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Systematics of *Polistes* (Hymenoptera: Vespidae), with a phylogenetic consideration of Hamilton's haplodiploidy hypothesis

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A review of previously published cladistic analyses of *Polistes* is presented. The two most recent analyses of *Polistes* are shown to be largely consistent phylogenetically. Although the taxonomy implied by each differs, this difference is shown to be mostly due to taxon sampling. After the review, a phylogenetic analysis of *Polistes* — the most data-rich yet undertaken — is presented. The analysis includes new data and the data from previously published analyses. The differing conclusions of the previous studies are discussed in light of the new analysis. After discussing the status of subgeneric taxonomy in *Polistes*, the new phylogeny is used to test an important hypothesis regarding the origin of social behavior: the haplodiploidy hypothesis of Hamilton.

Prior phylogenetic analyses within *Polistes*

Cladistic analysis of species-level relationships within *Polistes* was first carried out with a limited, specific objective: to determine relationships among the socially parasitic inquiline species and their host species; that is, to test Emery's Rule. Analyses of allozymes (Carpenter *et al*. 1993) and COI sequence data (Choudhary *et al*. 1994) thus treated nine species, the three inquilines, their hosts, and outgroups. Analysis of these data sources combined with morphology (Carpenter 1997) treated 11 species. The inquilines and their hosts are European, while the outgroups were North American species, so while these studies achieved their goal, with resolutions leading to rejection of Emery's Rule, they had little to say about broader phylogenetic patterns within the genus.

The first large-scale analysis within *Polistes* was Carpenter (1996), who studied the morphology of 144 species and an additional 43 subspecies, thus sampling most of the diversity within the genus (presently 204 species and an additional 99 subspecies). He presented three analyses of 33 morphological characters — first treating the 12 subgenera recognized by Richards (1973, 1978) as groundplan terminals, then subdividing these into subgenera and species groups invariant for informative characters (i.e., as summary terminals). Neither

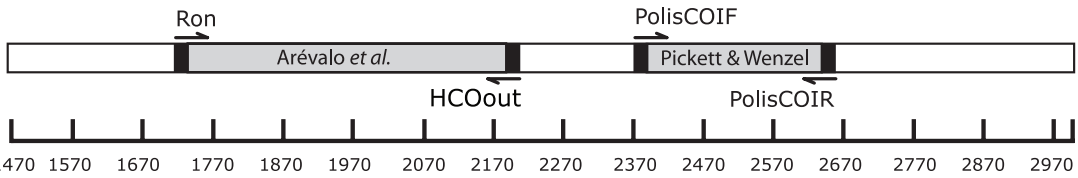

Fig. 1. A map of COI, numbered according to its position within the complete *Drosophila yakuba* mitochondrial genome. The map indicates the primers and regions of COI employed in previous analyses (*see* text).

of these first two analyses completely resolved relationships among these groups, but the second did show that the New World subgenus *Aphanilopterus* was paraphyletic in terms of the subgenera *Epicnemius*, *Fuscopolistes*, *Onerarius*, and *Palisotius* (all from the New World as well). In addition, this analysis found *Epicnemius* to be paraphyletic in terms of *Onerarius*, *Palisotius* and *Fuscopolistes*. The analysis also established all the New World species as a monophyletic lineage. Accordingly, Carpenter (1996) synonymized *Epicnemius*, *Onerarius*, *Palisotius*, and *Fuscopolistes* with *Aphanilopterus* (henceforth *Aphanilopterus*; cf. *Aphanilopterus sensu* Richards, henceforth *Aphanilopterus s. str.*). Thus *Aphanilopterus* in the present sense is a monophyletic subgenus. Carpenter (1996) also synonymized the subgenera *Polistella* with *Stenopolistes*, *Polistes s. str.* with *Sulcopolistes*, and *Gyrostoma* with *Megapolistes*, in each case after demonstrating paraphyly. A third analysis by Carpenter (1996) of the four subgenera resulting after synonymy of both New World and Old World subgenera did not resolve relationships among *Gyrostoma* and *Polistella*, but did show a sister-group relationship between *Polistes s. str.* and *Aphanilopterus*.

The first extensive molecular treatment was by Arévalo *et al*. (2004), which included 33 species of *Polistes*, along with 33 species of other tribes of Polistinae. The molecular data consisted of mitochondrial COI sequences, three nuclear DNA microsatellite flanking sequences, and the three repeat motifs for the microsatellites represented by gap-coding. These data were combined with the adult morphological characters of Carpenter (1996), with characters used by Carpenter *et al*. (2000) in their study of the genus *Polybia*, with larval characters taken from Kojima (1998) supplemented by other literature sources (Reid 1942, Dias Filho 1975, Wheeler & Wheeler 1979, Yamane & Okazawa 1981, Nelson 1982, Kojima & Yamane 1984, Kojima & Keeping 1985, Kojima 1987), and with characters of nest architecture from Wenzel (1993). In the combined analysis, as with Carpenter (1996) the clade for the New World species of *Polistes* was monophyletic, as were each of the Old World subgenera. However, among the Old World taxa *Polistes s. str.* was more basal than *Polistella* and *Gyrostoma* (*Megapolistes*). Moreover, within the New World clade, both *Aphanilopterus s. str.* and *Epicnemius* were monophyletic (however, the species sample was respectively just ten and two species).

Pickett and Wenzel (2004) subsequently analyzed 40 species of *Polistes* (33 of which were *Aphanilopterus* spp.) using slightly modified morphological data and a different, non-overlapping fragment of COI (Fig. 1). As with the preceding studies, the New World species of *Polistes* formed a monophyletic group. The arrangement among the Old World subgenera was different from both of the previous studies, but was based on just five species. In contrast to the study by Arévalo *et al*. (2004), the results of Pickett and Wenzel (2004) agreed with Carpenter's (1996) conclusions that neither *Aphanilopterus s. str.* nor *Epicnemius* are monophyletic. Pickett and Wenzel (2004) also indicated paraphyly of *Fuscopolistes*, which had not been suggested by previous work.

It may seem unexpected that the same locus (COI) might support different topologies. This finding, though, is not surprising when it is realized that the process of evolution can change through time, even within a single locus. This phenomenon of heterotachy (Lopez *et al*. 2002), in which rates of evolution change both across and within sites, is known to occur in many loci, especially those that are protein-coding (e.g. Fitch 1976). COI itself has recently been shown

to exhibit statistically significant heterotachy in lineages of social wasps (Pickett *et al*. 2005). Further, the half of the gene closest to the 5´ end exhibits more heterotachy than the half near the 3´ end, and that heterotachy is exhibited differentially across the lineages (i.e., some branches exhibit heterotachy in part of the gene, but not in others; Pickett *et al*. 2005). With this in mind, the different results obtained by Arévalo *et al*. (2004) and Pickett and Wenzel (2004) are not so unexpected after all.

Even considering issues of heterotachy, however, close examination of the topologies offered by Arévalo *et al*. (2004) and Pickett and Wenzel (2004) reveal less conflict than their differing taxonomic findings suggest. While it is true that Arévalo *et al*. (2004) found two subgenera to be monophyletic while Pickett and Wenzel (2004) did not, this is due mainly to differential taxonomic sampling within *Polistes*. For example, Arévalo *et al*. (2004) report that *Fuscopolistes* is monophyletic, whereas Pickett and Wenzel (2004) found it polyphyletic, in the form of two well-separated clades. One of the clades in Pickett and Wenzel (2004) comprised three taxa (*P. flavus*, *P. poeyi*, and *P. perplexus*) not included in the Arévalo *et al*. (2004) analysis. As such, had these taxa been omitted from Pickett and Wenzel, that study too would have reported the monophyly of *Fuscopolistes*. Also, if the preferred cladogram from Pickett and Wenzel (fig. 4) is pruned to include only those taxa found in Arévalo *et al*. (2004), the two species of *Epicnemius* would also form a monophyletic group, as in Arévalo *et al*. (2004). *Aphanilopterus sensu stricto*, however, is still rendered paraphyletic by representatives of Richards' (1973) subgenera *Onerarius* (*Polistes carnifex*) and *Palisotius* (*Polistes major*); on this last point, Arévalo *et al*. (2004) and Pickett and Wenzel (2004) disagree for reasons other than taxon sampling.

Because there are both taxon sampling and evidentiary differences between the two studies, conducting a new analysis that combines the two fragments of COI for as many taxa from the two studies as possible is the logical next step to resolving the differences. Herein, we perform such a combined analysis. We also include additional molecular data from the same locus (*see* "DNA extraction, amplification, and sequencing" below), adult morphological data as modified from Pickett and Wenzel (2004) and larval data from the literature (partly used by Arévalo *et al*. 2004; *see* below); the Pickett and Wenzel (2004) morphology set was chosen over that in Arévalo *et al*. (2004) because the latter data were compiled so as to represent variability across the Polistinae, whereas the dataset of Pickett and Wenzel (2004) was constructed to capture variability within *Polistes*. After presentation of the simultaneous analysis (Kluge 1989, Nixon and Carpenter 1996), we use the resulting phylogeny to investigate an historically important hypothesis of social evolution.

Materials and methods

In all, we treat 48 taxa (*see* Appendix). These include all *Polistes* species from Arévalo *et al*. (2004) and all taxa, both species and subspecies, from Pickett and Wenzel (2004) save those with substantial gaps in the molecular data (*see* below).

Morphological data

The morphological data all derive from data matrices used in previously published analyses, with adult morphology from Carpenter (1996) and Pickett and Wenzel (2004), with larval morphology from Kojima (1998) supplemented by Dias Filho (1975), Richards (1978), Wheeler and Wheeler (1979), Nelson (1982), Kojima and Yamane (1984), and Kojima (1987). The morphological and behavioral matrix appears in the Appendix. The matrix, containing 48 characters, differs from that of Pickett and Wenzel (2004) in the following ways:

1. The following characters from Pickett and Wenzel (2004) were not included here: Character 2: *jugal lobe*; Character 11, *male mandibular teeth*; and Character 21, *hindtrochanter*. These characters are pertinent to species not part of the present analysis.
2. Character 7, *occipital carina* (Character 12 in Pickett and Wenzel) is treated as additive.
3. Character 10, *dorsal groove* (Character 16 in

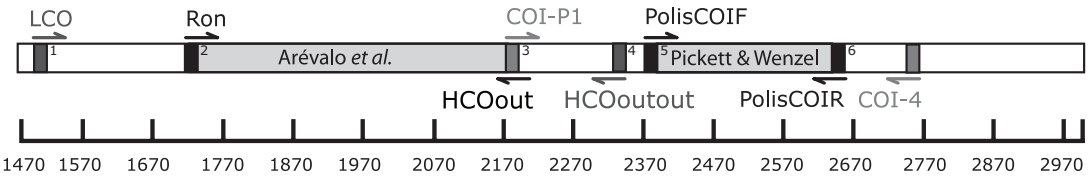

Fig. 2. A map of COI, as in Fig. 1, indicating the position of all primers and fragments used in the present analysis. The six fragments delimited by the eight primers are numbered from left to right.

Pickett and Wenzel) is treated as additive and *P. testaceicolor* is coded with state 1.

4. Character 7, *pronotal carina* (Character 13 in Pickett and Wenzel) is reworded and recoded here.
5. Character 22 of Pickett and Wenzel, *claws*, contained an error: *P. stigma bernardii* was coded as symmetrical. The error is corrected here (asymmetrical: Character 15).
6. Here we add 24 larval characters, included in Arévalo *et al*., with one character (Character 62 in Arévalo *et al*.), *mandibular teeth*, recoded as two states (here, Character 35).

DNA extraction, amplification, and sequencing

Arévalo *et al*. (2004) employed a fragment of COI delimited by the primers CI-J-1729, nicknamed "Ron", (5´-GGAGCTCCTGACATAGCATTCCC-3´), and CI-J-2191, nicknamed "HCOout", (5´-GAAGTTTATATTTTAATTTTACCTGG-3´; *see* Fig. 1). Pickett and Wenzel employed a fragment of COI delimited by the primers CI-J-2371, nicknamed "PolisCOIF" (5´-CGTGCATATTTTACCTCAGCAA-3´) and CI-J-2638, nicknamed "PolisCOIR", (5´-GCAGGATTTATCCATTGATTCC-3´). In order to minimize the undesirable effects of missing data, we attempted to obtain both (1) the Polis-COIF-PolisCOIR region for all the Arévalo *et al*. (2004) *Polistes* spp., and (2) the Ron-HCOout region for all *Polistes* and *Vespula* spp. included in Pickett and Wenzel (2004). These new fragments were added to the COI sequence data from Arévalo *et al*. (2004) and Pickett and Wenzel (2004); we did not include the microsatellite flanking sequences or the repeat motif data from Arévalo *et al*. (2004), the inclusion of which does not alter the results (data not shown). Whenever possible, DNA was extracted from alcohol-preserved specimens or from fresh (flash frozen) specimens. For these types of specimens, flight muscle is the optimal template. When pinned museum specimens were the only available source of template for a taxon, the terminal antennomere was used as the template. For very old specimens, extraction proceeded via a modified CTAB protocol (as discussed in Pickett and Wenzel [2004]). A standard Qiagen kit was used for fresh, EtOH-preserved, and recently-collected (< 5 years) pinned specimens. Most of the older dried specimens were extracted using the CTAB protocol.

Because many of the specimens used were pinned, museum specimens, we were only partially successful. In an effort to fill in these gaps of data, we employed a number of primer pairs that permit the amplification of fragmented DNA. In addition to the primers mentioned above, the following primers were used in various combinations: CI-J-1490, nicknamed "LCO" (5´-GGTCAACAAATCATAAAGATATTGG-3´); CI-J-2172, nicknamed "COI-PI" (5´-TTGATTTTTTGGTCAYCCWGAAGT-3´); CI-J-2329, nicknamed "HCOoutout" (5´-GTAAATATATGRTGDGCTC-3´); and CI-J-2763, nicknamed "COI-4" (5´-CCWVYTARDCCTARRAARTGTTG-3´). The linear arrangement of all primers used is indicated in Fig. 2.

The LCO-HCOoutout fragments were amplified using the following PCR program: Initial denature: 94 °C for 5 min.; denature 94 °C for 15 sec.; annealing: 50–42 °C for 5 sec.; extension: 68 °C for 30 sec.; repeat denature-extension 40 times; final extension: 72 °C for 7 min. The COI-PI to COI-4 fragments were amplified using a different program, optimized for this portion of the gene: Initial denature: 94 °C for 5 min.; denature 94 °C for 15 sec.; annealing: 50–42 °C for 30 sec.; extension: 68 °C for 30 sec.; repeat

denature-extension 40 times; final extension: 72 °C for 7 min.

Sequencing was performed using the dideoxy termination method with dye-labeled terminators using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA polymerase and run on the ABI Prism 377 DNA sequencer and ABI Prism 3700 DNA analyzer (Perkin-Elmer). Complementary strands were combined and edited with the computer program Sequencher 4.2 (Gene Codes Corporation).

Six fragments (numbered in Fig. 2) are delimited by the eight primers. To avoid serious problems due to missing data, we excluded any taxa for which more than 30% of the total molecular data were missing. That meant the removal of the following taxa, all of which appeared exclusively in the analysis by Pickett and Wenzel (2004): *P. actaeon*, *P. bahamensis*, *P. billardieri biglumoides*, *P. comanchus comanchus*, *P. consobrinus*, *P. flavus*, *P. infuscatus ecuadorius*, *P. major castaneicolor*, and *P. minor* (the extracts for these deriving from the oldest of the pinned specimens, all collected from 14 to 50 years ago; *see* Pickett and Wenzel 2004: table 1). Of the remaining 48 taxa sequenced, edited and treated here, 13 taxa lack the first fragment, three taxa lack the second fragment, 15 taxa lack the third fragment, 25 taxa lack the fourth fragment, 12 taxa lack the fifth fragment, and only 19 taxa contain the sixth fragment; some fragments are partially incomplete to primers. In the final matrix, all primer regions not sequenced using other primer pairs were removed.

Phylogenetic analysis

For the morphological data alone (and the analyses of the multiply aligned molecular data; *see* below) the following search strategy was implemented in TNT (Goloboff *et al*. 2003a). For each of 100 replicates, one Wagner tree (Kluge & Farris 1969) was built via random taxon addition sequence and the following search techniques and parameters employed: 40 parsimony ratchet (Nixon 1999) iterations (re-weighting 15% of the characters), 20 rounds of tree drifting, 5 rounds of tree fusing, and sectorial searching (Goloboff 1999). This search strategy was implemented using the following command line: "xmult = replications 100 ratchet 40 drift 20 fuse 5".

Both multiple sequence alignment and Direct Optimization ("Optimization Alignment" of Wheeler [1996]) + Iterative Pass Optimization (Wheeler 2003a) (hereafter DO-IPO) were employed when molecules were analyzed alone, and when they were analyzed simultaneously with the morphology. For multiple sequence alignment, the six orthologous fragments of COI amplified using the same primer pairs (*see* above) were aligned using default parameters in the program MALIGN (Wheeler & Gladstein 1994). These multiply-aligned fragments were then concatenated in their linear order by reference to their known positions in the *Drosophila yakuba* mitochondrial genome (Fig. 2). For DO-IPO, the same six fragments of COI were arranged in the same way, however character-state transformations within the five fragments were not treated as statically aligned. Instead, the fragments themselves were optimized dynamically and assigned during optimal tree search using the program POY (MPI version 3.0.12a-1116878497.04) (Wheeler *et al*. 2004). For all analyses — morphological, molecular, and combined — data were analyzed under the parsimony optimality criterion.

The DO-IPO search proceeded via the following strategy in POY (commands in parentheses follow the operation). The direct optimization (Wheeler 1996) method was implemented via the Implied Alignment approach (-staticapprox) which optimizes the same algorithm, but much more efficiently (Wheeler 2003b). All processes were parallelized (-parallel) via LAM Message Passing Interface across 22 hyperthreaded 2.8 GHz Pentium-class, Myrnet-linked Linux PCs (-np 21). One node was used as the master (by default) and the remaining nodes were set as slaves (-solospawn 20). One hundred parallelized replicates of 20 random addition sequences were implemented (-replicates 100, -multibuild, -buildsperreplicate 20); by default, each of these 2000 Wagner (Kluge & Farris 1969) builds were swapped via TBR, holding up to five trees per replicate (-maxtrees 5). Each build and round of swapping was followed by five parallelized rounds of the parsimony ratchet (Nixon 1999);

trees from the ratchet were also swapped via TBR (-ratchettbr 5). Trees resulting from this search were further refined by submitting them back to POY for a complete round of SPR-based tree fusing (Goloboff 1999; -treefusespr). Trees from that refinement stage were resubmitted to POY and refined further via IPO (Wheeler 2003a; -iterativepass -exact). The implied alignments from the DO-IPO analyses were submitted to TNT for additional swapping to ensure that all optimal trees for that homology scheme were discovered.

Both molecular homology schemes (whether *a priori* [via multiple alignment] or *a posteriori* [via DO-IPO]) resulted in the same topological results. The six fragments are available via Genbank under the following accession numbers (EF136414–EF136461).

Support

Numbers below branches are support values deriving from implementation of 10 000 pseudoreplicates of symmetric resampling, reported as GC scores (Goloboff *et al*. 2003b) in TNT (*see* Figs. 3–5). Traditional resampling measures (i.e., bootstrap and jackknife) are influenced by characters that are uninformative in a parsimony framework (i.e., invariant and autapomorphic characters), whereas symmetric resampling is not (Goloboff *et al*. 2003b).

Results and discussion of phylogenetic analysis

The results of the analysis of morphology alone, COI alone, and the simultaneous analysis of morphology and COI are shown in Figs. 3–5, respectively. After reporting and briefly discussing the results of these analyses of morphology and COI alone, only the simultaneous analysis will be considered further, as it is to be preferred for well established reasons (*see* Kluge 1989, Nixon and Carpenter 1996), not the least of which is that *a priori* exclusion of data not consistent with the investigator's favored views is antithetical to empiricism (*contra* Hunt 2006).

The minimum length of 132 (CI = 0.47, RI = 0.80) was found for the analysis of morphology

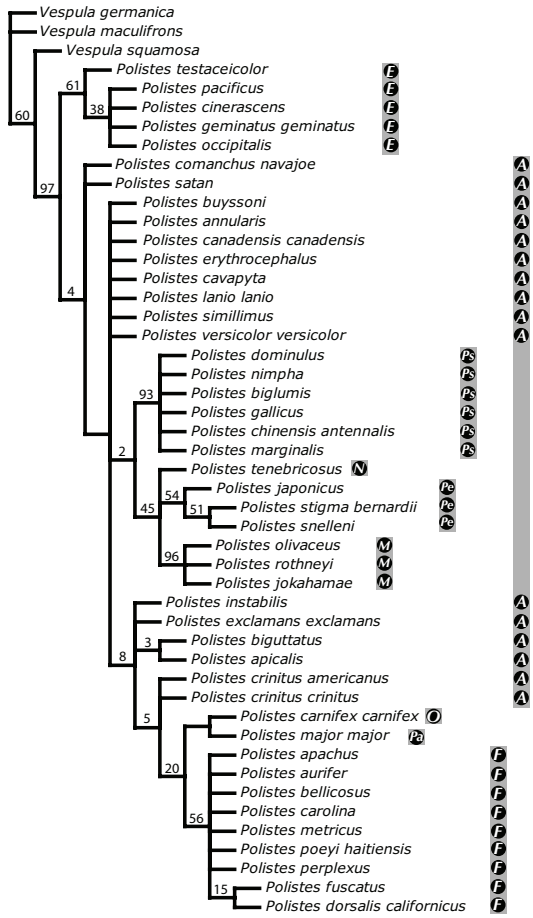

Fig. 3. The strict consensus of 7536 equally parsimonious trees resulting from the analysis of morphology alone. Grey rectangles with black circles indicate membership in Richards' (1973, 1978) subgenera. Key to letters in black circles are as follows: A = *Aphanilopterus s. str.*; E = *Epicnemius*; F = *Fuscopolistes*; M = *Megapolistes*; N = *Nygmopolistes*; O = *Onerarius*; Pa = *Palisotius*; Pe = *Polistella*; Ps = *Polistes sensu stricto*. Numbers above branches are symmetric resampling (Goloboff *et al*. 2003b) values.

alone. Of the 317 816 713 trees examined, 91 equally parsimonious trees resulted. The search process took 15 seconds on a 1.5 GHz G4 PowerPC Macintosh Powerbook. Swapping the trees to completion via the Tree Bisection Reconnection method (TBR; "bbreak = tbr") found a total of 7536 trees. This final swapping procedure took 30 seconds and examined 257 122 528 trees. The strict consensus (L = 145) of all trees is presented in Fig. 3. In the analysis of molecules alone, the tree searches in TNT and POY resulted in four

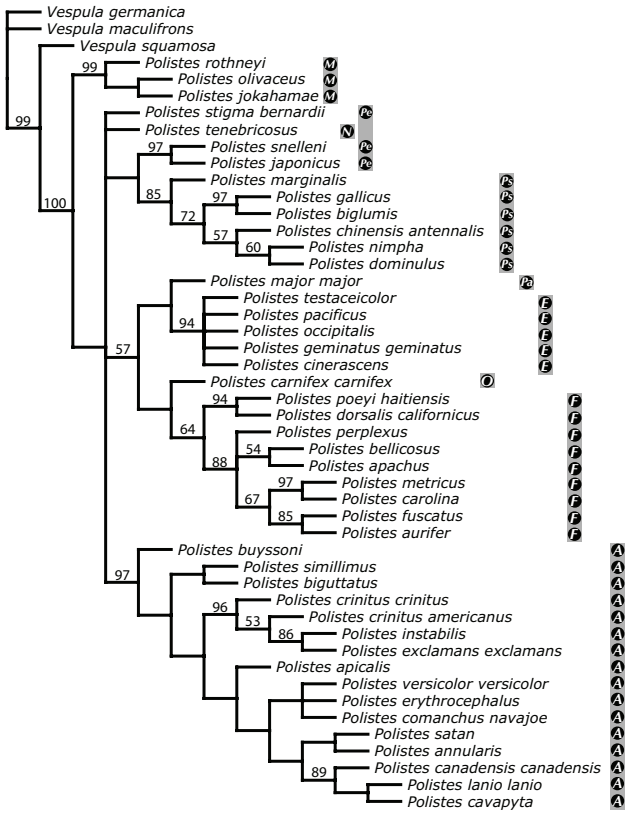

Fig. 4. The strict consensus of four equally parsimonious trees resulting from the analysis of the COI data alone. Grey rectangles with black circles indicate membership in Richards' (1973, 1978) subgenera. Key to letters in black circles are as follows: A = *Aphanilopterus s. str.*; E = *Epicnemius*; F = *Fuscopolistes*; M = *Megapolistes*; N = *Nygmopolistes*; O = *Onerarius*; Pa = *Palisotius*; Pe = *Polistella*; Ps = *Polistes sensu stricto*. Numbers above branches are symmetric resampling (Goloboff *et al.* 2003b) values.

trees (L = 2396. CI = 0.347, RI = 0.507). The strict consensus (L = 2452) of these four trees is presented in Fig. 4. For the simultaneous analysis of morphology and molecules, two trees (L = 2556, CI = 0.349, RI = 0.528) were found; TBR swapping (in TNT) did not find additional trees. The strict consensus of the 2 trees (L = 2558) is shown in Fig. 5.

The results from the analysis of morphology alone (Fig. 3) differ somewhat from those of Carpenter (1996) and Pickett and Wenzel (2004). Although *Polistella*, *Polistes s. str.*, and *Megapolistes* are monophyletic, these Old World groups unexpectedly render the New World *Aphanilopterus s. str*. paraphyletic. As mentioned above, Carpenter (1996) also reported a paraphyletic *Aphanilopterus s. str*., but in that study the paraphyly was due to *Epicnemius* and *Fuscopolistes*, only the latter of which renders *Aphanilopterus s. str*. paraphyletic here. In the analysis of COI alone, no Old World taxa render New World subgenera paraphyletic, but the relationships of the Old World subgenera are not well resolved. *Polistella* is polyphyletic, and *Nygmopolistes* is not sister to *Megapolistes*; both of these results are in conflict with both Carpenter (1996) and Arévalo *et al*. (2004) (but similar to the molecular-only tree of Arévalo *et al*. [2004: fig. 2]). As in the molecular-only tree of Pickett and Wenzel (2004: fig. 3), there is no New World clade, but the relationships among the New World subgenera are much more structured, with clades resolving the sister groups of *Palisotius*, *Epicnemius*, *Onerarius*, and *Fuscopolistes*.

The strict consensus of the analysis of both morphology and COI is the most resolved and most consistent with traditional taxonomy (Fig. 5). Single species represent *Nygmopolistes* (*P. tenebricosus*), *Onerarius* (*P. carnifex carnifex*) and *Palisotius* (*P. major major*), and so the monophyly of these groups is untested; however, all are in positions potentially consistent with their recognition (that is, not rendering other subgenera paraphyletic). Other than these, the monophyly of all of Richards' (1973, 1978) subgenera differs sharply from the situation pre-

Fig. 5. The preferred phylogeny: The strict consensus of two equally parsimonious trees resulting from the simultaneous analysis of COI data and morphology. Grey rectangles with black circles indicate membership in Richards' (1973, 1978) subgenera. Key to letters in black circles are as follows: A = *Aphanilopterus s. str.*; E = *Epicnemius*; F = *Fuscopolistes*; M = *Megapolistes*; N = *Nygmopolistes*; O = *Onerarius*; Pa = *Palisotius*; Pe = *Polistella*; Ps = *Polistes sensu stricto*. Numbers above branches are symmetric resampling (Goloboff *et al.* 2003b) values.

sented in the total evidence phylogeny of Pickett and Wenzel (2004). In Pickett and Wenzel (2004), none of the tested subgenera were monophyletic. The lack of monophyly in Pickett and Wenzel (2004) may well be simply due to the small amount of data analyzed, or it may be because of taxa included in that study that were not included here (*see* above). Analysis of additional taxa and data will be required to settle that question. In Arévalo *et al*. (2004), their tested subgenera were monophyletic, but the relationships of many of the subgenera were unresolved, and other subgeneric relationships are inconsistent with the present findings.

Old World subgenera

As mentioned above, the present phylogeny (Fig. 5) is inconsistent with previous studies regarding the relationships of the Old World subgenera. *Megapolistes* is monophyletic and sister to all other *Polistes*. No previous phylogenetic studies show this relationship. Arévalo *et al*. (2004) found a monophyletic *Megapolistes* (of two taxa), which was sister to *Nygmopolistes*. Pickett and Wenzel (2004) did not show these subgenera as sisters, nor was *Megapolistes* sister to the remaining *Polistes*.

Nygmopolistes in the present analysis is sister to a monophyletic *Polistella*, (*P. stigma bernardii* + (*P. japonicus* + *P. snelleni*)); the relationship

of that clade, and its position as sister to (*Polistes s. str.* + *Aphanilopterus*) are relationships that have not been recovered before in a phylogenetic analysis. Even if *Nygmopolistes* is ultimately not recognized (*see* discussions in Carpenter [1996] and Pickett and Wenzel [2004]), its synonymy with *Megapolistes* is not supported here (as discussed in Pickett and Wenzel [2004]). *Polistes s. str.* is monophyletic, as in Arévalo *et al.* (2004); Pickett and Wenzel (2004) included only one member of this subgenus, *P. dominulus*. However, the current placement of *Polistes s. str.* is inconsistent with the findings of both Pickett and Wenzel (2004) and Arévalo *et al.* (2004), in which the subgenus was found to be sister to (*Megapolistes* + *Aphanilopterus s. str.*) or sister to all other *Polistes*, respectively. The current placement of *Polistes s. str.* is as reported in Carpenter (1996).

New World subgenera

The resolution among the New World subgenera is better than any previous analysis. All tested subgenera are monophyletic. *Onerarius* (*P. carnifex carnifex*) and *Palisotius* (*P. major major*) are sister to a monophyletic *Fuscopolistes* and a monophyletic *Epicnemius*, respectively. This finding is not inconsistent with Arévalo *et al.* (2004), wherein the relationships were not resolved, but is inconsistent with Pickett and Wenzel (2004), who found *Onerarius* and *Palisotius* to render *Aphanilopterus s. str.* paraphyletic. Indeed, in Pickett and Wenzel (2004), *Aphanilopterus s. str.* was also rendered paraphyletic by components of the polyphyletic *Fuscopolistes* and *Epicnemius*. In the present analysis *Aphanilopterus s. str.* itself is supported, as in Arévalo *et al.* (2004).

As noted above, Epicnemius is monophyletic. Arévalo *et al.* (2004) included only two representatives of *Epicnemius* in their analysis (*P. cinerascens* and *P. pacificus*). Those two are shown as sisters here, and were part of one clade in Pickett and Wenzel (2004). However, other taxa included in Pickett and Wenzel (2004) did not form a clade of *Epicnemius*. Two of those taxa are excluded from the present study (*P. actaeon*, *P. billardieri biglumoides*; *see* above). However, the exclusion of these taxa here does not account entirely for the disagreement, as *P. occipitalis* was present both in Pickett and Wenzel (2004) and the present study, and it did not form a clade with other *Epicnemius* in the former, but does here. Carpenter's (1996) finding of paraphyly of *Epicnemius* was based on placement of a species not included here, *P. thoracicus*, and thus the current results cannot test that conclusion.

Similar statements can be made for *Fuscopolistes*. Arévalo *et al.* (2004) found a monophyletic *Fuscopolistes*. However, Pickett and Wenzel (2004) found two clades of the group, and some of those taxa are not included here (i.e., *P. flavus* and *P. poeyi poeyi*). However, *P. perplexus* was included in Pickett and Wenzel (2004) and it did not form a clade with the other *Fuscopolistes*, whereas it does here.

In short, the specific details of the phylogeny continue to change. The findings of subgeneric monophyly in Arévalo *et al.* (2004) are largely supported, though the relationships of the subgenera are either contradicted or much better resolved. Essentially all the findings of Pickett and Wenzel (2004) with respect to subgeneric relationships are contradicted. Such flux is to be expected, at least to some extent, for any phylogeny, especially when the amount of data brought to bear is still small (as is the case here). The addition of more data will surely continue to shape our understanding of the group. Again, although the present phylogeny treats more *Polistes* species than any previous molecular analysis, and is the most data-rich analysis to date, it does not include all of the species treated by Carpenter (1996). Moreover, although we believe this to be the best supported hypothesis to date, we do not consider the support especially robust. We are presently compiling a large phylogenetic dataset including all New World *Polistes*, and we will await the results of that analysis before considering taxonomic recommendations.

Testing Hamilton's haplodiploidy hypothesis

The phylogeny presented in Fig. 6 is the most data-rich phylogeny of *Polistes* presented to date. Certainly, more data will inform us further

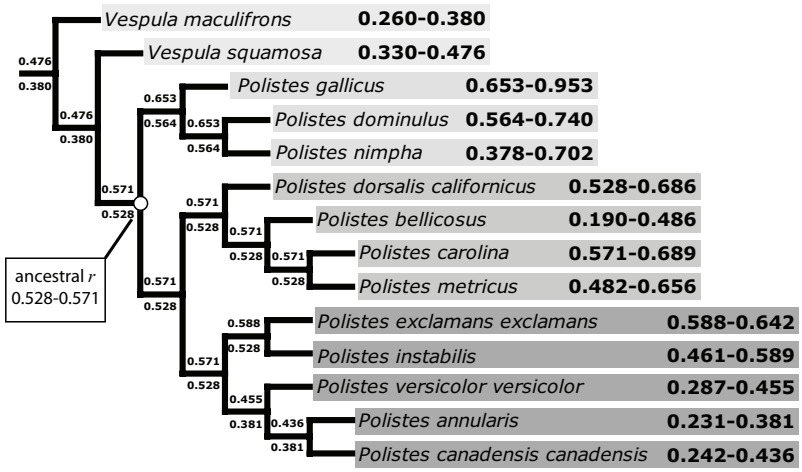

Fig. 6. Complete optimization of the continuous character "worker–worker relatedness". Relatedness data are optimized onto a taxon-reduced version of the strict consensus topology resulting from the simultaneous analysis of all data. Measured ± standard deviations were gathered from the literature (*see* text). The method optimizes ranges in terminal taxa via the same standard additive optimization calculus used to treat ranges in ancestors. Values above and below are up-pass optimizations of the standard error ranges of relatedness provided for the terminals.

of the relationships of these and other *Polistes* spp. As with all scientific questions, new data may alter views based on fewer data. However, perfect phylogenies are not needed to answer certain questions (Wenzel 1997). Certain aspects of this phylogeny are sufficiently resolved and sufficiently consistent with previous work to warrant its use to begin to address some of the many behavioral hypotheses that have been proposed. *Polistes* is both a model organism for understanding social evolution in general, and behaviorally interesting in its own right. As such, we will use the current phylogeny to address an important hypothesis of the evolution of social behavior: the so-called haplodiploidy hypothesis.

Hamilton's hypothesis

In what has become one of the most influential articles in modern biology, Hamilton (1964a) presented a cogent mathematical hypothesis now known as kin selection theory (Maynard Smith 1965). In his classic paper, Hamilton (1964a) described what he called a simple model for the evolution of a gene for altruism. Hamilton's model includes a number of parameters. A shorthand is frequently used to represent Hamilton's ideas, although Hamilton never stated his mathematics in this way: $Br > C$, where B is the benefit (in reproductive success) gained by the recipient of altruism, C is the cost (in reproductive success) incurred by the altruist, and r is the coefficient of relatedness between the recipient of altruism and the particular altruist. In his formulation, Hamilton referred to r as the *diluting effect*, because unless the relatedness is 1.0, as in clonal systems (or if metazoans are viewed as groups of individual cells), the relatedness will always reduce the benefit.

In part II of his paper, in a section entitled "A hypothesis concerning the social tendencies of the Hymenoptera", Hamilton (1964b) presents his now well-known hypothesis that the haplodiploid system of sex determination in Hymenoptera — in which sisters can potentially have a relatedness of 0.75 — can cause the benefit to the recipient of altruism to be *diluted less* than in the more typical diplo-diploid systems. Thus, haplodiploidy can favor the evolution of altruism.

Reasoning by throught experiment certainly can shed light on the plausibility of the haplodiploidy hypothesis. Sometimes, particular examples of high relatedness in colonies of extant taxa are used to infer that the ancestor of *Polistes* may have had similarly high within-colony related-

ness. Other arguments hold that high relatedness in other hymenopterans, or other social animals, suggests that we should conclude that Hamilton's hypothesis is correct. On the other hand, it is also argued that the scheme loses power when it is considered that, *on average*, wasp females are related to their siblings no more than diplo-diploid organisms, or that female-biased sex ratios render preferential investment in females less valuable. All of these arguments and others, many of which have cogent mathematical support, have been offered as inferential tools in the discussion of the applicability of Hamilton's haplodiploidy hypothesis.

Clearly, inference is the only available tool, but previous inferences tend to be heavy on theoretical argument and light on relevant empirical data. Direct inference of the ancestor of *Polistes* can be accomplished, however, using a phylogeny and via optimization of the relevant characters. The supported conclusion regarding the nature of the ancestor is needed to test Hamilton's hypothesis, as it is a hypothesis regarding the origin of sociality. While the potential of *Polistes* to realize this heightened relatedness is not in dispute, whether it in fact occurred in the ancestor of *Polistes* has never been addressed critically. If the ancestor is inferred to have had a within-colony relatedness above what is expected from diplo-diploid organisms, the hypothesis is supported; if, alternatively, the ancestor is shown to have relatedness values near 50%, then the hypothesis is not supported. Here we conduct a test of these two alternatives. With more data — both of the phylogeny and of within-colony relatedness — a more accurate image will take focus. This, however, is the first empirical attempt to answer this question so key to Hamilton's popular idea.

The optimization method

Wagner parsimony (Kluge & Farris 1969, Farris 1970), also known as additive character optimization, was the first quantitative cladistic optimization method, and it is still in wide use today. The method is primarily used to establish differential costs for transformations across character states arranged in linear order (usually according to similarity assessment). For example, if three character states {0,1,2} are observed, and 1 is deemed to be of intermediate similarity, then additive coding can be used to assert this judgment. The method permits the stipulation that transformations from 0 to 1 or from 1 to 2 cost the same (for example, a cost of 1), but the cost of transformation from 0 to 2 is equal to the costs of transforming from 0 to 1 plus the cost of transforming from 1 to 2 (hence, *additive*). This method, though usually employed for discrete morphological traits, permits the treatment of continuous characters in phylogenetic analysis. Additive optimization may be ideal for such characters as it specifically calculates homology across ranges of character states, it avoids entirely the problems of discretizing continuous characters, and it can accommodate the problem of significant differences between ranges (Goloboff *et al*. 2006).

Implementations for continuous characters have existed for some time (*see* Goloboff *et al*. 2006), but these do not permit the use of ranges of data nor optimization onto multifurcating topologies. A new implementation in TNT (Goloboff *et al*. 2003a) accommodates both of these concerns. This implementation allows the user to input ranges as values for terminal taxa and either involve them in the phylogenetic analysis or optimize them *post hoc* on a given tree. The use of ranges requires no change in the method, as the values of hypothetical ancestors are often ranges when characters are treated as additive. The use of ranges also has the advantage of using the data as they arise, as well as avoiding some of the unfortunate outcomes of attempting to discretize continuous data (*see* Farris 1990).

Relatedness data

Because *Polistes* has been so central to testing theories of social evolution, the nestmate relatedness of many species has been measured, primarily for investigations of kin selection theory. Different studies have investigated relatedness of different nestmates (e.g., Strassmann *et al*. 1989, Field *et al*. 1998, Queller *et al*. 2000) in *Polistes*. Hamilton's haplodiploidy hypothesis might be relevant to any of these, but we will focus

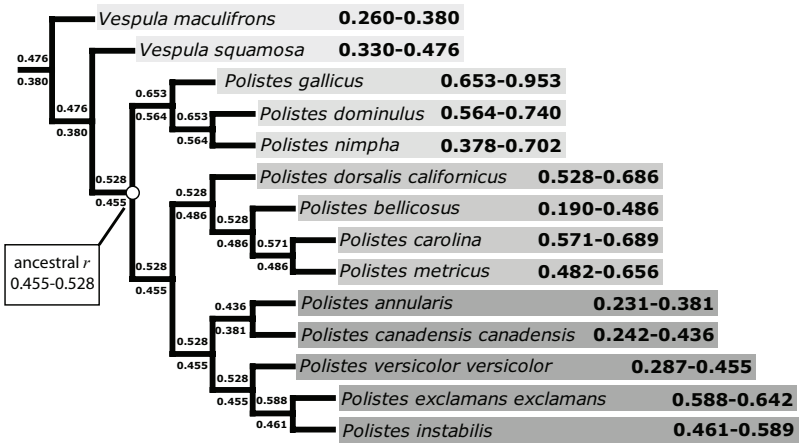

Fig. 7. Complete optimization of the continuous character "worker–worker relatedness". Relatedness data are optimized onto a taxon-reduced version of the summary topology implied by resampling (*see* text). Measured ± standard deviations were gathered from the literature (*see* text). The method optimizes ranges in terminal taxa via the same standard additive optimization calculus used to treat ranges in ancestors. Values above and below are up-pass optimizations of the standard error ranges of relatedness provided for the terminals.

here on worker–worker relatedness. The main reasons for this focus are simply that there are more measurements of sister–sister relatedness in the literature, and the desire to maximize the number of terminals. This procedure, however, could be applied to any relatedness measurements, although we think combination across category (e.g., sister–sister, daughter–sister, and co-foundress) would result in optimizations that are difficult to interpret. Here, we optimize data from Strassmann *et al*. (1989) and Ross (1986). Optimized values are reported standard errors about means. For *P. exclamans*, Strassmann *et al*. (1989) report values for two populations; for consistency, we use the intersection of those ranges here, as this is the method used in the down-pass of additive optimization.

Implementation and results

For the present analysis, relatedness data for only 14 species are treated. As such, the phylogeny from Fig. 5 must be either pruned to these 14, or reanalyzed with only these. Both treatments yield the same topology. This topology, we think, is an uncontroversial, albeit skeletal, representation of the phylogeny of *Polistes*, given the data available. However, there is no resampling support for one of the relationships — that of *P. versicolor* to the the other *Aphanilopterus s. str*. This is because the 50% majority rule consensus trees deriving from resampling support (whether symmetric or the more traditional [trees not shown]) resolve *P. versicolor* not as forming a clade with *P. annularis* and *P. canadensis canadensis* (as in the total evidence tree; *see* Fig. 5), but forming a clade with *P. instabilis* and *P. exclamans exclamans*. Although we consider the tree deriving from the simultaneous analysis of all the data the optimal tree, and do not consider 50% majority rule summaries (no matter what the source of the underlying trees) optimal solutions, given the lack of resampling support in the optimal tree, it may be worth examining the relatedness consequences of this alternate topology before making any strong pronouncements about the haplodiploidy hypothesis. As such, we present both optimizations here (Figs. 6 and 7). In both topologies, the Old World species are separated from the New World taxa, which form a clade, and all three traditional subgenera included (*Polistes s. str.*, *Fuscopolistes*, and *Aphanilopterus s. str.*) are monophyletic as the available data suggest the monophyly of these groups. The ancestral character-range optimizations were calculated in TNT on the skeletal *Polistes* topologies.

Although many of the terminal taxa treated have relatedness ranges far above $r = 0.5$ in both treatments (Figs. 6 and 7), confirming that hap-

lodiploidy can elevate within-colony relatedness, the optimization of these ranges on the inferred ancestor of *Polistes* does not show this elevated relatedness. The optimized range for the hypothetical ancestor of *Polistes* is 0.528–0.0571 in the reduced optimal topology (*see* Fig. 6), or 0.455–0.528 for the tree implied by resampling the matrix, representing the possible rearrangement of *P. versicolor* (*see* Fig. 7). In the former (Fig. 6), the range is slightly elevated, but not especially consistent with the expectations of elevated relatedness given by the haplodiploidy system. In the latter (Fig. 7), the range is well within the bounds of what could be expected from the common diplo-diploid system, showing no support for an elevated relatedness due to haplodiploidy. As such, the notion that haplodiploidy may have played an important role in the early evolution of *Polistes* is either weakly supported or unsupported. Of course, sociality did not evolve in the ancestor of *Polistes*, but earlier. However, the ancestral values for *Polistes* + *Vespula* (which, would correspond to the ancestor of Polistinae + Vespinae) shows an even lower relatedness range. As sociality in the Vespidae evolved in the ancestor of the Stenogastrinae + (Polistinae + Vespinae), more data are required to consider if haplodiploidy played a role in the initial evolution of sociality in the Vespidae. This first test, however, does not support the theory for *Polistes*, a model organism for its investigation.

Conclusions

Our knowledge of the detailed phylogeny of *Polistes* is still rudimentary. To date, no molecular analysis has included even a quarter of the species as terminals. In general, morphological variation is insufficient at the species level to resolve relationships, and so far, only relatively small fragments of molecular data have been combined with morphology. Both the inclusion of more data and more taxa will continue to improve our estimate of the phylogeny. Until anything resembling phylogenetic stability has been reached, we recommend (1) the continued recognition of but one New World subgenus, *Aphanilopterus*; and (2) the continued recognition of the Old World subgenera as suggested by Carpenter (1996).

Though rudimentary, the phylogeny of *Polistes* is sufficient to begin testing hypotheses of social behavior. We have begun this with the first phylogenetic test of Hamilton's haplodiploidy hypothesis. The results suggest marginal to no support of the idea that haplodiploidy played an important role in the early social evolution in the genus.

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Appendix. Morphological character matrix employed and description of states. The following symbols represent specific subset polymorphisms: * = {0,1}; $ = {1.2}; & = {1,3}; # = {1,2,3}. Question marks indicate unknown, and are treated as a complete subset polymorphisms.

	0 5 10 15 20 25 30 35
Vespula germanica	00000000002200000000000000000101000040101110100
Vespula maculifrons	00000000002200000000000?0000010?0000401??110000
Vespula squamosa	00000010002200000000000??????????????????????
Polistes annularis	00100012000202203020000011011101?1111#12?1?001?1
Polistes apachus	00101012000302302020101010011101?1*11&12???011?1
Polistes apicalis	00100012000302303000000??????????????????????
Polistes aurifer	00101012000302302020101010011101?1110?02???011?1
Polistes bellicosus	00101012000302302010101010011101?1110?02???011?1
Polistes biglumis	01100012010111102120000010011101111101020?00100
Polistes biguttatus	00100012000302303010000??????????????????????
Polistes buyssoni	00100012000202203020000??????????????????????
Polistes canadensis canadensis	00100012000202203020000011011101111110&02?0*01101
Polistes carnifex carnifex	20101112000302202020010011111101111111020?00111
Polistes carolina	00101012000302302010101010011101?1111&02????0???1
Polistes cavapyta	00100012000202203020000??????????????????????
Polistes chinensis antennalis	01100012010111102120000010111101111111&02?0?001*0
Polistes cinerascens	001000*20123020020000000111111011111110201100101
Polistes comanchus navajoe	00100012000202203000000??????????????????????
Polistes crinitus americanus	00100012000302302020000??????????????????????
Polistes crinitus crinitus	00100012000302302020000010011101111111102?0?0*1*1
Polistes dominula	01100012010111102120000010011101111110102?0?00100
Polistes dorsalis californicus	00101012000302302010101011011101?1111102???001?1
Polistes erythrocephalus	00100012000202203020000?0011101111111?0????00001
Polistes exclamans exclamans	00100012000302303020000010011101111110&02?0?001?*
Polistes fuscatus	00101012000302302010101011011101?1111?02???011?1
Polistes gallicus	01100012010111102120000010011101111101020?00100
Polistes geminatus geminatus	00100012012302002000000??????????????????????
Polistes instabilis	00100012000302303020000010011101?1110002???010?1
Polistes japonicus	10110011102001102020000010011101110101120?0?00121
Polistes jokahamae	20100011010001102021001210011101110100020010011
Polistes lanio lanio	00100012000202203020000011011101111111?2?0?01101
Polistes major major	001010120*03023020200000110111012111302?0?011?1
Polistes marginalis	01100012010111102120000??????????????????????
Polistes metricus	00101012000302302020101011011101111110&02?1?01101
Polistes nimpha	01100012010111102120000??????????????????????
Polistes occipitalis	00100012012302002000000??????????????????????
Polistes olivaceus	20100001010001102021001210011101210 10**2?0000021
Polistes pacificus	001000*20123020020000000111111011111302?0?00111
Polistes perplexus	00101012000302302020101 0?0111101?1111102???011?1
Polistes poeyi haitiensis	00101012002302302020101 0?0011101111111102???00101
Polistes rothneyi	20100001010001102021001210011101110101020010011
Polistes satan	00100012000202203000000??????????????????????
Polistes simillimus	00100012000202303020000010011101?1111?02???000?1
Polistes snelleni	00110011102001112020000011011101110110021?1?01111
Polistes stigma bernardii	00110011102001112000000010011101210103021000 01?1
Polistes tenebricosus	10101001000001102020000010011101110 10$0200000111
Polistes testaceicolor	00100002011302003000000??111???1101???????0???1
Polistes versicolor versicolor	00100012000202303020000010011101111111&02?0?0*1*1

Character list

0. Prestigma: no longer than half length of the pterostigma, along ventral part = 0; more than half the length of the pterostigma = 1; about equal to the length of the pterostigma = 2. [additive]
1. Male antennae: tapering apically = 0; hooked = 1.
2. Clypeal apex: truncate = 0; pointed = 1.
3. Clypeal dorsum: straight = 0; produced above tentorial pits = 1.
4. Male eyes: contacting clypeus = 0; separated = 1.
5. Malar space: shorter than wide = 0; longer = 1.
6. Occipital carina: complete to mandibular bases = 0; evanescent toward mandibular bases = 1.
7. Pronotal carina: absent = 0; present, lamellate into ventral angle = 1; shortened, reaching pronotal fovea = 2. [additive]
8. Pronotal fovea: present = 0; absent = 1.
9. Epicnemial carina: absent = 0; present = 1.
10. Dorsal Groove: present = 0; anterior portion present, not extending to scrobal sulcus = 1; absent = 2. [additive]
11. Mesepisternal punctation: coarse = 0; fine = 1; fine and well separated = 2; reduced = 3. [additive]
12. Punctation clathrate: absent = 0; present = 1.
13. Propodeal orifice: dorsally rounded = 0; dorsally acute = 1; acute and elongate = 2. [additive]
14. Propodeal striae: absent = 0; present = 1; fine = 2; laterally evanescent = 3. [additive]
15. Claws: symmetrical = 0; asymmetrical = 1.
16. Metasomal Segment I: transversely truncate = 0; petiolate = 1; conical, as wide or wider than long = 2; conical, longer than wide = 3. [nonadditive]
17. Metasomal Sternum I: ecarinate = 0; transversely carinate = 1.
18. Metasomal Sternum I Striae: astriate = 0; transversely striate near neck, extending weakly posteriorly = 1; transversely striate across entire expanded surface = 2. [additive]
19. Lateral process of male metasomal Sternum VII: absent = 0; present = 1.
20. Disc of male metasomal Sternum VII: medially slightly depressed = 0; tuberculate = 1.
21. Base of male metasomal Sternum VII: without anterior lobes = 0; lobed = 1.
22. Ventral margin of digitus: ventrally curved = 0; widened = 1.
23. Apex of digitus: narrow = 0; membranous = 1; membranous and saccate = 2. [additive]
24. Posterior frame of cranium: Posterior thickening of cranium well developed and tentorial = 0; Posterior thickening of cranium weak tentoria bridge thin and = 1.
25. Cranial shape frontal view: Subcircular or suboval with lateral sides uniformly curved = 0; Widest at or below level of line joining anterior tentorial pits = 1.
26. Cranial setae: Short sparse = 0; Dense long hairy = 1.
27. Head color: Hardly pigmented = 0; Extensively pigmented = 1.
28. Clypeus: Mid-point below level of mandibular base = 0; Mid-point at or above level of mandibular base = 1.
29. Labral width: Narrower than maximum width of clypeus = 0; As wide as or only slightly narrower than clypeus = 1.
30. Labrum except dorsal membraneous area: Narrowed where it joins clypeus = 0; Not narrowed where it joins clypeus = 1.
31. Labral shape: Bilobed ventrally = 0; Hardly emarginate ventrally = 1.
32. Spicules on palate: Present only ventrally and/or laterally = 1; Absent = 2.
33. Mandibular teeth: Strong well sclerotized = 0; Weak sclerotized as strongly as in basal area of mandible = 1.
34. Mandibular teeth: Three = 0; Two nearly equal size = 1; Two one shorter or rudimentary = 2. [additive]

35. Maxilla: Compressed hardly swollen basally = 0; Strongly basally swollen = 1.
36. Maxillary palpus: Thick flat apically = 0; Thick not flat apically = 1.
37. Galea: Simple cone with two apical sensilla = 0; Complex, with more than two sensilla = 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]
38. Prementum: Circular or subcircular = 0; Rounded quadrate = 1.
39. Setae behind each labial palpus: Single or two = 1; Many = 2.
40. Postmentum: Small = 0; Large = 1.
41. Spicules on postmentum: Absent = 0; Present ventrally and/or laterally absent in area ventral to prementum = 1.
42. Spicules on atrial wall: Absent = 0; Present = 1.
43. Processes at primary tracheal opening: Absent = 0; Simple not branching = 1.
44. Setae on venter of thoracic segment I: Minute or short = 0; Long hairy = 1.
45. Setae on venter of abdominal segment I: Minute or short = 0; Long hairy = 1.
46. Spicules on venter of 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]
47. Larval 10: flat = 0; tuberculate = 1.