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Molecular phylogeny of the paper wasp subgenus *Polistes* (*Polistella*) Ashmead, 1904 (Hymenoptera: Vespidae: Polistinae) from Vietnam

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ABSTRACT

This study provides the first molecular phylogeny of the social wasp subgenus *Polistella* (Hymenoptera: Vespidae: *Polistes*) from Vietnam. Fragments of the mitochondrial COI and 16S rRNA genes were used to reconstruct the phylogenetic trees among 38 *Polistes* species plus two out-group species (*Vespa soror* du Buysson and *Ropalidia fasciata* (Fabricius)). Our results support the existence of several species-groups, including two that are congruent with the previous *stigma* and *Stenopolistes* groups defined on the basis of morphology. Moreover, we recovered a clade including the *stigma* group and the two species *P. humilis* and *P. variabilis* that was sister to all other species of *Polistella*. However, the results also challenged the definition of other groups of *Polistella* based on morphological data, as well as the definition of two species: *P. brunus* and *P. affinis*. This first study calls for further analyses including morphological characters to clarify the taxonomy and the classification of the group.

Introduction

Social vespids are of central importance to studies of behavioral evolution because among them, the polistines (paper wasps) are considered to mark a clear, finely divided transition between primitively and highly eusocial behavior (Pardi, 1996). The vast majority of behavioral investigations of the Polistinae have focused on the cosmopolitan genus *Polistes* Latreille. Their relatively small colonies (usually with fewer than 100 individuals) and uncovered nests often made on human constructions allow us to make detailed behavioral observations. Therefore, *Polistes* has been the genus of particular interest for both experimental and theoretical investigations of social behavior (Turillazzi and West-Eberhard, 1996; Jandt et al., 2013). However, previous phylogenetic analyses of *Polistes* have included only a few species of *Polistella* (Pickett and Wenzel, 2004; Pickett et al., 2006; Arévalo et al., 2004; Pickett and Carpenter, 2010; Santos et al., 2015).

With more than 200 species, *Polistes* is subdivided into four monophyletic subgenera, with their relationships expressed as (*Gyrostoma* + (*Polistella* + (*Polistes* s. str. + *Aphanilopterus*))) (Carpenter, 1996a). *Gyrostoma*, *Polistella* and *Polistes* s.str. are primarily of the Old World (including Australia), and *Aphanilopterus* is exclusive to the New World. Of the four subgenera, *Polistella*, presently with 90 species and 34 subspecies distributed from Africa to Australia (Carpenter, 1996b, updated), has been less studied taxonomically, phylogenetically and biologically than other social wasps.

The phylogenetic relationships among *Polistes* species (including 230 species and 88 subspecies) have been studied by several authors. The first large-scale analysis within *Polistes* was Carpenter (1996a), who studied the morphology of 144 species and an additional 43 subspecies, thus sampling most of the diversity within the genus. The number of *Polistella* species used in that study was 36 species and 21 subspecies, the largest in number of *Polistella* species up to present (only a few *Polistella* species were used in the studies of Pickett and Wenzel, 2004, and Pickett et al., 2006). Carpenter (1996a) synonymized *Stenopolistes* under *Polistella*, and divided *Polistella* into 4 species groups, namely *Polistes adustus*, *P. stigma*, *P. sagittarius* and *Stenopolistes*, and those species groups were used until now.

The first extensive molecular treatment was by Arévalo et al. (2004), which included 33 species of *Polistes* (with only three species in *Polistella*), along with 33 species of other tribes of Polistinae. The

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molecular data consisted of mitochondrial CO1 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 (1996a), 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, and with characters of nest architecture from Wenzel (1993). In the combined analysis, each of the Old World subgenera was monophyletic including *Polistella*. And within the New World clade, both *Aphanilopterus* s.str. and *Epicnemius* (synonymized with *Aphanilopterus*) were monophyletic (however, these groups included ten and two species, respectively).

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 CO1. 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 (1996a) conclusions that neither *Aphanilopterus* s. str. nor *Epicnemius* are monophyletic.

Because there are both taxon sampling and evidentiary differences between Arévalo et al. (2004), Pickett and Wenzel (2004), Pickett et al. (2006) conducted a new analysis that combined the two fragments of CO1 for as many taxa from the two studies as possible. Pickett et al. (2006) treated 48 taxa, including all *Polistes* species from Arévalo et al. (2004) and all taxa, both species and subspecies from Pickett and Wenzel (2004). As the result, a phylogenetic tree based on combined morphological and molecular data was proposed. The resolution among the New World subgenera was better than any previous analysis. All tested subgenera were monophyletic. For the Old World subgenera, *Gyrostoma* was monophyletic and sister to all other *Polistes*. No previous phylogenetic studies showed this relationship.

Pickett and Carpenter (2010) presented a new phylogenetic analysis of Vespidae based on what was by far the largest taxon sample to include molecular data (using four genes CO1, 12S, 16S and 28S), and the largest phenotypic character dataset ever compiled, including 45 species of *Polistes* (with only four species in *Polistella*). The results from Pickett and Carpenter (2010) showed that only the subgenus *Aphanilopterus* is paraphyletic, rendered so by *Gyrostoma*, and three of these subgenera are monophyletic: *Gyrostoma*, *Polistella*, and *Polistes* s.str. In *Polistella stigma* formed a clade with a species from the *sagittarius* group (*P. sagittarius*).

Santos et al. (2015) presented the an analysis of the phylogeny of the genus *Polistes* using morphological and behavioral characters, as well as molecular data from six genes (COI, 12S, 16S, 28S, H3 and EF1-∂). A total of 58 species of *Polistes* were included in the analyses, with an emphasis on New World species (only 8 species were in *Polistella*). In the analysis of morphology data alone, *Polistella* was monophyletic, and *stigma* formed a clade with species from the *sagittarius* group (*P. sagittarius*) as sister to the other groups.

No phylogenetic analysis of *Polistella* species has been done until now. There are some reasons why the species-level phylogeny of *Polistella* has been neglected. First, despite behavioral salience, morphological variation within *Polistella* is slight. The other reason is most collections do not contain even a majority of the species, making it difficult for researchers to gain necessary experience with the group. As the morphology is so uniform in *Polistella*, it makes sense to seek other sources of data, such as molecular data. But DNA-grade specimens of *Polistella* are even scarcer than pinned specimens, and so obtaining molecular data is difficult.

Polistella had originally been subdivided into species groups (Das and Gupta, 1989), but Carpenter (1996a) mentioned that Das & Gupta's groups do not include all of the described species assignable to the subgenus. Das and Gupta treated only species occurring in India (15 species), dividing them into the *adustus* group, *stigma* group, and *maculipennis* group. No morphological features characterized their *adustus* group; rather, it was based on color, namely a black metasoma. The *stigma* group included two species, diagnosed principally by having the forewing marginal cell with a pigmented spot. The propodeal concavity was said to be shallower and the striae finer than in the third group, the *maculipennis* group, however these characters do not actually allow separation of the *maculipennis* group. Three species were included in the *maculipennis* group but one of these, *maculipennis* itself, has the forewing spotted apically - as stated by Das and Gupta (1989). Petersen (1987), cited by Das & Gupta, even treated *maculipennis* as a subspecies of *stigma*.

Das & Gupta's groups as constituted were thus unsuitable, and had been modified by Carpenter (1996a), who used 36 *Polistella* species in his study, as follows: The *adustus* group comprises those species having a tuberculate male sternum VII. The *stigma* group includes the species with an apically spotted forewing, while the *sagittarius* group includes all remaining species (*sagittarius* and *strigosus* were the other two species included by Das & Gupta in their *maculipennis* group). But as he (Carpenter, 1996a) pointed out all the species groups are as yet poorly defined. Thus far, the phylogenetic relationships of *Polistella* have not been evaluated in a study focused on this group.

This research, for the first time, provides a phylogenetic analysis among species of the subgenus *Polistella* based on molecular data, which can then allow us to revise the poorly established species group system of *Polistella* based on monophyly, and define the phylogenetic relationships among *Polistella* species.

Materials and methods

Taxon sampling

A total of 38 species of *Polistes* were included in the analyses, in addition to two outgroup species of *Vespa* and *Ropalidia*. Thirty-eight ingroup species representing four former species groups defined by Carpenter (1996a) (*Polistes adustus*, *P. stigma*, *P. sagittarius* and *Stenopolistes* groups) and one species group proposed by Nguyen et al. (2011) (group of basally strongly swollen second metasomal sternum) were included. Three specimens from Vietnam that could not be morphologically attributed to known species were included in the analysis (sp.1, sp.2 and sp.3).

DNA extraction and amplification

Total genomic DNA was extracted from leg tissues or body muscles using the DNAeasy Blood & Tissue Kit (Qiagen™).

Fragments of two mitochondrial genes, Cytochrome c subunit I (COI) and 16S rRNA, were amplified using polymerase chain reaction (PCR). The universal primer set (HCO-2190 and LCO-1498) (Folmer et al., 1994) was used to amplify a 680 bp fragment of COI while a primer set (16S-R1 (5′-TTA CGC TGT TAT CCC TAA-3′) and 16S-R2 (5′-GTA CCT TGT GTA TCA GGG TT-3′)) (Saito et al., 2007) was used for a 900 bp fragment of the 16S rRNA.

PCR conditions for amplification of the 16S rRNA gene was: an initial denaturation at 95 °C for 2 min followed by 36 cycles of 95 °C for 20 s, 45 °C for 40 s, and 72 °C for 1 min, and a final extension at 72° for 5 min. The annealing temperature was changed to 42 °C for 45 s when amplifying the COI gene.

PCR products were checked for potentially successful amplification of a fragment of each gene using electrophoresis in 1% Agarose-TBE 1X. Successfully amplified PCR products were purified using QIA quick PCR Purification Kit (Qiagen Inc.), and sequenced at Solgen, Inc. (Korea) on an Applied Biosystems automatic sequencer (ABI3130 XL).