of zethine traits is tenable. To begin with, the vast majority of species of *Zethus* known have "typical eumenine nest construction," using burrows. In fact, this is true of all species reported of the subgenus *Zethus* s.str. (see BOHART & STANGE 1965), to which both of the *Zethus* species analyzed by HINES et al. belong. Building nests of plant material is quite rare in *Zethus*, and found only in species in one subgenus, *Zethoides*; and in none of the other three subgenera of *Zethus*, in which only use of burrows has been reported. Nests of *Australozethus* (the other zethine analyzed by HINES et al.) are unknown, but no Australian zethine species (i.e., the *Australozethus* used by HINES et al.) have been reported to make nests of plant material, only burrowing is known (see CARDALE 1985).

Indeed, one does not even have to presume paraphyly of the subgenus *Zethus* s.str. in terms of *Zethoides* (STANGE 1979 characterized *Zethus* s.str. as the most primitive subgenus) to conclude that solitary nesting in burrows is ancestral in zethines. Aside from the genera already discussed, nests have been described in the following zethine genera: in *Discoelius*, all species nest in burrows in wood or reeds (see VAN DER VECHT & FISCHER 1972); in *Protodiscoelius*, nesting is in burrows (CLAUDE-JOSEPH 1930); in *Ischnocoelia*, nesting is in burrows (RAYMENT 1954). And while the nest of *Ctenochilus* has not been described, the female has a

psammophore (a basket-like modification of the labial palpi, used in carrying sand), and it therefore surely nests in burrows as well. Thus it is only *Calligaster* (two species reported; FORBES 1885; WILLIAMS 1919) and some species of the subgenus *Zethoides* (six species described in BOHART & STANGE 1965) that make nests of plant material – that is, 8 species out of 303 described in zethines (sensu STANGE 1979; the number of species is from Carpenter, unpubl.). While no cladogram of zethine genera has been published, BOHART & STANGE (1965) considered *Discoelius merula* (now in *Protodiscoelius*; see CARPENTER 1986) the most primitive taxon, and assumed (p. 19) that "the original ancestor of *Zethus* was a discoeliine wasp similar to *Discoelius merula*." Just from this, and the small percentage of species known to make nests of plant fibers, it is clear that the communal ancestor to highly social wasps required by HUNT & AMDAM's (2005) theory cannot be found in "Zethinae" – unless of course *Zethus* itself is paraphyletic in terms of highly social wasps.

In summary, none of the species included by HINES et al. exhibit the traits they claim their DNA data are indicating.

(7) HINES et al. (2007: 3297): "*Cowan (ref. 23, p. 73) notes that* Zethus *and* Calligaster *'are regularly cited as exemplifying the critical evolutionary stages of subsocial and communal behavior that connect solitary and eusocial wasps,' a perspective that dates to de Saussure (51)*."

This misrepresents DE SAUSSURE (1875), who (p. 12) stated: "Certain species of Odynerinae (*Zethus*) appear to manifest a tendency toward social habits; they form small agglomerations of nests which resemble a little the irregular nests of bumble bees (*Bombus*), but grouped yet more confusedly." All this seems to accord with the characterization by HINES et al. (2007). However, the very next sentences in DE SAUSSURE (1875) are: "But there always prevails this difference between the cells formed by the social and those made by the solitary Hymenoptera that the first have a cylindrical inner space, while the second are rather extended masses which are not in regular juxtaposition, so that they seem more like spheres and ellipsoids joined together, than cells constructed side by side on a general plan. In other words, the solitary species never seek to form a comb, although they sometimes form agglomerations of cells. The most part of them do not construct these rough cells one upon another, but disperse them into different positions." Far from considering *Zethus* as representing a critical stage in the evolution of eusocial wasps, de Saussure emphasized fundamental differences.

(8) HINES et al. (2007: 3298): "*Furthermore, stenogastrines use a wider diversity of construction materials (mud, masticated vegetation, or wood fibers) and nest design than polistines and vespines, possibly reflecting a more labile ancestral condition*."

This is incorrect: polistines use all those materials as well, and more (see WENZEL 1991, 1998).

9. Appendix 2: Morphological and behavioral characters

The characters of adult morphology are drawn primarily from published cladistic analyses by the junior author, *viz*. CARPENTER (1981, 1987, 1988, 1989, 1991, 1993, 1996, 1997, 2001, 2003), CARPENTER & CUMMING (1985), WENZEL & CARPENTER (1994), CARPENTER et al. (1996, 2000), ARÉVALO et al. (2004), CARPENTER & PERERA (2006) and CARPENTER & KIMSEY (2009). Most of these studies have treated subsets of the Vespidae, hence many of the characters are newly scored here for other taxa. A number of characters were discussed in the publications, but not scored at the time (see, for example, CARPENTER 1981: 14, where three other characters are described in the first paragraph under forewing plaiting: venational alignment, elongate discal cell and short cu-a); some of these were used in later work (e.g., the discal cell in CARPENTER 1989), but others are scored here for the first time. In the course of these studies, variation in other Vespidae was noted for some of the characters, and in some cases mentioned in passing (see, for example, the discussion of the axillary region in CARPENTER & CUMMING 1985), but not scored until now. A few characters were modified by PICKETT et al. (2006). Others have been modified in the present work to include more states, to accommodate variation in the terminals scored. Another source of characters has been as yet unpublished matrices. Finally, some features were observed during the course of the published work to be potentially informative within Vespidae but not scored until now. Some of these latter were introduced from the study by BROTHERS & CARPENTER (1993), for example the calcar characters. Others came from construction of identification keys (CARPENTER & GARCETE-BARRETT 2003; CARPENTER & NGUYEN 2003; CARPENTER 2004a,b, and unpublished). The remainder are the product of routine taxonomic work. The external characters were examined on pinned specimens of all the species

in the matrix. The single internal character (ovariole number) was extrapolated from the literature.

The characters of larval morphology are taken primarily from KOJIMA (1998), supplemented by other literature sources (see citations in PICKETT et al. 2006, and TORCHIO 1970, EVANS 1987 and KOJIMA 1990).

For behavior, the characters of nest architecture are garnered primarily from WENZEL (1991, 1993, 1998), supplemented by information for solitary Vespidae in taxonomic catalogs and other treatments (VAN DER VECHT & FISCHER 1972; KROMBEIN 1979; CARDALE 1985; GESS 1996; CARPENTER et al. 2006), and citations therein.

Other behavioral characters are derived partly from previously published studies. The few characters used in CARPENTER (1981) have been gradually expanded upon by subsequent authors, beginning with CARPENTER (1987, 1988, 1991), and followed by HUNT (1999) and then accelerated in CARPENTER (2003), ARÉVALO et al. (2004) and PICKETT et al. (2006). Partly this expansion has consisted of addition of new characters, but more of it has been by changing character circumscription. CARPENTER (1981) included one variable for "social behavior" and many more variables are used to encompass similarity in independent aspects of that gross phenomenon in more recent works. We have continued that expansion herein, bringing in variables used by NOLL (2002) and adding many new ones.

Multistate characters are treated as additive where similarity was observed to be nested.

The matrix for the 132 taxa treated here is included in the Electronic Supplement and also available for download at http://www.socialwasps.com.

1. Forewing longitudinal plaiting: absent = 0; present = 1.
2. Forewing vein alignment: absent = 0; M+CuA, CuA, m-cu1 and M aligned in straight bar = 1.
3. Forewing first discal cell: shorter than submedian cell = 0; at least the equal of the submedian cell = 1.
4. Forewing basal cell: apically subtruncate (wide) = 0; apically acute = 1.
5. Forewing cu-a position: distad of fork of M+CuA = 0; at fork = 1; basad = 2. [nonadditive]
6. Forewing cu-a curve: present = 0; straight = 1.
7. Forewing cu-a length: >0.3 length of M = 0; <0.3 length of M = 1.
8. Forewing subdiscal cell: not produced dorsoapically = 0; produced = 1.
9. Forewing submarginal cells: three = 0; two = 1.
10. Basal angle of first submarginal cell: broad = 0; acute = 1.
11. Forewing RS: vertical beneath prestigma = 0; oblique beneath prestigma = 1.
12. Forewing RS length: section below prestigma long = 0; section below prestigma short = 1.
13. Forewing second submarginal cell shape: narrowed above = 0; quadrate = 1.
14. Placement of forewing m-cu2: close to r-m2 = 0; far from r-m2 = 1.
15. Second submarginal cell: basal angle acute, M and Rs angled = 1; basal angle obtuse, M and Rs aligned = 2.
16. Third submarginal cell: apically rounded = 0; apically subtruncate = 1.
17. Forewing r-m3: gently curved to straight = 0; sigmoidal = 1.
18. Forewing recurrent veins: received in second and third submarginal cells = 0; received in second submarginal cell = 1.
19. First recurrent vein: angled sharply into second submarginal cell = 0; running straight into second submarginal cell = 1.
20. Prestigma length: shorter than pterostigma = 0; about equal in length to pterostigma = 1; longer than pterostigma = 2. [additive]
21. Pterostigma: truncate anteriorly = 0; subtruncate, basal angle of marginal cell acute = 1; pointed anteriorly = 2. [additive]
22. Forewing marginal cell: broadly rounded = 0; angled away from wing margin = 1; narrowed = 2; narrowed and pointed onto wing margin = 3. [additive]
23. Forewing marginal cell appendix: present = 0; absent = 1.
24. Marginal cell shape: Rs angled near middle of cell = 0; Rs angled close to pterostigma, then straight = 1.
25. Forewing preaxillary excision: present = 0; absent = 1.
26. Hamuli placement: beginning basad of fork of R1 and RS = 0; beginning at fork = 1.
27. Hindwing cell number: three = 0; two = 1.
28. Hindwing subbasal cell: broadest apically = 0; broadest subapically = 1.
29. Hindwing jugal lobe: long = 0; short = 1; absent = 2. [additive]
30. Hindwing axillary incision: shallow = 0; deep = 1; absent = 2. [additive]
31. Hindwing cu-a: transverse = 0; angled with A = 1; aligned with A = 2. [additive]
32. Hindwing CuA: diverging distad of cu-a = 0; diverging at cu-a = 1; diverging basad of cu-a = 2. [additive]
33. Hindwing A: free abscissa present = 0; absent = 1.
34. Hindwing preaxillary excision: absent = 0; shallow = 1; deep = 2. [additive]
35. Male hindwing margin: posterior margin hyaline = 0; posterior margin with pigmented seam = 1.
36. Eyes: without bristles = 0; with many bristles = 1.
37. Ocellar-eye distance: greater than distance between posterior ocelli = 0; less than distance between posterior ocelli = 1; less than diameter of ocellus = 2. [nonadditive]
38. Ocelli: smaller than distance between them = 0; as large as distance between them = 1.
39. Ocellar triangle: very broadly obtuse = 0; nearly equilateral to elongate = 1.
40. Ocellar-occipital distance: greater than length of ocellar triangle = 0; less than length of ocellar triangle = 1.
41. Vertex tubercles: absent = 0; present = 1.
42. Vertex declivity: distant from ocellar triangle = 0; pronounced behind ocellar triangle = 1.
43. Female cephalic foveae: absent = 0; two present, close = 1.
44. Foveal hairs: few = 0; profuse = 1.
45. Female cephalic depression: absent = 0; present = 1.
46. Antennal swelling: flagellomere 8 (9 in male) less than twice the width of 2 = 0; flagellomere 8 (9) much more than twice the width of 2 = 1; club = 2. [nonadditive]
47. Female antennal articles: 12 = 0; 11 = 1.
48. Male antennal articles: 13 = 0; 12 = 1.
49. Male antennal apex: simple = 0; hooked = 1; apical antennomeres buttonlike = 2; coiled = 3. [nonadditive]
50. Tyloids: present = 0; absent = 1.
51. Female first flagellomere: short, < half length of scape = 0; long, approaching length of scape = 1.

52. Frons: without seam or striae = 0; longitudinal seam present = 1.
53. Interantennal space: broad, rounded = 0; narrow, drawn up carinate = 1; projecting = 2; flat, triangular = 3. [nonadditive]
54. Interantennal distance: clearly more than antennal socket diameter = 0; about antennal socket diameter = 1.
55. Antennal separation: not lateral to posterior ocelli = 0; well lateral to posterior ocelli = 1.
56. Frontoclypeal suture: distinct = 0; indistinct = 1.
57. Antennal sockets: close to clypeus = 0; distant from clypeus by more than two socket diameters = 1.
58. Anterior tentorial pits: in contact with or close to antennal sockets = 0; far below antennal sockets = 1.
59. Clypeus dorsum: straight = 0; bisinuate = 1.
60. Female clypeus apex: broadly rounded = 0; narrowly emarginate = 1; sharply pointed = 2; truncate = 3; truncate depressed medially = 4; broadly emarginate = 5; feebly pointed = 6. [nonadditive]
61. Male clypeus apex: broadly rounded = 0; narrowly emarginate = 1; pointed = 2; truncate = 3; truncate-rounded = 4; broadly emarginate = 5. [nonadditive]
62. Clypeal lateral lobes: absent = 0; present, angular = 1; present, rounded = 2. [nonadditive]
63. Clypeus profile: convex = 0; dorsally flattened = 1.
64. Female clypeal-eye separation: touching = 0; not touching = 1.
65. Labrum: broad, partly concealed by clypeus = 0; narrow, well sclerotized = 1; broad, exposed = 2; very narrow and reduced = 3. [additive]
66. Mandibles decussate: tips overlapping = 0; tips projecting well beyond opposing mandible = 1.
67. Female mandibular teeth: two = 0; three = 1; four = 2; five = 3. [nonadditive]
68. Male mandibular teeth: one = 0; two = 1; three = 2; four = 3. [nonadditive]
69. Mandibular teeth placement: clustered towards tip of mandible = 0; along long axis of mandible = 1.
70. Mandibular teeth: pointed = 0; with elongate cutting edge, < twice length of apical part = 1; with elongate cutting edge, twice length of apical part = 2. [additive]
71. Mandibular interlock: absent = 0; third tooth inflected = 1.
72. Female mandibular flange: absent = 0; present = 1.
73. Mandibular ridges: absent = 0; present = 1.
74. Maxillary palp segments: 6 = 0; 5 = 1; 4 = 2; 3 = 3; 2 = 4. [nonadditive]
75. Maxillary palpomere 2: less than three times or more the length of palpomere 3 = 0; three times or more the length of palpomere 3 = 1.
76. Labial palp segments: 4 = 0; 3 = 1.
77. Labial palp bristles on basal palpomeres: absent = 0; present = 1.
78. Labial palpomere 1: approximately equal in length to segment 2 = 0; approximately equal to the combined length of segments 2–4 = 1.
79. Labial palpomere 3: with strong bristle = 0; without strong bristle = 1; with 2 bristles = 2. [nonadditive]
80. Ligula: as short as prementum, broad = 0; longer, attenuate = 1; longer than head length = 2. [nonadditive]
81. Ligula dorsal imbricate processes: absent = 0; present = 1; present, appressed = 2. [additive]
82. Acroglossal buttons: absent = 0; present = 1.
83. Glossa section basal to bifurcation: not longer than length of apical lobes = 0; longer = 1.
84. Paraglossae: long = 0; short = 1.
85. Anterior lingual plate: short = 0; long and narrow = 1.
86. Posterior lingual plate: narrow, distant from prementum = 0; broad, close to prementum = 1.
87. Posterior lingual plate sclerotization: fully sclerotized = 0; partially desclerotized = 1; fully desclerotized = 2. [additive]
88. Glossal sac: absent = 0; present = 1.
89. Prementum: whole = 0; basally emarginate = 1.
90. Malar space: short = 0; long = 1.
91. Gena width: widest dorsally = 0; widest ventrally = 1.
92. Dorsal occipital carina: complete to mandible = 0; incomplete, running toward mandible = 1; incomplete, running toward hypostoma = 2; complete to hypostoma = 3; absent = 4. [nonadditive]
93. Occipital carina forking behind hypostoma: absent = 0; present = 1; polished line = 2 .
94. Postocular carina: absent = 0; present = 1.
95. Hypostomal apodemes: absent = 0; present = 1.
96. Prothoracic lateral carinae: absent = 0; present = 1; grooved = 2. [additive]
97. Anterior pronotal face: punctation similar to rest of pronotum = 0; largely impunctate = 1.
98. Anterior pronotal carina: absent = 0; present = 1.
99. Dorsal pronotal carina: absent = 0; present = 1.
100. Dorsal pronotal carina length: elongate, running into ventral angle = 0; short, not running into ventral angle = 1.
101. Anterior pronotal foveae: absent = 0; present = 1.
102. Lateral pronotal fovea: absent = 0; present = 1.
103. Lateral pronotal fovea placement: posterior to dorsal carina = 0; anterior to dorsal carina = 1.
104. Humeral angles: absent = 0; carina slightly angular on humeri = 1; carina angled forward on humeri = 2. [nonadditive]
105. Humeral carina: absent = 0; present = 1.
106. Posterolateral angle of pronotum: dorsally produced and exceeding anterior margin of tegula slightly = 0; dorsally produced and forming acute lobe above tegula = 1.
107. Posterolateral margin of pronotum: running nearly vertically above spiracular operculum = 0; running horizontally above spiracular operculum = 1.
108. Pronotal groove: present = 0; absent = 1.
109. Pronotal striae: absent = 0; present in ventral angle = 1.
110. Pretegular carina: present = 0; absent = 1.
111. Furrow in front of pretegular carina, spiracular operculum: absent = 0; present, crenate = 1.
112. Pronotal lobe: close to tegula = 0; separated by several times its length = 1.
113. Secondary spiracular entrance: absent = 0; present = 1.
114. Mesopleural basalar area: with elongate excavation = 0; with shallow, short excavation = 1; flat = 2. [additive]
115. Mespisternum: anteriorly angular, accommodating legs when folded = 0; anteriorly rounded = 1.
116. Mesepisternal crenulae: absent = 0; crenulate behind posteroventral angle of pronotum = 1.
117. Epicnemium: ecarinate = 0; carinate = 1.
118. Dorsal groove: present = 0; absent = 1.
119. Scrobal sulcus: present = 0; absent = 1.
120. Scrobal sulcus crenulae: absent = 0; present = 1.
121. Scrobal sulcus curvature: straight = 0; arcuate dorsally = 1.
122. Mesepimeron: strongly bulging = 0; weakly convex = 1.
123. Mesepisternum: strongly bulging, sloping steeply posteriorly to pleural suture = 0; little bulging, sloping little posteriorly to pleural suture = 1.
124. Tegula shape: longer than broad, narrowed posteriorly = 0; about as broad as long, widest posteriorly = 1; pyriform = 2; 2–3x as long as broad, outer margin con-

cave = 3; convex, longer than broad = 4; short, evenly convex = 5; campanulate = 6; shortened, truncate posteriorly = 7; short, semicircular = 8. [nonadditive]

125. Tegula anterior angle: absent = 0; present = 1.
126. Tegula rim: absent = 0; present = 1.
127. Tegula interior emargination: absent = 0; present = 1.
128. Anterior scutal depression: absent = 0; present = 1; with posterolateral extension = 2. [additive]
129. Median notal suture: absent = 0; present = 1.
130. Notauli: absent = 1; prescutal sutures = 2.
131. Parapsidal furrows: present = 0; incomplete posteriorly = 1; absent = 2. [nonadditive]
132. Mesoscutal lamella: present adjoining tegula = 0; reduced = 1; parategula = 2. [nonadditive]
133. Scuto-scutellar suture: crenate = 0; smooth = 1.
134. Scutellum shape: rounded posteriorly = 0; pointed posteriorly = 1.
135. Scutellum profile in lateral view: largely flat = 0; bulging = 1; angled = 2. [additive]
136. Scutellum: smooth = 0; with impressed or pigmented median line = 1.
137. Axillary lobes: not separate from scutellum = 0; demarcated by sulcus = 1.
138. Axillary surface: horizontal = 0; vertical = 1.
139. Axillary fossa: broad = 0; slitlike = 1.
140. Transcutellar carina: running laterally = 0; running anteriorly = 1.
141. Scutellar crest: flat behind axillary fossa = 0; vertical, carinate behind fossa = 1.
142. Metapleuron: depressed well below level of mesopleuron = 0; at almost same level of mesopleuron in large part = 1.
143. Metapleural basalar area: broadly excavated below second peritreme = 0; narrow carina = 1; broadly raised posterior to second peritreme = 2. [additive]
144. Endophragmal pit placement: well anterior to spiracle = 0; almost below spiracle = 1.
145. Endophragmal pit depression: in small depression = 0; in broad, deep depression = 1.
146. Secondary metapleural sulcus: running into and coincident with pleural suture = 0; not coincident with pleural suture = 1.
147. Metapleural-propodeal suture: distinct posteroventrally of endophragmal pit = 0; indistinct = 1.
148. Metapleural-propodeal suture sculpture dorsal to endophragmal pit: smooth = 0; crenulate = 1; striate = 2. [nonadditive]
149. Metanotum: flat or curving in lateral view = 0; angulate = 1.
150. Metanotal orientation in lateral view: horizontal = 0; partly vertical = 1; largely vertical (dorsal surface reduced) = 2. [additive]
151. Metanotal crenation: absent = 0; present = 1.
152. Metanotal excavation: flat laterally = 0; excavated, crenate laterally = 1; fossa = 2; carinate beside disc of metanotum, crenate lateral to this = 3; carina distant from metanotal disc, sculpture weak = 4. [nonadditive]
153. Metanotal lobe: absent = 0; posteromedial lobe present = 1.
154. Metanotal tubercle: absent = 0; present = 1.
155. Metasternum: depressed anteriorly = 0; entirely depressed = 1.
156. Propodeal length: moderate = 0; shortened = 1.
157. Propodeal spiracle: dorsal = 0; lateral = 1.
158. Propodeal shelf: absent = 0.
159. Propodeal processes: absent = 0; present = 1.
160. Propodeal carinae: ecarinate = 0; posterior face margined by partial carinae = 1.
161. Propodeal concavity: posterior face slightly depressed = 0; deep, narrow furrow = 1; shallow furrow = 2; posterior only = 3; wide and shallow = 4; convex = 5; deep = 6. [nonadditive]
162. Propodeum posterior face: medially flat = 0; with impressed medial line = 1; with medial ribbonlike carina = 2; polished medially = 3. [nonadditive]
163. Propodeal orifice: dorsally broad = 0; dorsally narrowed = 1; dorsally acute = 2. [additive]
164. Cuticular ridge above propodeal orifice: continuous above orifice = 0; absent above orifice = 1.
165. Propodeal valvula: not differentiated = 0; membranous valvula present = 1.
166. Propodeal valvula shape: little projecting = 0; elongate, quadrate = 1; large lobe = 2; attenuate posteriorly = 3. [nonadditive]
167. Submarginal carina: absent = 0; low ridge = 1; produced = 2. [additive]
168. Forecoxa: laterally rounded, little produced = 0; laterally strongly produced = 1.
169. Foretibial calcar: spatulate = 0; slightly curved = 1; slightly curved, tip broad = 2; slightly curved, chitinous expansion = 3. [nonadditive]
170. Forebasitarsus: excavated basally opposite calcar = 0; not excavated = 1.
171. Forebasitarsus length: about as long as other foretarsal segments = 0; longer than any other foretarsal segments = 1.
172. Female foretarsi: symmetrical = 0; segments 2–4 asymmetrical = 1.
173. Female foretarsal brush: absent = 0; present = 1.
174. Female foretarsal hairs: straight = 0; curving, hooked = 1.
175. Midcoxa: laterally rounded, little produced = 0; laterally strongly produced = 1.
176. Mesocoxae: separated = 0; contiguous = 1.
177. Midfemoral basal ring: present = 0; absent = 1.
178. Female midtibial spurs: two = 0; one = 1.
179. Male midtibial spurs: two = 0; one = 1; absent = 2. [additive]
180. Male midtarsi: tarsomeres symmetrical = 0; basal tarsomeres dilated = 1; apical tarsomeres asymmetrical = 2. [nonadditive]
181. Hindcoxa: about as broad as long = 0; longer than broad = 1.
182. Hindcoxa carina: smooth = 0; carinate = 1; carina toothed = 2. [additive]
183. Hindtibia: with long erect hairs (bristles) = 0; without long erect hairs = 1.
184. Hindtibial calcar: absent = 0; short chitinous comb along length of spur = 1; long chitinous comb along most of spur = 2. [additive]
185. Hindtibial inner spur tip: single point = 0; subdivided = 1.
186. Hindtibial inner spur: straight = 0; curved = 1.
187. Claws: simple = 0; toothed = 1; bifid = 2. [nonadditive]
188. Metasomal segment I width and shape: segment I > half the width of segment II = 0; segment I < half the width of segment II, campanulate = 1; segment I < half the width of segment II, flasklike = 2; segment I < half the width of segment II, bulbous apically = 3; segment I < half the width of segment II, elongate apically = 4; segment I < half the width of segment II, parallel-sided = 5; segment I < half the width of segment II, flaring = 6; segment I < half the width of segment II, nodose = 7. [nonadditive]
189. Metasomal Tergum I expansion to maximum width: basal, abrupt = 0; medial, abrupt = 1; very gradual = 2; even curve in dorsal view = 3. [nonadditive]

190. Metasomal Tergum I maximum width placement: posterior = 0; subapical = 1.
191. Metasomal Tergum I declivity: angular in lateral view = 0; little pronounced = 1; even curve = 2. [nonadditive]
192. Metasomal segment I spiracles: at expansion of segment = 0; well anterior to expansion = 1.
193. Metasomal Tergum and Sternum I: unfused = 0; fused = 1.
194. Metasomal Tergum I margins: lateral margins not meeting ventrally = 0; lateral margins closely approximated ventrally = 1.
195. Metasomal Tergum I transverse carina: absent = 0; present = 1.
196. Longitudinal furrow of metasomal Tergum I: absent = 0; present = 1.
197. Metasomal Tergum I lamella: absent = 0; transverse apical thickening with caudal lamella = 1.
198. Metasomal Sternum I sculpture: smooth = 0; anterior ridges, coarse punctation or striae = 1; finely striate = 2; anterior carina = 3. [nonadditive]
199. Metasomal Segment II: sessile = 0; petiolate basally = 1.
200. Metasomal Tergum II: not constricted basally = 0; constricted basally = 1.
201. Tergal thyridium: absent = 0; transverse, basal = 1; elongate = 2. [additive]
202. Metasomal Tergum II apical lamella: absent = 0; present = 1.
203. Metasomal retraction: absent = 0; present = 1.
204. Metasomal Sternum II transverse furrow: present = 0; absent = 1.
205. Metasomal Sternum II basal ridges: absent = 0; present = 1; traces = 2. [nonadditive]
206. Metasomal Sternum II declivity: gradual = 0; truncate = 1.
207. Metasomal Sternum II sulcus: absent = 0; basomedian, longitudinal sulcus present = 1.
208. Sternal II thyridium: absent = 0; elongate = 1; indented = 2. [additive]
209. Male sternal processes: absent = 0; present = 1.
210. Female Sternum VI: rounded = 0; with small apical depression = 1; flat = 2; notched = 3. [nonadditive]
211. Female Sternum VI margins: flat = 0; elongate, curving up = 1.
212. Male Sternum VII: convex = 0; flat = 1; broadly emarginate, laterally carinate = 2; depressed = 3; emarginate = 4. [nonadditive]
213. Basal ring: elongate = 0; short = 1.
214. Parameral spines: absent = 0; present = 1; elongate = 2; dilated = 3. [nonadditive]
215. Parameral spine curvature: absent = 0; strongly recurved = 1.
216. Paramere process: absent = 0; broad inflection = 1; pointed = 2; fingerlike = 3. [nonadditive]
217. Paramere base: not emarginate dorsally = 0; emarginate dorsally = 1.
218. Volsella: cuspis elongate, digitus absent = 0; cuspis truncate, digitus broad = 1; cuspis rounded, digitus acute = 2; fused = 3. [nonadditive]
219. Cuspis: cuspis and lamina not fused = 0; fused, sclerotized = 1; fused, sclerotizaton reduced = 2. [nonadditive]
220. Volsellar apodeme: absent = 0; present = 1.
221. Aedeagus: broad, blunt = 0; narrow, attenuate = 1.
222. Aedeagal apical indentation: deep = 0; shallow = 1; absent = 2. [additive]
223. Van der Vecht's gland: absent = 0; external modified area present = 1; externally absent = 2. [nonadditive]
224. Ovariole number: three per ovary = 0; four or more per ovary = 1.
225. Posterior frame of larval cranium: well developed = 0; weak tentorial bridge thin = 1.
226. Larval cranial shape in frontal view: subcircular or suboval with lateral sides uniformly curved = 0; lateral sides weakly recurved near mandibular bases = 1; widest at or below level of line joining anterior tentorial pits = 2. [nonadditive]
227. Position of larval anterior tentorial pit: at or slightly below level of tentorial bridge = 0; above level of tentorial bridge = 1.
228. Larval hypostomal ridge: nearly straight or weakly and smoothly curved = 0; ventral margin sinuate near mandibular base = 1.
229. Larval cranial setae: short sparse = 0; dense long hairy = 1; rather strong bristles = 2. [nonadditive]
230. Larval head color: hardly pigmented = 0; extensively pigmented = 1.
231. Larval antenna size: small = 0; large = 1.
232. Larval antenna: nearly flat = 0; with elongate papilla = 1.
233. Larval antenna-anterior tentorial pit distance: distinctly more than diameter of antenna = 0; close = 1.
234. Dorsal margin of larval clypeus: well defined by an internal thickening = 0; thickening weak or nearly disappearing = 1.
235. Larval clypeus: mid-point below level of mandibular base = 0; mid-point at or above level of mandibular base = 1; mid-point about at level of mandibular base = 2. [nonadditive]
236. Larval labral width: narrower than maximum width of clypeus = 0; as wide as or only slightly narrower than clypeus = 1.
237. Larval labrum-clypeus junction: labrum narrowed where it joins clypeus = 0; labrum not narrowed where it joins clypeus = 1.
238. Larval labral shape: bilobed ventrally = 0; hardly emarginate ventrally = 1; with lateral projections trilobed = 2. [nonadditive]
239. Larval labral papillae: weak, low and simple cone = 0; nearly absent = 1; strong elongate = 2. [nonadditive]
240. Spicules on larval palate: present nearly over its surface or absent only mediodorsally = 0; present only ventrally and/or laterally = 1; absent = 2. [nonadditive]
241. Shape of spicules on larval palate: pointed apically = 0; scale-like = 1.
242. Spicules on larval mandibular corium: absent = 0; present = 1.
243. Larval mandibular teeth sclerotization: strong, well sclerotized = 0; weak, sclerotized as strongly as in basal area of mandible = 1.
244. Larval mandibles: touching or slightly separate when closed = 0; elongate attenuate crossed when closed = 1; reduced in size, widely separated when closed = 2; reduced in size, with tooth short or nearly disappearing = 3. [nonadditive]
245. Larval mandibular teeth: tridentate = 0; bidentate = 1; unidentate = 2. [additive]
246. Larval teeth arrangement: All teeth distinctly separated in nearly the same plane = 0; one tooth set back from dorsal margin = 1; two upper teeth rudimentary = 2. [nonadditive]
247. Larval mandibular cusps: absent = 0; present = 1.
248. Larval mandibular setae: absent = 0; present = 1.
249. Larval maxilla: compressed hardly swollen basally = 0; strongly basally swollen = 1.
250. Larval maxillary spicules: present on upper surface and/or extending apically = 0; present in basal (or lateral) half = 1.

251. Larval maxillary palpus: thick flat apically = 0; thick not flat apically = 1; slender, elongate = 2. [nonadditive]
252. Larval galea: simple cone with two apical sensilla = 0; complex, usually with more than two = 1; bilobed apically with single sensillum on each lobe = 2; bilobed with two sensilla on one of lobes or trilobed = 3; thick flat apically = 4. [nonadditive]
253. Larval labial palpus: thick flat apically = 0; slender elongate = 1.
254. Setae behind each larval labial palpus: absent = 0; single or two = 1; many = 2. [nonadditive]
255. Spicules on larval postmentum: absent = 0; present ventrally and or laterally = 1; dense on nearly entire surface = 2. [nonadditive]
256. First larval spiracle: as large as or slightly larger than succeeding spiracles = 0; larger (about 1.5×) = 1; distinctly larger (>2.0×) = 2. [additive]
257. Spicules on larval atrial wall: absent = 0; present = 1.
258. Processes at primary tracheal opening in larvae: absent = 0; simple not branching = 1; branching = 2. [additive]
259. Abdominal segment I ventral lobes in larvae: absent = 0; present = 1.
260. Setae on venter of thoracic segment I in larvae: minute or short = 0; long, hairy = 1; thick bristles = 2. [nonadditive]
261. Setae on venter of abdominal segment I in larvae: minute or short = 0; long, hairy = 1; thick bristles = 2. [nonadditive]
262. Spicules on venter of larval thoracic segments II and III: simple pointed apically = 0; simple blunt apically or minutely dentate ridges = 1; absent at least area between leg-bud plates = 2. [nonadditive]
263. Setae on dorsum of thoracic segment I in larvae: minute or short = 0; long = 1.
264. Spicules on dorsum of thoracic segment I in larvae: absent = 0; present = 1.
265. Larval tenth abdominal segment: flat = 0; tuberculate = 1.
266. Pupal scutal prongs: absent = 0; present = 1.
267. Pupal metasomal bending: absent = 0; bent ventrally at junction of I and II = 1.
268. Nest number: multiple = 0; single = 1.
269. Nest construction: closed cell = 0; specialized nest constructed = 1.
270. Nest architecture: burrow in soil = 0; renting pre-existing cavities = 1; separate mud cells = 2; comb = 3. [nonadditive]
271. Comb shape: rectinidal = 0; laterinidal = 1.
272. Comb propagation: expanding gradually = 0; built suddenly or in successive blocks = 1.
273. Free, aerial nests: absent = 0; present = 1; enclosed = 2. [nonadditive]
274. Envelope: none = 0; single sheet from substrate, secretion = 1; single sheet from substrate, paper = 2; nested spheres from pedicel region = 3; single sheet from margin of comb = 4. [nonadditive]
275. Entrance: simple = 0; long downward spout = 1; short peripheral collar = 2. [nonadditive]
276. Envelope shape: flask-shaped = 0; dome-shaped = 1.
277. Envelope expansion: remodeled to allow comb to grow beyond initial periphery = 0; prefabricated restricting comb growth to initial diameter = 1.
278. Envelope closure: most cells laid before envelope closes = 0; envelope closes during cell outlining = 1.
279. Envelope reinforcement: by blots = 0; secretion = 1; imbricate = 2. [nonadditive]
280. Comb pedicel: absent = 0; flattened pedicel of friable paper = 1; rodlike pedicel = 2; felt platform = 3; pulp foundation = 4. [nonadditive]
281. Pedicel placement: cell-marginal = 0; cell-central = 1.
282. Secondary combs: absent = 0; present = 1.
283. Secondary envelopes: absent = 0; present = 1.
284. Suspensoria: absent = 0; present, ribbonlike = 1; present, pillarlike = 2. [nonadditive]
285. Nest material: soil = 0; paper = 1.
286. Brood cells: independent cells = 0; sharing walls = 1.
287. Cell shape: ellipitical = 0; hexagonal = 1.
288. Brood cell construction: performed from start to finish by a single female = 0; various individuals engaged at the same time = 1.
289. Brood cell construction: each cell is followed by oviposition = 0; more than one cell can be built before oviposition = 1.
290. Open brood cells: single = 0; multiple = 1.
291. Cell construction and oviposition: always by the same female = 0; in part in cells built by other females = 1.
292. Queen cells: no special cells constructed for rearing queens = 0; special queen cells constructed = 1.
293. Oviposition: direct = 0; indirect = 1.
294. Oviposition timing: onto prey = 0; into empty cell = 1.
295. Oviposition in the presence of more than one female: more than one female can lay eggs at the same time = 0; only the queens lay eggs = 1.
296. Egg and provision platform: absent = 0; metasomal secretion = 1.
297. Provisions: Coleoptera = 0; Lepidoptera = 1; arthropod generalist = 2; pollen = 3. [nonadditive]
298. Prey number: one = 0; many = 1.
299. Prey: live = 0; also carrion = 1.
300. Prey capture: with sting = 0; with mandibles = 1.
301. Prey site: external = 0; concealed = 1.
302. Malaxation of prey: absent = 0; present = 1.
303. Flight time: diurnal = 0; nocturnal = 1.
304. Timing of provisioning: prior to egg hatch = 0; after egg hatch = 1.
305. Progressive provisioning: absent = 0; present, amount sufficient to last a day or more = 1; present, amount not sufficient to last a day = 2. [additive]
306. Cell provisioning: always by the same female that oviposited in the cell = 0; by the same female that oviposited in the cell in the solitary phase = 1; always by other females than that which oviposited = 2. [additive]
307. Division of labor: solitary = 0; temporary eusociality = 1; permanant sterility = 2. [additive]
308. Morphological differences between castes: no castes = 0; caste differences statistical = 1; castes differ strikingly = 2. [additive]
309. Worker number: "small" (<800 maximum at peak) = 0; "large" (>5,000 maximum at peak) = 1.
310. Nesting cycle: determinate = 0; indeterminate = 1.
311. Number of queens: absent = 0; short-term monogyny = 1; matrifilial monogyny = 2; polygyny = 3. [additive]
312. Colony foundation: solitary = 0; swarm with single queen = 1; swarm with multiple queens = 2. [nonadditive]
313. Extended brood care: absent = 0; present = 1.
314. Nest sharing: absent = 0; present = 1.
315. Nest size: single cell = 0; "small" (<3,500 cells maximum at peak) = 1; "large" (>10,000 cells maximum at peak) = 2. [additive]
316. Overlap of adult generations: absent = 0; present = 1.
317. Cell re-use: absent = 0; present = 1.

318. Meconium extraction: absent = 0; present, through cell entrance = 1; present, through cell back = 2. [nonadditive]
319. Adult-adult trophallaxis: absent = 0; present = 1.
320. Cocoon: complete = 0; incomplete = 1.
321. Cell closure: present = 0; narrowing of cell = 1; absent = 2. [nonadditive]
322. Larval diapause: present = 0; absent = 1.
323. Adult emergence: protandry = 0; protogyny = 1.
324. Larval-adult trophallaxis: absent = 0; licking of secretion = 1; direct = 2. [additive]
325. Antivertebrate venom: absent = 0; present = 1.
326. Nest defense: absent = 0; present = 1.
327. Ant repellent: absent = 0; present = 1.
328. Foraging behavior: all individuals forage = 0; all individuals forage but dominants stay in the nest more = 1; queens never forage = 2. [additive]
329. Time of cell closure: immediately after provisioning = 0; upon pupation = 1.
330. Thermoregulatory behavior: absent = 0; wing fanning = 1.
331. Water collection for construction: absent = 0; present = 1.
332. Unevenly-aged brood: absent = 0; present = 1.
333. Dominance hierarchies: absent = 0; present = 1.

Electronic Supplement Files

at http://www.arthropod-systematics.de/ (“Contents”)

File 1: Pickett_and_Carpenter_2010_morph.ss.
Morphological character matrix.