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Molecular and morphological differentiation within the *Gonatocerus fuscicornis* species complex (Hymenoptera Mymaridae)

Serguei V. TRIAPITSYN[1], Paul F. RUGMAN-JONES[1], Tetsuya ADACHI-HAGIMORI[2]
[1]*Department of Entomology, University of California, Riverside, California, USA*
[2]*Laboratory of Applied Entomology, University of Miyazaki, Japan*

Abstract

The fairyflies *Gonatocerus aegyptiacus* Soyka, *G. cincticipitis* Sahad, *G. fuscicornis* (Walker), *G. minor* Matthews stat. rev., and *G. saipanensis* (Doutt) stat. rev. (Hymenoptera Mymaridae), which constitute the *G. fuscicornis* species complex, are separated based on morphology and the available genetic evidence for the four of these nominal species. *G. minor* and *G. saipanensis* are reinstated as valid taxa from the previous synonymy with *G. aegyptiacus*. *G. cincticipitis* and *G. saipanensis* are egg parasitoids of green rice leafhoppers *Nephotettix* spp. (Hemiptera Cicadellidae) in Asia; *G. fuscicornis* and *G. minor* are common in Europe where these rice pests do not occur. An updated key to females of the Palaearctic species of *Gonatocerus* is given; *G. cincticipitis* is redescribed and rediagnosed. *Lymaenon tarae* Narayanan et Subba Rao syn. nov., *Gonatocerus miurai* Sahad syn. nov., and *G. alami* Shamim et Shafee syn. nov. are synonymized with *G. saipanensis* from the previous synonymy with *G. aegyptiacus*.

Key words: Cicadellidae, rice pest, *Nephotettix* spp., egg parasitoid, Mymaridae, *Gonatocerus*, integrative taxonomy.

Introduction

The recent proliferation of studies using an integrative taxonomic approach in dealing with morphologically similar and genetically closely related nominal species of some Mymaridae (Hymenoptera Chalcidoidea) has helped to untangle some of the most difficult species complexes within several genera, in which misidentifications and/or oversplitting of taxa were historically common (e.g., Triapitsyn *et al.*, 2018a).

One of the many remaining unresolved, taxonomically difficult species complexes in Mymaridae is the *Gonatocerus fuscicornis* (Walker) complex within the genus *Gonatocerus* Nees ab Esenbeck (*sensu stricto*), as defined by Huber (2015). Besides the mostly Palaearctic *G. fuscicornis*, which is very common in Europe on grassy vegetation and also known from several other zoogeographical regions (Triapitsyn, 2013), this complex also includes two other currently valid species, *Gonatocerus aegyptiacus* Soyka and *Gonatocerus cincticipitis* Sahad. The latter two are economically important egg parasitoids of green rice leafhoppers *Nephotettix* spp. (Hemiptera Cicadellidae) in Asia, particularly of the common rice pest *Nephotettix cincticeps* (Uhler) (Sahad, 1982a; 1982b; 1982c; Sahad and Hirashima, 1984; Triapitsyn, 2013; Triapitsyn *et al*., 2018b; 2021). The only known host of *G. fuscicornis* is *Capsus ater* (L.) (Hemiptera Miridae) (Triapitsyn, 2013). While the identity of *G. fuscicornis* is now well established (Triapitsyn, 2013), that of *G. cincticipitis*, which was known from Japan and the Republic of Korea (Sahad, 1982c; Sahad and Hirashima, 1984; Triapitsyn, 2013), has been problematic (Triapitsyn, 2018) even after its redescriptions, illustrations and diagnoses provided in Sahad and Hirashima (1984) and Triapitsyn (2013). Taxonomic history and recognition of *G. aegyptiacus* is longer than that of *G. cincticipitis* and even more confusing, particularly from the biological point of view. *G. aegyptiacus* was rather poorly described, without any illustrations, from Egypt (Soyka, 1950). Furthermore, its holotype is lost and this nominal species had been in obscurity and practically unrecognizable for a long time, until Triapitsyn (2013) redescribed it based on the slide-mounted female paratypes and other apparently conspecific, non-type specimens from the Palaearctic Region. Triapitsyn (2013) also synonymized with *G. aegyptiacus* the following nominal species: the European *Gonatocerus minor* Matthews, *Gonatocerus saipanensis* (Doutt) from Saipan Island, Northern Mariana Islands in Micronesia, and three nominal species from Asia, *Gonatocerus alami* Shamim et Shafee, *Gonatocerus tarae* Narayanan et Subba Rao from India, and *Gonatocerus miurai* Sahad from Japan and the Republic of Korea, which were clearly conspecific. These synonymies were made solely based on the almost identical adult morphology of both sexes. However, the first author of this communication has never been fully satisfied with one of them because their host associations could not be the same: whereas species of the genus *Nephotettix* Matsumura are widely distributed in Africa (including Egypt), Asia, Australasia and Oceania (Duan and Zhang, 2014; Dmitriev, 2019), they do not occur in Europe, so conspecificity of *G. minor*, which was originally described from England, UK (Matthews, 1986), with *G. aegyptiacus* from these other regions remained doubtful from the biological perspective. By themselves, however, different host associations of some polyphagous or oligophagous species of Mymaridae in different zoogeographical regions do not necessarily imply that these populations are not conspecific: for instance, *Anagrus* (*Anagrus*) *incarnatus* (Haliday) is a known egg parasitoid of various planthoppers (Hemiptera Delphacidae) in Europe and North America and also of rice planthopper pests in Asia (Triapitsyn *et al*., 2018a). However, the confirmed host associations of *G. aegyptiacus sensu* Triapitsyn (2013) in Taiwan and elsewhere in Asia seem to be restricted to rice leafhoppers *Nephotettix* spp. and *Maiestas*

dorsalis (Motschulsky) (Hemiptera Cicadellidae) (Triapitsyn *et al.*, 2021), thus casting a strong doubt that it can also occur in northern Europe. So, to test the current synonymy of *G. minor* with *G. aegyptiacus sensu* Triapitsyn (2013), and also to figure out the true identity of *G. cincticipitis* and its separation from both *G. aegyptiacus* and *G. fuscicornis*, we used molecular methods by extracting DNA from freshly collected specimens of these nominal species from both Europe and East Asia and by conducting genetic analyses to complement the existing morphological data in Triapitsyn (2013). An additional problem to be solved is that the true identity of *G. aegyptiacus* from Egypt and its conspecificity with morphologically very similar congeneric egg parasitoids of *Nephotettix* spp. in Asia are not well established. Unfortunately, obtaining either ethanol-preserved or dry-mounted specimens from Egypt, India, and Saipan Island was not feasible due to funding, collecting permissions, international mailing restrictions for insects, and other issues.

Materials and methods

Sources of specimens

For genetic studies (table 1), ethanol-preserved *G. aegyptiacus sensu* Triapitsyn (2013) from Japan (morphologically definitely conspecific with the former species *G. miurai*) were collected by a Malaise trap in an organic rice field in Miyazaki City, Miyazaki Prefecture, Kyushu Island, Japan, and those from Taiwan were voucher specimens of the recent study on egg parasitoids of rice leafhoppers (Triapitsyn *et al.*, 2018b; 2021). That same study also provided a specimen of the outgroup taxon, *Gonatocerus longicornis* Nees ab Esenbeck 1834, which was presumed not to be a member of the *G. fuscicornis* species complex {Taiwan, Taichung, Wufeng, Taiwan Agricultural Research Institute, 24°01'52.4"N 120°41'34.2"E, 76 m a.s.l., 25.ix-11.x.2017, H.-T. Shih, Malaise trap in organic rice field [1 female, UCRC] (molecular voucher PR20-242, UCRC_ENT 00536081)}. Specimens of *Gonatocerus* spp. from Japan were donated by P. Jałoszyński and H. Kusuhara. Those of *G. cincticipitis* were collected by S. V. Triapitsyn, T. Adachi-Hagimori, N. Kado and Y. Narai by sweeping and yellow pan traps in rice fields of Shimane Prefecture, Honshu Island, Japan, including in its type locality (Shimane University Honjo Farm, Kamihonjocho, Matsue), in October 2019. Specimens of *G. fuscicornis* and *G. minor* from Finland (all in ethanol) were donated by J. Paukkunen.

For the morphological studies, slide-mounted primary molecular voucher specimens of P. F. Rugman-Jones were used; each of them was assigned his PR number (table 1) and an Entomology Research Museum, University of California, Riverside, California, USA (UCRC) database UCRC_ENT number. In addition, numerous specimens of all four nominal species treated here were examined in the UCRC, many of which were identified and listed by Triapitsyn (2013). Those of *G. aegyptiacus sensu* Triapitsyn (2013) and *G. cincticipitis* were also examined in October and November 2019 during the first author's visits to the three major collections of Mymaridae in Japan.

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

	Molecular voucher	Country	Locality	COI	28S-D2	ITS2[1]
G. cincticipitis	PR19-497	Japan	Izumo	MW810965	MW843404	MW843432-34
G. cincticipitis	PR19-498	Japan	Matsue	MW810966	–	MW843435-37
G. fuscicornis	PR20-064	Finland	Parikkala	MW810967	MW843405	MW843438
G. fuscicornis	PR20-065	Finland	Parikkala	MW810968	MW843406	MW843439
G. fuscicornis	D4367	Ireland	Ballynafagh Lake	MW810961	MW843399	MW843419
G. minor	PR20-066	Finland	Parikkala	MW810969	MW843407	MW843440
G. minor	PR20-067	Finland	Parikkala	MW810970	MW843408	MW843441
G. minor	PR20-068	Finland	Parikkala	MW810971	MW843409	MW843442
G. longicornis	PR20-242	Taiwan (China)	Taichung	MW810976	MW843414	MW843447
G. saipanensis	PR21-142	Japan	Miyazaki	MW810978	MW843416	MW843449
G. saipanensis	PR21-143	Japan	Miyazaki	MW810979	MW843417	MW843450
G. saipanensis	PR19-496	Japan	Okinawa Island	–	MW843403	MW843428-31
G. saipanensis	PR20-239	Taiwan (China)	Minxiong	MW810973	MW843411	MW843444
G. saipanensis	PR20-243	Taiwan (China)	Minxiong	MW810977	MW843415	MW843448
G. saipanensis	PR19-493	Taiwan (China)	Taichung	MW810962	MW843400	MW843420-23
G. saipanensis	PR19-494	Taiwan (China)	Taichung	MW810963	MW843401	MW843424-25
G. saipanensis	PR20-241	Taiwan (China)	Taichung	MW810975	MW843413	MW843446
G. meghalayanus	PR20-238	Taiwan (China)	Taichung	MW810972	MW843410	MW843443
G. meghalayanus	PR20-240	Taiwan (China)	Taichung	MW810974	MW843412	MW843445
G. sp.	PR19-495	Japan	Okinawa Island	MW810964	MW843402	MW843426-27
G. sp.	– (A1)	Japan	Fukuoka	MW810959	MW8434397	–
G. sp.	– (A2)	Japan	Fukuoka	MW810960	MW843398	–
G. sp.	PR21-144	Japan	Miyazaki	MW810980	MW843418	MW843451

[1]Multiple accession numbers are the result of having sequenced several clones of the PCR product (see text).

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).

Selected specimens of *G. cincticipitis*, all dry-mounted on points and borrowed from the Entomological Laboratory, Faculty of Agriculture, Kyushu University, Fukuoka, Japan, were further dissected and slide-mounted in Canada balsam using a slightly modified technique described in Huber (2015). All slide mounts were examined under a Zeiss Axioskop 2 plus compound microscope (Carl Zeiss Microscopy, LLC, Thornwood, New York, USA).

The following acronyms are used to designate depositories of specimens:

ELKU - Entomological Laboratory, Faculty of Agriculture, Kyushu University, Fukuoka, Japan;

ITLJ - Insect Museum, National Institute for Agro-Environmental Sciences, NARO, Tsukuba, Japan;

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

ZLMU - Laboratory of Entomology, Faculty of Agriculture, Meijo University, Tempaku, Nagoya, Japan.

DNA extraction, amplification, and sequencing

At the University of California, Riverside (hereafter UCR), 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.

The polymerase chain reaction (PCR) was used to amplify a section of the mitochondrial cytochrome oxidase subunit 1 gene (COI) utilizing the primers C1-J-1718 with C1-N-2191 (Simon *et al*., 1994), or, if those initial primers failed, LCO1490 with HCO2198 (Folmer *et al.*, 1994). COI reactions were performed in 25 µL volumes containing 1× Thermopol PCR buffer (New England BioLabs, Ipswich, Massachusetts, USA), an additional 1.0 mM $MgCl_2$, 20 µg BSA (NEB # BS9000S), 1U *Taq* polymerase (NEB), 0.2 µM of each primer, 200 µM each dATP, dCTP, dGTP, and 400 µM dUTP. After an initial denaturing step of 2 minutes at 94 °C, thermocycling conditions were: five cycles of 30 seconds at 94 °C, 1 minute 30 seconds at 45 °C, and 1 minute at 72 °C; followed by a further 35 cycles in which the annealing temperature was increased to 51 °C; and a final extension of 5 minutes at 72 °C. We also amplified and sequenced two regions of ribosomal RNA (rRNA); the D2 domain of 28S (28S-D2) and the internal transcribed spacer 2 (ITS2), using the respective primers and protocols detailed in Morse *et al*. (2016). Amplifications were confirmed by gel electrophoresis and PCR products were purified using a DNA Clean & Concentrator™-5 kit (Zymo Research, Irvine, California, USA). Purified products were direct sequenced in both directions at the Institute for Integrative Genome Biology, UCR.

A handful of the ITS2 PCR products proved difficult to direct-sequence and in each case, sequencing was subsequently facilitated by inserting the PCR product into a plasmid vector (pGEM-T Easy Vector System; Promega, Madison, WI). Plasmids were transformed in JM109 competent cells and insert-positive clones were identified by blue-white screening. One to three clones of the ITS2 PCR product were amplified and sequenced from each problematic specimen using M13 primers.

At the University of Miyazaki Kibana campus, one female and one male of *Gonatocerus* sp., which are voucher specimens of the colony maintained by H. Kusuhara using *N. cincticeps* eggs on rice plants in a greenhouse at the Kyushu University Ito Campus, Motooka, Fukuoka City, Fukuoka Prefecture, Japan (table 1), were selected for DNA extraction using a DNeasy Blood & Tissue Kit (Qiagen), following the manufacturer's destructive protocol. PCR was used to amplify the COI and 28S gene regions using the same primers listed above, but using GoTaq® G2 Hot Start Green Master Mix (Promega). Amplicons were purified using a Monofas® DNA purification kit I (GL Sciences, Inc.) and sequenced by FASMAC (http://fasmac.co.jp/).

All sequences were deposited in GenBank (Clark *et al.*, 2016; see table 1 for accession numbers).

Genetic analyses

Phylogenetic analysis of DNA sequence variation was performed on the COI and 28S-D2 data separately. We obtained COI sequences from 22 of 23 specimens, data on which is summarized in table 1. 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 9 sequences retrieved from BOLD and GenBank, which represented haplotypes of the European *G. fuscicornis* [= *Gonatocerus sulphuripes* (Foerster)] from Belarus, Bulgaria, Germany, Russia, and Canada, and three further sequences representing *Gonatocerus* spp. haplotypes reared from *Nephotettix virescens* (Distant) and *Nephotettix nigropictus* (Stal) in the Philippines (Sann *et al.*, 2018; see figure 23 for accession numbers). At least one of them was identified, based on morphology, by Sann *et al*. (2018) as *Gonatocerus orientalis* Zeya, which is now called *Gonatocerus meghalayanus* Zeya (Zeya, 2011; Huber, 2015) and was known previously only from India (Zeya and Hayat, 1995). *G. meghalayanus* is also a member of *G. fuscicornis* complex. 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 35 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 21 of the 22 specimens for which COI was available, plus the one specimen for which COI could not be amplified (table 1). These were combined with four sequences representing *Gonatocerus* spp. reared from *N. virescens* and *N. nigropictus* in the Philippines (Sann *et al.*, 2018) and a 28S-D2 sequence of *C. 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 27 terminal taxa and 515 nucleotide positions. Phylogenetic reconstruction was performed using PhyML as detailed for COI.

More or less complete sequences of the ITS2 were obtained for 20 of the 23 specimens (table 1). We were only able to obtain a partial ITS2 sequence for the remaining specimen (D4367). The *G. aegyptiacus sensu* Triapitsyn (2013) specimens proved particularly difficult to sequence due to the occurrence of back-to-back monomeric runs of Ts and As at the 5' end of the locus (see GenBank accessions). In an attempt to get a full ITS2 sequence for this species, the PCR amplicons of three specimens were cloned. The ITS2 of the two *G. cincticipitis* specimens (PR19-497 and PR19-498) and of *Gonatocerus* sp. (PR19-495) also required cloning. 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.

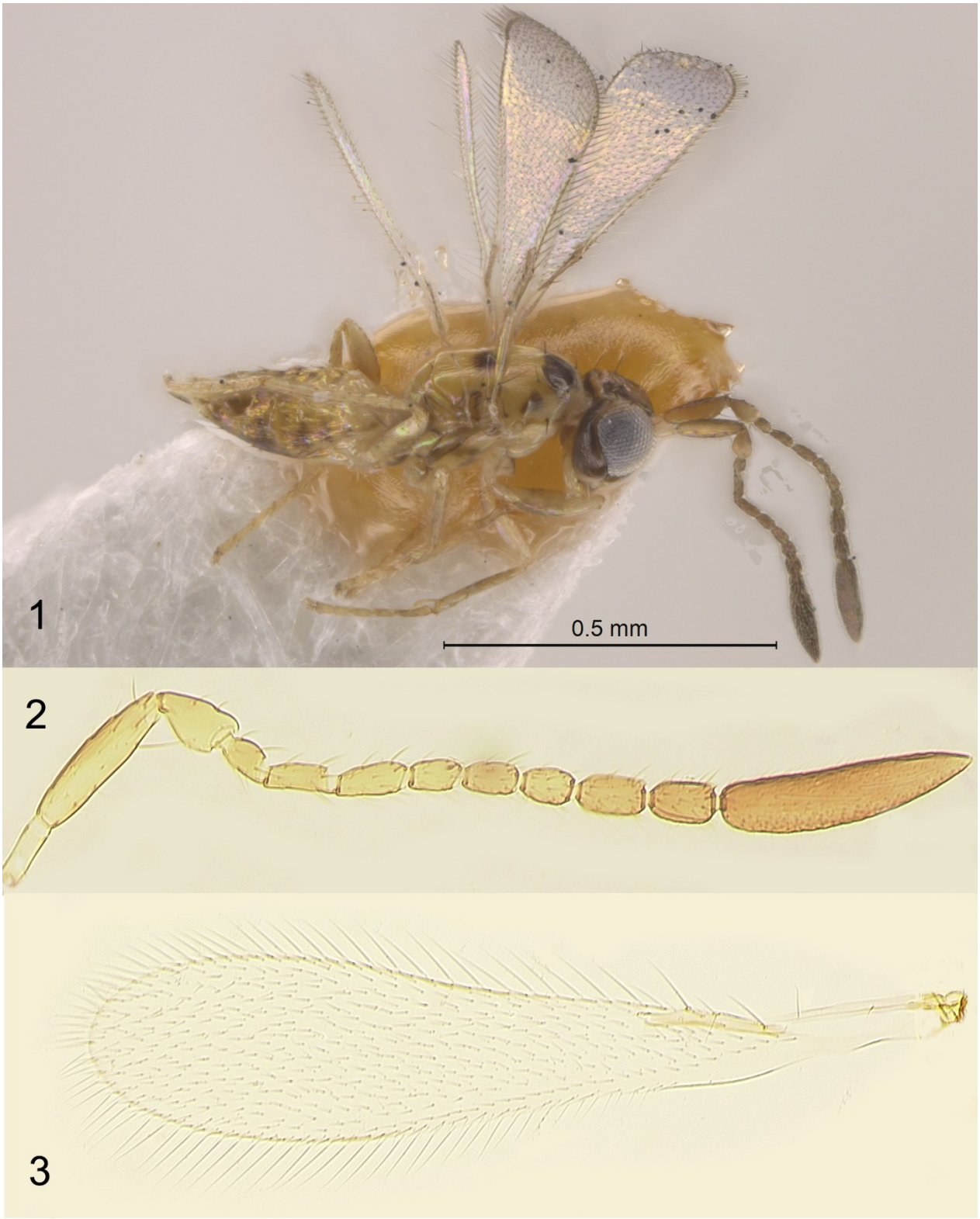
Genetic separation between the members of the *G. fuscicornis* complex was further estimated by calculating average pairwise uncorrected p-distances within and between the COI sequences of the different species. The analysis was conducted in MEGA version 7 using only COI sequences generated in this study.

Results

An updated key to females of the Palaearctic species of *Gonatocerus*

(derived from Triapitsyn, 2013)

1	-	Mps only on F8 or F7 and F8 2
--	-	Mps also on funicular segments other than F7 and F8, on at least one antenna 6
2 (1)	-	Mps almost always only on F8 [known from Egypt only] . . . . . *G. aegyptiacus* Soyka (*sensu stricto*)
--	-	Mps also on F7, at least 1 mps on at least one antenna 3
3 (2)	-	Body colour mostly brown to dark brown, with some yellow or light brown patches, especially on mesosoma and base of metasoma (figures 5-6, 15) 4
--	-	Body colour mostly yellow to light brown, with some brown or dark brown patches (figures 1, 19) 5
4 (3)	-	Fore wing 3.6-3.8× as long as wide, with discal microtrichia originating behind about middle of submarginal vein (figure 17) . . . . . *G. fuscicornis* (Walker)
--	-	Fore wing 4.0-4.3× as long as wide, with discal microtrichia originating behind apex of submarginal vein (figure 10) . . . . . *G. cincticipitis* Sahad (part)
5 (3)	-	Fore wing with discal microtrichia originating behind base of marginal vein (figure 3) [eastern Palaearctic, Oriental, Australasian, and Oceanean, egg parasitoid of *Nephotettix* spp. (best identified using molecular methods, as presented below)] . . . . . *G. saipanensis* (Doutt), stat. rev.
--	-	Fore wing with discal microtrichia originating behind very apex of submarginal vein (figure 21) [mainly European (best identified using molecular methods, as presented below)] . . . . . *G. minor* Matthews, stat. rev.
6 (1)	-	F5 and F6 each with 2 mps 7
--	-	F5 with 0 or 1 mps and F6 with 0-2 mps; or if F5 with 2 mps, then F6 without mps 8
7 (6)	-	F4 with at most 1 mps; ovipositor at least 1.9× as long as mesotibia . . . . . *G. longicornis* Nees ab Esenbeck
--	-	F4 with 2 mps; ovipositor at most 1.4× as long as mesotibia . . . . . *G. bukashka* Triapitsyn
8 (6)	-	F5 without mps . . . . . *G. pictus* (Haliday)
--	-	F5 with 1 or 2 mps at least on one antenna 9
9 (8)	-	Clava at least 3.6× as long as wide; funicular segments relatively longer, funicle length at least 2.4× clava length; fore wing with discal microtrichia relatively shorter and much more numerous . . . . . *G. koziavka* Triapitsyn
--	-	Clava at most 3.2× as long as wide; funicular segments relatively shorter, funicle length at most 2.1× clava length; fore wing with discal microtrichia relatively longer and less numerous (figure 10) . . . . . *G. cincticipitis* Sahad (part)



Figures 1-3. *Gonatocerus saipanensis*, female. **1.** habitus in dorsolateral view (Wufeng, Taichung, Taiwan); **2-3.** Gukeng, Yunlin County, Taiwan (**2**) antenna, (**3**) fore wing.

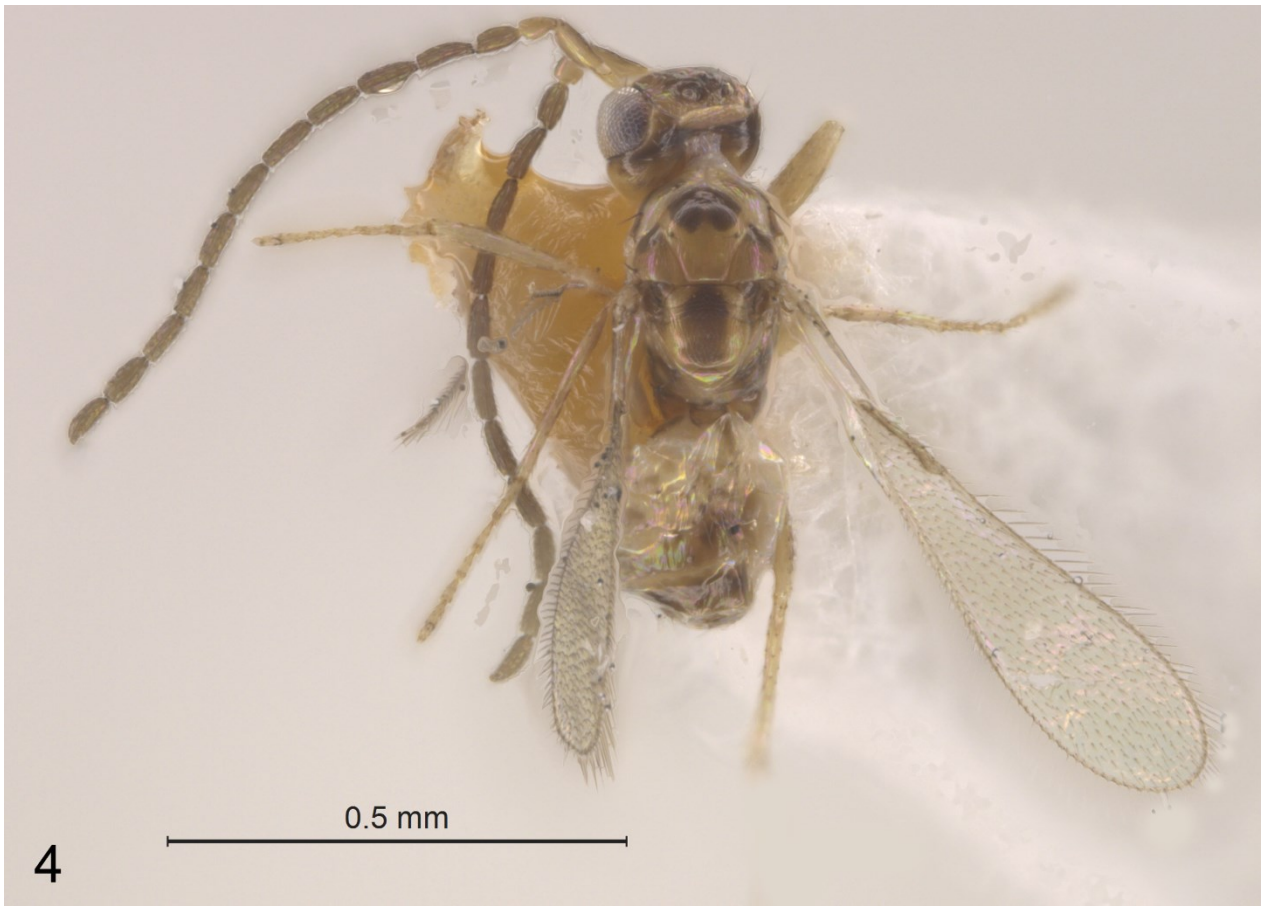


Figure 4. *Gonatocerus saipanensis*, male (Wufeng, Taichung, Taiwan). Habitus in dorsal view.

Gonatocerus aegyptiacus Soyka 1950 (*sensu stricto*)

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

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

D i a g n o s i s

The little known *G. aegyptiacus* (*sensu stricto*) may be difficult to distinguish morphologically from other members of the *G. fuscicornis* species complex, particularly from *G. minor* and *G. saipanensis*. Most females from Egypt lack mps on F7 of the antenna (Triapitsyn, 2013).

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

Palaearctic Region: Egypt (Soyka, 1950).

Records of *G. aegyptiacus sensu* Triapitsyn (2013) from the Palaearctic part of China and also from Primorskiy Kray in the Russian Far East, as well as those from New York (USA) in the Nearctic Region and from the Republic of South Africa and United Arab Emirates in the Afrotropical Region (Triapitsyn, 2013), need confirmation using molecular methods as some of them could belong to either *G. minor* or *G. saipanensis* rather than to *G. aegyptiacus* (*sensu stricto*).

H o s t s

Unknown. The hosts indicated for *G. aegyptiacus* in Triapitsyn (2013) apply only to *G. saipanensis*, as treated here.

Gonatocerus cincticipitis Sahad 1982

(figures 5-14)

Gonatocerus sp. y: Sahad, 1982a: 246-259 (biology, previous references in Japanese as *Gonatocerus* sp. or *Lymaenon* sp.); Sahad, 1982b: 467-476 (morphology, immature stages).

Gonatocerus cincticipitis Sahad, 1982c: 192-195. Type locality: Matsue, Shimane Prefecture, Honshu Island, Japan.

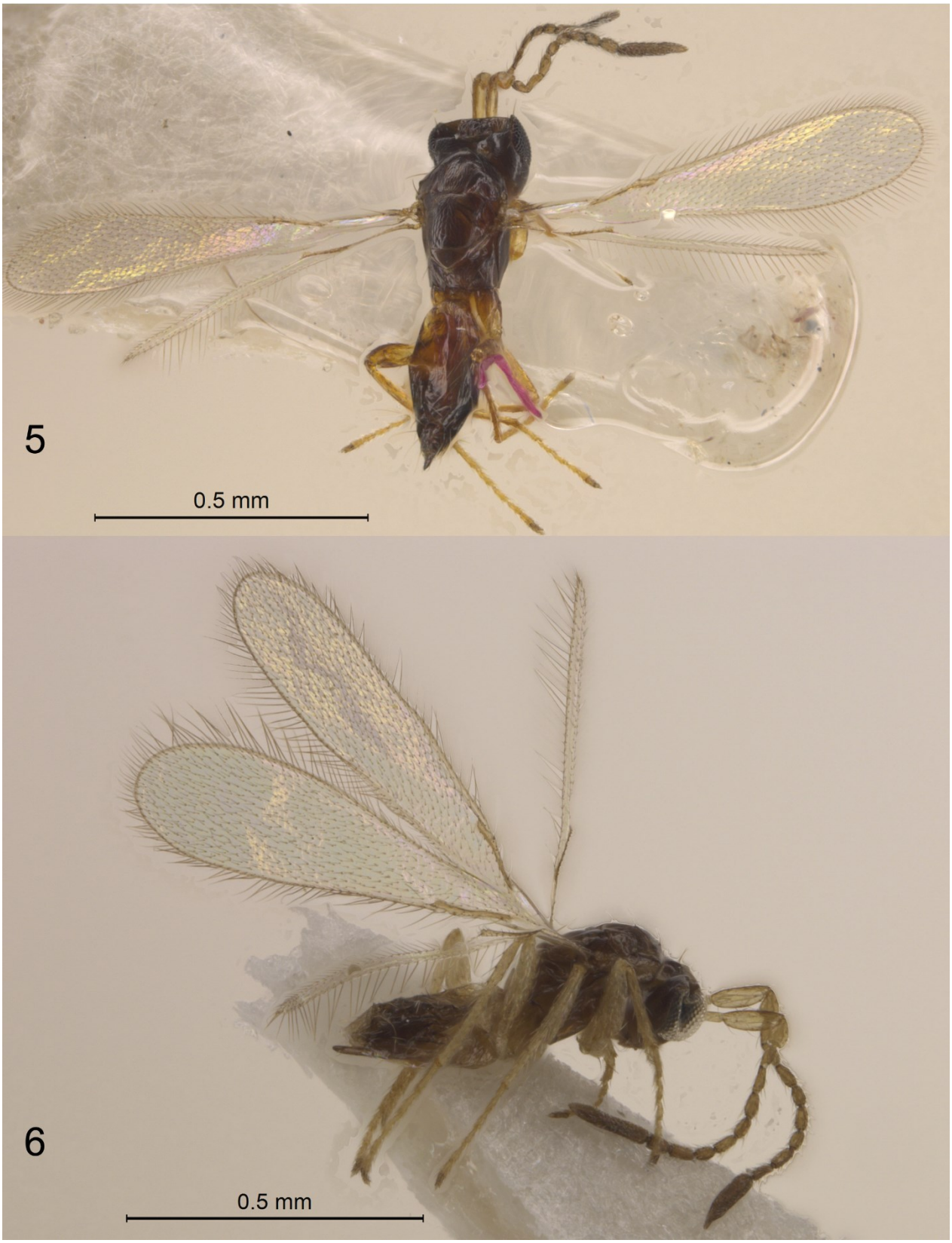
Gonatocerus sulphuripes (Foerster): Sahad and Hirashima, 1984: 3 (list), 22-25 (redescription, illustrations, distribution in Japan). Misidentified.

Gonatocerus cincticipitis Sahad: Sahad and Hirashima, 1984: 3 (list), 37-43 (redescription, illustrations, distribution, host association, biology); Huber, 2015: 30 (list); Triapitsyn, 2018: 144 (similarity with *G. aegyptiacus*, record from Japan).

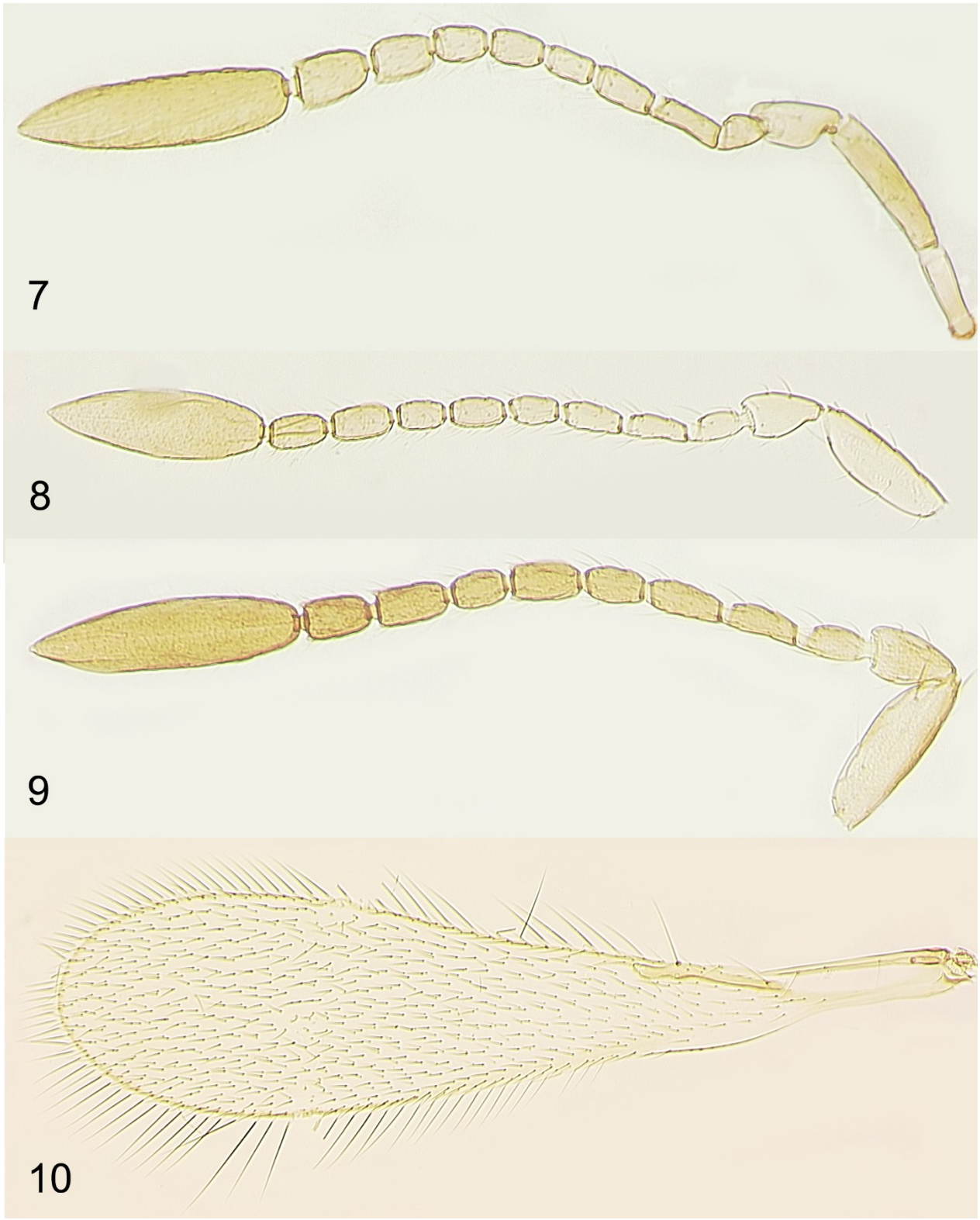
Gonatocerus (*Gonatocerus*) *cincticipitis* Sahad: Triapitsyn, 2013: 15-17 (taxonomic history, distribution, redescription, illustrations, diagnosis, host).

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

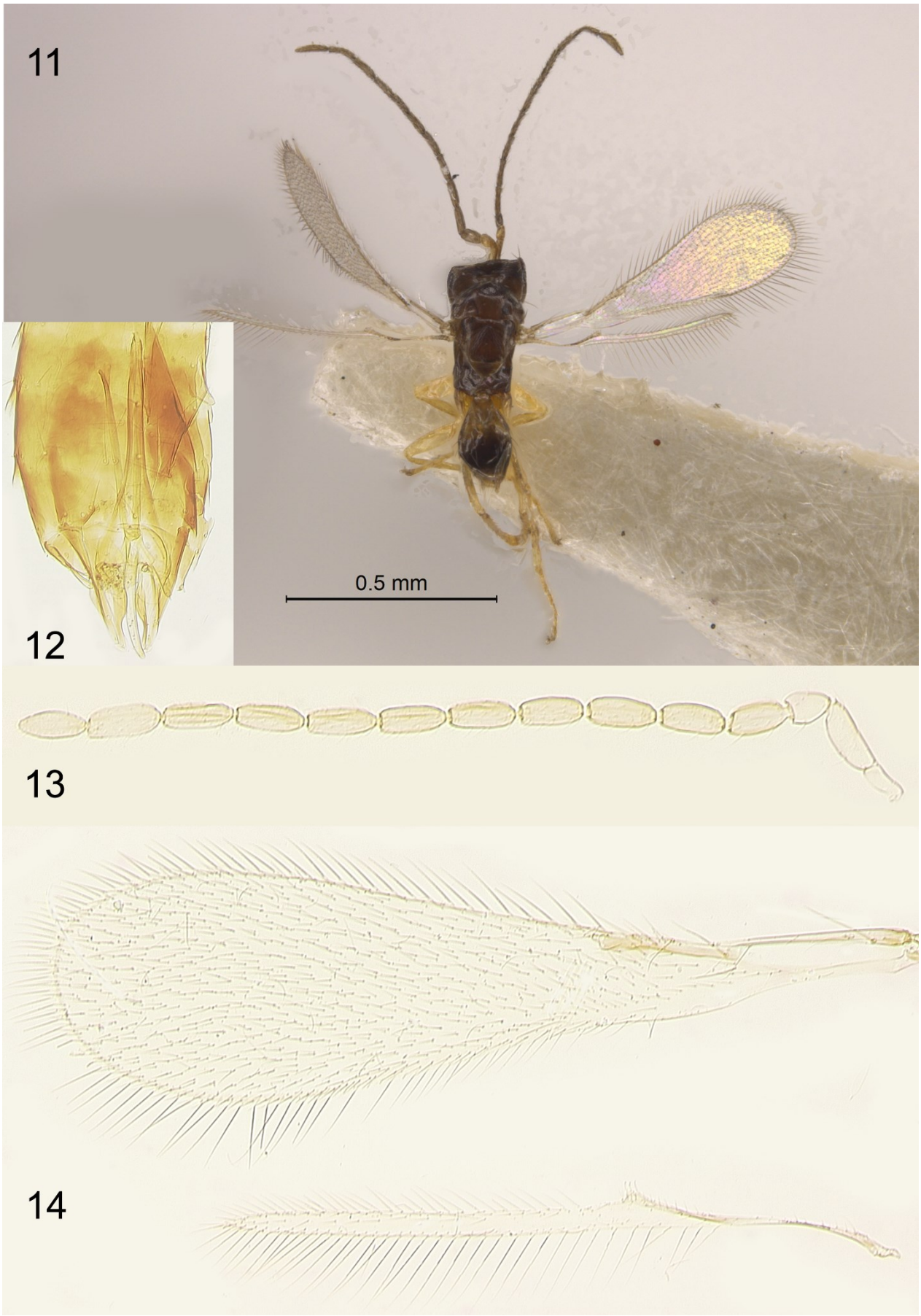
Japan: Honshu Island: Aichi Prefecture: Ichinomiya, 2-8.ix.2006, C. Ueshima, paddy field [2 females, ZLMU]. Toyota, Kojima, 3-7.x.1999, Y. Murata, flight intercept trap [1 female, ZLMU]. Gifu Prefecture, Kani, Katabita, K. Yamagishi, Malaise trap: 19-25.vi.2004 [2 females, ZLMU]; 3-9.ix.2004 [1 female, ZLMU]. Niigata Prefecture, Nagaoka, Teradomari, 23.viii.1980, K. A. Sahad



Figures 5-6. *Gonatocerus cincticipitis*, female. **5.** habitus in dorsal view (Harumachi, Fukuoka, Fukuoka Prefecture, Kyushu Island, Japan); **6.** habitus in lateral view (Tsuyahonmachi, Fukuoka).



Figures 7-10. *Gonatocerus cincticipitis*, female. **7-9.** antennae (**7**) Izumo, Shimane Prefecture, Honshu Island, Japan (**8-10)** Teradomari, Nagaoka, Niigata Prefecture, Honshu Island, Japan (**10**) fore wing.



Figures 11-14. *Gonatocerus cincticipitis*, male. **11. 13.** Teradomari, Nagaoka, Niigata Prefecture, Honshu Island, Japan; **12. 14.** Hakozaki, Fukuoka, Fukuoka Prefecture, Kyushu Island, Japan; (**11**) habitus in dorsal view (**12)** genitalia (**13**) antenna (**14**) fore and hind wings.

[numerous females and males, ELKU] (identified as *Gonatocerus* sp. y by K. A. Sahad). Osaka Prefecture, Amano [Village], 11.xi.1933, Shinkai [4 females, 2 males, ITLJ]. Shimane Prefecture: Izumo, Shimane Agricultural Technology Center, 35°20'04"N 132°43'59"E 7 m a.s.l., 3-5.x.2019, S. V. Triapitsyn, N. Kado, T. Adachi-Hagimori, yellow pan traps in organic rice field [1 female, UCRC] (molecular voucher PR19-497, UCRC ENT 311773). Matsue: Kamihonjocho, Shimane University Honjo Farm, 35°30'59"N 133°06'31"E, 50 m a.s.l., 2.x.2019, S. V. Triapitsyn, T. Adachi-Hagimori, N. Kado, Y. Narai, sweeping in a small, non-organic rice plot [1 female, UCRC] (molecular voucher PR19-498, UCRC_ENT 00528728); Sada, 20-25.vii.1978, K. A. Sahad [numerous females and males, ELKU]. Kyushu Island: Fukuoka Prefecture, Fukuoka: x.1914, Mochizuki (from eggs of *N. cincticeps*) [10 females, 1 male, ITLJ]; Hakozaki, 30.viii.1979, 24.viii.1981, and 31.viii-1.ix.1981, K. A. Sahad [numerous females and males, ELKU] (identified as *Gonatocerus* sp. y by K. A. Sahad). Harumachi, K. A. Sahad: 30.viii.1979 [1 female, 1 male, ELKU] (both mislabeled as a 'Holotype" in red ink by K. A. Sahad); 23.viii.1980, "Plot A" [numerous females and males, ELKU] (one male also labeled: "Emerged from the egg of *N. cincticeps* K. A. Sahad leg."); 8.ix.1980, "plot B" [numerous females and males, ELKU]. Tsuyahonmachi (near Hakozaki), C. Okuma: 26.x.1971, rice paddy [1 female, ELKU]; 22.viii.1972, small stream [1 female, ELKU]; 2.x.1972, rice paddy [1 male, ELKU]; 16.x.1972, small stream [1 female, ELKU]; 13.xi.1972, small stream [1 female, ELKU]; 9.v.1973, rice paddy area [1 female, ELKU]; 4.vi.1973, rice paddy area [1 female, ELKU].

D i a g n o s i s

G. cincticipitis is morphologically similar to *G. aegyptiacus* (*sensu stricto*), *G. fuscicornis*, *G. minor* and *G. saipanensis* from which some females differ by the presence of usually 1 mps on F5 on at least one antenna (figure 8) [less often having 2 mps (figure 9), as stated by Sahad (1982c) and Sahad and Hirashima (1984)]. It also differs from *G. saipanensis*, which also has a narrow fore wing (figure 3), by the discal setae on the fore wing (figure 10) originating behind apex of the submarginal vein (behind base of the marginal vein in *G. saipanensis*). From *G. fuscicornis*, which has a similar body colour, all individuals of *G. cincticipitis* (including those which often lack an mps on F5 of both female antennae) in having a relatively narrower fore wing, as indicated in the key above. From *G. minor*, in which discal setae on the fore wing (figure 21) also originate behind apex of the submarginal vein, *G. cincticipitis* (figures 5-6) differs by the darker general body colour (in the former, it is mostly yellow to light brown, with some brown or dark brown patches, figure 19).

R e d e s c r i p t i o n

FEMALE (non-type specimens from Japan and the Republic of Korea, including those listed in Triapitsyn (2013) [UCRC]). Body length 600-950 µm (dry-mounted specimens, n = 25). Head dark brown; often scape, pedicel, and F1 light brown, remainder of flagellum brown but sometimes only radicle light brown and the rest of antenna brown; mesosoma mostly dark brown, sometimes with lighter patches; gaster mostly brown or dark brown except light brown basally; legs yellow to light brown (figures 5-6).

Antenna (figures 7-9) with radicle 0.35-0.36× total length of scape, rest of scape 2.7-3.2× as long as wide; pedicel longer than F1; F1 a little shorter than F2 and about as long as F4 and F6, F2 about as long as F3, F5, F7, and F8 except when F5 slightly shorter when lacking mps; F5 either without mps (figure 7) or with 1 mps (figure 8) on one antenna and 0 or 1 on the other antenna, or with 2 mps (figure 9) on one or both antennae, F7 and F8 each with 2 mps; clava with 7 or 8 mps, 3.1-3.6× as long as wide, about as long as combined length of F5-F8 or slightly shorter when F5 lacks mps.

Mesosoma shorter than gaster. Propodeum with fine submedian lines. Fore wing (figure 10) 4.0-4.3× as long as wide; longest marginal seta 0.43-0.54× maximum wing width. Fore wing disc slightly infumate throughout, mostly bare behind submarginal vein and setose behind apex of submarginal vein and beyond. Hind wing 27-29× as long as wide; disc bare except for rows of setae along margins and a few additional setae, slightly infumate throughout; longest marginal seta 3.6-4.4× maximum wing width.

Metasoma with petiole wider than long. Ovipositor 0.7-0.8× length of gaster, at most barely exserted beyond its apex; ovipositor 1.3-1.5× length of mesotibia.

MALE (non-type specimens from Japan including those listed in Triapitsyn (2013) [UCRC]). Body length 630-920 µm (dry-mounted specimens, n = 16). Similar to female in general body colour (figure 11); scape and pedicel light brown, flagellum brown. Antenna (figure 13) with scape minus radicle 2.25-2.6× as long as wide. Fore wing (figure 14) 3.7-4.2× as long as wide; hind wing (figure 14) 24-30× as long as wide. Genitalia (figure 12) length 220-380 µm.

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

Palaearctic Region: Japan, and Republic of Korea (Sahad and Hirashima, 1984; Triapitsyn, 2013; 2018).

H o s t

Cidadellidae: *N. cincticeps* (Uhler) (Sahad, 1982a [as *G.* sp. y], 1982b [as *G.* sp. y], 1982c; Sahad and Hirashima, 1984; Triapitsyn, 2013).

B i o l o g y

Biological traits of *G.* (*Gonatocerus*) *cincticipitis* were reported by Sahad (1982a) [as *G.* sp. y], Miura (1990a; 1990b), and Sahad and Hirashima (1984).

R e m a r k s

Sahad and Hirashima (1984) recorded and illustrated *G. sulphuripes* (Foerster) collected by a suction trap in *Imperata cylindrica* field in Hakozaki, Fukuoka City, Fukuoka Prefecture, Kyushu Island, Japan, but that was an obvious misidentification because its fore wing was 4.05× as long as wide and the discal microtrichia did not originate behind the middle of submarginal vein. Zeya and Hayat (1995) commented that this record could be due to

a misidentification of *G. tarae*, so later Triapitsyn (2013) listed that record under *G. aegyptiacus*. But that was actually highly unlikely for the following reason. As described by Sahad and Hirashima (1984), its general body colour was dark brown, and the female fore wing length: width ratio was well within the known range of *G. cincticipitis*, which is 4.0-4.3× as long as wide (Triapitsyn, 2013). Thus, Sahad and Hirashima's (1984) record of *G. sulphuripes* from Japan was undoubtedly a misidentification of those individuals of *G. cincticipitis* that often lack mps on F5 of both female antennae. Indeed, *G. cincticipitis* is well known from the same locality (see "Material examined" above), and was collected there by K. A. Sahad himself. Interestingly, he did not recognize his own species, *G. cincticipitis*, because it was described and illustrated (Sahad, 1982; Sahad and Hirashima, 1984) as always having "a pair of" mps on F5 of the female antenna. Unfortunately, during a visit to ELKU, S. V. Triapitsyn could not find any of Sahad's voucher specimens identified by him as *G. sulphuripes*.

The holotype female of *G. cincticipitis*, examined by S. V. Triapitsyn during a visit to ELKU in October 2019, is complete, mounted dorsoventrally on a slide. F5 bears 1 mps on both antennae. Its ovipositor occupies nearly entire length of gaster (about 90%), a little exerted beyond gastral apex.

Before examining in person, the holotype and other specimens of *G. cincticipitis* collected by K. A. Sahad and others in ELKU and other collections of Mymaridae in Japan, the first author of this communication was also confused about its identity: Triapitsyn (2018, p. 144) incorrectly supposed that this species could be a synonym of *G. aegyptiacus*. However, the true *G. cincticipitis* has never been recorded from Taiwan, despite numerous rearings of egg parasitoids from sentinel eggs of its host, *N. cincticeps*, exposed in various rice fields (Triapitsyn *et al*., 2021). Thus, it appears to be an eastern Palaearctic species which has not yet been recorded from the Oriental Region.

Re-collecting *G. cincticipitis* in its type locality was a challenge because all rice fields at the Shimane University Honjo Farm in Kamihonjocho, Matsue, Shimane Prefecture had been treated with pesticides long before our brief visits in the beginning of October 2019, when it was already late in the growing season and no *N. cincticeps* were present. However, we were able to collect, by sweeping, one female of *G. cincticipitis* in a very small, supposedly organic plot of ripe but still unharvested rice at the same locality, and also another female at the organic, partially harvested experimental rice plot at the Shimane Agricultural Technology Center in Izumo, Shimane Prefecture using yellow pan traps, where *N. cincticeps* leafhoppers were also absent at that time.

Gonatocerus fuscicornis (Walker 1846)

(figures 15-18)

Lymaenon fuscicornis Walker, 1846: 51. Type locality: unknown (?UK).

Gonatocerus (*Gonatocerus*) *fuscicornis* (Walker): Triapitsyn, 2013: 17-26 (taxonomic history, synonyms, type material, distribution, redescription, illustrations, diagnosis, host).

Gonatocerus fuscicornis (Walker): Huber, 2015: 30-31 (list); Triapitsyn, 2018: 144 (record from Taiwan).

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

Finland, South Karelia, Parikkala, Melkoniemi, Kallioniemi, 61.5232°N 29.3675°E, 77 m a.s.l., 25.vii.2019, J. Paukkunen, yellow pan traps in garden/dry meadow [2 females, UCRC] (molecular vouchers PR20-064, PR20-065, UCRC_ENT 00536083, UCRC_ENT 00536186, respectively). Ireland, County Kildare, Ballynafagh Lake shore, 53°18'20"N 6°46'58"W, 87 m a.s.l., 26.viii.2015, S. V. Triapitsyn, total sweeping [2 females, UCRC] (including C. Dominguez' molecular voucher D4367 [a database number of J. M. Heraty Laboratory at the University of California, Riverside], UCRC_ENT 00418137).

D i a g n o s i s

Besides the body colour, which is mostly brown to dark brown with some yellow or light brown patches (especially on mesosoma and base of metasoma), *G. fuscicornis* is characterized by the combination of its female antenna (figure 16) with mps almost always only on F7 and F8 and the fore wing disc setose from about middle of the submarginal vein (figure 17).

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

Palaearctic Region: Armenia, Austria, Belgium, Bulgaria, China, Czech Republic, Denmark, Finland, France, Georgia, Germany, Greece, Hungary, Ireland, Italy, Kyrgyzstan, Netherlands, North Macedonia, Norway, Poland, Republic of Korea, Romania, Russia, Spain, Sweden, Turkey, and UK (England, Scotland, and Wales); Afrotropical Region: United Arab Emirates; Nearctic Region: USA (Alaska) (Triapitsyn, 2013); Oriental Region: India (Zeya and Hayat, 1995) and Taiwan (Triapitsyn, 2018).

H o s t

Miridae: *Capsus ater* (Triapitsyn, 2013).

Gonatocerus meghalayanus Zeya 2011

Gonatocerus orientalis Zeya in Zeya and Hayat, 1995: 89-91, 144, 159-160 (illustrations). Type locality: "Botanical garden" (no locality indicated, but likely Shillong), Meghalaya, India.

Gonatocerus meghalayanus Zeya, 2011: 33 (replacement name for *G. orientalis* Zeya, 1995 *nec G. orientalis* Girault, 1917).

Gonatocerus zeyai Özdikmen, 2011: 840 (duplicate, unnecessary replacement name for *G. orientalis* Zeya, 1995 *nec G. orientalis* Girault, 1917).

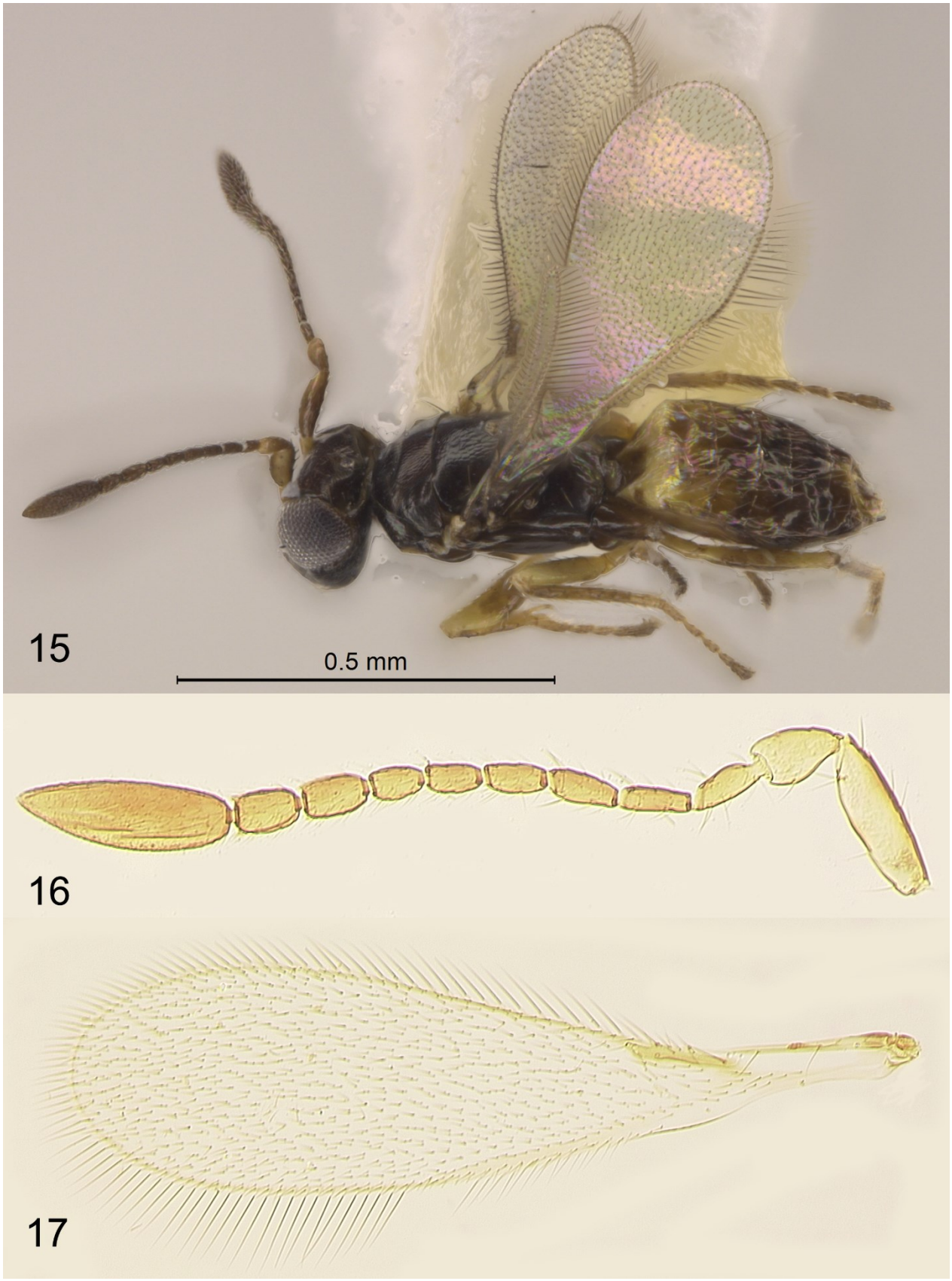
Gonatocerus meghalayanus Zeya: Huber, 2015: 31-32 (list).

Gonatocerus orientalis Zeya: Sann *et al.*, 2018: 4 (hosts in the Philippines).

Gonatocerus aegyptiacus Soyka [*sensu* Triapitsyn (2013)]: Triapitsyn *et al.*, 2021: 83 (misidentification, in part).

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

India, Karnataka, near Dandeli Reserve, 15°20'36"N 74°37'19"E, 530 m a.s.l., 17.xi.2003, J. M. Heraty [1 female, UCRC]. Taiwan, Taichung, Wufeng, Taiwan Agricultural



Figures 15-17. *Gonatocerus fuscicornis*, female. **15.** habitus in dorsolateral view (Ouchamps, Département Loir-et-Cher, France); **16.** antenna (Parikkala, South Karelia, Finland); **17.** fore wing (Richmond Park, London Borough of Richmond upon Thames, England, UK).

Figure 18. *Gonatocerus fuscicornis*, male (Ouchamps, Département Loir-et-Cher, France). Habitus in lateral view.

Research Institute, 24°01'52.4"N 120°41'34.2"E, 76 m a.s.l., 25.ix-11.x.2017, H.-T. Shih, Malaise trap in organic rice field [2 females, UCRC] (molecular vouchers PR20-238 and PR20-240, UCRC_ENT 00536088 and UCRC_ENT 00536089, respectively).

D i a g n o s i s

G. meghalayanus is very similar to *G. saipanensis*, from which it differs mainly in having clava of the female antenna shorter than the combined length of F5-F8 (Zeya and Hayat, 1995 [compared with *G. tarae*]).

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

Oriental Region: India (Zeya and Hayat, 1995 [as *G. orientalis*]), Philippines (Sann *et al*., 2018 [as *G. orientalis*]), and Taiwan [new record] (Triapitsyn *et al*., 2021 [misidentified as *G. aegyptiacus*, in part]).

H o s t

Cidadellidae: *N. nigropictus* and *N. virescens* in the Philippines (Sann *et al.*, 2018 [as *G. orientalis*]).

R e m a r k s

As revealed by our genetic analyses, some misidentified specimens of *G. meghalayanus* were mixed in with those of *G. saipanensis* [as *G. aegyptiacus*] in Malaise trap samples collected in the organic rice field at the Taiwan Agricultural Research Institute, Wufeng, Taichung, Taiwan (Triapitsyn *et al.*, 2021). Specimens of *N. nigropictus* were also collected in the same trap a year earlier, in October 2016 (Triapitsyn *et al.*, 2021: 83), so this leafhopper could be the likely host of *G. meghalayanus* there.

Gonatocerus minor Soyka, 1950, stat. rev.
(figures 19-22)

Gonatocerus minor Matthews, 1986: 220 (member of the *sulphuripes* species group). Type locality: Hatfield Forest, Essex County, England, UK. Stat. rev. (reinstated as a valid taxon from the previous synonymy with *G. aegyptiacus* Soyka by Triapitsyn, 2013: 9).

Gonatocerus (*Gonatocerus*) *aegyptiacus* Soyka: Triapitsyn, 2013: 9-13 (in part; taxonomic history, synonymy, type material, distribution, redescription, illustrations, diagnosis).

Gonatocerus aegyptiacus Soyka *sensu* Triapitsyn (2013): Huber, 2015: 30 (list, as a synonym of *G. aegyptiacus* Soyka).

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

Finland, South Karelia, Parikkala, Melkoniemi, Kallioniemi, 61.5232°N 29.3675°E, 77 m a.s.l., 25.vii.2019, J. Paukkunen, yellow pan traps in garden/dry meadow [2 females, 1 male, UCRC] (molecular vouchers PR20-066–PR20-068, UCRC_ENT 00536082, UCRC_ENT 00536084, UCRC_ENT 00536080, respectively).

D i a g n o s i s

In some small specimens from Europe F7 of the female antenna rarely either lacks mps or bears just 1 mps (Triapitsyn, 2013). It is almost identical morphologically to *G. aegyptiacus* (*sensu stricto*) and *G. saipanensis*, from which it can be separated mainly geographically or using molecular methods, as presented below for both *G. minor* and *G. saipanensis*, besides the minor morphological differences indicated in the key. Fore wing (figure 21) in females from Europe is 3.9-4.3× as long as wide (n = 9, specimens from Finland as well as France, UK (England), and the European part of Russia, as listed in Triapitsyn (2013) [UCRC]).

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

Palaearctic Region: Austria, Bulgaria, Denmark, France, Greece, Hungary, Italy, Kyrgyzstan, Mongolia, Russia, Spain, and UK (England); Nearctic Region: USA (Triapitsyn, 2013).

H o s t

Unknown. The hosts indicated for *G. aegyptiacus* in Triapitsyn (2013) apply only to *G. saipanensis*, as treated here.

Gonatocerus saipanensis (Doutt, 1955), stat. rev.
(figures 1-4)

Lymaenon saipanensis Doutt, 1955: 13-15. Type locality: Saipan Island, Northern Mariana Islands. Previously synonymized with *G. aegyptiacus* by Triapitsyn, 2013: 9.

Lymaenon tarae Narayanan and Subba Rao, 1961: 657-659. Type locality: Delhi, India. Synonymized with *G. aegyptiacus* by Triapitsyn, 2013: 9. Syn. nov.

Gonatocerus sp. r: Sahad, 1982a: 246, 257 (mentioned).

Gonatocerus miurai Sahad, 1982c: 195-198 (= *G.* sp. r of Sahad 1982a). Type locality: Matsue, Shimane Prefecture, Honshu Island, Japan. Previously synonymized with *G. tarae* (Narayanan et Subba Rao) by Zeya and Hayat, 1995: 84 and with *G. aegyptiacus* by Triapitsyn, 2013: 9. Syn. nov.

Gonatocerus alami Shamim and Shafee, 1984: 623-624. Type locality: Aligarh, Uttar Pradesh, India. Previously synonymized with *G. tarae* by Zeya and Hayat, 1995: 84 and with *G. aegyptiacus* by Triapitsyn, 2013: 9. Syn. nov.

Gonatocerus miurai Sahad: Sahad and Hirashima, 1984: 3 (list), 34-37 (redescription, illustrations, distribution, host association).

Gonatocerus (*Gonatocerus*) *aegyptiacus* Soyka: Triapitsyn, 2013: 9-13 (in part; taxonomic history, distribution, redescription, illustrations, diagnosis, hosts).

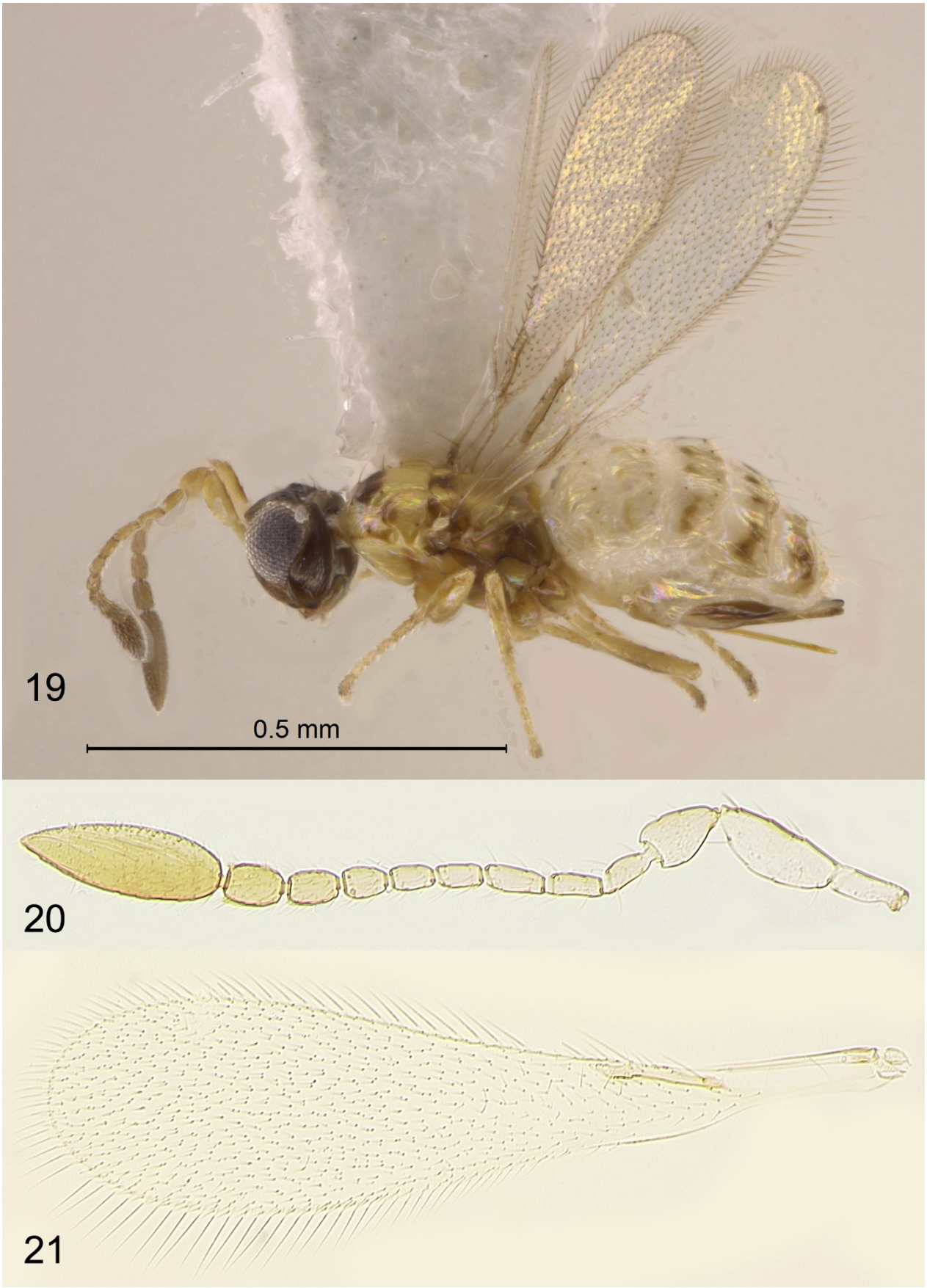
Gonatocerus aegyptiacus Soyka [*sensu* Triapitsyn (2013)]: Huber, 2015: 29-30 (list, in part); Triapitsyn, 2018: 143 (records from Japan and Taiwan, host associations), 144 (similarity with *G. cincticipitis*); Triapitsyn *et al.*, 2018b: 7 (host in Taiwan), 14 (illustration); Triapitsyn *et al.*, 2021: 82 (illustrations), 83 (distribution and hosts in Taiwan), 97 (key).

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

Japan: Kyushu Island: Fukuoka Prefecture, Fukuoka City, Tsuyahonmachi (near Hakozaki), 25.ix.1972, C. Okuma, rice field [1 female, ELKU]. Miyazaki Prefecture, Miyazaki City, Ooaza, Atoe, 31°56'50.9"N 131°22'55.8"E, 7 m a.s.l., T. Adachi-Hagimori, T. Minami, K. Matsui (Malaise trap in in organic rice field): 24.viii-1.ix.2020 [1 female, UCRC] (molecular voucher PR21-142, UCRC_ENT 00541268); 17.ix-1.x.2020 [3 females, UCRC] (including molecular voucher PR21-143, UCRC_ENT 00541270). Ryukyu Islands, Okinawa Islands, Okinawa Prefecture: Ishigaki Island, Sokobaru, 17.i.2017, P. Jałoszyński, forest [1 male, UCRC]. Okinawa Island: Todoroki Waterfall, 21.ii.2019, P. Jałoszyński [1 male, UCRC]. Motobu Peninsula, near Nakijin Castle, ca. 50 m, 25.ii.2019, P. Jałoszyński, humid forest [1 female, UCRC] (molecular voucher PR19-496, UCRC_ENT 00517340). Taiwan: Chiayi County, Minxiong Township, 8-22.vi.2017, S.-H. Huang, Malaise trap in rice field [2 females, UCRC] (molecular vouchers PR20-239 and PR20-243, UCRC_ENT 00536085 and UCRC_ENT 00536086, respectively). Taichung, Wufeng, Taiwan Agricultural Research Institute, 24°01'52.4"N 120°41'34.2"E, 76 m a.s.l., organic rice field: 31.v-13.vi.2017, H.-T. Shih, Malaise trap [2 females, UCRC] (molecular vouchers PR19-493 and PR19-494, UCRC_ENT 00517339 and UCRC_ENT 00517337, respectively); 6-8.vi.2017, M.-J. Tseng, yellow pan traps [1 male, UCRC] (molecular voucher PR20-241, UCRC_ENT 00536087).

D i a g n o s i s

It may be difficult to distinguish *G. saipanensis* from *G. fuscicornis* (especially the darker-colored specimens and females in which F7 of the antenna bears 2 mps) but in the latter species the discal setae originate behind about middle of the submarginal vein (figure 17) whereas in the former the fore wing disc is bare behind the entire submarginal vein (figure 3). In most specimens from Egypt identified by W. Soyka as *G. aegyptiacus*, F7 of the female antenna lacks mps, only occasionally bearing



Figures 19-21. *Gonatocerus minor*, female. **19.** habitus in lateral view (La Gard ou Gardon, Département Gard, France); **20.** antenna (Parikkala, South Karelia, Finland); **21.** fore wing (Sainte Colombe, Département Gironde, France).

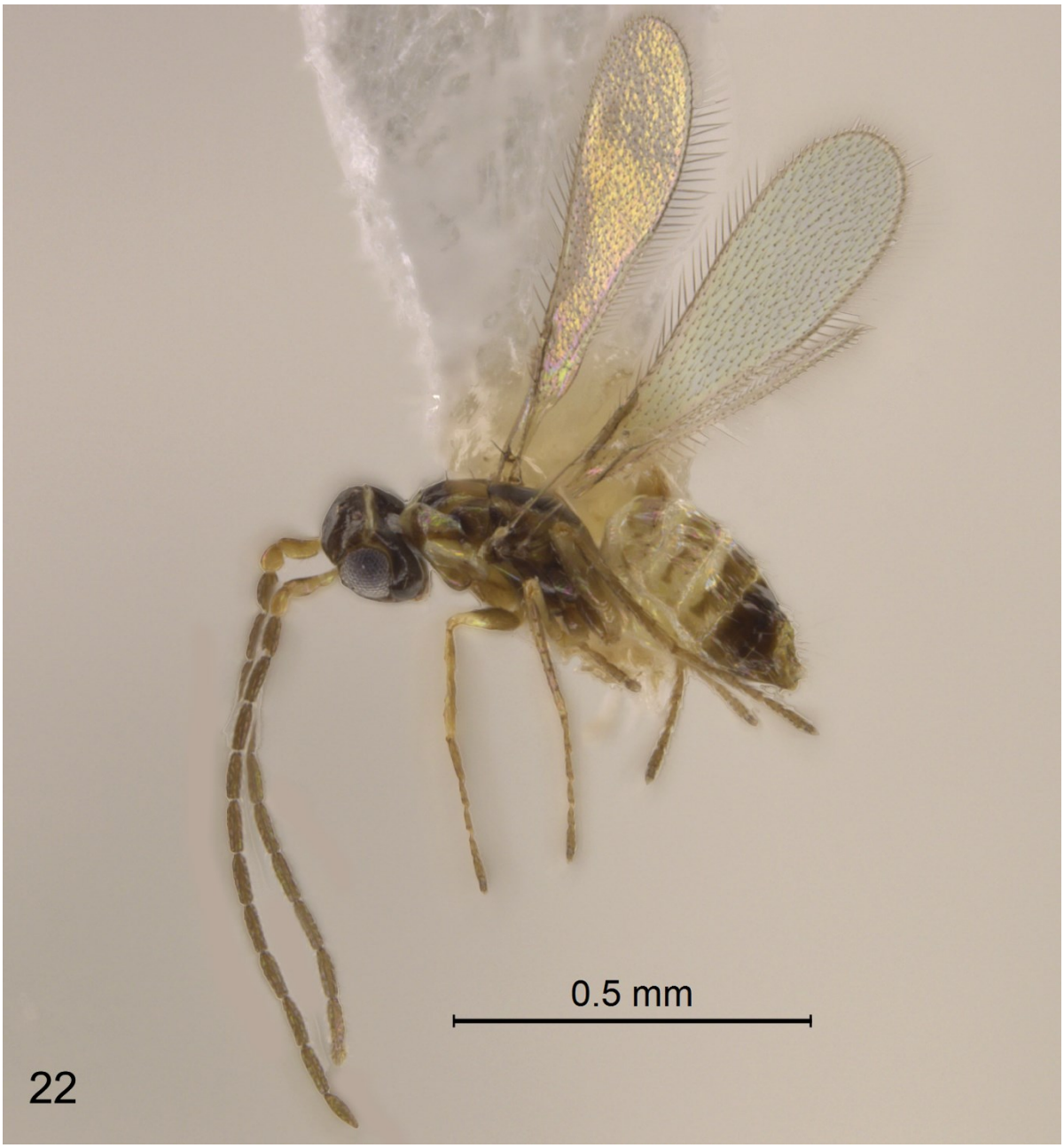


Figure 22. *Gonatocerus minor*, male (5.5 km E of Monte Romano, Viterbo Province, Lazio, Italy). Habitus in lateral view.

1 mps. *G. saipanensis* is almost identical morphologically to *G. minor* stat. rev., from which it can be separated mostly geographically or, preferably, using molecular methods, as presented below, besides the minor but more or less consistent morphological difference indicated in the key. Clava of the female antenna in *G. saipanensis* is either slightly longer than or about as long as the combined length of F5-F8 (Zeya and Hayat, 1995 [for *G. tarae* (Narayanan and Subba Rao)]).

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

Palaearctic Region: Japan (Sahad, 1982c [as *G. miurai*]; Sahad and Hirashima, 1984 [as *G. miurai*]; Triapitsyn, 2013 [as *G. aegyptiacus*]), Republic of Korea (Sahad and Hirashima, 1984 [as *G. miurai*]); Australasia: Australia (Triapitsyn, 2013 [as *G. aegyptiacus*]). Oceania: American Samoa, Fiji (Triapitsyn, 2013 [as *G. aegyptiacus*]), Northern Mariana Islands (Doutt, 1955 [as *G. saipanensis*]); Oriental Region: India (Zeya and Hayat, 1995 [as *G. tarae*]; Triapitsyn, 2013 [as *G. aegyptiacus*]), Nepal, Pakistan, Thailand (Triapitsyn, 2013 [as *G. aegyptiacus*]), and Taiwan (Triapitsyn, 2018 [as *G. aegyptiacus*]; Triapitsyn *et al.*, 2018b [as *G. aegyptiacus*]).

H o s t

Cidadellidae: *M. dorsalis* (Triapitsyn *et al.*, 2021 [as *G. aegyptiacus*]), *N. cincticeps* (Sahad, 1982a [as *G.* sp. r], 1982c [as *G. miurai*]; Sahad and Hirashima, 1984 [as *G. miurai*]; Triapitsyn, 2018 [as *G. aegyptiacus*]; Triapitsyn *et al.*, 2018b [as *G. aegyptiacus*]) and *Nephotettix* spp. (Triapitsyn, 2018 [as *G. aegyptiacus*]; Triapitsyn *et al.*, 2021 [as *G. aegyptiacus*]). Its records

from eggs of Delphacidae (Hemiptera), such as *Nilaparvata lugens* (Stal) and *Sogatella furcifera* (Horvath), by Zeya and Hayat (1995) [as *G. tarae*] need confirmation and are probably erroneous because of the likely flawed rearing method: as shown by the thorough rearings using a sentinel eggs method, this species seems to parasitize only Cicadellidae (Triapitsyn *et al.*, 2021 [as *G. aegyptiacus*]).

R e m a r k s

It is extremely rare that some individuals (at most one in more than 700 specimens examined) may have an mps on F5 of just one of the female antennae (Triapitsyn, 2018 [as *G. aegyptiacus*]). Because of that, this feature is not regarded in the key above.

The holotype female of *G. miurai*, examined by S. V. Triapitsyn during a visit to ELKU in October 2019, is mounted laterally on a slide and is complete except lacking most of one hind wing.

Gonatocerus sp.

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

Japan: Kyushu Island: Fukuoka Prefecture, Fukuoka City: Nishi-ku (Nishi Ward), Motooka, Kyushu University Ito Campus, from a colony in an experimental greenhouse reared on *N. cincticeps* eggs, H. Kusuhara (originally collected from a rice field at Susenji, 33°34'15"N 130°14'50"E, 11 m a.s.l., H. Kusuhara): 6th generation, 8.xii.2019 [5 females, ELKU (2), UCRC (2); 1 used for destructive DNA extraction]; 8th generation, 14.i.2020 [5 males, ELKU (2), UCRC (2); 1 used for destructive DNA extraction]. Miyazaki Prefecture, Miyazaki City, Ooaza, Atoe, 31°56'50.9"N 131°22'55.8"E, 7 m a.s.l., T. Adachi-Hagimori, T. Minami, K. Matsui (Malaise trap in in organic rice field): 30.vii-11.viii.2020 [1 female, UCRC]; 24.viii-1.ix.2020 [1 female, UCRC] (molecular voucher PR21-144, UCRC_ENT 00541272). Ryukyu Islands, Okinawa Islands, Okinawa Prefecture, Okinawa Island, Oura Bay, Oura, E of river, 16.ii.2019, P. Jałoszyński [1 female, UCRC] (molecular voucher PR19-495, UCRC_ENT 00517338).

R e m a r k s

This enigmatic species, first recognized from Okinawa Island based on molecular voucher specimen PR19-495, does not fit any of the five aforementioned nominal species of *Gonatocerus* from the Palaearctic Region nor *G. meghalayanus* from the Oriental Region. It is somewhat close to *G. saipanensis* morphologically although its antennal clava is distinctly shorter than the combined length of F5-F8, and COI sequences placed it at the base of the *saipanensis* clade (figure 23). However, its COI sequence is actually genetically more distant from *G. saipanensis* (7.2%) than it is from any other member of the complex (figure 23, table 2). Furthermore, a closer relationship between it and the other members of the complex was also suggested by the 28S-D2 sequences (figure 24; see below for further discussion). Unlike the typical *G. saipanensis*, which almost always lacks mps on F5 of the female antenna but very rarely may have an mps on just one of the antennae in the same individual, this *Gonatocerus* sp. has an mps on F5 on both antennae (like in some *G. cincticipitis*) and is relatively darker coloured.

Based on COI sequences, the recently collected specimens from Kyushu Island, both from Miyazaki City (including molecular voucher PR21-144) and those from H. Kusuhara's colony in Fukuoka City (A1 and A2, figure 23), are closely related to *Gonatocerus* sp. specimen PR19-495 from Okinawa Island (figure 23) and are likely conspecific with it. However, there are some noted differences in their 28S-D2 and ITS2 sequences that need to be further investigated, in combination with their morphometric analysis, to reveal the true identity of this apparently undescribed species. Specimens from Kyushu

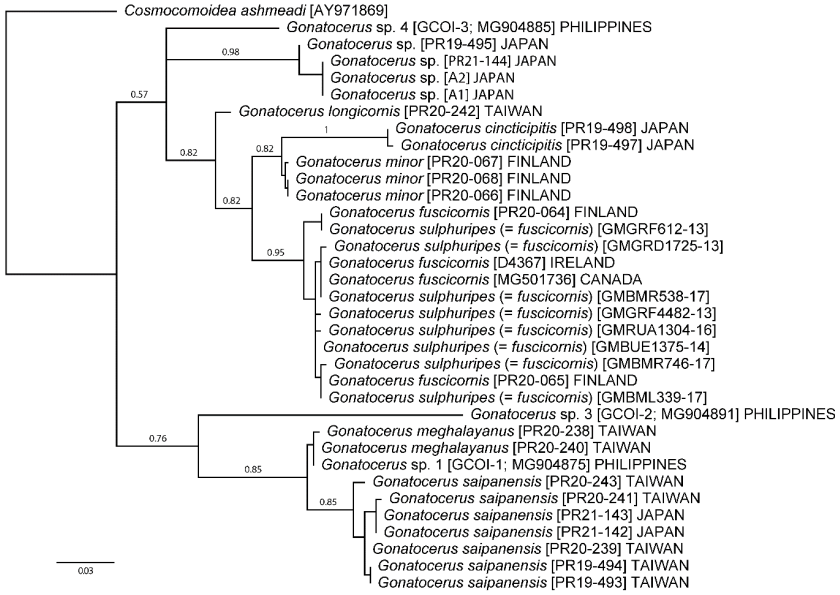


Figure 23. Relationships among members of the *Gonatocerus fuscicornis* complex based on DNA sequences of the mitochondrial COI. Maximum-likelihood analyses were conducted in PhyML and branch support was assessed using approximate likelihood ratio tests. Support for major branches is shown above the branch.

Table 2. Genetic divergence between members of the *G. fuscicornis* complex, based on DNA sequences of the mitochondrial COI gene. Diagonal element shows intraspecific variation (only when more than one sequence was considered). Average pairwise uncorrected p-distances calculated using MEGA 7.0.21.

Species of *Gonatocerus*	*saipanensis*	*meghalayanus*	*cincticipitis*	*fuscicornis*	*minor*	sp.	*longicornis*
saipanensis	0.007						
meghalayanus	0.026	0.002					
cincticipitis	0.098	0.094	0.002				
fuscicornis	0.082	0.081	0.057	0.012			
minor	0.086	0.088	0.041	0.038	0.002		
sp.	0.074	0.071	0.073	0.059	0.063	0.005	
longicornis	0.044	0.076	0.061	0.044	0.032	0.065	-

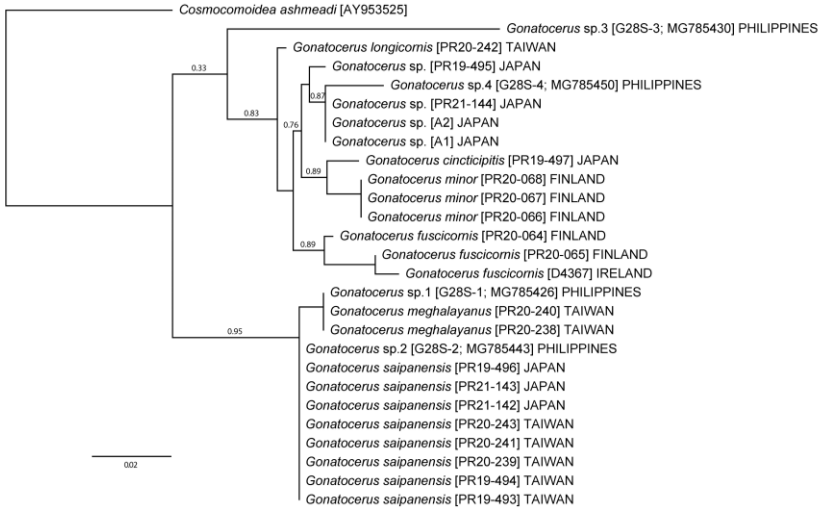


Figure 24. Relationships among members of the *Gonatocerus fuscicornis* complex based on DNA sequences of the nuclear ribosomal 28S-D2. Maximum-likelihood analyses were conducted in PhyML and branch support was assessed using approximate likelihood ratio tests. Support for major branches is shown above the branch.

Island lack mps on F5 of the female antenna and are similar in body colour to both the *Gonatocerus* sp. specimen PR19-495 from Okinawa Island and *G. cincticipitis*. Thus, identity of the previously collected *G. cincticipitis* specimens from Kyushu Island that lack mps on F5 on the female antenna, including those collected by K. A. Sahad, is now in doubt because so far these can only be recognized using molecular methods.

Genetic analyses and discussion

DNA sequences of the mitochondrial COI and nuclear ribosomal 28S-D2 loci provided clear and corroborating support for the five Palaearctic species plus the Oriental *G. meghalayanus* discussed above (figures 23 and 24). Furthermore, the actual relationships among those taxa were almost identical between the two gene trees with the only difference being the placement of the enigmatic specimen PR19-495 (*Gonatocerus* sp. from Okinawa Island) and the likely conspecific *Gonatocerus* sp. specimens from Fukuoka and Miyazaki prefectures, Kyushu Island of Japan, and two further unknown species from the Philippines (Sann *et al.*, 2018), referred to in figures 23 and 24 as *Gonatocerus* sp. 3 and sp. 4. This is likely just an artefact of sampling so few species and/or loci. *Gonatocerus* currently contains 38 extant, valid species worldwide (Huber, 2015) and a realistic assessment of the evolutionary relationships within the genus would require additional loci and a level of taxon sampling far beyond the scope of the current study. Instead, we have essentially utilized COI and 28S-D2 as diagnostic markers, and most importantly for our purpose, they collated specimens of the *fuscicornis* complex into six identical groups (figures 23 and 24). The same six groups were also evident in sequences of the ITS2 gene (not shown). In addition to corroborating support from the ribosomal loci, levels of inter- and intra-specific divergence in the COI gene alone (table 2) were consistent with those typical of valid species (Hebert *et al.*, 2003). Within the *fuscicornis* complex, COI and 28S-D2 both suggest that our specimens of *G. saipanensis* from Japan (Kyushu and Okinawa islands) and Taiwan are relatively divergent from the other members of the complex. Based on the analysis of 28S-D2 sequences (figure 24), *Gonatocerus* sp. 2 reared from *N. virescens* and *N. nigropictus* in the Philippines (Sann *et al.*, 2018) is conspecific with *G. saipanensis* from Japan and Taiwan. Furthermore, the molecular data suggested that the Taiwan specimens in fact represented more than one species. Sequences of the COI

and 28S-D2 (and ITS2; not shown) corroborated in splitting the specimens PR20-238 and PR20-240 from the remaining specimens. These two specimens came from the same rice field as, and are almost identical morphologically to, those firmly identified as *G. saipanensis* (figures 23, 24), except for having the clava of the female antenna slightly shorter than the combined length of F5-F8 and a few setae behind apex of the submarginal vein on one of the fore wings but not on the other. In light of the molecular evidence, these slight morphological differences tend to suggest that PR20-238 and PR20-240 in fact key to *G. orientalis* (now *G. meghalayanus*) in Zeya and Hayat (1995). These specimens were also genetically identical to the most-common of the *Gonatocerus* species from Sann *et al.* (2018) which we call *Gonatocerus* sp. 1 (figures 23, 24); *Gonatocerus* spp. 1-4 from the Philippines were all identified by them as *G. orientalis*. Thus, this warrants a further, integrative taxonomic study of the genetic composition of *G. aegyptiacus* (*sensu stricto*) from Egypt, *G. saipanensis*, and also of *G. meghalayanus* and some other, morphologically quite similar species of *Gonatocerus* described from the Oriental Region and Australia with unknown host associations, with a broad sampling from multiple countries and localities, and from different leafhopper hosts, combined with a thorough morphometric analysis based on statistically significant number of specimens. Preferably, such a study would also integrate reciprocal cross-breeding experiments between the genetically discrete populations of these species.

Given the relative degree of divergence between *G. saipanensis* and the other members of the *fuscicornis* complex, it was surprising to find how genetically similar those other members were to the one congener included from outside of the complex, *G. longicornis* (table 2; figure 23). Morphologically, *G. longicornis* is a very well defined and easily recognizable common species (Triapitsyn, 2013). However, genetically, it appears to be no more removed from the *fuscicornis* complex than the complex members are from each other. This highlights the importance of incorporating molecular data into the taxonomic/diagnostic process.

It is unfortunate that we did not have molecular voucher specimens of *G. aegyptiacus* (*sensu stricto*) from its country of origin, Egypt, to make a direct comparison with our Japan and Taiwan material of *G. saipanensis* and the European material of *G. minor*. However, it is more likely that the true *G. aegyptiacus* from Egypt could be genetically akin to our specimens of *G. saipanensis* from Japan and Taiwan and its synonyms from Asia, previously identified as *G. miurai* in Japan and the Republic of Korea and as *G. tarae* in India, which are egg parasitoids of *Nephotettix* spp. rice pests, than to those previously identified as *G. minor* from northern Europe. Indeed, *Nephotettix modulatus* Melichar occurs in Egypt and also in Tunisia, Morocco, and the Afrotropical Region (Ghauri, 1968; Duan and Zhang, 2014), so it could be a likely host of the Egyptian *G. aegyptiacus*. Therefore, we are rather confident in reinstating *G. minor* stat. rev. from its previous synonymy with *G. aegyptiacus* by Triapitsyn (2013). However, we would definitely like to test this using molecular methods if such an opportunity arises and *G. aegyptiacus* can be recollected in Egypt. The same applies to *G. saipanensis* from Saipan Island, although both morphologically and biogeographically its conspecificity with *G. miurai* from Japan and the Republic of Korea and *G. tarae* from India is not in doubt. In the unfortunate absence of supporting genetic evidence, resurrection of *G. saipanensis* from its previous synonymy with *G. aegyptiacus* is based on the fact that the former nominal species always bears at least 1 mps on F7 of the female antenna, which the latter usually lacks.

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Authors' addresses: Tetsuya ADACHI-HAGIMORI (corresponding author: tadachi@cc.miyazaki-u.ac.jp), Laboratory of Applied Entomology, University of Miyazaki, 1-1, Gakuenkibanadainishi, Miyazaki, 8892192, Japan; Serguei V. TRIAPITSYN, Paul F. RUGMAN-JONES, Department of Entomology, University of California, 3401 Watkins Drive, Riverside, California, 92521, USA.

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