Cladistics 31 (2015) 535–549

Cladistics

10.1111/cla.12103

Phylogeny and historical biogeography of the paper wasp genus *Polistes* (Hymenoptera: Vespidae): implications for the overwintering hypothesis of social evolution

Bernardo F. Santos[a,b], Ansel Payne[a,b], Kurt M. Pickett[†] and James M. Carpenter[a,]*

[a]*Division of Invertebrate Zoology, American Museum of Natural History, Central Park West at 79th street, New York, NY 10024-5192, USA;* [b]*Richard Gilder Graduate School, American Museum of Natural History, New York, NY, USA*

Accepted 26 October 2014

Abstract

The phylogeny of the paper wasp genus *Polistes* is investigated using morphological and behavioural characters, as well as molecular data from six genes (*COI*, *12S*, *16S*, *28S*, *H3*, and *EF1-α*). The results are used to investigate the following evolutionary hypotheses about the genus: (i) that *Polistes* first evolved in Southeast Asia, (ii) that dispersal to the New World occurred only once, and (iii) that long-term monogyny evolved as an adaptation to overwintering in a temperate climate. Optimization of distribution records on the recovered tree does not allow unambiguous reconstruction of the ancestral area of *Polistes*. While the results indicate that *Polistes* dispersed into the New World from Asia, South America is recovered as the ancestral area for all New World *Polistes*: Nearctic species groups evolved multiple times from this South American stock. The final tree topology suggests strongly that the genus first arose in a tropical environment, refuting the idea of monogyny as an overwintering adaptation.

The genus *Polistes* is one of the most species-rich, familiar and widespread groups of social wasps. The genus currently comprises over 200 species, and is found on all six habitable continents (Carpenter, 1996a). The group has also served as a model organism in both experimental and theoretical studies of the evolution and maintenance of social behaviour (Reeve, 1991; Pickett and Wenzel, 2004). In many ways, *Polistes* is ideally suited to such a role, given (i) the abundance and conspicuousness of species' nests, (ii) their efficient exploitation of anthropogenic habitats, (iii) the relatively small size of their colonies, allowing detailed observation and manipulation (< 100 individuals; Pardi, 1996), and (iv) their relative ease of collection (few homeowners object to the wholesale removal of paper wasp colonies from their properties).

Given this interest and the wealth of data available on the group's behaviour, it is somewhat disappointing that the *Polistes* phylogeny remains poorly understood. The lack of a phylogenetic framework obviously makes it difficult to place distributional and behavioural observations within their proper evolutionary context. For example, while there have been numerous speculations about the centre of origin for *Polistes*, none of these ideas has been rigorously tested against a phylogenetic hypothesis. The general notion is that *Polistes* first evolved in the Oriental tropics (Wheeler, 1922; Yoshikawa, 1962; van der Vecht, 1965) from whence it dispersed to the rest of the globe, with New World invasion occurring via Beringia (van der Vecht, 1965; Richards, 1971; Reeve, 1991).

These shortcomings also hinder the evaluation of popular hypotheses regarding behavioural evolution

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*Corresponding author:
E-mail address: polistes@verizon.net

[†]Deceased, formerly Department of Biology, University of Vermont, Burlington, VT 05405, USA.

Table 1
Summary of the dataset used in the present work, with the number of unaligned base pairs for each genetic loci and the number of morphological and behavioral characters (*M&B*) for each species

Taxon	12S	16S	28S	COI	EF1-α	H3	M&B	Total
Agelaia pallipes	347	518	1048	1078	0	328	48	3367
Apoica thoracica	349	513	509	1078	0	0	48	2497
Metapolybia cingulata	350	345	1036	1072	1028	0	48	3879
Mischocyttarus carinulatus	344	513	1018	1078	0	328	24	3305
Polistes actaeon	350	510	544	1002	0	0	24	2430
Polistes annularis	354	517	994	1078	1052	328	45	4368
Polistes apachus	0	0	994	1078	0	0	44	2116
Polistes apicalis	358	516	994	1078	588	328	24	3886
Polistes arizonensis	0	0	0	658	0	0	24	682
Polistes aurifer	354	513	544	1065	0	328	43	2847
Polistes bahamensis	0	0	0	658	0	0	24	682
Polistes bellicosus	354	514	994	1234	0	328	43	3467
Polistes bicolor	342	515	996	1078	1101	328	24	4384
Polistes biglumis	0	0	0	439	0	0	46	485
Polistes biguttatus	0	0	531	1081		328	24	1964
Polistes buyssoni	350	513	994	1234	1101	328	24	4544
Polistes canadensis	0	0	531	997	0	0	47	1575
Polistes carnifex	0	0	0	997	0	0	46	1043
Polistes carolina	354	513	993	1234	1101	328	42	4565
Polistes cavapyta	354	517	994	1069	1100	328	24	4386
Polistes chinensis antennalis	0	808	996	1082	0	328	47	3261
Polistes cinerascens	352	515	546	1078	423	329	48	3291
Polistes comanchus navajoe	353	515	996	1234	882	329	24	4333
Polistes crinitus	350	514	998	1078	1101	0	46	4087
Polistes dominula	354	524	980	1234	1101	328	47	4568
Polistes dorsalis californicus	352	512	994	1234	1101	328	44	4565
Polistes erythrocephalus	0	0	0	421	0	0	45	466
Polistes exclamans	351	352	1005	1078	877	328	46	4037
Polistes flavus	0	303	0	0	0	0	42	345
Polistes formosanus	0	766	0	605	0	0	24	1395
Polistes fuscatus	354	513	994	1234	872	328	43	4338
Polistes gallicus	354	524	544	1078	491	0	47	3038
Polistes geminatus	339	514	1000	1078	876	328	24	4159
Polistes goeldii	355	517	994	1078	1101	328	39	4412
Polistes humilis	0	808	421	658	0	0	46	1933
Polistes instabilis	0	0	0	439	0	0	44	483
Polistes japonicus	353	798	994	1234	333	328	47	4087
Polistes jokahamae	0	784	994	605	0	0	48	2431
Polistes lanio lanio	346	517	994	437	1101	328	46	3769
Polistes major major	0	517	994	658	852	328	46	3395
Polistes marginalis	343	522	992	1234	816	0	24	3931
Polistes melanotus	354	514	531	815	0	328	24	2566
Polistes metricus	354	791	994	1234	1101	328	48	4850
Polistes myersi myersi	366						24	390
Polistes nimpha	347	518	996	812	1101	328	24	4126
Polistes nipponensis	0	790	0	605	0	0	48	1443
Polistes occipitalis	347	509	996	1078	0	0	24	2954
Polistes olivaceus	0	0	0	1123	0	0	48	1171
Polistes pacificus	352	510	994	1123	0	328	47	3354
Polistes poeyi haitiensis	356	435	775	815	0	0	46	2427
Polistes riparius	0	811	0	605	0	0	47	1463
Polistes rothneyi	0	812	0	605	0	0	48	1465
Polistes rubiginosus	354	414	994	1234	1101	328	44	4469
Polistes sagittarius	351	298	551	1077	1101	328	24	3730
Polistes satan	0	0	531	245	0	0	24	800
Polistes simillimus	355	516	995	1078	0	0	43	2987
Polistes snelleni	358	786	994	815	0	328	47	3328
Polistes stigma bernardii	350	0	811	1078	333	328	47	2947
Polistes tenebricosus	353	514	993	1078	0	0	48	2986
Polistes testaceicolor	344	511	992	1123	333	328	36	3667
Polistes variabilis	0	801	0	605	0	0	47	1453

Table 1
(Continued)

Taxon	12S	16S	28S	COI	EF1-α	H3	M&B	Total
Polistes versicolor	0	352	0	658	0	0	47	1057
Pseudopolybia compressa	341	440	966	812	0	329	24	2912
Vespula germanica	347	434	646	1078	0	0	48	2553
Vespula maculifrons	347	510	646	1078	0	328	48	2957
Vespula squamosa	350	508	993	1078	0	328	24	3281

Table 2
Values of K used for each partition in the phylogenetic analysis

Partition	Value of K
Morphology and behavior	21.962891
12S	7.500000
16S	8.906250
28S	5.781250
COI	11.674804
EF1-α	1.992187
H3	4.765625

within the group. It has been suggested, for example, that long-term monogyny in *Polistes*, i.e. the existence of a single egg-laying female per colony, living long enough to overlap adult generations, appeared as an adaptation to the demands of overwintering (West-Eberhard, 1978; Itô, 1986). According to that idea, large, well-nourished queens capable of surviving a long winter set up the conditions necessary for the maintenance of monogyny over multiple generations. Colonies of *Polistes* can be founded by one or by a group of females. In the latter case, one of the females eventually becomes dominant (queen) through differential oophagy and physical aggression, while the remaining females become subordinates (workers) or leave the colony (Pardi, 1948; West-Eberhard, 1967). Although there is evidence for multiple egg-layers on occasion (e.g. Nagamati Junior et al., 2010), all species of *Polistes* can be considered at least predominantly monogynous. The obvious assumption made by the overwintering hypothesis is that the ancestral *Polistes* evolved within a temperate habitat, a conclusion at odds with the idea of a tropical Oriental centre of origin.

Previous work on the phylogeny of *Polistes* has dealt either with taxonomic issues regarding subgeneric classification and species delimitations (Carpenter, 1996b; Pickett and Wenzel, 2004; Pickett et al., 2006), with the relationships among socially parasitic inquiline species and their hosts (Carpenter et al., 1993; Choudhary et al., 1994; Carpenter, 1997), or else in the context of a larger assessment of Vespidae as a whole (Pickett and Carpenter, 2010). Here we provide the most extensive investigation yet of phylogenetic relationships within *Polistes*, and use the results to test three evolutionary hypotheses relevant to the biogeography and social evolution of this important model genus: (i) that *Polistes* first evolved in Southeast Asia, (ii) that dispersal to the New World occurred only once, and (iii) that permanent monogyny evolved as an adaptation to overwintering in a temperate climate.

Material and methods

Dataset

A total of 58 species of *Polistes* were included in the analyses, as well as eight outgroup species. The outgroup includes three species of *Vespula* (Vespinae), plus representatives of five other genera of Polistinae. The character matrix (see Table 1) includes morphological and behavioural data presented by Pickett et al. (2006), augmented with 23 newly scored species, and sequences for three mitochondrial and three nuclear loci: *Cytochrome Oxidase I* (*COI*), *12S rRNA* (*12S*), *16S rRNA* (*16S*), *28S rRNA* (*28S*), *Histone 3* (*H3*), and the F2 copy of *Elongation factor I alpha* (*EF1-α*), respectively. Most of the data for *COI*, *12S*, *16S,* and *28S* are from Pickett and Carpenter (2010), with a few additional sequences obtained from other projects on GenBank (accession numbers in Supplementary File 1). The sequences for *H3* (328 bp) and *EF1-α* (~1101 bp, but see below) are all original. Primer sequences for the *EF1-α* fragment were taken from Danforth et al. (1999); primers for the H3 fragments were adopted from Colgan et al. (1998).

Sequences obtained from GenBank, as well as those for *EF1-α*, were variable in length; in the latter case, this reflects the heterogeneous degree of amplification success using different sets of primers. The introns for *EF1-α* were removed based on the reference translations of the gene available for *Polistes dominula* and *Metapolybia cingulata*. Removal of introns was done in light of the misleading effects that such sequences can have in phylogenetic analyses (e.g. Payne, 2014; see also Lecointre and Deleporte, 2005).

Table 3
Distributional data for the species of *Polistes* and outgroup species used in this work

Taxon	NW	Afr	WeP	EaA	SEA	Aus
Agelaia pallipes	X					
Apoica thoracica	X					
Metapolybia cingulata	X					
Mischocyttarus carinulatus	X					
Polistes actaeon	X					
Polistes annularis	X					
Polistes apaches	X					
Polistes apicalis	X					
Polistes arizonensis	X					
Polistes aurifer	X					
Polistes bahamensis	X					
Polistes bellicosus	X					
Polistes bicolor	X					
Polistes biglumis			X			
Polistes biguttatus	X					
Polistes buyssoni	X					
Polistes canadensis	X					
Polistes carnifex	X					
Polistes carolina	X					
Polistes cavapyta	X					
Polistes chinensis antennalis				X		
Polistes cinerascens	X					
Polistes comanchus navajoe	X					
Polistes crinitus	X					
Polistes dominula			X	X		
Polistes dorsalis californicus	X					
Polistes erythrocephalus	X					
Polistes exclamans	X					
Polistes flavus	X					
Polistes formosanus					X	
Polistes fuscatus	X					
Polistes gallicus			X	X		
Polistes geminatus geminatus	X					
Polistes goeldii	X					
Polistes humilis						X
Polistes instabilis	X					
Polistes japonicus				X	X	
Polistes jokahamae				X	X	
Polistes lanio lanio	X					
Polistes major major	X					
Polistes marginalis		X				
Polistes melanotus	X					
Polistes metricus	X					
Polistes nimpha			X	X		
Polistes nipponensis				X		
Polistes occipitalis	X					
Polistes olivaceus			X	X	X	
Polistes pacificus	X					
Polistes perplexus	X					
Polistes poeyi haitiensis	X					
Polistes riparius				X		
Polistes rothneyi			X	X	X	
Polistes sagittarius					X	
Polistes satan	X					
Polistes simillimus	X					
Polistes snelleni				X		
Polistes stigma bernardii						X
Polistes tenebricosus					X	
Polistes testaceicolor	X					
Polistes variabilis						X
Pseudopolybia compressa	X					
Vespula germanica			X	X		

Table 3
(Continued)

Taxon	NW	Afr	WeP	EaA	SEA	Aus
Vespula maculifrons	X					
Vespula squamosa	X					

ENA, Eastern North America; NW, New World; Afr, Afrotropics; WeP, Western Palearctic, including Europe, Mediterranean, Middle East and Central Asia; EaA, Eastern Asia; SEA, Southeast Asia; Aus, Australia

Table 4
Numerical data for the analysis of different data partitions

Partition	*R*	Fit	MPTs	Support
Morphology and behavior	4.039	4.08397	217	19.3
Mitochondrial	4.783	702.89978	6	40.2
Nuclear	2.470	75.24653	75	25.8
Ribosomal	3.326	322.08442	58	41.2
Protein coding	4.615	447.86827	8	44.2
All molecular	4.993	788.13886	7	43.9
Total evidence	4.582	817.96637	1	52.1

R, number of rearrangements tried, in billions; Fit, the best score; MPTs, number of most parsimonious trees recovered; Support, average group support recovered from 10 000 pseudoreplicates of symmetrical resampling.

Sequence alignment and phylogenetic analyses

Multiple sequence alignment was conducted in MAFFT v.5 (Katoh et al., 2002). Default parameters were used for *COI*, *H3*, and *EF1*-α, for which the alignment is relatively trivial. For the three ribosomal loci, however, the E-INS-I algorithm was used, which is suitable for sequences with large unalignable regions (Katoh et al., 2005).

Phylogenetic analyses were conducted in TNT v1.1 (Goloboff et al., 2008a), under maximum parsimony and using implied weighting (Goloboff, 1993). Goloboff et al. (2008b) suggested that the concavity value *K* should be calculated as a function of *N*, which is the ratio of a single extra step to the cost of the most homoplastic character. We used the TNT script *setk.run*, written by Salvador Arias (Instituto Miguel Lillo, San Miguel de Tucumán, Argentina), to calculate the appropriate value of *K* under $N = 15$. The most appropriate value of *K* was chosen for each partition independently (Table 3) and implemented using the extended implied weighting function in TNT (Goloboff, 2013). Tree search was performed using 200 replications, each with one hit to minimum length, 100 parsimony ratchet (Nixon, 1999a) iterations (with upweighting probability of 8%, downweighting probability of 4%), 50 rounds of tree drifting, 20 rounds of tree fusing, and sectorial searching (Goloboff, 1999). Clade support was estimated using 10 000 pseudoreplicates of symmetric resampling (Goloboff et al., 2003) in TNT, reported as frequency differences (GC scores). All matrices are available as supplementary files to the online version of this work (Files 2–8).

Both the ancestral area for *Polistes* and its dispersal event pathway were inferred by optimizing distributional data directly onto the cladogram using Winclada (Nixon, 1999b). Nine areas were delimited based on distributional patterns observed in the genus (Table 4); using data retrieved from Carpenter (1996a), each species was assigned to one or more of these distributional realms (Table 4). The delimitation of areas remains one of the foremost problems in historical biogeography (Ree and Sanmartín, 2009; Arias et al., 2011); in this case, as usual, the delimitation is somewhat arbitrary. Southeast Asia, for example, is geologically a composite area (see below), and climatically heterogeneous, as its northern part has distinct seasonality. However, because this was the framework in which existing biogeographical hypotheses were proposed (van der Vecht, 1965; Richards, 1971; Reeve, 1991), the areas defined herein can serve as a test of those hypotheses in light of available data.

Results

Comparison of data partitions

Analysis of the morphological and behavioural matrix produced a consensus that was not well resolved (Fig. 1; see Table 4). While the analysis of molecular data alone yielded a well-resolved consensus

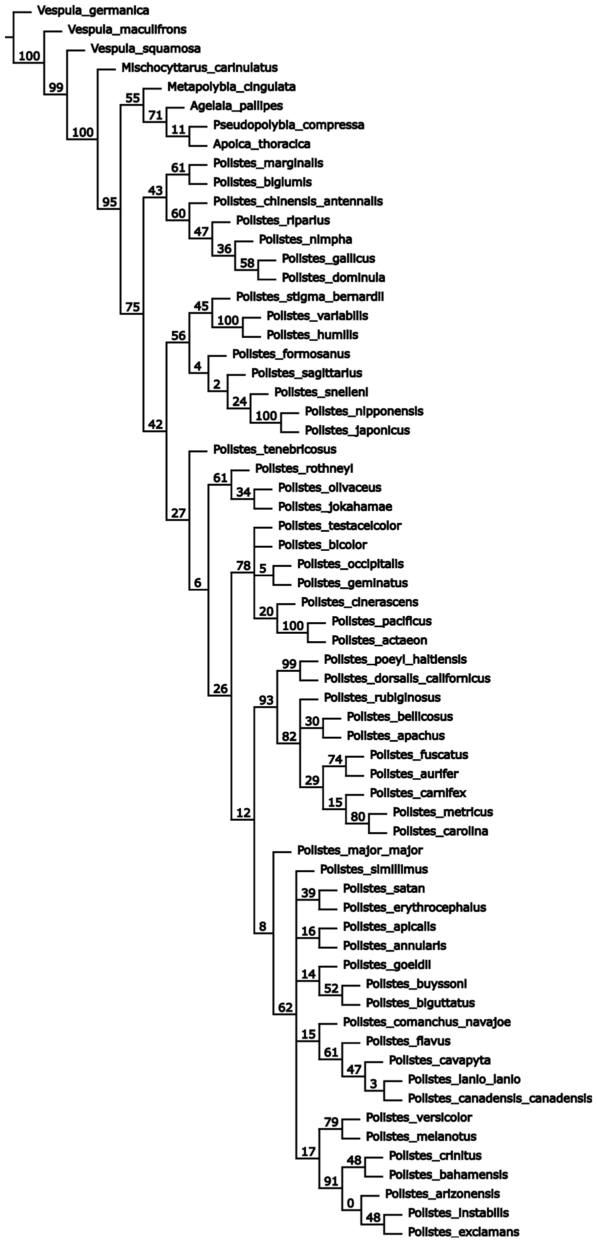

Fig. 1. Strict consensus of 217 most parsimonious trees obtained in TNT for the analysis of morphological and behavioural data alone. Numbers above the branches are symmetrical resampling support values.

topology (Fig. 2), it did not recover the New World taxa as a monophyletic group, with a basal polytomy and the group *P. rothneyi* (*P. olivaceus* + *P. jokahamae*) placed inside the *P. actaeon* clade. Among the different molecular partitions, the analysis of protein-coding genes alone (Fig. 3) produced a consensus that was strikingly more informative than the analyses based on ribosomal genes (Fig. 4). The former analysis also yielded results that were most similar to those of

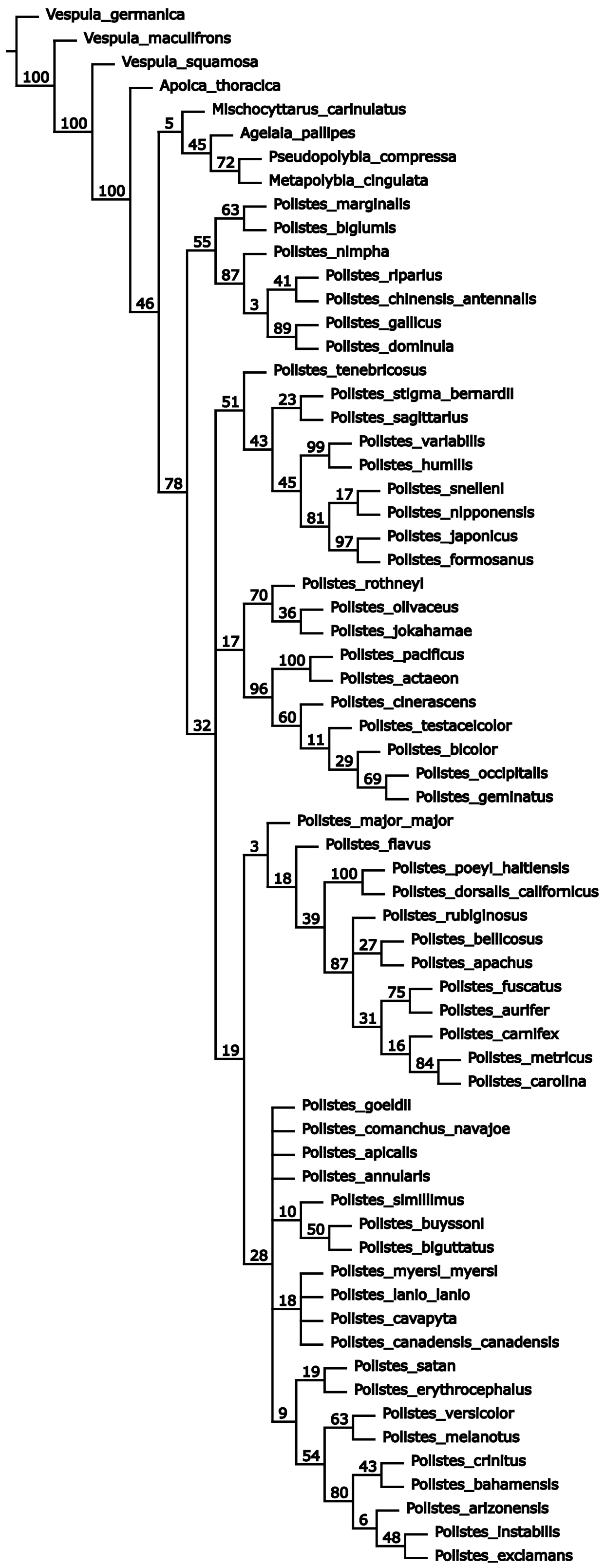

Fig. 2. Strict consensus of seven most parsimonious trees obtained in TNT for the analysis of molecular data alone. Numbers above the branches are symmetrical resampling support values.

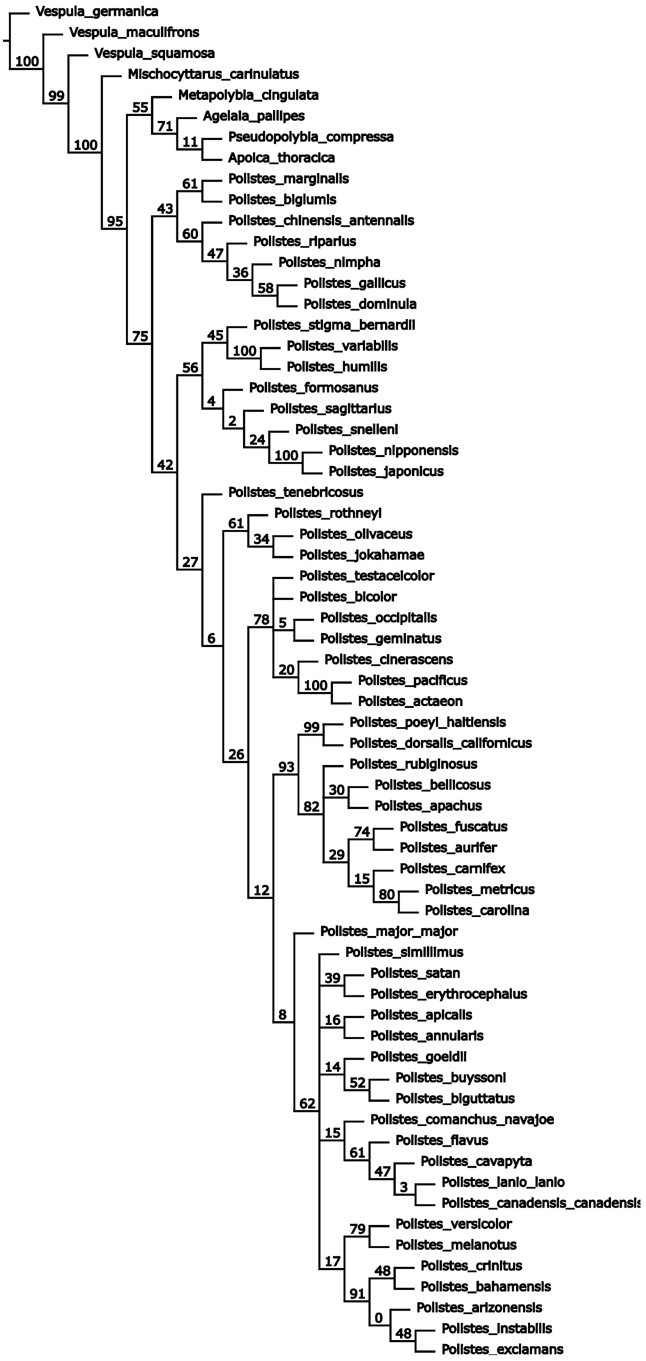

Fig. 3. Strict consensus of eight most parsimonious trees obtained in TNT for the analysis of data from protein-coding genes alone. Numbers above the branches are symmetrical resampling support values.

the total evidence analyses, indicating that the protein-coding genes may provide the majority of the total evidence phylogenetic signal. Although much more complete (see Table 1), the ribosomal dataset seemed mostly to provide confounding signal.

When the topologies produced by the mitochondrial (Fig. 5) and nuclear (Fig. 6) partitions are compared,

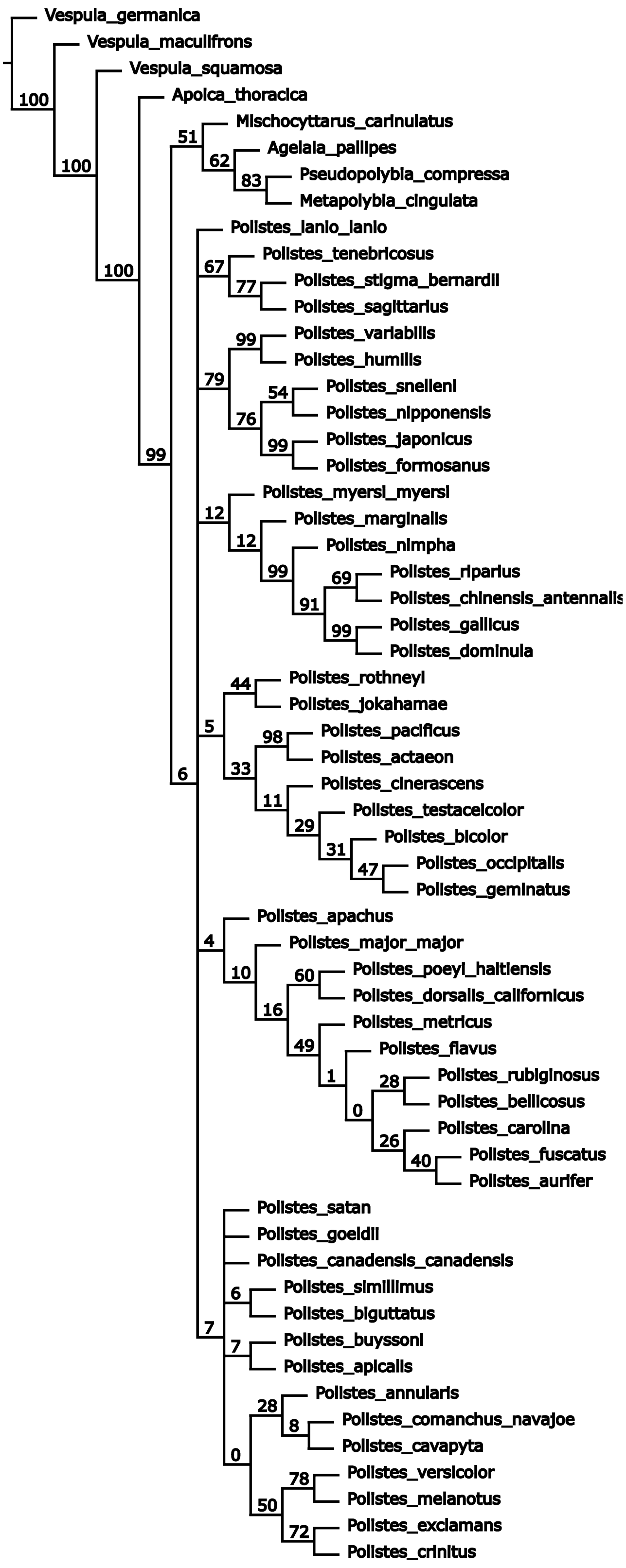

Fig. 4. Strict consensus of 58 most parsimonious trees obtained in TNT for the analysis of data from ribosomal genes alone. Numbers above the branches are symmetrical resampling support values.

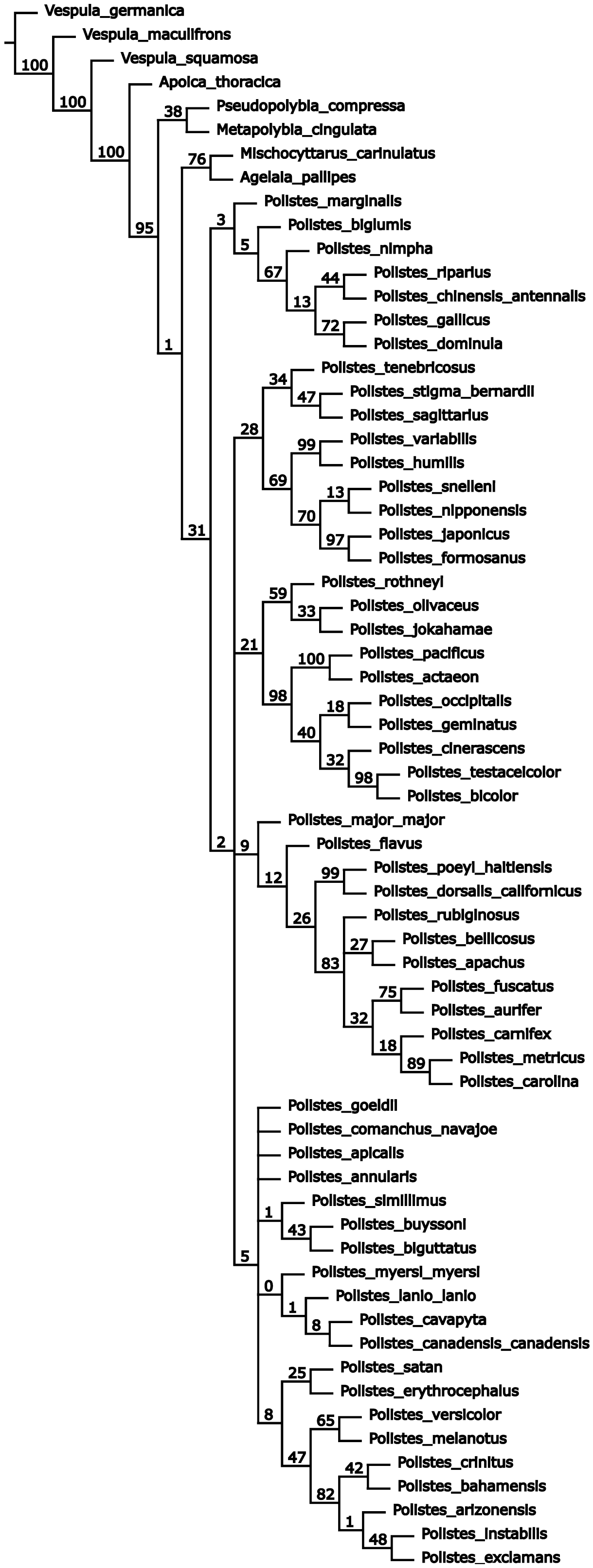

Fig. 5. Strict consensus of six most parsimonious trees obtained in TNT for the analysis of data from mitochondrial genes alone. Numbers above the branches are symmetrical resampling support values.

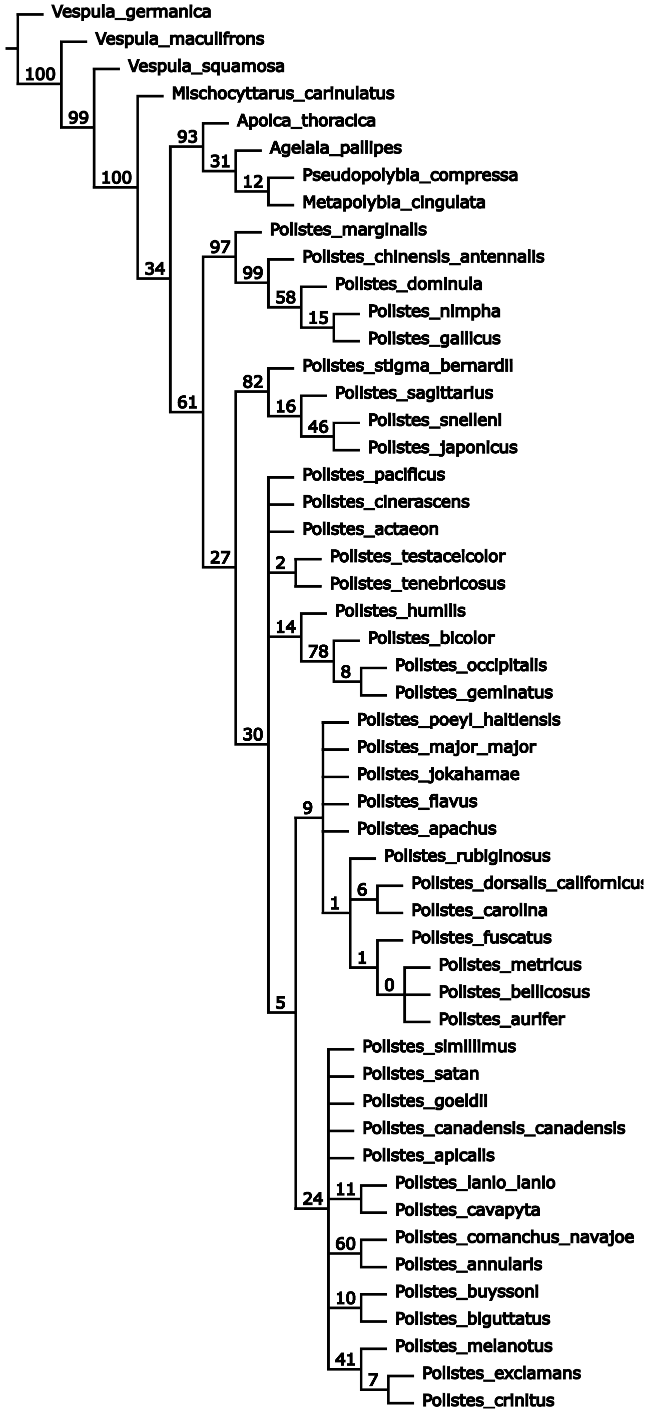

Fig. 6. Strict consensus of 75 most parsimonious trees obtained in TNT for the analysis of data from nuclear genes alone. Numbers above the branches are symmetrical resampling support values.

the nuclear genes have a much less resolved consensus with less support, probably because of the poor performance of *28S*. When analysed individually, the mitochondrial, nuclear, and ribosomal partitions all resulted in trees that were either largely collapsed or considerably different from the total evidence results. None of these three partitions recovered the New World *Polistes* as monophyletic, and the relationships were not sufficiently resolved to evaluate the biogeography of South American versus North American groups. Although only marginally informative when analysed on their own, morphological data were important to increase the resolution and level of support of the final analysis, reducing the number of most parsimonious trees from seven (in the molecular dataset) to one (in the total evidence analysis).

The total evidence dataset yielded the most resolved and well-supported results. Average group support was high (52.1), and most of the nodes relevant for this discussion were well supported (Fig. 7). All analyses recovered *Polistes* as a monophyletic group, in agreement with previous results. Moreover, currently recognized subgenera (Carpenter, 1996b) were recovered as monophyletic. Of the New World subgenera recognized by Richards (1973) (and synonymized by Carpenter, 1996b), *Fuscopolistes* is paraphyletic, in terms of *Polistes carnifex* (in the monotypic subgenus *Onerarius* in Richards, 1971). Unlike in Pickett and Carpenter (2010), the New World species form a clade, as was the case in Carpenter (1996b), Arévalo et al. (2004), Pickett and Wenzel (2004) and Pickett et al. (2006). Among the Old World species, the nominotypical subgenus is sister-group to all remaining *Polistes*, as was also the case in Pickett and Carpenter (2010), but not in any of the other studies.

Historical biogeography

Optimizing the current distribution of *Polistes* species on the final tree does not allow unambiguous inference of the ancestral area for the genus (Fig. 8). While *Polistes* clearly first evolved in the Old World (see below), it is not possible to estimate precise ancestral areas by optimizing the distributions on the tree.

All New World species were recovered as a single clade, while Old World taxa formed a paraphyletic assemblage, suggesting a single colonization event for the Americas (Figs 7 and 8). The sister group for all New World *Polistes* was a large clade for which the inferred ancestral area is Southeast Asia. Within the New World clade, the topology suggests strongly that the invasion of the Americas occurred via South America, with a number of independent colonization events in North America (Fig. 9). One of the main New World clades is composed almost entirely of North American species, including at least two secondary invasions of South America by range extension in *P. carnifex* and *P. fuscatus*. The Caribbean was colonized independently by *P. poeyi* and by the *P. crinitus* clade.

Discussion

Topology

The topology recovered here is similar to that from Pickett and Carpenter (2010); the main difference is that in the previous work, the clade with the New World species also contained the Old World clade *P. tenebricosus* + *P. jokahamae*. The same result is recovered here in some of the trees based on molecular data alone (Fig. 2). This is not surprising, given that Pickett and Carpenter's morphological matrix was focused mostly on resolving the higher-level phylogeny of Vespidae, with few characters informative for *Polistes*. Adopting the characters from Pickett et al. (2006) resulted in a monophyletic New World *Polistes*; in that work, however, the clade *P. rothney* (*P. tenebricosus* + *P. jokahamae*) appeared at the base of *Polistes*, probably due to the signal provided by COI sequences. In Pickett et al. (2006), the clade composed of *Polistes sensu stricto* species also appeared as sister-group to the New World clade. With the inclusion of data from additional genes, these two clades were swapped: *Polistes sensu strictu* was recovered as the basal-most group, while the *P. tenebricosus* clade was recovered as part of another assembly of Old World species.

Centre of origin for Polistes

The idea that *Polistes* evolved in the Oriental tropics is an old one (van der Vecht, 1965; Richards, 1971; Reeve, 1991), and has been justified mostly by the perception of this area as a centre of diversity for social wasps as a whole. Another source of support for the idea is the presence in Southeast Asia of putatively primitive forms of nest architecture (West-Eberhard, 1969). In addition, *Polistes* species found in Europe and North America appear to have a much greater number of chromosomes than their Southeast Asian counterparts (Reeve, 1991). Although this observation is based on only a small sample of species, it nevertheless led Reeve (1991) to suggest that the spread of *Polistes* from Southeast Asia across other regions was related to an increase in chromosome number and environmental opportunity.

The results of the present work do not provide unambiguous support to the Oriental tropics as the center of origin for *Polistes*. The ancestral area for *Polistes*, as optimized from the total evidence tree, is ambiguous. Southeast Asia is recovered as the ancestral area for the clade of Old World taxa that is sister to the New World species (Fig. 8). While the ancestral area for the basal-most clade of the genus—composed of species of *Polistes sensu stricto*—was ambiguous, it is noteworthy that none of those species occurs in Southeast Asia, although the southern limits of the

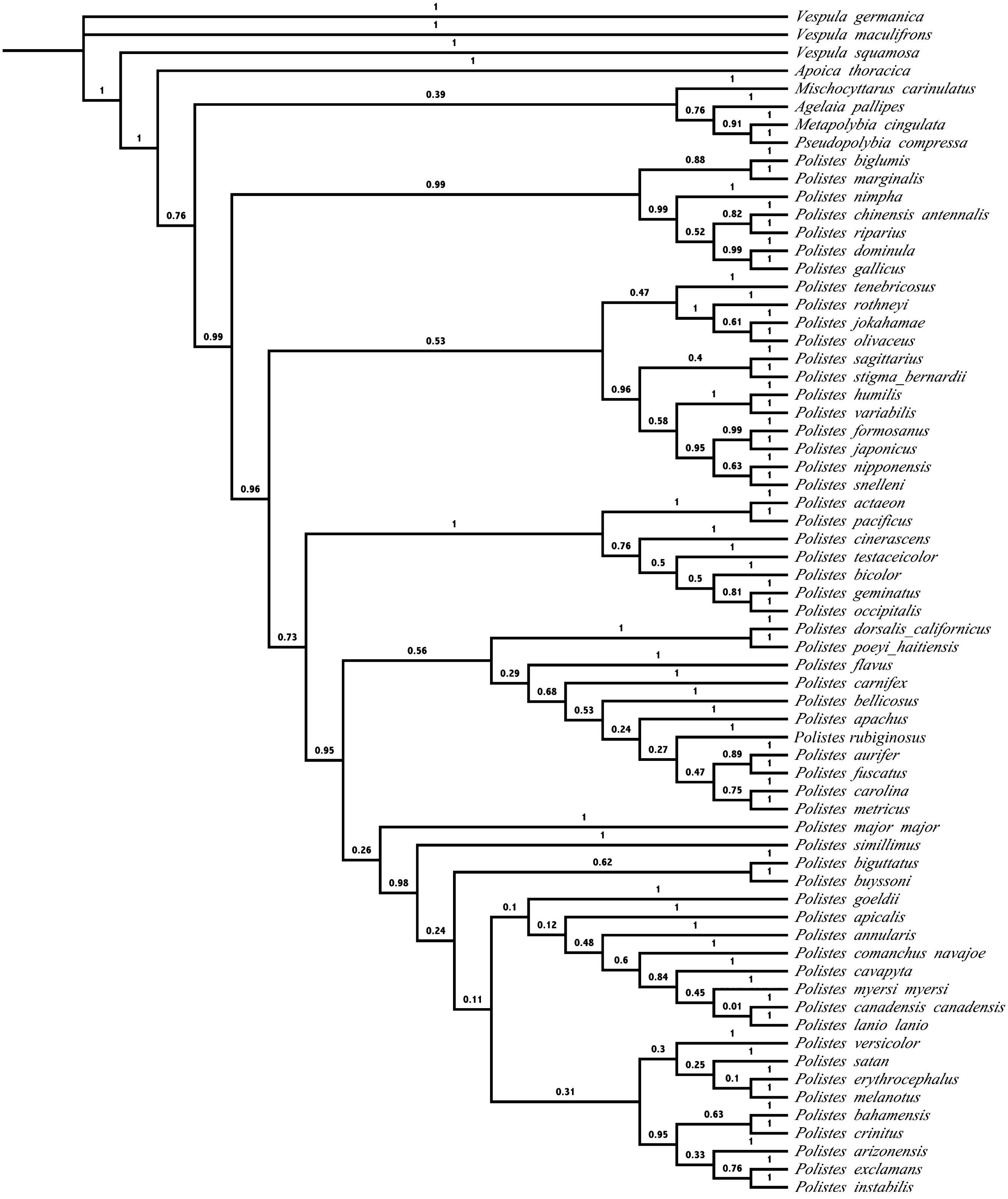

Fig. 7. The preferred phylogeny: the single most parsimonious tree obtained in TNT for the combined analysis of morphological, behavioural and molecular data. Numbers above the branches are symmetrical resampling support values, reported as frequency differences.

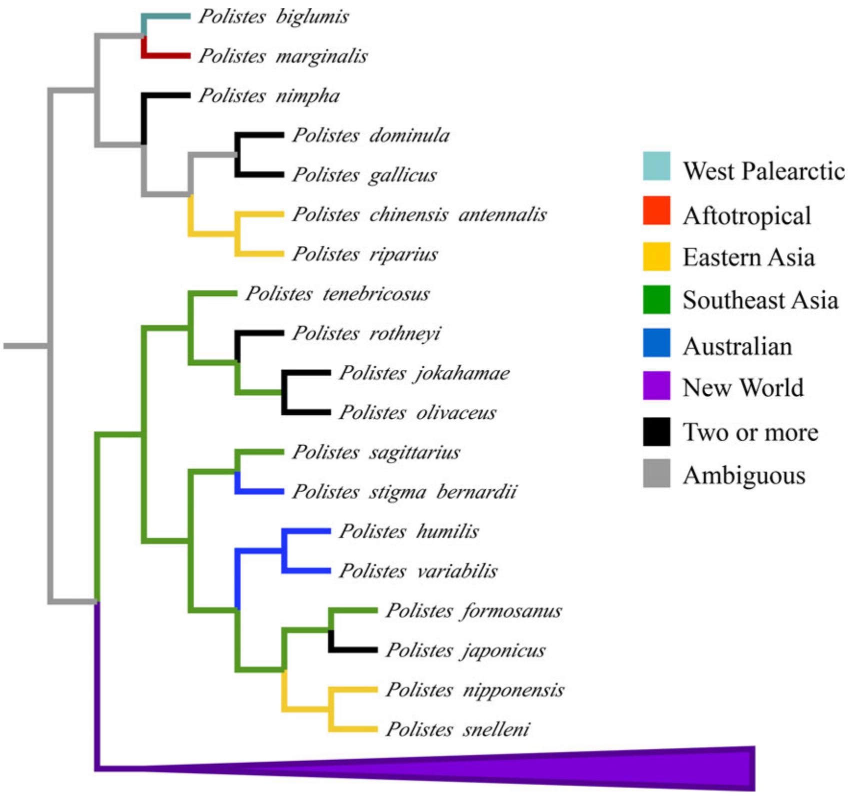

Fig. 8. Ancestral distributions for species of *Polistes* as optimized in the total evidence cladogram.

distribution of *P. chinensis chinensis* fall under the "Oriental" realm. Clearly, additional taxon sampling among the Old World species is needed for a better evaluation of the centre of origin of *Polistes*.

Dispersal to the New World

The suggestion that the New World *Polistes* originated in South America conflicts with previous assumptions of dispersal across the Bering Bridge (van der Vecht, 1965; Richards, 1971; Reeve, 1991). In fact, species from North America appear mostly nested within a paraphyletic grade of South American species, and optimizing the presence in South *versus* North America as a single character will render South America as the ancestral area (Fig. 9).

The ancestral area for the sister-group to the New World species was recovered as Southeast Asia, which also conflicts with the Beringia hypothesis. A scenario that seems more consistent with the available evidence would be transoceanic dispersal from Southeast Asia to South America. Such a phenomenon is usually dismissed as unlikely due to the seemingly overwhelming scale of the geographical barrier involved (i.e. the Pacific Ocean). However, there is strong evidence for at least some cases of cross-Pacific colonization (Winkworth et al., 2002; Howarth et al., 2003; Mummenhoff et al., 2004; Sharma and Giribet, 2012), as well as a surprisingly high frequency of oceanic dispersal across the world (for a review see de Queiroz, 2005). Considering the clear dispersal ability of *Polistes*, as evidenced by its cosmopolitan distribution, as well as its high colonization success following human introductions, a scenario of transoceanic dispersal remains plausible.

Some alternatives to oceanic dispersal also remain. One of them is relictualism: vicariant events followed by range contraction of a clade due to extinction. Another possibility would be overland dispersal via trans-Antarctic connections between the Australian plate and South America. However, species of the genus are absent (except for human introductions) in many of the areas usually associated with the trans-Antarctic connection, such as New Zealand and the southern extremity of South America. A third alternative would be incomplete taxon sampling: species from other areas being more related to the New World clade but not included in the present analyses.

There is a general perception that Polistinae as a whole may have a Gondwanan origin (Carpenter, 1981, 1993), a possibility that cannot be ruled out under the results of the current analyses. Note that only one species from the Afrotropics was represented in the analysed matrix. The inclusion of further Afri-

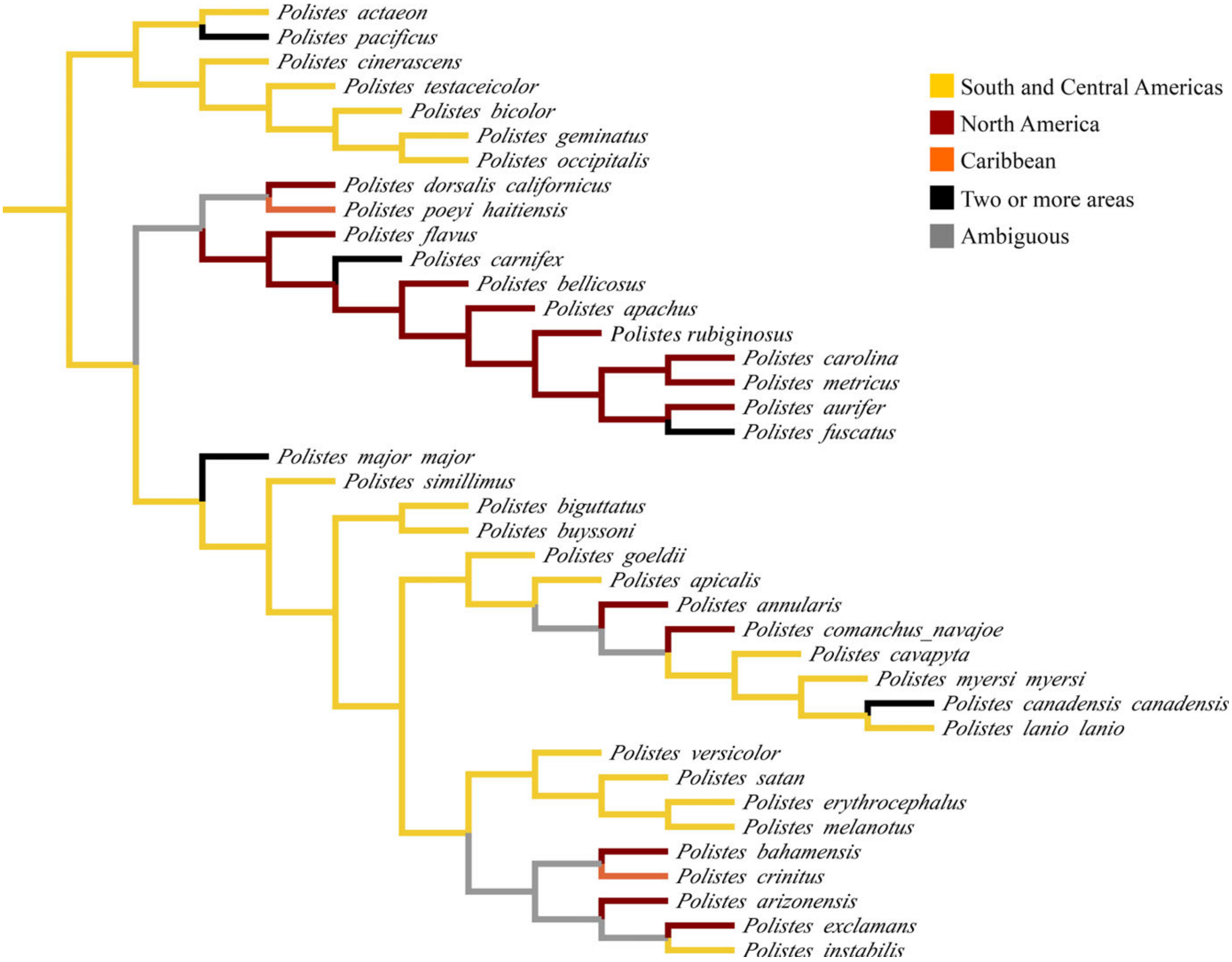

Fig. 9. Ancestral distributions for New World taxa as optimized in the total evidence cladogram.

can species could lead to Africa as the ancestral area for the *Polistes sensu stricto* clade. At the same time, in terms of Earth history, Southeast Asia is a composite area—most of its area has Laurasian origin, but India was part of Gondwana. If the Southeast Asian species derive from an ancestor originally from India, all three main clades of *Polistes* could have Gondwanan origin, in which case a vicariant scenario would better explain the distributional patterns of the genus. Future work with further taxon sampling will help to elucidate the trajectory of *Polistes* across the globe.

Monogyny and overwintering

The topology of the tree presented here suggests strongly that the ancestor of all *Polistes* lived in the tropics. This is the most parsimonious inference when the temperate versus tropical distribution for each species is optimized on the tree (Fig 10). This inference is fundamentally inconsistent with the notion that long-term monogyny was an adaptation to overwintering, as proposed by previous authors (West-Eberhard, 1978; Itô, 1986). In the absence of severe winters, other determining factors must be sought for the emergence of a permanently monogynous lifestyle.

Conclusions

Our results show that there is still not enough evidence to determine the ancestral area for *Polistes*, although there is support for the idea that the early evolution of the genus took place in the Old World tropics. The unambiguously tropical-living ancestor recovered here refutes the notion that monogyny evolved as an adaptation to severe winters. In agreement with previous assumptions, the genus colonized the New World only once—via South America and

Fig. 10. Tropical and temperate distributions optimized in the tree to reconstruct the ancestral area for *Polistes*.

not Beringia—with multiple subsequent incursions into North America.

Acknowledgements

B.F.S. and A.P. were supported by fellowships from the Richard Gilder Graduate School. Mark Siddall (AMNH) provided useful comments and incentive in an early stage of this work. TNT is made available through the sponsorship of the Willi Hennig Society. Parts of the research were supported by National Science Foundation Grant DEB-0542640 to K.M.P. and J.M.C.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

File 1. Genbank_numbers. Accession numbers in GenBank for sequences used in the analyses.

File 2. Santos_etal_2014_morph.txt. Matrix with morphological and behavioural characters used in the analyses.

File 3. Santos_etal_2014_molecular.txt. Matrix with all molecular data used in the analyses.

File 4. Santos_etal_2014_coding.txt. Matrix with all data from protein-coding loci used in the analyses.

File 5. Santos_etal_2014_ribosomal.txt. Matrix with all data from ribosomal loci used in the analyses.

File 6. Santos_etal_2014_mitochondrial.txt. Matrix with all data from mitochondrial loci used in the analyses.

File 7. Santos_etal_2014_nuclear.txt. Matrix with all data from nuclear loci used in the analyses.

File 8. Santos_etal_2014_combined.txt. Matrix with the combined data including all morphological, behavioural and molecular characters used in the analyses.