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Molecular phylogeny and identification of the Egyptian wasps (Hymenoptera: Vespidae) based on COI mitochondrial gene sequences

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Abstract

The Hymenoptera is one of the vital and biggest insect orders comprising the bees, wasps, sawflies, and ants. Wasps are important to natural and biological pest control because they are predators or parasitoids of pest arthropods. This study investigated the genetic diversity among the three wasps, *Vespa orientalis* Linnaeus, *Polistes bucharensis* Erichson, and *Polistes mongolicus* du Buysson, collected from three different governorates in Egypt, using cytochrome oxidase subunit I (COI) DNA barcoding. PCR was performed to amplify COI fragment. The amplified COI regions (710 bp) were sequenced and analyzed. All novel nucleotide sequences of COI gene were deposited into the GenBank database. The genetic distances were estimated using Kimura two-parameter model. In spite of the wide geographical range, minor genetic diversity was observed between some populations of the three wasp species, revealing unrestricted gene flow between them. Phylogenetic relationship analysis was performed, using maximum likelihood (ML) method. The results of the phylogenetic analyses recovered *P. bucharensis* more closely related to *P. dominula* and *P. gallicus*. *P. mongolicus* collected from Menofia Governorate formed a distinct branch with 99% support. *V. orientalis* was sister to the yellowjacket *Dolichovespula adulterine*, with 84% support. It can be concluded that DNA barcode is a powerful tool for rapid and accurate identification of Egyptian wasp species.

Keywords: COI, Egypt, Hymenoptera, Vespidae, Phylogeny

Background

Wasps are predators or parasitoids of pest arthropods, so they are imperative to normal and biological control of pests (Hunt 2007). These insects have an essential part in pollination, a few of them in wax and honey production (Grissell 2010). The Vespidae are a large (about 5000 species), assorted cosmopolitan family of wasps, counting about all the known eusocial wasps (such as *Polistes* spp., *Vespa orientalis*, and *Vespula germanica*) and numerous single wasps (Grissell 2010). *V. orientalis* represents enormous issue for beekeepers (Haddad et al. 2005). The paper wasp genus *Polistes* Latreille, 1802, is an imperative model group for behavioral and developmental studies (Tibbetts 2007 and Jandt et al. 2014). It incorporates numerous eusocial species that show

different shapes of social organization. In addition, the comparatively little colony size of *Polistes* species and their uncovered nests encourage both field perceptions and tests (e.g., Cervo et al. 2008). Right now, over 220 species are recognized around the world (Arens 2011; Buck et al. 2012; Nugroho et al. 2012). Species identification is a crucial portion of recognizing and portraying biodiversity. Customarily, identification has been based on morphological diagnoses provided by taxonomic studies. Specialists such as taxonomists and prepared technicians can distinguish taxa accurately, since it requires extraordinary aptitudes acquired through extensive experience. Consequently, elective and accurate identification strategies that non-experts can use are required. Progresses in DNA sequencing innovations, advance in biotechnology, and the scientific classification crisis itself played a large role in the creation of DNA barcoding. Identification based on DNA barcode is very

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compelling at discriminating a constrained set of species, such as species occurring in a little range, agricultural pest species, and invasive species (Meier et al. 2008; Kress et al. 2009). Insects have been a major target of DNA barcoding due to their extraordinary diversity and their economic, agricultural, and epidemiological importance. However, DNA barcoding has several preferences. One advantage is its accessibility. The standard DNA barcode region, a part of COI, is exceptionally efficient for species identification. This locale has great discrimination power for most animal groups. The originally designed universal primer can be applied to all animal phyla (Hebert et al. 2003a,b, 2004). A short stretch of COI has enough information and can be straightforwardly sequenced with a sequencer. This is a protein-coding region so the alignment process is not difficult. Blunders can be identified by checking whether the obtained sequence is translatable. Sequence diversity in this gene could be utilized to make a “barcoding” framework that would enable the diagnosis of species of all animal life (Waugh 2007 for review; Hebert et al. 2010). These valuable highlights are the reason why the COI region was chosen as the standard DNA barcode. DNA barcoding can be a straightforward but effective strategy for non-experts, particularly those who routinely identify a large number of samples. Holometabolous insect orders, such as O:Hymenoptera, are greatly variable, and various endeavors have been made to relate their life stages using molecular markers (Miller et al. 2005; Ahrens et al. 2007; Sutou et al. 2007; Hayashi and Sota 2010; Kathirithamby et al. 2010; Murría et al. 2010; Pauls et al. 2010).

This study aimed to identify the three Egyptian wasp species by direct sequencing of the COI fragments and to estimate the differences of the wasp population based on their geographic aspects.

Methods

Sample collection

A total number of 120 specimens of wasps, representing three species (60 *Vespa orientalis*, 20 *Polistes monogolicus*, and 40 *Polistes bucharensis*) were collected from six different sites across three governorates in Egypt, with relatively different Egyptian agro-ecosystems: Menofia representing middle of the Delta (Lower Egypt), Giza representing the west bank of the Nile, and El-Fayoum representing Middle Egypt in September, 2017. Thirty-six specimens, out of 120 (three specimens of each species from each site), were randomly selected and used in this study (Table 1).

DNA extraction

Total genomic DNA was isolated from the head of each individual adult wasp, using QIAamp DNA mini kit (Qiagen, Valencia, CA, USA), according to the manufacturer's instructions. A final 100 µl DNA extract was eluted and stored at -20 °C until molecular evaluation.

PCR amplification

The barcoding region of cytochrome c oxidase I gene was amplified by using primers: LCO1490 forward 5'-TT TCAACWAATCATAAAGATATTGG-3' and HCO2198 reverse 5'-TAA ACT TCW GGR TGW CCA AAR AAT CA- 3' (Cruaud et al. 2010). PCR was performed in triplicate using Master Mix (Qiagen). A 25 µl reaction mixture contained 12.5 µl of Master Mix, 2 µl DNA template, 10 pmol of each primer. Using 3PrimePCR thermocycler (Techne, UK), reaction mixtures were initially incubated at 94 °C for 2 min, followed by 35 cycles of amplification (94 °C for 30 s, 42 °C for 50 s, and 72 °C for 35 s), followed by final extension at 72 °C for 5 min. PCR products were separated on 1.5% agarose gel, and bands visualized with GelRed staining (Biotium) and UV transillumination.

Table 1 List of species, collection sites, and GenBank accession numbers of COI

Name	Accession number	Collection site	Species
CU_1	MG736622	Giza (El-Mansoria)	<i>Vespa orientalis</i>
CU_2	MG736623	Giza (Abo-Rawash)	<i>Vespa orientalis</i>
CU_3	MG736624	Menofia (Ashmoon)	<i>Vespa orientalis</i>
CU_4	MG736625	Menofia (Quesna)	<i>Vespa orientalis</i>
CU_5	MG736626	El-Fayoum (El-Asfar)	<i>Vespa orientalis</i>
CU_6	MG736627	El-Fayoum (Abo-Dawood)	<i>Vespa orientalis</i>
CU_7	MG736628	Giza (El-Mansoria)	<i>Polistes bucharensis</i>
CU_8	MG736629	Giza (Abo-Rawash)	<i>Polistes bucharensis</i>
CU_9	MG736630	Menofia (Ashmoon)	<i>Polistes mongolicus</i>
CU_10	MG736631	Menofia (Quesna)	<i>Polistes mongolicus</i>
CU_11	MG736632	El-Fayoum (El-Asfar)	<i>Polistes bucharensis</i>
CU_12	MG736633	El-Fayoum (Abo-Dawood)	<i>Polistes bucharensis</i>

DNA sequencing

Positive bands were purified using QIAquick Gel extraction kit (Qiagen™, Germany), according to the kit instructions. Cycling sequence was performed with Big Dye Terminator Version 3.1 kit (Applied Biosystems, Foster City, CA), following manufacturer's instructions. The sequence-loaded plate run on automated machine (3500 analyzer) by colors laboratory (Cairo, Egypt), using the aforementioned primers.

Phylogenetic analyses

Sequence reads were edited and assembled, using the DNASTAR software (Lasergene, Madison, WI). Sequence similarity searches were confirmed, using BLASTN and BLASTX (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The sequences were aligned, using Clustal W version 1.8 (Altschul et al. 1997). MEGA 7 software (Kumar et al. 2016) was used to perform the phylogenetic analysis. Phylogenetic trees of the cytochrome oxidase subunit I sequences were conducted with, using the maximum likelihood (ML) method (Felsenstein 1981; Kishino et al. 1990), and 1000 bootstrap replication was used to evaluate the branching confidence. Pairwise genetic distances were estimated using Kimura two-parameter model (Kimura 1980).

Nucleotide sequence submission and GenBank accession numbers

Accession numbers for each novel nucleotide sequence of COI gene of the Egyptian *V. orientalis*, *P. mongolicus*, and *P. bucharensis* are given in Table 1.

Results and discussion

This is the first time that the molecular data of wasps from the regions of El-Mansoria and Abo-Rawash (Giza), Ashmoon and Quesna (Menofia), and El-Asfar and Abo-Dawood (El-Fayoum) are presented, compared, and analyzed (Table 1). One specimen of each species from each site was sequenced. The results indicated that the amplified PCR products with COI primers were 710 nucleotides in length (Fig. 1). The 710 bp target fragment incorporates the DNA barcode region of the animal taxa. Sequence diversity in this region was used as a tool for species discrimination (Hebert et al. 2003b). The nucleotide sequences of the COI for the three species of wasps under study were submitted to GenBank and subjected to a homology search using BLASTX. According to the sequence similarity, the local isolates could be identified as members of *V. orientalis* Linnaeus, *P. bucharensis* Erichson, and *P. mongolicus* du Buysson. Multiple sequence alignment was performed, using Clustal W (MEGA 7). A bootstrap phylogenetic tree based on COI sequences of the local isolated wasps and other species was constructed using neighbor joining (NJ) and maximum likelihood (ML) methods with Kimura two-parameter model. Both trees resulted in the same topological structure and confirmed the identification of all specimens based on COI gene. So, only ML tree is represented here. At the species level, ML tree topology reflected a strong relation between *V. orientalis*, collected from different localities in Egypt, which clustered together with 100% support with other *V. orientalis* isolates found in GenBank and with 84% support with *Dolichovespula adulterine* (Fig. 2). The genus-level relationships of vespine wasps have been studied by many

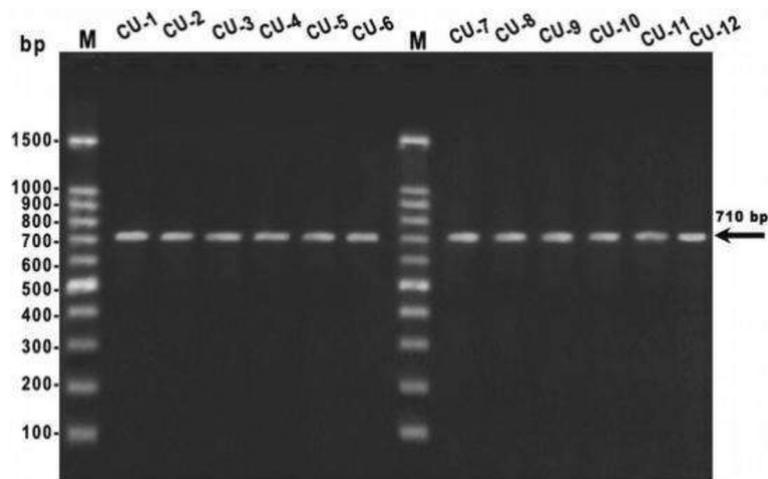
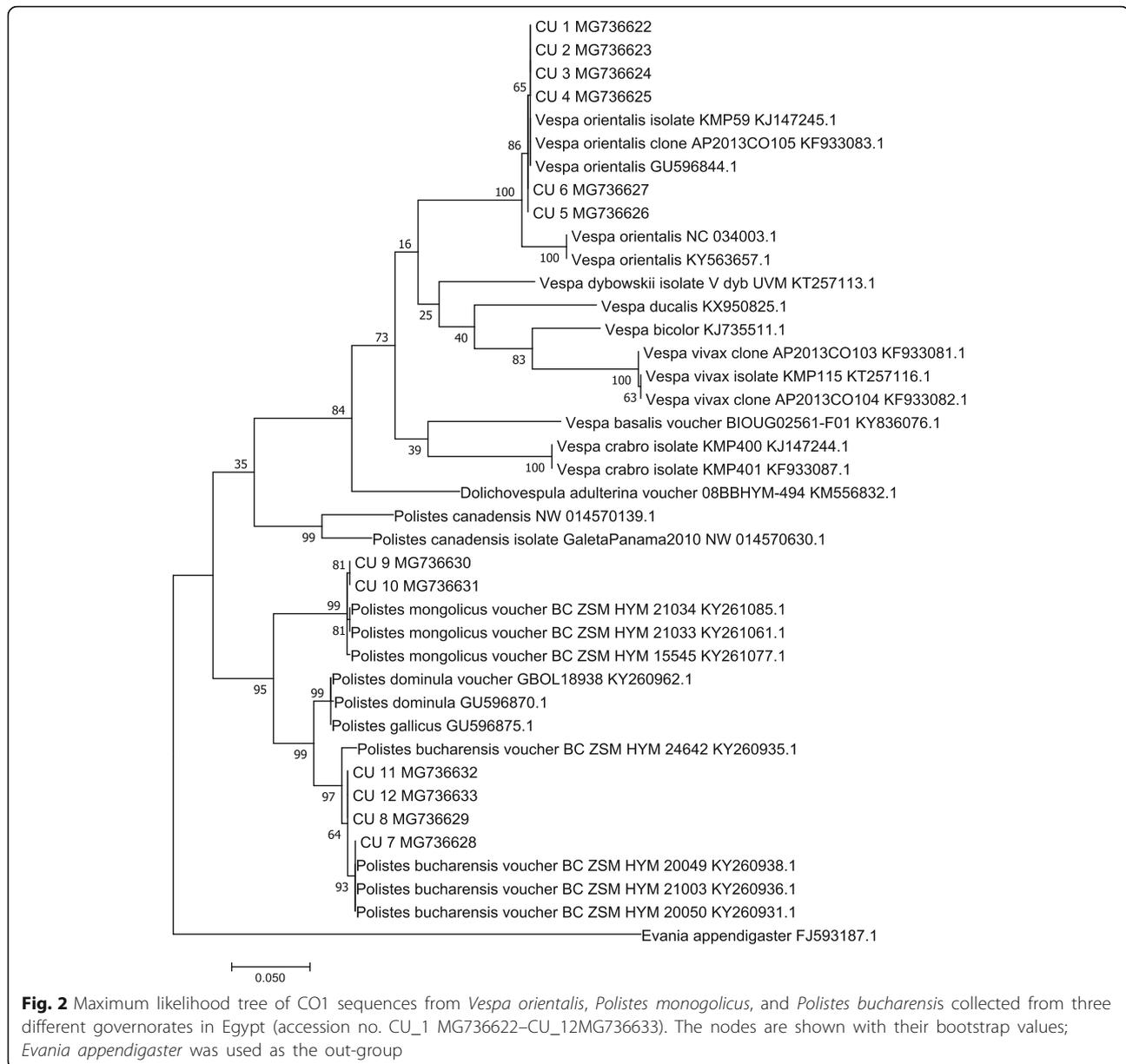


Fig. 1 Agarose gel showing polymerase chain reaction (PCR) results using DNA templates from different governorates with the primer sets LCO1490/HCO2198 for COI. M, marker (100 bp DNA Ladder RTU); Lanes CU_1 and CU_2 *Vespa orientalis* and CU_7 and CU_8 *Polistes bucharensis* collected from Giza; CU_3 and CU_4 *Vespa orientalis* and CU_9 and CU_10 *Polistes mongolicus* collected from Menofia. CU_5 and CU_6 *Vespa orientalis* and CU_11 and CU_12 *Polistes bucharensis* collected from Fayoum, respectively of the head of the adult individual



authors. They have recovered yellowjackets as a monophyletic group (Carpenter 1987; Pickett and Carpenter 2010; Saito and Kojima 2011; Perrard et al. 2016). Recently, Lopez-Osorio et al. (2017) found that the hornet genus *Vespa* is sister to the yellowjacket genus *Dolichovespula*. This result agreed with our results (Fig. 2). One of the vital factors related with population differentiation is the geographical isolation; the greater the geographical distance between populations, the less chance of gene flow; thus, there should be more differentiation between them (Zhang and Kang 2005). To test for a topographically based distribution of genetic diversity, we carried out the pairwise distance inside the *V. orientalis* group which showed that strains isolated from Giza and Menofia Governorates,

CU_1, CU_2, CU_3, and CU_4, were exactly the same. Moreover, they showed no difference with the Egyptian reference strain isolated in 2013 (Carpenter et al. 2013) and strain isolated from Cyprus (Pickett and Carpenter 2010). These results could be explained by the findings of Kumar et al. (2001) who reported that gene flow between populations was independent of geographic distance. Moreover, in the phylogenetic tree, the results indicated that isolated *V. orientalis* strains from Jordan (accession numbers: NC034003 and KY563657) were closely related to the Egyptian strains, collected from the three different governorates with 100% support (Fig. 2). This might be due to the wide distribution of *V. orientalis* in Egypt, Jordan, and Israel, where the social wasp fauna (Vespinae,

Polistinae) in Jordan is similar to faunas of Israel and Egypt (Zalat 1992; Haddad et al. 2007) while strains isolated from El-Fayoum Governorate, CU_5 and CU_6, had minor sequence variation from the other collected species (Table 2, A). This may be due to that El-Fayoum Governorate includes different climate and the type of cultivated crops differs from those of the Nile Valley. This suggested that other than geographical distances, neighborhood conditions had a high impact on the *V. orientalis* population structure and may strongly influence gene flow between sites, even on a very little scale. Based on our COI analysis, gene flow among the four populations of *V. orientalis* collected from Giza and Menofia had apparently occurred. Meanwhile, restricted gene flow might exist between them and the populations collected from EL-Fayoum. In addition, the tree topology reflects a strong relation between *P. bucharensis*, collected from Giza and El-Fayoum Governorates, which lie in close proximity and are included within a single cluster in the phylogenetic tree, regardless the collection sites with 97% support with the isolated strain from Greece (accession number: KY260935). The pairwise distance calculation inside the *P. bucharensis* group showed that the collected *P. bucharensis* species were identical with a minor sequence variation to strain CU_7. This variation might be due to host plant differences and geographic locations. Strain CU_7 was identical to the reference strains, isolated from Cyprus (accession numbers: KY260936 and KY260938) (Schmid-Egger et al. 2017) (Table 2, B). *P. bucharensis* showed a strong relation with *P. dominula* and *P. gallicus* (Fig. 2). In

a similar study, *P. bucharensis* was settled inside the *P. dominula* cluster in both the phylogenetic analysis and the neighbor joining (Schmid-Egger et al. 2017). From the tree of this study, it is evident that *P. mongolicus*, collected from Menofia Governorate (CU_9 and CU_10), formed a distinct branch with 81% support which was previously reported by Schmid-Egger et al. (2017) that in N Africa *P. mongolicus* is restricted to Egypt. In addition, the tree reflects a strong relation between CU_9 and CU_10 strains which clustered together with 99% support with the isolated strains from different localities in Cyprus (accession numbers: KY261085, KY261061, and KY261077) (Fig. 2). These results indicated that minor genetic diversity was observed between some populations of the three wasp species collected from different sites across the three governorates, revealing unlimited gene flow between them. Migration resulted in unrestricted gene flow between populations of the moth *Scirpophaga incertulas* (Kumar et al. 2001). *Evania appendigaster*, which was taken as an out-group, creates a clear branch outside for reference. All nodes in the phylogenetic tree were well supported with strong bootstrap values. As far as we know, this is the first study regarding the molecular identification of the Egyptian wasps observed among some diverse areas across the country. In this study, the target species, *V. orientalis* and *P. bucharensis* and *P. mongolicus* were identified based on homology and phylogenetic investigations conducted for COI mitochondrial gene. Molecular analysis utilizing fundamental computer program was effective in their identification and distinguishing them from other

Table 2 Pairwise genetic distances of the analyzed COI gene inside the *Vespa orientalis* group (A) and inside the *Polistes bucharensis* group (B). The pairwise distance was calculated using the Kimura two-parameter model

A		1	2	3	4	5	6	7	8
1	CU_1_MG736622	0.000							
2	CU_2_MG736623	0.000	0.000						
3	CU_3_MG736624	0.000	0.000	0.000					
4	CU_4_MG736625	0.000	0.000	0.000	0.000				
5	CU_5_MG736626	0.002	0.002	0.002	0.002	0.000			
6	CU_6_MG736627	0.002	0.002	0.002	0.002	0.000	0.000		
7	<i>Vespa orientalis</i> _KF933083.1	0.000	0.000	0.000	0.000	0.002	0.002	0.000	
8	<i>Vespa orientalis</i> _GU596844.1	0.000	0.000	0.000	0.000	0.002	0.002	0.000	0.000
B		1	2	3	4	5	6		
1	CU_7_MG736628	0.000							
2	CU_8_MG736629	0.005	0.000						
3	CU_11_MG736632	0.005	0.000	0.000					
4	CU_12_MG736633	0.005	0.000	0.000	0.000				
5	<i>Polistes bucharensis</i> _KY260938.1	0.000	0.005	0.005	0.005	0.000			
6	<i>Polistes bucharensis</i> _KY260936.1	0.000	0.005	0.005	0.005	0.000	0.000		

common species of wasps. Identification of many species based on COI and 16S rDNA has been reported previously (Boyer et al. 2011; Liu et al. 2011; Mc Donnell et al. 2011). Additionally, Li et al. (2010) used both barcode and non-barcode sequences to reveal actual host utilization of fig-associated *Sycophila* wasps (Hymenoptera: Eurytomidae). Moreover, 430,000 barcodes representing about 50,000 species of butterflies have been collected (Silva-Brandão et al. 2009; International Barcode of Life 2010b). On the other hand, nuclear genes, are required for discrimination between species when two groups of organisms have diverged very recently, may share the same DNA barcode(s), but may not belong to the same species (e.g., Hebert et al. 2003b; Kaila and Ståhls 2006; Langhoff et al. 2009; Burns et al. 2010; Žurovcová et al. 2010). Further work should be aimed at expanding sample sizes, taxa differences, and geographic populations to empower the creation of a DNA-based identification framework for wasps in Egypt.

Conclusions

The study supported the efficiency of COI DNA barcoding for accurate identification of *V. orientalis*, *P. bucharensis*, and *P. mongolicus*, collected from three different governorates in Egypt. Minor genetic variation was observed between some populations of the three wasp species under study, revealing unlimited gene flow between them. In the phylogenetic tree, the isolated *V. orientalis* strains from Jordan (accession numbers: NC034003 and KY563657) were closely related to the Egyptian strains collected from the three different governorates with 100% support. Also, the tree topology reflected a strong relation between *P. bucharensis* collected from Giza and El-Fayoum Governorates (with 97% support) and other strain isolated from Greece (accession number: KY260935). In addition, *P. mongolicus* collected from two different sites of Menofia Governorates were more closely related to the isolated strains from different localities in Cyprus (accession numbers: KY261085, KY261061, and KY261077) with 99% support.

Abbreviations

COI: Cytochrome oxidase subunit I; ML: Maximum likelihood method; NJ: Neighbor joining; *P.*: *Polistes*; *V.*: *Vespa*

Acknowledgements

We thank the Scientific Research Sector, Faculty of Science, Cairo University for funding this study. We extend special thanks to the anonymous reviewer for his careful reading and concerned comments on the previous version of this article.

Funding

This work was financially supported by Scientific Research Sector, Faculty of Science, Cairo University.

Availability of data and materials

The datasets generated and analyzed during the current study are available in GenBank repository (<https://www.ncbi.nlm.nih.gov/genbank/>).

Authors' contributions

EMA designed the study, supervised the work, and wrote the manuscript with input from all authors. IE and MO carried out the experiments. AA analyzed the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Received: 17 January 2018 Accepted: 23 February 2018

Published online: 05 April 2018

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