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On the genetic identity of *Gonatocerus aegyptiacus* (Hymenoptera Mymaridae) from Egypt

Serguei V. TRIAPITSYN[1], Paul F. RUGMAN-JONES[1], Medhat I. ABUL-SOOD[2], Neveen S. GADALLAH[3]
[1]*Department of Entomology, University of California, Riverside, California, USA*
[2]*Zoology Department, Faculty of Science (Boys), Al-Azhar University, Nasr City, Cairo, Egypt*
[3]*Entomology Department, Faculty of Science, Cairo University, Giza, Egypt*

Abstract

Genetic identity of the fairyfly species *Gonatocerus aegyptiacus* Soyka (Hymenoptera Mymaridae), known only from Egypt, is revealed and compared to other relevant members of the *Gonatocerus fuscicornis* (Walker) species complex. *G. aegyptiacus* is shown to be particularly morphologically similar to the European *Gonatocerus minor* Matthews but clearly different from that and other members of the complex based on comparison of the sequences of the nuclear 28S-D2 and ITS2 DNA loci, and the mitochondrial COI gene. Illustrations of the female *G. aegyptiacus* are provided to facilitate its recognition.

Key words: Mymaridae, *Gonatocerus aegyptiacus*, Egypt, genetic identity, integrative taxonomy.

Introduction

This is a follow up to the recent study by Triapitsyn *et al.* (2021a) on the *Gonatocerus fuscicornis* (Walker) species complex within the genus *Gonatocerus* Nees ab Esenbeck (*sensu stricto*) (Hymenoptera Mymaridae), as defined by Huber (2015). Some species of *Gonatocerus* are economically important egg parasitoids of the leafhopper pests of agricultural crops, particularly of rice in Asia (Triapitsyn, 2013; Triapitsyn *et al.*, 2021b). Because of the lack at that time of freshly collected specimens of *Gonatocerus aegyptiacus* Soyka from Egypt suitable for DNA extraction, molecular data on this little-known southern Palaearctic species, originally rather poorly described by Soyka (1950), could not be included in their genetic analysis. Since then, *G. aegyptiacus* was found not to be uncommon in Egypt where it can be rather easily collected (Abul-Sood *et al.*, 2022), who redescribed (morphologically only) it in detail based on freshly captured specimens. Some of those were preserved in ethanol to be used for DNA extraction and consequent molecular analyses to reveal its genetic identity including comparison with other members of the complex, some of which are very similar morphologically (Triapitsyn *et al.*, 2021a). That morphological similarity resulted in the proposed synonymy of the European *Gonatocerus minor* Matthews, *Lymaenon saipanensis* Doutt from Micronesia, as well as *Gonatocerus alami* Shamim et Shafee, *Gonatocerus miurai* Sahad and *Lymaenon tarae* Narayanan et Subba Rao from Asia under *G. aegyptiacus* by Triapitsyn (2013). Triapitsyn *et al.* (2021a) reversed that taxonomic decision, albeit without supporting genetic evidence that was lacking at that time for some of the nominal species, and took *G. minor* and *Gonatocerus saipanensis* (Doutt) from Saipan Island (along with its synonyms from Japan and India) out of synonymy under *G. aegyptiacus*, applying a more conservative approach to the issue about its identity. They predicted that the latter would be unlikely to be conspecific with other members of the *G. fuscicornis* species complex, which also includes *Gonatocerus cincticipitis* Sahad, *Gonatocerus longicornis* Nees ab Esenbeck, and *Gonatocerus meghalayanus* Zeya, at least, along with several undescribed/unidentified species from East Asia (Triapitsyn *et al.*, 2021a).

Here we present results of the genetic study on *G. aegyptiacus* and comment on its molecular identity in relation to other members of the complex for which relevant sequences are available.

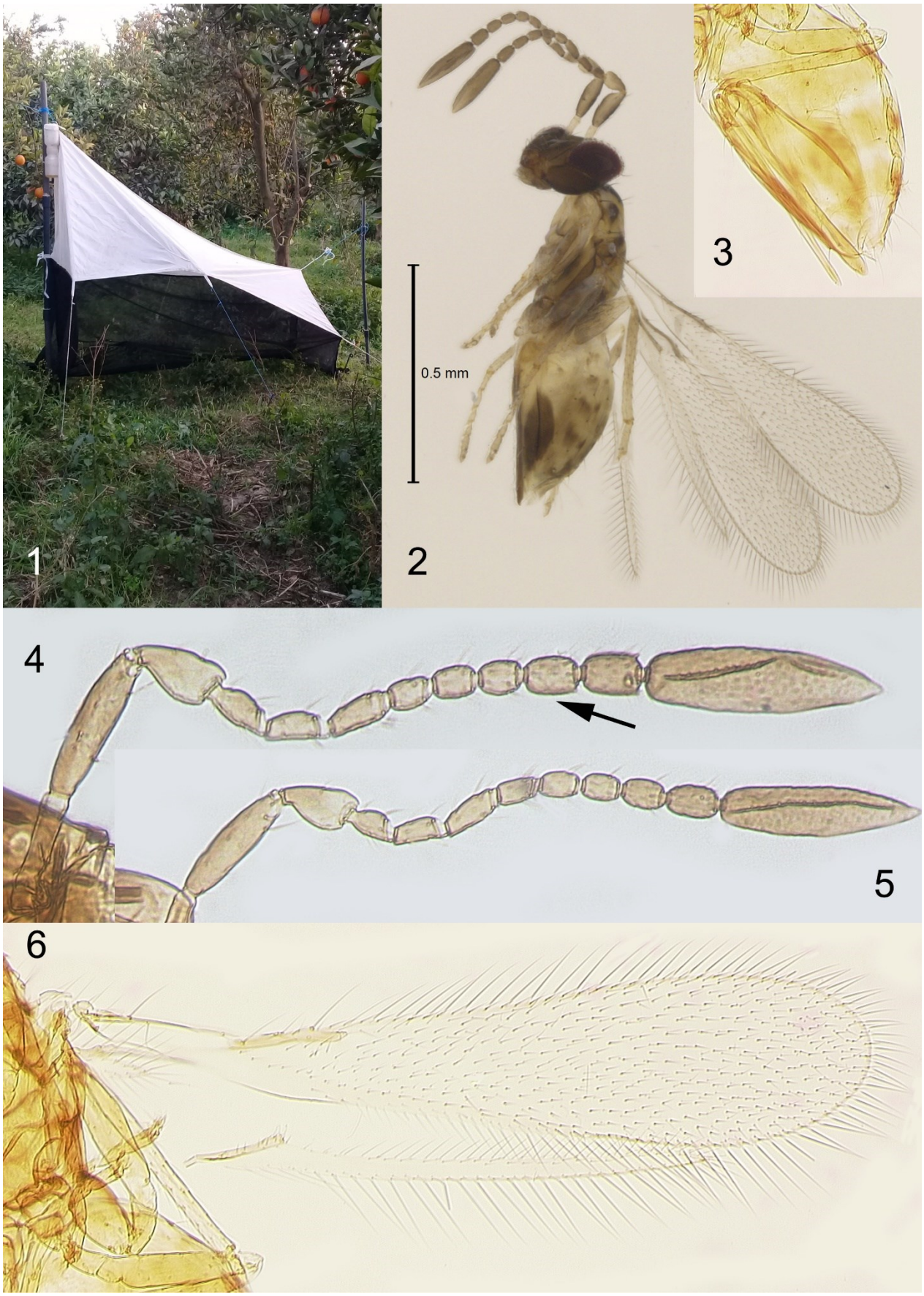
Materials and methods

Source of specimens

Specimens of *G. aegyptiacus* were collected in Egypt by a Malaise trap (figure 1), as indicated under "Material examined" section below, in a non-organic citrus orchard at Munsah (Monufia Governorate, 30°21'41"N 30°55'26"E, 13 m) in which only natural fertilizers were used. These were preserved in 95% ethanol and used for DNA extraction. For the identification and morphological studies, three slide-mounted primary molecular voucher specimens of P. F. Rugman-Jones were used; each of them was assigned his PR number and an Entomology Research Museum, University of California, Riverside, California, USA (UCRC) database UCRC_ENT number.

Taxonomic studies

For morphological terminology we follow that of Triapitsyn (2013) and Huber (2015). Abbreviations for some morphological features used in the text are: F = funicular segment of female antenna; mps = multiporous plate sensillum or sensilla on the antennal flagellar segments (= longitudinal sensillum or sensilla, or sensory ridge(s) of other authors). All slide mounts were examined under a Zeiss Axioskop 2 plus compound microscope (Carl Zeiss Microscopy, LLC, Thornwood, New York, USA).



Figures 1-6. (1) Malaise trap in a citrus orchard in Munsah, Monufia Governorate, Egypt; **(2-6)** *G. aegyptiacus*, female (Munsah): (**2**) habitus in lateral view, (**3**) ovipositor, (**4**) antenna (F7 with 1 mps, pointed to by an arrow), **(5)** antenna (F7 without mps), (**6**) fore and hind wings.

Table 1. Molecular voucher specimens of *G. aegyptiacus* (present study) used in genetic analyses, and GenBank accession numbers for the gene regions successfully sequenced.

Molecular voucher	Country	Locality	COI	28S-D2	ITS2
PR19-574	Egypt	Munsah	OP958794	OP962142	OP962139
PR19-575	Egypt	Munsah	OP958795	OP962143	OP962140
PR20-576	Egypt	Munsah	OP958796	OP962144	OP962141

The following acronym is used for the depository of the molecular voucher specimens:

UCRC - Entomology Research Museum, University of California, Riverside, California, USA

DNA extraction, amplification, and sequencing

DNA was extracted from individual wasps using the non-destructive HotSHOT method of Truett *et al*. (2000) in a total volume of 80 µL. Following DNA extraction, all specimens were retrieved and slide-mounted in Canada balsam for morphological examination. Extracted DNA was stored at −20 °C. Amplification of the DNA and sequencing was done using exactly the same methods as for other members of the *G. fuscicornis* species complex, as described in detail in Triapitsyn *et al*. (2021a). We amplified and sequenced a section of the mitochondrial cytochrome oxidase subunit 1 gene (COI) and two regions of ribosomal RNA (rRNA); the D2 domain of 28S (28S-D2) and the internal transcribed spacer 2 (ITS2). Purified products were direct sequenced in both directions at the Institute for Integrative Genome Biology, University of California, Riverside, California, USA. All sequences were deposited in GenBank, with the accession numbers indicated in table 1.

Genetic analyses

Phylogenetic analysis of DNA sequence variation was performed on the COI and 28S-D2 data separately. We obtained COI sequences from 3 specimens of *G. aegyptiacus*. Each COI sequence was translated into its amino acid chain (http://www.ebi.ac.uk/Tools/emboss/transeq/index.html) to confirm the absence of nuclear pseudogenes (Song *et al.*, 2008). The COI sequences were then combined with 34 sequences of other members of the *G. fuscicornis* species complex as presented in Triapitsyn *et al*. (2021a; see their table 1 for GenBank accessions). We also added a haplotype of *Cosmocomoidea ashmeadi* (Girault) (AY971869), which is from the same tribe Gonatocerini Ashmead as *Gonatocerus* spp., to use as an outgroup taxon. The combined sequence file was aligned in MAFFT version 7.050 (http://mafft.cbrc.jp/alignment/software/) using the "auto" setting to select the best alignment strategy (Katoh and Standley, 2013). Aligned sequences were trimmed to a uniform length, removing nucleotide deficient "overhangs" from the 5' and 3' ends. The result was a final data matrix containing 38 terminal taxa, 404 nucleotide positions, and no gaps. Phylogenetic reconstruction was performed by conducting a maximum likelihood (ML) analysis using PhyML 3.1 via the phylogeny.fr platform (Dereeper *et al.*, 2008). The nucleotide substitution model HKY85 was chosen and branch support was assessed by conducting approximate likelihood ratio tests (Anisimova and Gascuel, 2006), within PhyML. The resulting tree was redrawn using FigTree v.1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

Sequences of 28S-D2 were obtained for each of the three specimens and combined with 26 homologous sequences of other members of the *G. fuscicornis* species complex as presented in Triapitsyn *et al*. (2021a), and one sequence of *Cosmocomoidea ashmeadi* retrieved from GenBank (AY953525). The combined sequence file was again aligned in MAFFT, this time using the Q-INS-I iterative strategy (Katoh and Standley, 2013). Aligned sequences were trimmed to a uniform length, removing nucleotide deficient "overhangs" from the 5' and 3' ends. The result was a final, gapped data matrix containing 30 terminal taxa and 520 nucleotide positions. Phylogenetic reconstruction was performed using PhyML as detailed for COI.

Sequences of the ITS2 were also obtained for the 3 specimens and simply compared with each other, and with those of other members of the *G. fuscicornis* species complex as presented in Triapitsyn *et al*. (2021a), in search of corroborating support for the results of the analyses of COI and 28S. Due to large interspecific differences in the size of the ITS2 region in our *Gonatocerus* specimens (600-800 bp), and the subsequent ambiguity inherent in any alignment of such a dataset, no formal analysis was conducted on ITS2. Instead, we simply clustered the sequences manually in BioEdit and looked for corroboration between those clusters and the results of the analyses of COI and 28S.

Finally, the extent of genetic separation between *G. aegyptiacus* and other members of the *G. fuscicornis* complex was estimated by calculating average pairwise uncorrected p-distances within and between the COI sequences of the different named species. The analysis was conducted in MEGA version 11 (Tamura *et al.*, 2021) using only COI sequences generated in this study and that of Triapitsyn *et al*. (2021a).

Results

Taxonomy

Gonatocerus aegyptiacus Soyka 1950 (figures 2-6)

Gonatocerus aegyptiacus Soyka, 1950: 125-126. Type locality: Shareh El-Haram, Giza, Egypt.

Lymaenon aegyptiacus (Soyka): Heqvist, 1960: 430 (list, *de-facto* generic transfer).

Gonatocerus (*Gonatocerus*) *aegyptiacus* Soyka: Triapitsyn, 2013: 9-13 (in part; taxonomic history, *de-facto* transfer to *Gonatocerus*, distribution [in part, Egypt only], redescription of some paratypes, diagnosis).

Gonatocerus aegyptiacus Soyka: Huber, 2015: 29 (list,

Table 2. Genetic divergence between named members of the *G. fuscicornis* complex, based on DNA sequences of the mitochondrial COI gene (Triapitsyn *et al.*, 2021a) with an addition of *G. aegyptiacus* and exclusion of *G. cincticipitis*. Diagonal element shows intraspecific variation (only when more than one sequence was considered). Average pairwise uncorrected p-distances calculated using MEGA 11.0.8.

Species of *Gonatocerus*	*aegyptiacus*	*fuscicornis*	*longicornis*	*meghalayanus*	*minor*	*saipanensis*
aegyptiacus	0.005					
fuscicornis	0.079	0.012				
longicornis	0.078	0.044	-			
meghalayanus	0.077	0.081	0.076	0.002		
minor	0.079	0.038	0.032	0.088	0.002	
saipanensis	0.072	0.082	0.086	0.026	0.086	0.007

unnecessary new combination, type information); Al-Azab, 2020: 8 (list); Triapitsyn *et al.*, 2021a: 184 (key), 186 (*sensu stricto*; brief diagnosis, distribution in Egypt only), 199 (discussion, possible host); Abul-Sood *et al.*, 2022: 327-332 (type information, redescription, distribution, illustrations), 347 (key).

M a t e r i a l e x a m i n e d

Egypt, Monufia Governorate, Munsah, 30°21'41"N 30°55'26"E, 13 m, viii.2021, M. I. Abul-Sood, Malaise trap in citrus orchard [4 females, UCRC, including 3 molecular vouchers: PR21-574 (UCRC_ENT 00528729), PR21-575 (UCRC_ENT 00541274), and PR21-576 (UCRC_ENT 00541279)].

D i a g n o s i s

Gonatocerus aegyptiacus females (figure 2, particularly those that have 1 mps on F7, figure 4) may be difficult to distinguish morphologically from other, quite similar members of the *G. fuscicornis* species complex, particularly from some small specimens of *G. minor* that occasionally may either lack or have just 1 mps on F7, and also from some *G. saipanensis* (Triapitsyn *et al.*, 2021a). Most females of *G. aegyptiacus* from Egypt lack mps on F7 of the antenna (figure 5) (Triapitsyn, 2013; Abul-Sood *et al.*, 2022). Out of four females *G. aegyptiacus* examined here, one has F7 with 1 mps (figure 4), two have F7 without mps (figure 5), and one has incomplete antennae. Unlike in females of other species in the complex including *G. minor* and *G. saipanensis*, F8 of *G. aegyptiacus* bears just 1, not 2, mps (Abul-Sood *et al.*, 2022). To further facilitate recognition of this species, illustrated here are also female ovipositor (figure 3) and fore wing (figure 6) of *G. aegyptiacus*, which has discal microtrichia originating behind base of marginal vein, like also in *G. saipanensis* (Triapitsyn *et al.*, 2021a).

D i s t r i b u t i o n

Palaearctic Region: Egypt (Soyka, 1950; Abul-Sood *et al.*, 2022).

H o s t s

Unknown. The hosts indicated for *G. aegyptiacus* in Triapitsyn (2013) apply only to *G. saipanensis* (Triapitsyn *et al.*, 2021a).

R e m a r k s

The holotype female of *G. aegyptiacus* is lost, but the remaining paratypes allow for a positive recognition of this species (Triapitsyn, 2013; Abul-Sood *et al.*, 2022).

Genetic analyses and discussion

DNA sequences of the mitochondrial COI and nuclear ribosomal 28S-D2 loci provided corroborating support of *G. aegyptiacus* being a good, valid species which is genetically clearly distinct (table 2; figures 7 and 8) from all other members of the *G. fuscicornis* species complex, both described and undescribed, analysed in Triapitsyn *et al*. (2021a), including the morphologically very similar *G. minor* from Europe and *G. saipanensis* from Asia (figures 7 and 8, respectively). Thus, the mostly intuitive taxonomic decision by Triapitsyn *et al*. (2021a) to take *G. minor* and *G. saipanensis* out of synonymy under *G. aegyptiacus*, as proposed by Triapitsyn (2013) solely based on a morphological assessment of a few available W. Soyka's specimens on slides, has proven to be correct. Furthermore, utilizing COI and 28S-D2 as diagnostic markers, we determined that *G. aegyptiacus* clearly is more closely related to *G. meghalayanus* and *G. saipanensis* that it is to the other members of the *G. fuscicornis* species complex (figures 7 and 8). The same groupings were also evident in sequences of the ITS2 gene (not shown, but see GenBank accessions OP962139-41 and those from Triapitsyn *et al*., 2021a). In addition to corroborating support from the ribosomal loci, levels of inter- and intra-specific divergence in the COI gene alone were consistent with those typical of valid species (Hebert *et al.*, 2003). It should be noted however, that the majority of species included in this study were each sampled from a geographically restrictive range, often a single location. Our estimates of intraspecific variation are therefore unlikely to capture the full range of variation present within each species. To do so would require a much broader geographic sampling, beyond the scope of the present study.

The demonstrated relative genetic proximity of *G. aegyptiacus* to *G. meghalayanus* and *G. saipanensis* (figures 7 and 8), both of which are known egg parasitoids of *Nephotettix* Matsumura (Hemiptera Cicadellidae) species in Asia, provides an indirect support to an educated guess that *G. aegyptiacus* might also be an egg parasitoid

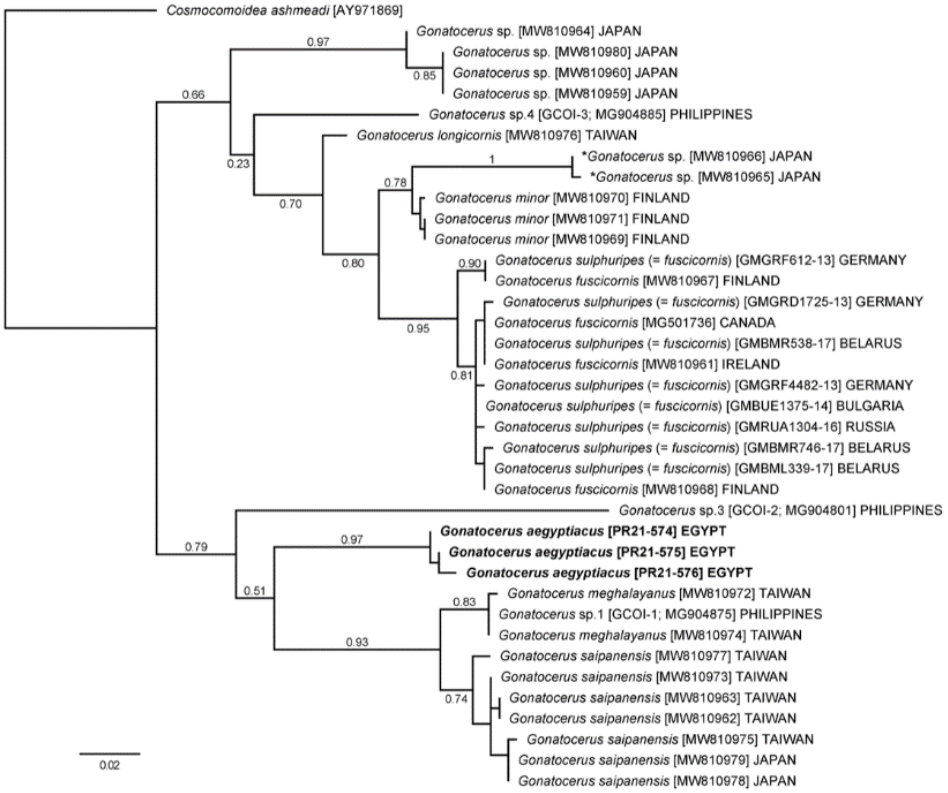


Figure 7. Relationships among members of the *G. fuscicornis* species complex based on DNA sequences of the mitochondrial COI. Maximum-likelihood analyses were conducted in PhyML and branch support was assessed using approximate likelihood ratio tests. Support for major branches is shown above the branch. An asterisk denotes *Gonatocerus* sp. from Japan that was misidentified as *G. cincticipitis* in Triapitsyn *et al.* (2021a).

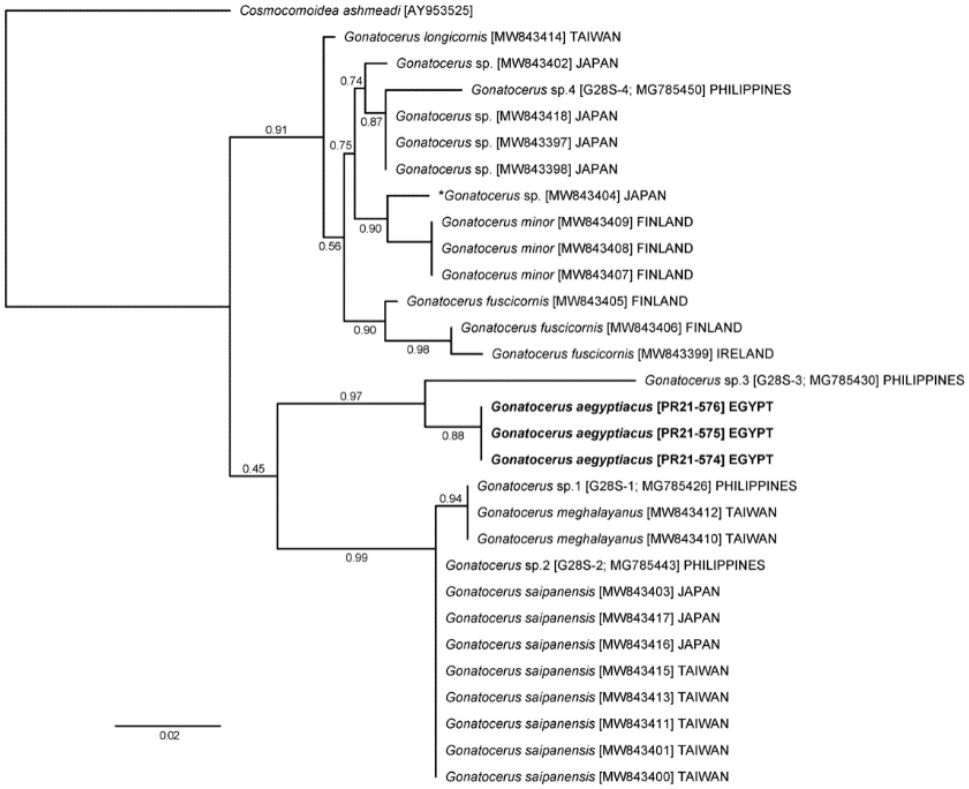


Figure 8. Relationships among members of the *G. fuscicornis* complex based on DNA sequences of the nuclear ribosomal 28S-D2. Maximum-likelihood analyses were conducted in PhyML and branch support was assessed using approximate likelihood ratio tests. Support for major branches is shown above the branch. An asterisk denotes *Gonatocerus* sp. from Japan that was misidentified as *G. cincticipitis* in Triapitsyn *et al.* (2021a).

of a *Nephotettix* species, most likely of *Nephotettix modulatus* Melichar since their distributions overlap (Triapitsyn *et al.*, 2021a). Indeed, *N. modulatus* occurs in Egypt and also in Tunisia, Morocco, and the Afrotropical Region (Ghauri, 1968). Interestingly, *G. aegyptiacus* is genetically more remotely related to its geographical neighbour, the European *G. minor* (figures 7 and 8), to which it is most similar morphologically.

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Authors' addresses: Serguei V. TRIAPITSYN (corresponding author: serguei.triapitsyn@ucr.edu), Paul F. RUGMAN-JONES, Department of Entomology, University of California, 3401 Watkins Drive, Riverside, California, 92521, USA; Medhat I. ABUL-SOOD, Zoology Department, Faculty of Science (Boys), Al-Azhar University, P.O. Box 11884, Nasr City, Cairo, Egypt; Neveen S. GADALLAH, Entomology Department, Faculty of Science, Cairo University, Giza, Egypt.

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