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Phylogeny of hornets: a total evidence approach (Hymenoptera, Vespidae, Vespinae, *Vespa*)

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Abstract

The only previous comprehensive phylogenetic analysis of the 22 species of the genus *Vespa* was based on just 11 morphological characters and resulted in only limited resolution. In order to improve the phylogenetic inference, we carried out a simultaneous analysis with 45 morphological characters and data from four mitochondrial and two nuclear genes. The results support a number of the previously found relationships. The monophyly of the genus *Vespa* and the existence of a main clade excluding *V. basalis* and *V. binghami* are confirmed. The *tropica* group is supported. The *affinis* group is not supported; molecular data relate the previously unresolved *V. orientalis* to *V. affinis* + *V. mocsaryana*.

Keywords

Hornet, Phylogeny, Total Evidence, Vespidae

Introduction

The genus *Vespa* is one of the four genera of the subfamily Vespinae; it is composed of 22 extant species of hornets (Archer 1991, Nguyen et al. 2006). Most species have a distribution restricted to Asia, with the highest diversity found in northern Indo-

Malaya (Matsuura and Yamane 1990, Carpenter and Kojima 1997). Two species are also naturally distributed outside of Asia: *Vespa crabro* is found in Europe and around the Black Sea and the Caspian Sea and *V. orientalis* in the north of Africa, Mediterranean regions and across the Middle-East. In the mid-19th century *V. crabro* was introduced into North America where it is now established (de Saussure 1898), while more recently, *V. velutina* was accidentally introduced into Europe, where it became invasive (Villemant et al. 2011). Several hornet species have been the subject of various biological studies, either because of their social habits (Matsuura 1991, Foster et al. 2000, Ishay et al. 2008), their threat to human health (Vetter et al. 1999), their impact on apiculture (Abrol 1994, Ranhabat et al. 2009) or even their interest as edible insects (Ying et al. 2010). However, the phylogeny of this genus is not yet well resolved.

The first cladistic study of *Vespa* was that of Archer (1994a), based on 11 morphological characters (10 binary and one multistate). It distinguished a main clade comprising most *Vespa* species, and two species unresolved at the base of the entire tree (Fig. 1). One of them, *V. binghami*, is the only *Vespa* species presenting morphological adaptation to nocturnal habits (van der Vecht 1959). The other unplaced species of Archer's cladogram, *V. basalis*, is a small hornet readily distinguishable by its reduced punctation, especially on the clypeus. The main clade found by Archer (1994a) presented an unresolved basal node with four lineages, one comprising a single species (*V. orientalis*) and a second he termed the *crabro* group (*V. crabro* and *V. dybowskii*). The third lineage, or *tropica* group, included five species in two clades: one with *V. mandarinia* + *V. soror*, and the other with three species (*V. ducalis, V. philippinensis* and *V. tropica*). Archer's last lineage, called the *affinis* group (Fig. 1), was composed of the 12 remaining species including an unresolved clade of four species which have very similar morphology (*V. bicolor*, *V. simillima*, *V. velutina* and *V. vivax*). This unresolved clade was termed the *bicolor* group by Archer in another paper (1994b) and was sister-group of two unresolved species from so-called Sundaland: *V. bellicosa* and *V. multimaculata*.

Archer's study was based mostly on male characters, which are known to be reliable phylogenetic characters (e. g. Song and Bucheli 2010), but his cladogram has many unresolved relationships. Furthermore, some of the lineages are only supported by single characters from female morphology, such as the *tropica* group, which is characterized by the female clypeus shape. Corroborating such groups thus requires further analyses.

This study presents results from an ongoing project on the evolution of vespine wasps, focusing on the genus *Vespa*. Our aims are to confirm the monophyly of the genus *Vespa*, a question not addressed by Archer (1994a), and to clarify relationships among the species based on a more extensive morphological matrix and combining molecular data.

Methods

Phylogenetic relationships were inferred for the 22 species of the genus *Vespa* and five other species of Vespidae as outgroup: *Dolichovespula media* (Retzius, 1783), *Provespa anomala* (de Saussure, 1854), *Provespa barthelemyi* (du Buysson, 1905) and *Vespula ger-*

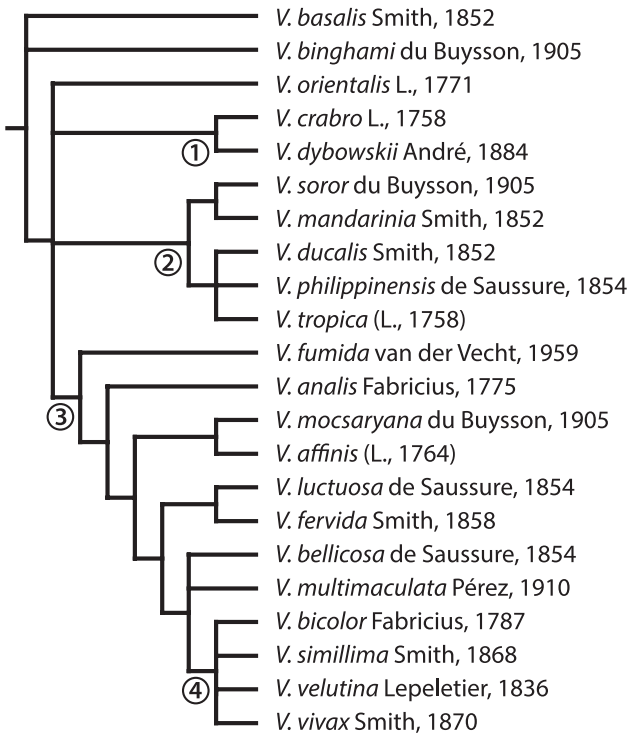

Figure 1. Phylogeny of the genus *Vespa* after Archer (1994a: figure 8). Tree updated for the current classification (Nguyen et al. 2006). The species groups discussed in this paper are as follows: **1** *crabro* **2** *tropica* **3** *affinis* **4** *bicolor sensu* Archer (1994b).

manica (Fabricius, 1793) from the subfamily Vespinae and *Polistes dominula* (Christ, 1791) from the Polistinae.

The morphological matrix was scored from specimens in the following natural history collections: American Museum of Natural History, Muséum National d'Histoire Naturelle, Nationaal Natuurhistorisch Museum and United States National Museum of Natural History. Specimens for molecular study were collected by J.K. and J.M.C. and preserved in 95 - 99% ethanol.

DNA was extracted from one leg and one antenna per specimen using QIAGEN "DNeasy tissue Kit". Genes were amplified using PCR with PuReTaq Ready-To-Go beads in a total volume of 25μL including primers (Appendix 1) and DNA. Amplification cycles were specific to genes (Appendix 2). AMPures and CleanSEQ procedures were used for DNA purification and sequencing was performed on ABI PRISM 3730xl machines by Agencourt Biosciences (Beverly, USA). One missing gene fragment of *V. basalis* was obtained from Genbank database (accession number: AB585949).

The analyses are based on 45 morphological characters and multiple nuclear and mitochondrial loci, comprising: 374 sites of 12S, 528 sites of 16S, 2231 sites of 28S (sequenced in 4 fragments), 1442 sites of CO1 (sequenced in 2 fragments), 880 sites of elongation factor 1α (EF1α), and 328 sites of H3. Each of these genes was aligned

separately using the MAFFT software with the L-INS-i algorithm (Katoh et al. 2005). Morphological characters were coded with Winclada V. 1.00.08 (Nixon 2002).

Phylogenetic analyses were performed in a parsimony framework using TNT (Goloboff et al. 2008). Analyses were first conducted on the morphological matrix and on the different molecular datasets separately, then on a matrix of all the data combined. Sequence alignments were merged with Winclada, and the final composite molecular matrix contained 5783 aligned nucleotides. Each analysis was performed with the new technology search algorithms including sectorial searches, the parsimony ratchet, tree drifting and tree fusing, default parameters except: 200 ratchet iterations, upweighting percentage 8, downweighting 4; 50 cycles of drift; minimum length hit 25 times. Molecular gaps were treated as missing data. Node supports were computed as GC-values of a symmetric resampling of 1000 replicates (group supported/contradicted values; Goloboff et al. 2003). GC values range from -1 to 1 and are the differences in group frequency between the group found in most parsimonious trees and the most frequent contradictory group. Only supported groups (support value above zero, scaled by TNT 0-100) are shown.

Results

Morphological analysis

Analysis of the 45 morphological characters (Appendix 3) resulted in a single most parsimonious tree (not shown; Length: 107, Consistency Index (CI): 0.617, Retention Index (RI): 0.784). The support tree is shown in Fig. 2. The only difference is that the most parsimonious cladogram resolves *V. orientalis* as the sister species to the clade composed of *mandarinia* + *soror* and the *affinis* group; the support tree (Fig. 2) does not include this node. On both trees the genus *Vespa* appears as monophyletic, and *V. basalis* the sister species of the rest of the genus, followed by *V. binghami*. The 20 remaining species are grouped in three clades, relationships among which are not resolved (Fig. 2): a clade with only *V. orientalis*, the second comprising *crabro* + *dybowskii* together with *V. tropica* and its two closely related species, and the third clade with the remaining 14 species. The *tropica* group *sensu* Archer (1994a) is thus not monophyletic according to these morphological data: *V. mandarinia* + *V. soror* are not closely related to *V. tropica*, rather they form the sister group of what corresponds to the *affinis* group of Archer. Finally, *V. bellicosa* and *V. multimaculata* are internal components of a clade including the four species of the *bicolor* group *sensu* Archer (1994b).

Molecular analyses

Of the 27 studied species, specimens relatively recently collected were available for 17 species (including the outgroups). Due to low quality DNA templates and the use

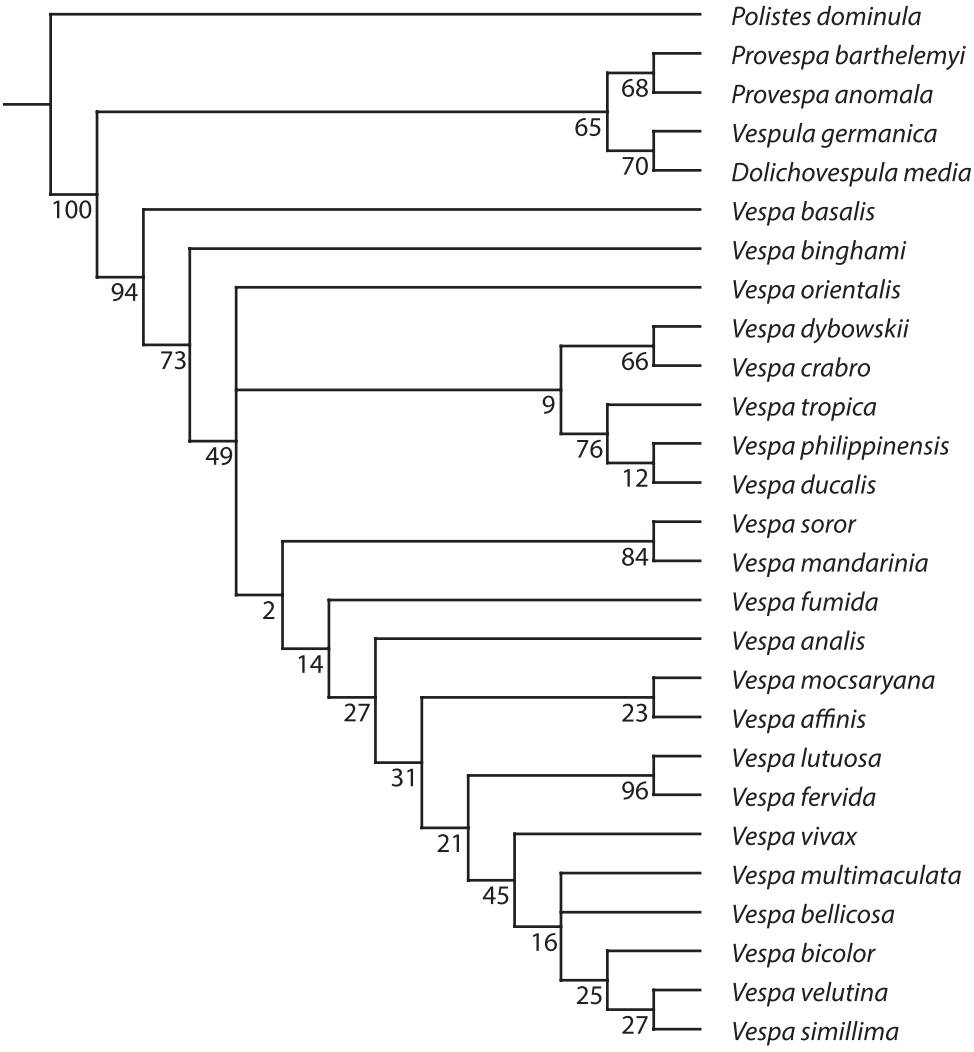

Figure 2. Support tree for relationships among the 22 *Vespa* species based on 45 morphological characters. Supports for nodes are given in GC-values (see text for explanation) when they are greater than zero.

of non-specific primers, molecular data are not homogeneous across the genus. The monophyly of the genus *Vespa* was found in most analyses based on single genes and in the analysis of merged alignments (Table 1, Fig. 3). The main species-groups appear monophyletic over most analyses except for the low variation 28S. *Vespa basalis* remains the basal species in the genus. Two main clades diverge in the remaining species: the monophyletic *tropica* group is the first clade, while the *bicolor* group and a clade of *V. crabro*, *V. mocsaryana* and *V. affinis* form the second. The *bicolor* group *sensu* Archer (1994b) is supported in all molecular analyses, with *V. vivax* being resolved as the sister species of *V. velutina*.

Table 1. Results of phylogenetic analyses of molecular data for the genus *Vespa*. Marker: gene used in the analysis. "Multi-locus" marker is a combination of all genes used. N tree: number of most parsimonious trees. Length: length of the most parsimonious trees. *Vespa*, *bicolor*, *mandarinia*, *tropica*: monophyly of the considered clade when it is tested.

marker	N tree	length	CI	RI	*Vespa*	*bicolor*	*mandarinia*	*tropica*
12S	5	261	0.709	0.651	yes	yes	yes	-
16S	2	332	0.654	0.545	yes	yes	yes	yes
28S	>1000	145	0.909	0.750	no	yes	no	no
CO1	1	1431	0.508	0.369	yes	yes	yes	-
EF1a	1	219	0.886	0.819	yes	yes	yes	-
H3	1	81	0.889	0.690	no	yes	-	-
Multi-locus	207	2494	0.620	0.468	yes	yes	yes	yes

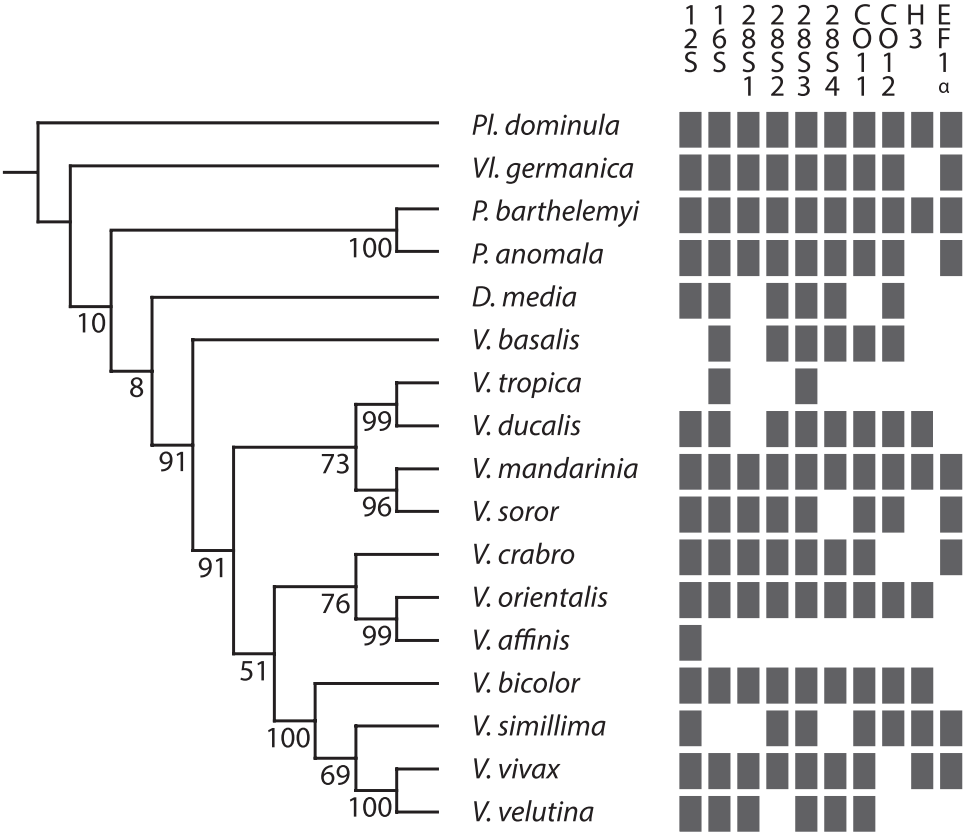

Figure 3. Support tree for the relationships among 13 *Vespa* species based on the six genes. Supports for nodes are given in GC-values. Grey rectangles show the molecular markers available for each species.

Total evidence analysis

The combined analysis of morphological and molecular data returned eight equally parsimonious trees all showing *Vespa* monophyletic with *V. basalis* as the sister species of the rest of the genus (not shown; Length: 2665, CI: 0.608, RI: 0.488). The consensus tree (not shown) is completely resolved except for a node including the *bicolor* group, *V. bellicosa* and *V. multimaculata*. In the support tree (Fig. 4), two internal nodes are collapsed, because of a different position of *V. fumida*.

The monophyly of the genus is supported by five synapomorphies: the long prestigma, the developed vertex, the strongly elevated interantennal space, the presence of a carina on the hindcoxa, and the projection at the apex of the digitus in males.

The addition of molecular data to the morphological matrix resulted in stronger support of the *tropica* group and resolved the position of *V. orientalis*, as part of a clade with two morphologically different species, *V. affinis* and *V. mocsaryana*, close to *V. crabro*. The *affinis* group *sensu* Archer (1994a) thus did not appear monophyletic. These changes resulted in fewer steps for the morphological character of the clypeal apical margin, a synapomorphy of the *tropica* group, while the CIs of eight other morphological characters (five pronotal and head characters and three male characters) diminished.

In the combined analysis tree, most of the clades of *Vespa* species are supported by morphological characters, five of which are uncontroverted synapomorphies. *Vespa basalis* is distinguished from the other species on the basis of the aedeagal apical lobes in males and the edges of the interantennal space. The main clade excluding *V. basalis* and *V. binghami* is supported by the clypeal punctures dense mesally in females and the emarginated apical margins of the metasomal sterna VI and VII in males.

Within the main *Vespa* clade, the *tropica* group is morphologically supported by the triangular apico-lateral angles of female clypeus only, but molecular data confirms this homology. Within this group, *V. mandarinia* + *V. soror* is defined by two uncontroverted characters: the spade-shape of the aedeagus apex in males and the expansion of the gena behind the eyes. The three other species of the *tropica* group share the presence of pronotal striae, long first metasomal segment and marked scutal and metapleural punctures.

The two species of the *crabro* group share two secondary reversions: the straight apical margin of the male metasomal sternum VI and the loss of digital apical process. *Vespa affinis* and *V. mocsaryana* share the posteromedially deeply emarginated male metasomal sterna VI and VII and long first metasomal segment. The clade consisting of *V. orientalis* and *V. affinis* + *V. mocsaryana* is supported by the short malar space, a homoplastic synapomorphy found also in the clade of *V. analis*, *V. luctuosa* + *V. fervida*, *V. multimaculata*, *V. bellicosa* and the *bicolor* group. This latter clade is also supported by the bulbous aedeagal shaft in males. The clade of these species but *V. analis* is morphologically supported by a pretegular carina ventrally effaced, the presence of a few ventral striae on the female pronotum and the apical margin of the male metasomal sternum VII deeply emarginate. *Vespa fervida* + *V. luctuosa* is supported by the well

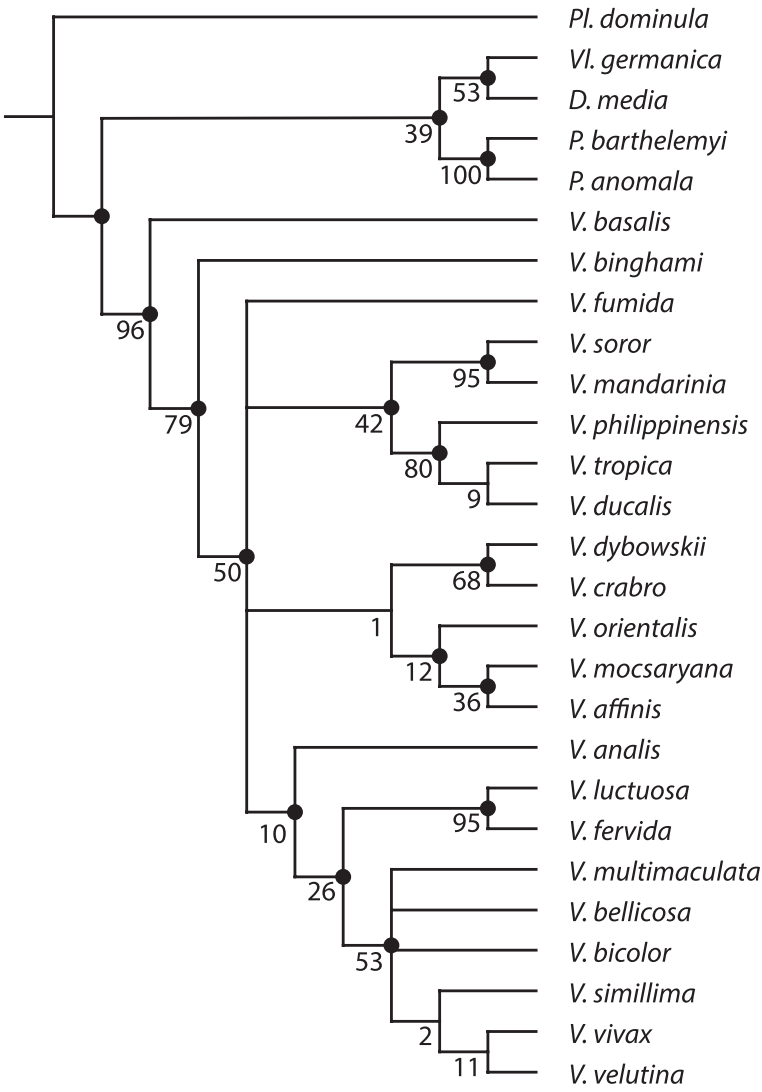

Figure 4. Support tree for the relationships within the genus *Vespa* based on a combined analysis. Support tree based on 45 morphological characters and six genes. Black nodes indicate clades supported by morphological characters. Absence of mark on nodes indicates clades diagnosed by molecular data only. Supports for nodes are given in GC-values.

defined punctures on metapleura and lateral faces of the metasomal tergum II as well as the uncontroverted synapomorphy of the median process in the apical margin of the metasomal sternum VII. Finally, the clade of the *bicolor* group and *V. multimaculata* and *V. bellicosa* is supported by the distinct interruption of the pronotal carina by the pronotal pit. The four remaining clades within the genus *Vespa* are not diagnosed by morphological characters. These latter clades also have low support under symmetric resampling (Fig. 4).

Discussion

Our analyses of both morphological and molecular characters confirm the monophyly of the genus *Vespa*. This genus was first diagnosed from other Vespidae on the basis of the shape of the head (Thomson 1869) and especially the vertex length, which is congruent with the other synapomorphies of the genus. In our morphological and total evidence analyses, the genus *Vespa* is the sister-group to the other vespine genera with similar relationships to those described by Carpenter (1987). The results of Carpenter (1987) based on morphology and ours based on combined analysis contradict Pickett and Carpenter (2010).

While our molecular sample is incomplete, it nonetheless confirms the monophyly of two of Archer's species groups within *Vespa* based on the morphology (*crabro* and *tropica* groups). Molecular data help to place *V. orientalis,* which both Archer's and our morphological analyses failed to resolve well. *Vespa orientalis* is the only *Vespa* species distributed in arid areas in central Asia and the Middle-East. A close relationship of this species to *V. affinis* + *V. mocsaryana* despite obvious morphological differences begs the question of morphological adaptations to arid climates in *V. orientalis* that may have blurred the morphological phylogenetic signal. However, the close relationship of *V. orientalis* and *V. affinis* is suggested only by the 12S gene in the molecular data. Further gene sequences for this last species and for *V. mocsaryana* are necessary to clarify whether the clade consisting of *V. orientalis* and *V. affinis* + *V. mocsaryana* is definitively supported.

Our results are also consistent with previous authors regarding the close relationships of *V. luctuosa* and *V. fervida*, which are very similar in their morphology (van der Vecht 1957, Archer 1994a, 1999). On the other hand, *V. bellicosa* and *V. multimaculata*, for which close relationships to *V. luctuosa* were suggested based on their distribution and morphological similarities (Bequaert 1934), are not closely related to *V. luctuosa*. They appear to form a clade together with Archer's *bicolor* group. Relationships within this last clade are still poorly resolved with low node supports. Molecular data showed a closer relationship between *V. vivax* and *V. velutina* (Fig. 3), while the morphological characters placed *V. simillima* and *V. velutina* as sister species. Such a discrepancy may have resulted from the fact that no molecular data were available for *V. multimaculata* and *V. bellicosa*.

Archer's finding of a main clade of *Vespa* excluding *V. basalis* and *V. binghami* has been confirmed both by morphological and molecular data. Our results also suggest that the nocturnal species *V. binghami* is closer to the main clade of *Vespa* than is *V. basalis*. Morphological adaptations to nocturnal habits in *V. binghami* such as enlarged ocelli are thus autapomorphies. Recognition of the subgenus *Nyctovespa*, with *V. binghami* as sole included species (van der Vecht 1959), would thus render the subgenus *Vespa* paraphyletic, and the synonymy of *Nyctovespa* (Carpenter 1987) is justified on that basis.

Our extended morphological matrix and the molecular sequences partly support Archer's results, and this analysis confirms that male characters such as the shape of the last metasomal sterna and the genitalia are reliable phylogenetic characters in Vespidae.

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Appendix 1

Primers used for sequencing the six genes.

Marker	Primer	Name	Sequence (5'-3')
12S	F	12S AI	AAACTAGGATTAGATACCCTATTAT
12S	R	12S BI	AAGAGCGACGGGCGATGTGT
16S	F	16S A	CGCCTGTTTATCAAAAACAT
16S	R	16S B	CTCCGGTTTGAACTCAGATCA
28S-1	F	28S 1A	CCCSCGTAAYTTAGGCATAT
28S-1	R	28S 4 BR	CCTTGGTCCGTGTTTCAAGAC
28S-2	F	28S 3.2a	AGTACGTGAAACCGTTCASGGGT
28S-2	R	28S Br	TCGGAAGGAACCAGCTACTA
28S-3	F	28S 4a	GGAGTCTAGCATGTGYGCAAGTC
28S-3	R	28S 5b	CCACAGCGCCGATTCTGCTTACC
28S-4	F	28S 4.8a	ACCTATTCTCAAACTTTAAATGG
28S-4	R	28S 7b1	GACTTCCCTTACCTACAT
CO1-1	F	LCO	GGTCAACAAATCATAAAGATATTGG
CO1-1	R	HCO out out	GTAAATATATGRTGDGCTC
CO1-2	F	Jerry	CAACATTTATTTTGATTTTTTGG
CO1-2	R	Pat	TCCAATGCACTAATCTGCCATATTA
EF1α	F	HaF2For	GGGYAAAGGWTCCTTCAARTATGC
EF1α	R	F2Rev1	AATCAGCAGCACCTTTAGGTGG
H3	F	H3-AF	ATGGCTCGTACCAAGCAGACVGC
H3	R	H3-AR	GTCACYATYATGCCYAAGGATAT

Appendix 2

PCR program of each marker with temperature and time (°C – minute). Den. = Denaturing phase. Anneal. = Annealing phase. Elong. = Elongation phase. N = number of cycles of Denaturing + Annealing + Elongation phases.

Marker	Den.	Anneal.	Elong.	N
12S	97 – 0.5	42 – 0.75	68 – 0.5	40
16S	94 – 0.5	42 – 0.5	72 – 0.5	40
28S-1	94 – 1	43.5 – 0.5	72 – 1	40
28S-2	94 – 1	43.5 – 0.5	72 – 1	40
28S-3	94 – 1	43.5 – 0.5	72 – 1	40
28S-4	94 – 1	40 – 0.5	72 – 1	40
CO1-1	94 – 0.5	36 / 51 – 0.5	72 – 0.5	5 / 35
CO1-2	94 – 0.5	36 / 48 – 1	72 – 1	5 / 35
EF1α	94 – 1	54 – 1	72 – 1.5	35
H3	94 – 0.4	51 – 0.5	72 – 0.75	40

Appendix 3

Matrix of morphological characters. Outgroup species are in grey.

Character	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 2 3 3 3 3 3 3 3 3 3 4 4 4 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 3 4 5
P. dominula	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0
D. media	1 1 1 1 1 1 0 0 0 0 1 1 2 0 0 0 1 1 1 0 0 2 2 1 1 1 1 1 1 1 1 0 1 0 1 0 2 0 5 0 2 0 0 0
V. germanica	1 1 1 1 1 1 2 0 0 0 1 1 2 0 0 0 1 1 3 - - 2 0 1 1 1 1 1 1 1 1 1 1 0 0 0 1 0 6 0 1 0 0 0
P. anomala	1 1 1 1 1 1 2 0 0 1 1 1 1 0 0 0 1 0 2 - 0 2 0 0 1 1 1 1 1 1 1 1 0 0 1 0 0 0 0 7 1 1 0 0 0
P. barthelemyi	1 1 1 1 1 1 0 0 0 1 1 1 1 0 0 0 1 0 2 - 0 2 0 0 1 1 1 1 1 1 1 1 0 0 1 0 0 0 0 4 0 1 0 0 0
V. affinis	2 0 1 0 1 1 0 1 0 0 3 1 1 0 1 0 0 1 0 0 0 0 0 1 0 1 1 1 1 1 1 2 1 1 1 0 2 2 0 1 1 1 1 1 0
V. analis	2 0 1 0 1 1 0 1 0 0 3 1 1 1 1 0 / 1 0 1 0 0 1 0 0 0 0 2 1 1 1 1 1 1 1 1 1 0 0 1 2 0 1 1 1 1 1 0
V. basalis	2 0 1 0 1 1 0 1 1 0 2 1 1 0 0 0 1 1 0 0 1 0 1 0 0 1 1 1 1 1 1 1 1 1 1 0 0 0 0 0 2 0 1 0 1 0
V. bellicosa	2 0 1 0 1 1 0 1 0 0 2 1 1 0 1 0 0 1 0 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 0 2 2 0 1 1 1 1 1 0
V. bicolor	2 0 1 0 1 1 0 1 0 0 2 1 1 0 0 0 0 1 0 0 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 0 2 2 0 1 1 1 1 1 0
V. binghami	2 0 1 0 1 1 1 1 1 0 1 3 1 1 0 0 0 0 1 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 0 0 0 0 0 1 1 1 0 1 0
V. crabro	2 0 1 0 1 1 0 1 0 0 3 1 1 0 1 1 0 / 1 1 0 0 0 0 0 1 0 1 1 1 1 1 1 1 1 1 1 1 0 0 0 1 0 1 1 1 0 0 0
V. ducalis	2 0 1 0 1 1 0 1 0 0 3 1 2 0 1 1 0 0 0 0 1 0 2 1 1 1 1 1 2 1 1 1 0 1 1 0 1 0 1 1 1 0 1 0
V. dybowskii	2 0 1 0 1 1 0 1 0 0 3 1 1 0 1 1 0 0 0 0 1 0 1 1 1 1 1 1 1 1 1 1 1 0 0 0 1 0 1 1 1 0 0 0
V. fervida	2 0 1 0 1 1 0 1 0 0 3 1 1 0 1 0 0 1 0 0 1 0 1 1 0 2 1 1 1 1 1 2 1 1 0 1 2 1 1 1 1 1 1 1 0
V. fumida	2 0 1 0 1 1 0 1 0 0 3 1 1 0 0 1 1 0 0 1 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 0 0 1 2 0 1 1 1 0 1 1
V. luctuosa	2 0 1 0 1 1 0 1 0 0 2 1 1 0 1 0 0 1 0 0 1 0 1 1 0 2 1 1 1 1 1 2 1 1 0 1 2 1 1 1 1 1 1 1 0
V. mandarinia	2 0 1 0 1 1 0 2 0 0 3 1 2 0 1 1 0 0 1 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 0 0 1 1 0 3 1 1 0 1 0
V. mocsaryana	2 0 1 0 1 1 0 1 0 0 3 1 1 0 1 0 0 1 0 0 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 1 0 2 2 0 1 1 1 0 1 0
V. multimaculata	2 0 1 0 1 1 0 1 0 0 2 1 1 0 1 0 0 1 0 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 0 2 2 0 1 1 1 1 1 0
V. orientalis	2 0 1 0 1 1 0 1 0 0 3 1 1 0 1 0 / 1 0 1 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 1 0 0 1 1 0 1 1 1 0 1 0
V. philippinensis	2 0 1 0 1 1 0 1 0 0 3 1 2 0 1 0 0 1 0 0 0 0 1 0 2 1 1 1 1 2 1 1 1 0 1 1 0 1 1 1 0 1 0
V. simillima	2 0 1 0 1 1 0 1 0 0 3 1 1 0 0 0 1 0 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 0 2 2 0 1 1 1 1 1 0
V. soror	2 0 1 0 1 1 0 2 0 0 3 1 2 0 1 1 0 0 1 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 0 0 0 1 0 3 1 1 0 1 0
V. tropica	2 0 1 0 1 1 0 1 0 0 3 1 2 0 1 1 0 0 0 0 0 1 0 2 1 1 1 1 1 2 1 1 1 0 0 1 0 1 1 1 0 1 0
V. velutina	2 0 1 0 1 1 0 1 0 0 2 1 1 0 0 1 0 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 0 2 2 0 1 1 1 1 1 0
V. vivax	2 0 1 0 1 1 0 1 0 0 3 1 1 0 1 0 0 1 0 0 1 1 1 0 0 1 1 1 1 1 1 1 1 1 1 0 0 2 2 0 1 1 1 1 1 0

Morphological codes
(F / M): character restricted to Females / Males

1. Prestigma length: (0) prestigma shorter than pterostigma, (1) prestigma longer than pterostigma, (2) prestigma length 3X pterostigma length.
2. Base of second submarginal cell: (0) M obliquely oriented with respect to m-cu1, (1) M vertically oriented (angle at M noticeable).
3. Placement of forewing m-cu2: (0) close to r-m2, (1) far from r-m2.
4. Hamuli placement: (0) beginning basad of fork of R1&Rs, (1) beginning at fork R1&Rs.
5. Hindwing jugal lobe: (0) present, (1) absent.
6. Hindwing axillary incision: (0) present, (1) absent.

7. (M) Tyloides: (0) two on apical flagellomeres, (1) one on apical flagellomeres, (2) absent.
8. Vertex length: (0) ocelloccipital distance short, < length of ocellar triangle, (1) ocelloccipital distance long, > length of ocellar triangle, (2) ocelloccipital distance long, gena produced behind eye.
9. Vertex indentation: (0) absent, (1) present.
10. Ocelli diameter: (0) less than distance between posterior ocellus and eye, (1) greater than this distance.
11. Interantennal space: (0) broad, rounded, (1) defined triangular area, (2) strongly elevated, with rounded edges, (3) defined triangular area strongly elevated, with sharp edges.
12. Clypeus dorsum: (0) straight, (1) bisinuate.
13. (F) Apex of clypeus: (0) pointed, (1) shallowly emarginated, anterior angles blunt, broad, (2) shallowly emarginated, anterior angles triangular.
14. (F) Mesal clypeal tooth: (0) absent, (1) present.
15. (F) Clypeal punctures: (0) sparse, superficial mesally, (1) dense mesally.
16. (M) Clypeal-eye contact: (0) touching, (1) gap.
17. (F) Malar space: (0) short, (1) long, > length of penultimate flagellomere.
18. Mandibular teeth: (0) pointed, (1) with elongate cutting edge, twice length of apical part.
19. Labial palpus third segment: (0) with strong seta, (1) without this seta, but with hairs.
20. Pronotal carina: (0) present, (1) dorsally reduced, (2) lateral remnants, (3) absent.
21. Pronotal carina dorsally: (0) largely transverse before scutum, (1) deeply U-shaped before scutum.
22. Pronotal carina laterally: (0) little interrupted by the pronotal fovea, (1) widely interrupted by the pronotal fovea.
23. Pretegular carina: (0) complete, (1) ventrally effaced, (2) absent.
24. (F) Pronotal striae: (0) absent, (1) few ventral striae, (2) dense ventral and dorsal striae.
25. Scutal lamella: (0) present, (1) absent.
26. Scutal punctures: (0) dense micropunctures, (1) superficial, (2) dense and deep micropunctures.
27. Epicnemial carina: (0) present, (1) absent.
28. Dorsal groove: (0) present, (1) absent.
29. Scutellum profile in lateral view: (0) bulging, (1) largely flat.
30. Metanotal orientation: (0) partly vertical, (1) largely vertical (dorsal surface reduced).
31. Metanotal lobe: (0) absent, (1) posteromedial lobe present.
32. Metapleural sculpture: (0) striae, (1) superficial punctures ventrally, (2) well-defined punctures ventrally.
33. Hind coxa carina: (0) absent, (1) present.
34. Metasomal segment I: (0) rounded in lateral view, (1) sharply angled between anterior and dorsal faces.
35. Metasomal segment I length: (0) short, (1) long.

36. Metasomal tergum II lateral macropunctures: (0) superficial to sparse, (1) dense, well defined.
37. (M) Apical margin of metasomal sternum VI: (0) almost straight, (1) shallowly emarginated, (2) deeply emarginated.
38. (M) Apical margin of metqasomal sternum VII: (0) convex, (1) shallowly emarginated, (2) deeply emarginated.
39. (M) Median process of metasomal sternum VII: (0) absent, (1) present.
40. (M) Aedeagal apex: (0) little differentiated, (1) transverse, projecting laterally, (2) rounded with subapical processes, (3) spade-shaped, (4) elongate, (5) narrow, (6) subcircular, (7) triangular.
41. (M) Aedeagal apical lobes: (0) absent, (1) apex forming expanded lobes.
42. (M) Aedeagus width: (0) narrow throughout, (1) as wide or wider apically as medially, (2) narrower apically than medially.
43. (M) Aedeagal shaft: (0) non-bulbous, (1) bulbous.
44. (M) Digital apical processes: (0) absent, (1) present.
45. (M) Inner apical margin of paramere: (0) obtusely angled with aedeagus, (1) forming right angle to aedeagus.