

Systematics, Morphology and Biogeography

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

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## 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 genitalic 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.

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## 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 *Pachymenes* 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

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**Table 1**  
Morphological character list for the cladistic analyses of *Alphamenes*. Figures that do not appear in the text are available in Supplementary Material.

Head
(1) Clypeal teeth: (0) carinate (Figs. 16a, 19 and 21); (1) ecarinate (Fig. 17).
(2) Clypeal apex, middle portion: (0) strongly emarginate (Figs. 16b, 18b); (1) weakly emarginate (Fig. 17b).
(3) Clypeal dimensions: (0) wider than long (Fig. 18c); (1) longer than wide (Fig. 16c).
(4) Labrum apex: (0) truncated (Fig. 19e); (1) rounded (Fig. 20e).
(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).
(6) Frons medially: (0) with furrow (Figs. 16d, 17d and 4d); (1) without furrow (Fig. 21d).
(7) Male antennae: (0) with tyloids (Figs. 22a, 23a and 27a); (1) without tyloids.
(8) Shape of male tyloids (applicable only to terminals that received state 0 for character 7): (0) narrow (Figs. 22a and 23a); (1) wide (Fig. 27a).
(9) F11 of male antennae: (0) long, reaching or almost reaching the apex of F9 (Fig. 22b); (1) short, clearly not reaching the apex of F9 (Fig. 25b).
(10) Shape of male F11: (0) pointed (Fig. 24c); (1) rounded (Fig. 26c).
Mesosoma
(11) Lateral surface of pronotum: (0) depressed (Hermes and Oliveira, 2016, p. 191, Fig. 13); (1) flat to convex (Hermes and Oliveira, 2016, p. 191, Fig. 14).
(12) Pronotal carina of pronotum: (0) complete (Fig. 30a); (1) incomplete (Fig. 31a).
(13) Pronotal carina, humeral region: (0) slightly sinuous (Fig. 32b); (1) strongly sinuous (Fig. 33b).
(14) Male pronotum: (0) without fovea (Fig. 34a); (1) with fovea (Fig. 30c).
(15) Mesepisternum I: (0) without epicnemial carina; (1) with epicnemial carina (Fig. 32).
(16) Mesepisternum II: (0) with transverse sculpture adjacent to meso-metapleural carina (Figs. 35 and 36b); (1) without transverse sculpture adjacent to meso-metapleural carina (Fig. 34b).
(17) Mesepisternum III: (0) strongly punctate (Figs. 35c and 36c); (1) weakly punctate or unpunctate (Fig. 34c).
(18) Longitudinal mesepisternal sulcus: (0) without crenulae (Fig. 29d); (1) with crenulae (Fig. 28d).
(19) Tegulae, posterior margin: (0) slightly convex, posterior lobe well defined (Fig. 37d); (1) strongly convex, rounded, posterior lobe ill-defined (Fig. 38d).
(20) Dorsal surface of tegulae: (0) reticulate (Figs. 37e and 40); (1) slightly punctate (Fig. 38e); (2) strongly punctate (Fig. 39e).
(21) Sulcus between mesoescutum and metanotum: (0) highly developed (Figs. 42a and 33a); (1) little or not developed (Fig. 41a).
(22) Metanotum: (0) with lateral longitudinal carina adjacent to disc (Figs. 42b and 43b, 44b); (1) without lateral longitudinal carina adjacent to disc (Fig. 45b).
(23) Punctures on lateral region of propodeum: (0) evident; dense (Fig. 46a); (1) obsolete or absent (Fig. 34f).
(24) Propodeum: (0) swollen dorsolaterally (Figs. 50a and 53); (1) not swollen dorsolaterally (Figs. 51a and 52a).
(25) Median sulcus on posterior surface of propodeum, lower portion: (0) deeply depressed (Fig. 49b); (1) not deeply depressed (Fig. 47b).
(26) Median concavity on posterior surface of propodeum: (0) long, reaching half of propodeum (Fig. 48c); (1) short, not reaching half of propodeum (Fig. 49c).
Metasoma
(27) T1 in dorsal view: (0) gradually expanding towards apex (Figs. 51b, 52b and 54c); (1) abruptly expanding medially (Fig. 57c); (2) abruptly expanding towards apex, campanulate (Figs. 50b and 53c); (3) expanded all along (Fig. 56c).
(28) T1: (0) strongly punctate (Figs. 50c, 52c, 53a, 54a and 55a); (1) slightly punctate (Figs. 51c and 57c).
(29) Apical depression on T1: (0) strongly developed (Fig. 57b); (1) slightly developed or absent (Figs. 53b and 56b).
(30) T2 shape in dorsal view: (0) rounded, as wide as long (Fig. 58a); (1) elongate (Fig. 59a).
(31) Punctuation on T2: (0) dense and deep (Fig. 58b); (1) scarce and shallow (Fig. 59b).
(32) Apex of T2: (0) with lamella (Fig. 60c); (1) without lamella (Fig. 61c).
(33) Female S2: (0) densely setose (Fig. 62d); (1) sparsely setose (Fig. 63d).
(34) Male S7: (0) without carina (Fig. 4 and 65); (1) with carina (Figs. 2, 3, 5, 6, 7 and 64).
(35) Male S7, shape of carina (applicable only to terminals that received state 1 for character 34): (0) with longitudinal median carina (Figs. 2 and 3); (1) with transverse median carina (Figs. 6 and 7).
Male genitalia
(36) Ventral lobi 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 and Carpenter, 2012, p. 15, Fig. 37).
(37) Apex of the aedeagus: (0) with sulcus (Fig. 67 (Hermes and Oliveira, 2016)); (1) without sulcus (Fig. 66 (Hermes and Oliveira, 2016)).
(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).
(39) Ventral margin of aedeagus II: (0) with accessory lobe (Fig. 9e); (1) without accessory lobe (Hermes and Oliveira, p. 193, Fig. 32).
(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).
(41) Digitus: (0) without median projection adjacent to lamella (Hermes and Oliveira, 2016, p. 193, Fig. 34); (1) with median projection adjacent to lamella (Fig. 8a).
(42) Cuspis: (0) without median strong setae (Hermes and Oliveira, 2016, p. 193, Fig. 34); (1) with median strong setae (Fig. 8b).

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 Villemanant); MNHNPY – 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 Moure, 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).

**Table 2**

Character matrix used for phylogenetic analyzes. The symbols ‘-’ and ‘?’ correspond to inapplicable and unobserved data, respectively.

	Characters																																																	
Terminals	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	4	4	4						
	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2								
<i>Monobia angulosa</i>	1	1	1	1	1	0	1	-	0	1	1	0	0	1	1	1	0	1	0	0	0	0	0	1	1	1	3	1	1	0	1	1	1	1	-	5	0	0	1	1	0	0								
<i>Minixi brasiliannum</i>	0	0	1	0	0	0	0	0	0	0	0	1	1	1	1	0	1	1	0	1	1	0	1	1	0	0	1	0	0	0	1	-	0	0	0	1	1	0	0	1	1	0	0							
<i>Minixi mexicanum</i>	1	0	1	0	0	0	0	1	0	0	0	0	1	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	0	1	0	0	1	1	?	?	0	1	1	0	0					
<i>Minixi suffusum</i>	1	0	1	0	0	0	0	1	0	0	0	0	1	1	1	1	0	1	1	0	1	1	0	1	1	1	0	0	1	0	0	0	0	1	-	0	0	0	1	1	0	0	1	1	0	0				
<i>Minixi tricoloratum</i>	0	0	1	0	1	0	0	1	1	0	0	0	1	1	1	0	1	0	1	2	1	1	0	1	1	0	0	1	1	1	0	0	0	-	0	1	0	1	1	0	1	1	0	0						
<i>Minixi arechavaletae</i>	0	0	1	0	1	0	0	1	0	0	0	0	1	0	0	0	1	1	0	1	0	1	1	0	0	1	2	0	1	1	1	1	0	0	0	1	1	1	1	1	0	0	0	1	0	1	1	0	0	
<i>Minixi sumichrasti</i>	0	0	1	0	1	0	0	0	0	0	0	0	1	0	1	0	1	0	1	1	0	1	1	0	0	0	1	2	0	1	1	1	1	1	1	-	0	1	0	1	1	0	0	1	1	0	0			
<i>Minixi uruguayense</i>	0	0	0	0	1	0	?	?	?	?	0	0	0	1	0	0	0	1	0	1	0	1	1	0	0	0	2	0	1	1	1	0	1	?	-	?	?	?	?	?	?	?	?	?	?	?	?			
<i>Minixi bifasciatum</i>	1	0	1	0	1	0	0	1	0	0	0	1	0	0	0	0	1	1	0	1	0	0	0	1	2	0	1	1	1	1	1	1	-	4	1	0	1	1	0	0	1	1	0	0						
<i>Minixi jorgenseni</i>	0	0	1	0	1	0	0	1	0	0	0	0	1	0	1	0	1	1	1	1	0	0	0	1	2	0	1	1	1	1	1	1	-	0	1	0	1	1	0	0	1	1	0	0						
<i>Minixi brethesi</i>	0	0	1	0	0	0	0	1	0	0	0	0	1	0	1	0	1	1	0	0	1	0	0	0	2	0	1	1	1	1	0	1	-	?	?	?	?	?	?	?	?	?	?	?	?					
<i>Minixi atrum</i>	0	0	1	0	?	?	0	0	0	?	0	?	?	?	?	?	?	?	1	0	?	?	?	0	0	1	?	0	0	1	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?				
<i>Minixi mariachii</i>	0	0	1	0	0	1	0	1	0	0	0	0	1	0	1	0	1	1	0	1	1	0	0	1	1	0	1	1	1	1	0	1	1	-	0	0	0	1	1	0	0	1	1	0	0					
<i>Alphamenes campanulatus</i>	0	0	1	0	1	0	0	0	0	0	1	0	0	1	1	1	0	0	1	0	1	0	0	1	1	0	1	1	0	1	1	0	1	0	0	1	0	0	1	0	0	1	0	0	1	1	0	0		
<i>Alphamenes incertus</i>	0	0	1	0	1	0	0	0	0	0	1	0	0	1	1	1	0	0	1	0	?	0	0	1	1	1	0	1	1	0	1	1	?	?	0	1	2	0	1	0	0	1	1	0	0	1	1			
<i>Alphamenes insignis</i>	0	0	1	0	0	0	0	0	0	0	1	0	0	1	1	1	0	0	1	0	0	1	1	0	0	1	1	0	0	1	1	1	1	-	3	0	1	0	0	0	1	1	0	0	1	1				
<i>Alphamenes richardsi</i>	0	0	1	0	0	1	0	0	0	0	1	0	0	1	1	1	0	0	1	1	0	0	0	1	1	0	0	1	1	1	1	1	1	-	0	0	1	0	0	1	1	0	0	1	1	0				
<i>Alphamenes usitatus</i>	0	1	1	0	0	0	0	0	0	1	0	0	1	1	1	0	0	1	0	1	0	0	1	1	1	0	1	1	1	1	1	1	0	1	2	0	1	0	0	1	1	0	0	1	1	0	0			
<i>Alphamenes convexus</i>	0	?	?	?	?	?	0	0	0	0	1	0	?	1	1	?	?	?	1	?	?	?	?	0	1	1	?	0	1	1	0	1	?	?	0	0	1	0	0	0	0	0	1	1	0	0				
<i>Alphamenes semiplanus</i>	0	0	1	?	?	?	0	0	0	0	1	0	?	1	?	0	?	1	0	?	?	?	0	1	1	?	0	1	1	1	1	?	?	?	0	0	1	0	0	0	0	0	1	1	0	0				
<i>Pachymenes ater</i>	0	0	1	1	1	1	0	0	0	0	1	1	-	0	0	1	1	0	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	0	0	1	0	1	1	1	0	0	1	1	0	0		
<i>Pachymenes sericeus</i>	0	0	1	1	1	0	?	?	?	?	1	1	-	0	0	1	1	0	1	0	1	1	1	1	1	1	1	1	1	0	1	1	0	?	?	0	1	0	1	1	0	0	1	1	0	0				
<i>Pachymenes picturatus</i>	1	0	1	0	0	1	0	1	0	0	1	1	-	0	0	1	1	0	0	0	1	1	1	1	1	0	1	1	1	1	1	0	1	0	0	0	0	1	0	1	0	1	1	0	1	1	0	1		
<i>Laevimenes laevigatus</i>	0	0	1	0	1	0	0	0	0	0	1	0	0	1	1	1	0	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	0	0	1	0	1	
<i>Laevimenes morbilosus</i>	0	0	1	0	1	0	0	0	0	0	1	0	0	1	1	1	1	0	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	0	0	1	0	1	

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 stereoscope 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 *Alphamenes* 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 *Alphamenes* 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 *Laevimenes* Giordani Soika, 1978 and *Pachymenes* de Saussure, 1852 were included in the analyzes. The inclusion of these taxa in the analyzes aimed at testing the monophyly of the ingroup genera (mainly *Alphamenes* 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

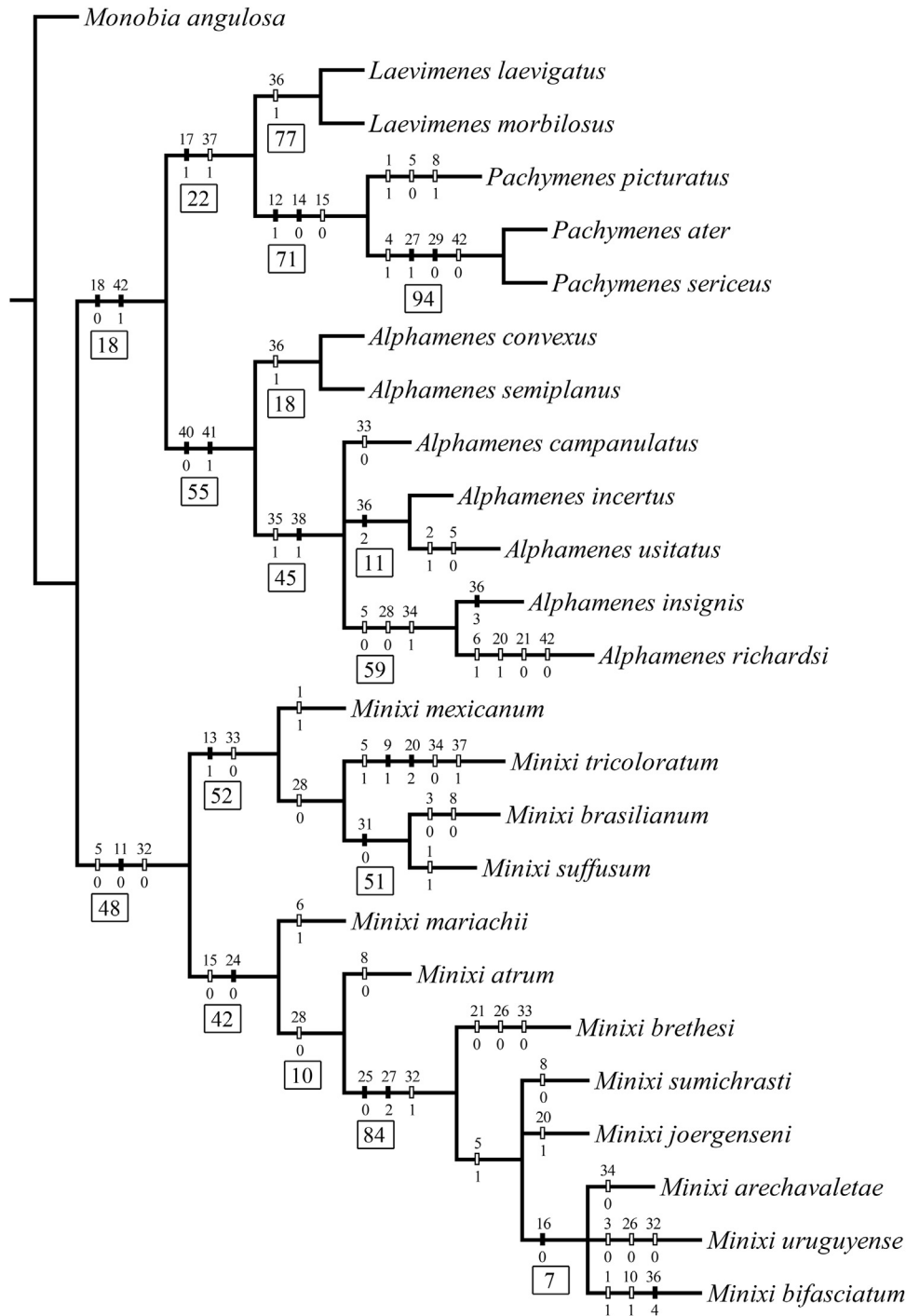
character weighing (Goloboff, 1993; Goloboff et al., 2008). The value for the constant *k* was calculated with the script *setk.run* (*k*=3.046875). In TNT the “New Technology Search” option was used following Hermes et al. (2014) and Hermes and Oliveira (2016).

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., 2003) 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 *Pachyminixi* of Giordani Soika (1978), with the



**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.

inclusion of *M. mariachii* 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 Schulthess, 1904) clade ([Fig. 1](#)) and the transitional morphological condition of *M. mariachii* [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.





**Figs. 2–7.** Male sternum 7 (arrows): 2. *Alphamenes convexus* (Fox, 1899); 3. *A. semiplanus* Giordani Soika, 1978; 4. *A. insignis* (Fox, 1899); 5. *A. campanulatus* (Fabricius, 1804); 6. *A. incertus* (de Saussure, 1875); 7. *A. usitatus* (Fox, 1899). Figs. 2, 3, 4, 5 and 7 modified from Oliveira et al., 2017. Scale bars for Figs. 2, 3, 4, 5 = 1 mm; Figs. 6, 7 = 0.5 mm.

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.

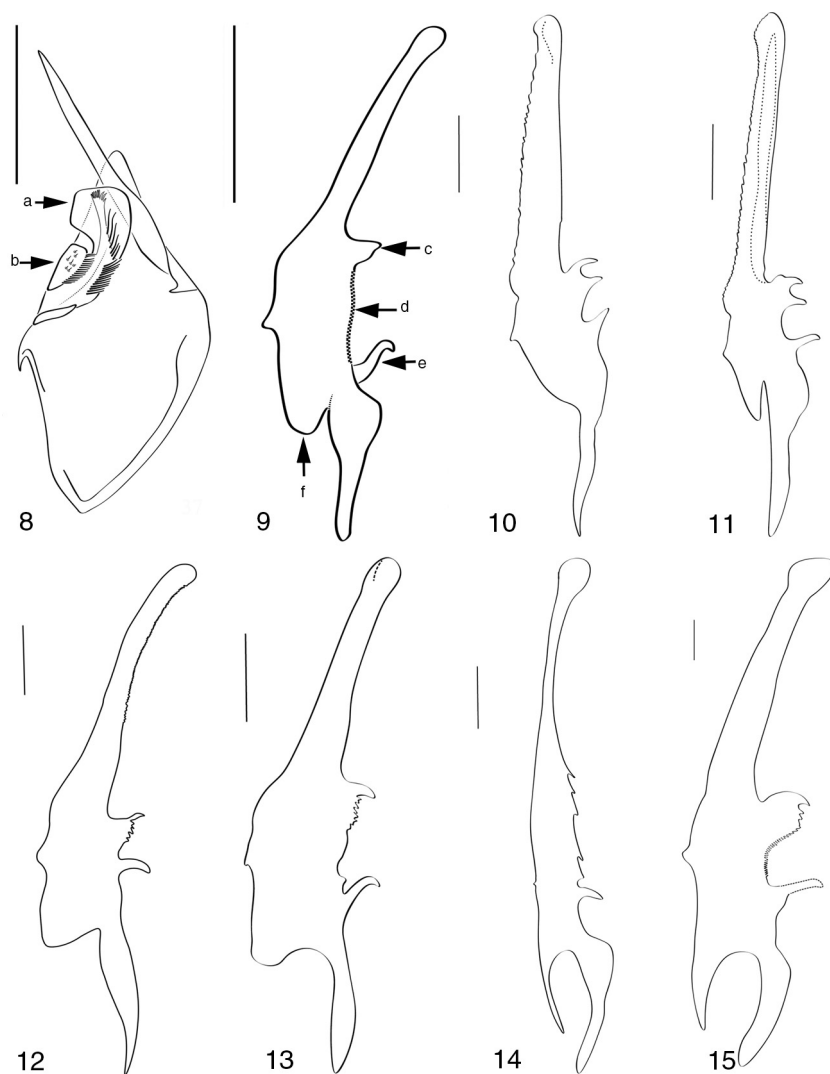
## Conclusion

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.



**Figs. 8–15.** Male genitalia. Figs. 8 and 9. *A. campanulatus* (Fabricius, 1804): 8. Gonocoxite and volsela in lateral view; 9. Aedeagus in lateral view (a. digitus; b. cuspsis; c. ventral lobi of the penis valves; d. ventral marg in of aedeagus; e. accessory lobe; f. basal apodeme of aedeagus;) (modified from [Hermes and Oliveira, 2016](#)). Figs. 10–15. Ventral lobi of the penis valves (aedeagus): 10. *A. convexus* (Fox, 1899); 11. *A. semiplanus* [Giordani Soika, 1978](#); 12. *A. incertus* (de Saussure, 1875); 13. *A. usitatus* (Fox, 1899); 14. *A. richardsi* [Giordani Soika, 1978](#); 15. *A. insignis* (Fox, 1899). Scale bars for Figs. 10–12 and 14–15 = 0.2 mm; Fig. 13 = 0.5 mm.

### Conflicts of interest

The authors declare no conflicts of interest.

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### 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](https://doi.org/10.1016/j.rbe.2018.11.006).

### References

Bank, S., Sann, M., Mayer, C., Meusemann, K., Donath, A., Podsiadlowski, L., Kozlov, A., Petersen, M., Krogmann, L., Meier, R., Rosa, P., Schmitt, T., Wurdack, M., Liu, S., Zhou, X., Misof, B., Peters, R., Niehuis, O., 2017. [Transcriptome and target](#)

[DNA enrichment sequence data provide new insights into the phylogeny of vespid wasps \(Hymenoptera: Aculeata: Vespidae\)](#). *Mol. Phylogenet. Evol.* 116, 213–226.

Bitsch, J., 2012. [Morphologie comparée des derniers segments du gastre et des genitalia mâles des Vespidae. 1. Sous-famille des Eumeninae \(Hymenoptera\)](#). *Bull. Soc. Entomol. France* 117 (2), 199–218.

Carpenter, J.M., 1982. [The phylogenetic relationships and natural classification of the Vespoidea \(Hymenoptera\)](#). *Syst. Entomol.* 7, 11–38.

Carpenter, J.M., Garcete-Barrett, B.R., 2003. [A key to the Neotropical genera of Eumeninae \(Hymenoptera: Vespidae\)](#). *Bol. Mus. Nac. Hist. Nat. Paraguay* 14 (1–2), 52–73.

Ferreira, W.D., Grandinete, Y.C., Lopes, R.B., Hermes, M.G., 2015. [A new contribution to the knowledge of Neotropical Eumeninae \(Hymenoptera Vespidae\)](#). *Zootaxa* 3981 (1), 117–124.

Ferreira, W.D., Oliveira, L.A., Inez, T.G., Hermes, M.G., 2017. [A new species of \*Pirhosigma\* Giordani Soika, 1978 \(Hymenoptera, Vespidae: Eumeninae\), with additional notes and a key to the species in the genus](#). *Zootaxa* 4300 (2), 269–278.

Ferreira, W.D., Oliveira, L.A., Hermes, M.G., 2018. [A revision of the Neotropical eumenine wasp genus \*Stenosigma\* Giordani Soika, 1978 \(Hymenoptera, Vespidae\)](#). *Zootaxa* 4455 (3), 499–512.

Fitch, W.N., 1971. [Toward defining the course of evolution: minimum change for a specified tree topology](#). *Syst. Zool.* 20, 406–416.

Giordani Soika, A., 1978. [Revisione degli Eumenidi neotropicali appartenenti ai generi \*Eumenes\* Latr., \*Omicron\* \(Sauss.\), \*Pararhaphidoglossa\* Schulth. ed affini](#). *Boll. Mus. Civ. Stor. Nat. Venezia* 29, 1–420.

Goloboff, P.A., 1993. [Estimating character weights during tree search](#). *Cladistics* 9, 83–91.

Goloboff, P.A., Farris, J.S., Källersjö, M., Oxelmann, B., Ramírez, M., Szumik, C., 2003. [Improvements to resampling measures of group support](#). *Cladistics* 19, 324–332.

- Goloboff, P.A., Carpenter, J.M., Arias, J.S., Esquivel, D.R.M., 2008. [Weighting against homoplasy improves phylogenetic analysis of morphological data sets](#). *Cladistics* 24, 758–773.
- Goloboff, P.A., Catalano, S.A., 2016. [TNT version 1.5, including a full implementation of phylogenetic morphometrics](#). *Cladistics* 32, 221–238.
- Grandinete, Y.C., Hermes, M.G., Noll, F.B., 2015. [Systematics and phylogeny of the Neotropical \*Pachymenes\* de Saussure and \*Santamenes\* Giordani Soika \(Hymenoptera, Vespidae Eumeninae\)](#). *Syst. Entomol.* 40, 365–384.
- Hermes, M.G., Carpenter, J.M., 2012. [Are \*Monobia\* and \*Montezumia\* monophyletic? A cladistic analysis of their species groups based on morphological data \(Hymenoptera, Vespidae, Eumeninae\)](#). *Am. Mus. Nov.* 3733, 1–23.
- Hermes, M.G., Melo, G.A.R., Carpenter, J.M., 2014. [The higher level phylogenetic relationships of the Eumeninae \(Insecta, Hymenoptera Vespidae\), with emphasis on \*Eumenes\* sensu lato](#). *Cladistics* 30, 453–484.
- Hermes, M.G., Oliveira, L.A., 2016. [Morphological cladistics analysis resolves the generic limits of the Neotropical potter wasps genera \*Minixi\* Giordani Soika and \*Pachyminixi\* Giordani Soika \(Hymenoptera: Vespidae: Eumeninae\)](#). *Invertebr. Syst.* 30, 187–200.
- Hines, H.M., Hunt, J.H., O'Connor, T.K., Gillespie, J.J., Cameron, S.A., 2007. [Multigene phylogeny reveals eusociality evolved twice in vespid wasps](#). *Proc. Natl. Acad. Sci. U.S.A.* 104 (9), 3295–3299.
- Nixon, K.C., 1999–2002. *Winclada*, ver. 1.0000. Published by the author. Ithaca, New York.
- Nixon, K.C., Carpenter, J.M., 1993. [On outgroups](#). *Cladistics* 9, 413–426.
- Oliveira, L.A., Ferreira, W.D., Inez, T.G., Hermes, M.G., 2017. [Contributions to the taxonomy of \*Minixi\* Giordani Soika and \*Alphamenes\* van der Vecht \(Hymenoptera, Vespidae Eumeninae\), with an identification key to the species of the latter](#). *Zootaxa* 4317 (3), 530–540.
- Pickett, K.M., Carpenter, J.M., 2010. [Simultaneous analysis and the origin of eusociality in the Vespidae \(Insecta: Hymenoptera\)](#). *Arthropod Syst. Phylo.* 68 (1), 3–33.
- Piekarski, P.K., Carpenter, J.M., Lemmon, A.R., Lemmon, E.M., Sharanowski, B.J., 2018. [Phylogenomic evidence overturns current conceptions of social evolution in wasps \(Vespidae\)](#). *Mol. Biol. Evol.* 35 (9), 2097–2109.
- Selis, M., 2017. [A new species of \*Minixi\* Giordani Soika, 1978 from Argentina \(Hymenoptera Vespidae, Eumeninae\)](#). *Linzer Biol. Beitr.* 49 (1), 687–690.