A phylogenetic investigation of the Neotropical genus *Alphamenes* van der Vecht, 1977 (Hymenoptera, Vespidae, Eumeninae)

Letícia A. de Oliveira, Tiago G. Inez, Wellington D. Ferreira, Marcel G. Hermes

**Article Info**

**Article history:**
Received 24 October 2018
Accepted 27 November 2018
Available online 19 December 2018
Associate Editor: James Carpenter

**Keywords:**
Male genitalia
Minixi
Systematics
Taxonomy

**Abstract**

Advances in potter wasp systematics have been achieved recently, with classificatory changes resulting from analyses based upon large scale molecular datasets. For the Neotropics, recent hypotheses point to the occurrence of an exclusive clade recognized within the tribe Eumenini. In this group, several contributions regarding taxonomy and systematics have been proposed in the last five years, including the genus *Alphamenes*. This taxon contains seven described species whose distribution is exclusively Neotropical. Females are morphologically homogeneous, and characters related to copulatory organs are useful in male diagnosis. This contribution forms the first phylogenetic approach to include all species of *Alphamenes*, hence the first to strongly test for group monophyly. Our cladistic results recovered *Alphamenes* as a monophyletic group supported by male genital features. Relationships among included species also rely upon genital characters, highlighting the importance of these attributes for eumenine systematics. Recent phylogenetic investigations applied to the Neotropical fauna of potter wasps represent desirable advancements towards a natural classification for the group.

© 2018 Published by Elsevier Editora Ltda. on behalf of Sociedade Brasileira de Entomologia. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

**Introduction**

Phylogenetic studies based upon molecular data have recently changed our understanding of the evolutionary relationships within the eumenines, the so-called potter wasps (Bank et al., 2017; Hines et al., 2007; Piekarski et al., 2018). These studies contradict our traditional view of the vespid wasp – and by extent, relationships within the eumenines – phylogenetic relationships based on morphological data (Carpenter, 1982; Hermes et al., 2014) and a total evidence approach (Pickett and Carpenter, 2010), rendering Eumeninae paraphyletic.

In the Neotropical region, advances in the systematics of the group were achieved only recently (e.g. Hermes and Carpenter, 2012; Hermes and Oliveira, 2016). Despite the fact that the tribal classification proposed by Hermes et al. (2014) was challenged by molecular studies, their results indicate that a subgroup of Eumeninae may indeed be an exclusive Neotropical clade. This clade was recognized as “Clade 4” in their contribution, which contains the majority of the taxonomic diversity within their tribe Eumenini.

Within the Neotropical Eumenini sensu Hermes et al. (2014), some contributions encompassing a wide range of taxa were recently published (e.g. Grandinete et al., 2015; Hermes and Oliveira, 2016). The eumenine classification within this region has been continually investigated under cladistic approaches, with generic synonymy resulting in the cases of *Pachymeres* de Saussure, 1852 and *Santamenes* Giordani Soika, 1990 (Grandinete et al., 2015) and *Minixi* Giordani Soika, 1978 and *Pachyminixi* Giordani Soika, 1978 (Hermes and Oliveira, 2016). Regarding the latter, a special case of a closely related taxon, namely *Alphamenes* van der Vecht, 1977, remains to be carefully addressed. Sexual association for members of this genus is particularly difficult, with females of some species being morphologically similar (Giordani Soika, 1978; Oliveira et al., 2017). Also, no phylogenetic study to date have included all species of *Alphamenes*, thus its monophyly is still open to further investigation.

The present contribution enhances our knowledge about the Neotropical fauna of eumenine wasps, aiming at testing the monophyly of *Alphamenes* and recovering the phylogenetic relationships among its species. This forms our final contribution to a series of papers dealing with the systematics of *Alphamenes* and *Minixi* (Hermes and Oliveira, 2016; Oliveira et al., 2017), whose generic status have been traditionally questioned by eumenine workers.
Table 1

Morphological character list for the cladistic analyses of Alphamenes. Figures that do not appear in the text are available in Supplementary Material.

<table>
<thead>
<tr>
<th>Head</th>
<th>Male genitalia</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Clypeal teeth: (0) carinate (Figs. 16a, 19 and 21); (1) ecarinate (Fig. 17).</td>
<td>(36) Ventral lobes of the penis valves; shape: (0) single, one thin tooth; (Hermes and Oliveira, 2016, p. 193, Fig. 32); (1) bifid, two teeth (Figs. 10 and 11); (2) more than two teeth, margin serrate (Figs. 12 and 13); (3) single, one enlarged tooth (Fig. 15); (4) single, one tooth somewhat enlarged (Giordani Soika, 1978, p. 406, Fig. 485); (5) slightly evident, very short (Hermes; Carpenter, 2012, p. 15, Fig. 37).</td>
</tr>
<tr>
<td>(2) Clypeal apex, middle portion: (0) strongly emarginate (Figs. 16b, 18b); (1) weakly emarginate (Fig. 17b).</td>
<td>(37) Apex of the aedeagus: (0) with sulcus (Fig. 67 (Hermes and Oliveira, 2016)); (1) without sulcus (Fig. 66 (Hermes and Oliveira, 2016)).</td>
</tr>
<tr>
<td>(3) Clypeal dimensions: (0) wider than long (Fig. 18c); (1) longer than wide (Fig. 16c).</td>
<td>(38) Ventral margin of aedeagus I: (0) smooth at medial enlargement (Hermes and Oliveira, 2016, p. 193, Fig. 32); (1) serrate at medial enlargement (Fig. 9d).</td>
</tr>
<tr>
<td>(4) Labrum apex: (0) truncated (Fig. 19c); (1) rounded (Fig. 20e).</td>
<td>(39) Ventral margin of aedeagus II: (0) with accessory lobe (Fig. 9e); (1) without accessory lobe (Hermes and Oliveira, 1913, Fig. 32).</td>
</tr>
<tr>
<td>(5) Interantennal region: (0) raised and rounded (Hermes and Oliveira, 2016, p. 190, Fig. 6); (1) raised and longitudinally cariniform (Hermes and Oliveira, 2016, p. 190, Fig. 7).</td>
<td>(40) Basal plate of aedeagus: (0) shorter (sometimes very short) than basal apodemes (Figs. 9–15); (1) elongate, longer or reaching base of basal apodemes (Hermes and Oliveira, 2016, p. 193, Fig. 32).</td>
</tr>
<tr>
<td>(6) Frons medially: (0) with furrow (Figs. 16d, 17d and 4d); (1) without furrow (Fig. 21d).</td>
<td>(41) Digitus: (0) without medium projection adjacent to lamella (Hermes and Oliveira, 2016, p. 193, Fig. 34); (1) with medium projection adjacent to lamella (Fig. 8a).</td>
</tr>
<tr>
<td>(7) Male antennae: (0) with tyloids (Figs. 22a, 23a and 27a); (1) without tyloids.</td>
<td>(42) Cuspis: (0) without medium strong setae (Hermes and Oliveira, 2016, p. 193, Fig. 34); (1) with medium strong setae (Fig. 8b).</td>
</tr>
</tbody>
</table>

in the last couple of decades (see Carpenter and Garcete-Barrett, 2003).

Material and methods

Material examination and morphological studies

We examined 376 specimens belonging to Alphamenes and Minixi (Table 3 in Supplementary Material). The specimens were borrowed from the following institutions: AMNH – American Museum of Natural History, New York, USA (Dr. James M. Carpenter); MCZ – Museum of Comparative Zoology, Harvard University, Cambridge, USA (Dr. Jignasha Rana); MNHN – Muséum National d’Histoire Naturelle, Paris, France (Dr. Claire Villemant); MHNHP – Museo Nacional de Historia Natural del Paraguay, San Lorenzo, Paraguay (Dr. Bolívar R. Garcete-Barrett); CMNH – Carnegie Museum of Natural History, Pittsburgh, USA (Dr. John Rawlins); NHM – Natural History Museum, London, England (Dr. Gavin Broad); ZMHB – Museum für Naturkunde der Humboldt-Universität, Berlin, Germany (Dr. Frank Koch); MSNVE – Museo di Storia Naturale di Venezia, Venice, Italy (Dr. Marco Uliana); HYMSJRP – Coleção de Hymenoptera, Departamento de Zoologia e Botânica, Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista ‘Júlio de Mesquita Filho’, São José do Rio Preto, Brazil (Dr. Fernando B. Noll); UFMG – Coleções Taxonômicas da Universidade Federal de Minas Gerais, Belo Horizonte, Brazil (Dr. Fernando A. Silveira); DZUP – Coleção de Entomologia Pe. Jesus Santiago Moura, Departamento de Zoologia da Universidade Federal do Paraná, Curitiba, Brazil (Dr. Gabriel Melo); CEUFLA – Coleção Entomológica da Universidade Federal de Lavras, Lavras, Brazil (Dr. Marcel G. Hermes).
The morphological study of the specimens was carried out with the stereoscopic microscope Leica S8 APO. Images of the structures of phylogenetic and taxonomic interest were obtained with a Leica 104 45929 digital camera coupled to the Leica DFC295 stereo microscope. Terminology for male genitalia followed Bitsch (2012) and for external morphology Carpenter and Garcete-Barrett (2003). The male genitalia was detached from the metasoma and clarified on 10% NaOH solution for 24–36 h, then neutralized with acetic acid, washed in water and stored in glycerol.

**Cladistic analyses**

For the reconstruction of the phylogenetic relationships, all recognized species within *Alphameses* and *Minixi* were included as ingroup (sensu Hermes and Oliveira, 2016). In the case of *M. atrum* Selis, 2017, the information was extracted from the literature (Selis, 2017). We based our analyses upon our previous matrix (Hermes and Oliveira, 2016), with the inclusion of all species of *Alphameses* and adjusting character coding accordingly. This resulted in a matrix with 42 morphological characters, an increase of eight characters from Hermes and Oliveira (2016). Species of the closely related *Laevimines* Giordani Soika, 1978 and *Pachymenes* de Saussure, 1852 were included in the analyses. The inclusion of these taxa in the analyses aimed at testing the monophyly of the ingroup genera (mainly *Alphameses* and *Minixi*). Despite the fact that *Minixi* was already investigated phylogenetically (Hermes and Oliveira, 2016), we opted for including its species to perform a stronger test (more ingroup taxa and more characters) of the monophyly of the taxa under scrutiny. For the rooting of the trees the outgroup method was used (Nixon and Carpenter, 1993) and the species *Monobia angulosa* de Saussure, 1852 was selected.

Fitch’s parsimony (Fitch, 1971) was the optimization criterion used in the present study. Character matrix construction was carried out with Winclada v. 1.00.08 (Nixon, 1999–2002). Heuristic searches for the most parsimonious cladograms were performed with TNT v. 1.5 (Goloboff and Catalano, 2016), using implied character weighing (Goloboff, 1993; Goloboff et al., 2008). The value for the constant k was calculated with the script *setk.run* (k = 3.046875). In TNT the “New Technology Search” option was used following Hermes et al. (2014) and Hermes and Oliveira (2016).

The visualization and editing of the cladograms, as well as optimization of the characters, were performed in Winclada, with only unambiguous changes shown. Support for the branches was investigated through Symmetric Re-sampling (Goloboff et al., 2005) using TNT 1.5, with 1000 replications for traditional (TBR) tree search and 10,000 re-sampling replications.

**Results and discussion**

The morphological character list is shown in Table 1. The phylogenetic analysis with implied character weighing of the matrix (Table 2) containing 42 characters returned one most-parsimonious tree (Fig. 1), with length (L) = 100, consistency index (CI) = 49, and retention index (RI) = 72. However, the resampling analysis presented small changes when compared to the implied weighting tree. These changes are related to the placement of *M. tricoloratum* (Zavattari, 1911), *M. mexicanum* (de Saussure, 1857) and *M. brethesi* (Bertoni, 1927) (Fig. 1).

The generic relationships recovered herein are identical to the hypothesis presented by Hermes et al. (2014). However, only one synapomorphy (vs. five synapomorphies recovered by Hermes and Oliveira (2016)) and two homoplasies (vs. one recovered by Hermes and Oliveira (2016)) support *Minixi* as a natural group. Of these, the interantennal region raised and rounded (char 5[0]) and the lateral surface of pronotum depressed (char 11[0]) were recovered in both studies. It is worthy of mentioning the fact that the relationships among species included in *Minixi* changed from our previous results (see Hermes and Oliveira, 2016). The hypothesis recovered herein allows the recognition of two major clades, which would also allow their treatment as separate genera (for example, returning to the concept of *Minixi* and *Pachyminxii* of Giordani Soika (1978), with the
inclusion of *M. mariachi* and *M. atrum* in the latter). Nevertheless, we advocate the concept of Hermes and Oliveira (2016) and recognize only one genus, based on the low resampling support for the *M. atrum*–*M. bifasciatum* (von Schultess, 1904) clade (Fig. 1) and the transitional morphological condition of *M. mariachi* Hermes and Oliveira, 2016 (see Hermes and Oliveira, 2016 for details).

*Alphamenes* was recovered as monophyletic with a somewhat high support value. Also, this is the first study to include all recognized species within the taxon in a phylogenetic investigation. Two synapomorphies related to male genitalia support the clade: basal plate of aedeagus short (char 40 [0]) and digitus with median projection adjacent to lamella (char 41 [1]) (Figs. 8–15). The male genitalia has already been pointed as providing strong evidence for the monophyly of *Alphamenes* (Hermes and Oliveira, 2016; Oliveira et al., 2017). Furthermore, features associated with the male last visible sternum (Figs. 2–7) are taxonomically important and reliable (Giordani Soika, 1978). On the other hand, identification to species level of females of *Alphamenes* is quite difficult, and only recently Oliveira et al. (2017) provided further morphological evidence to distinguish some species within the genus.

---

**Fig. 1.** Most parsimonious tree obtained with implied weighing of the characters (*k* = 3.046875), with length (*L*) = 100; consistency index (CI) = 49; retention index (RI) = 72. Black rectangles correspond to unique transformations (synapomorphies) and white rectangles correspond to homoplastic transformations. Support values (symmetric resampling) are given below branches (GC values) (where values are missing, branches collapsed during the resampling analysis). Only unambiguous changes are shown.
Within the Alphamenes, two major clades may be readily recognized. Nevertheless, these are both supported only by copulatory features, such as the shape of the last metasomal sternum’s carina (Figs. 2–7) and features related to the aedeagus (Figs. 8–15). In general, few synapomorphies were recovered to support internal relationships within Alphamenes, especially because most characters were extracted from females. As mentioned previously (Giordani Soika, 1978; Hermes and Oliveira, 2016; Oliveira et al., 2017), female homogeneity hampers taxonomy of the genus, but is also another evidence for monophyly of the group.

Conclusion

The character matrix and the taxon sampling in the present study were increased from previous investigations, which resulted in a more robust test for the monophyly of the included taxa. This is the first contribution to include all species recognized within Alphamenes, and the monophyly of the taxon was recovered with somewhat strong support. As mentioned in previous studies, characters of male genitalia were important in recovering both the monophyly of Alphamenes as well as internal relationships among its species.

The taxonomy of Alphamenes may be regarded as satisfactorily resolved, despite some females are still difficult to differentiate (e.g. A. semiplanus Giordani Soika, 1978, A. incertus (de Saussure, 1875), A. campanulatus (Fabricius, 1804) and A. convexus (Fox, 1899)). Some efforts may allow the resolution of these matters, such as fieldwork in order to recover nests of Alphamenes for males and females may be reared (Oliveira et al., 2017). From there, several morphological approaches may be conducted to sexually associate individuals of a given species, such as internal anatomy of the exoskeleton, female sting, and mouthparts. Finally, an integrative approach for Alphamenes taxonomy would most certainly serve as a solid basis for robust species hypotheses within the genus.

A series of recent contributions were recently published by the authors (e.g. Ferreira et al., 2015, 2017, 2018; Hermes and Oliveira, 2016; Oliveira et al., 2017) regarding Neotropical eumenine taxonomy and phylogeny. The continuing practice of investigating the generic limits of Neotropical taxa using phylogenetic tools is a desirable one, especially after the expansion of generic names conducted by Giordani Soika between the 1970s and 1990s. Examples of obscure and/or dubious recognition of genera are Ancistroceroides de Saussure, 1855, Hypancistrocerus de Saussure, 1855, Hypodynerus de Saussure, 1855 (especially the eastern Neotropical fauna), Parancistrocerus Bequaert, 1925, and Stenodynerus de Saussure, 1863. Phylogenetic investigations followed by modern taxonomic revisions are desired for these taxa, for the somewhat simple task of describing new species be conducted with clear concepts about generic limits within the Neotropical eumenines.
Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

We thank the curators cited in the Material and Methods section for the loaning of the specimens. Thanks to Dr. Salvador Arias for providing the setk.run script and the Willi Hennig Society for allowing the use of the software TNT. LAO and WDF are supported by FAPEMIG and CNPq scholarships, respectively. Financial support was provided by FAPEMIG Process CRA-APQ-02784-17.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.rbe.2018.11.006.

References


Weighting against homoplasy improves phylogenetic analysis of morphological data sets. Cladistics 24, 758–773.


