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A new *Gonatocerus* (Hymenoptera: Mymaridae) from Argentina, with taxonomic notes and molecular data on the *G. tuberculifemur* species complex

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Abstract

Gonatocerus deleoni Triapitsyn, Logarzo & Virla **sp. n.**, reared from sentinel eggs of *Tapajosa rubromarginata* (Signoret) (Cicadellidae: Cicadellinae: Proconiini) on citrus plants, a new member of the *ater* species group of *Gonatocerus* Nees (Mymaridae), is described from the state of Mendoza, Argentina. Taxonomic notes and host association data are provided to help differentiate this new species from the morphologically similar but genetically distinct taxon, *G. tuberculifemur* (Ogloblin). The female of the latter is redescribed and the male is newly described. *Gonatocerus deleoni*, *G. tuberculifemur*, and three forms (different molecular clades) comprise the *G. tuberculifemur* complex. These forms are identified but not formally described because of lack of morphologically distinguishing features. The taxonomic conclusions are supported by molecular data, and by results of reciprocal cross-breeding experiments between most of them.

Key words: Mymaridae, *Gonatocerus*, taxonomy, Cicadellidae, Proconiini, egg parasitoid, Argentina

Introduction

Gonatocerus Nees is a common and speciose genus of Mymaridae (Hymenoptera), particularly in the Neotropics. Huber (1988) provided an overview of *Gonatocerus* and defined its species groups. In the New World, many members of the *ater* species group are known to be egg parasitoids of various proconiine sharpshooters (Hemiptera: Cicadellidae: Cicadellinae: Proconiini) (Triapitsyn 2002a,b, 2006a). During surveys for a neoclassical (i.e., a form of inoculative biological control in which natural enemies are imported from elsewhere and released in small numbers in attempt to establish a permanent population to control a pest with which they have not co-evolved) biological control program against the glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar), in California, USA, at least 15 species of *Gonatocerus* were recently reared and identified in Argentina, mostly from sentinel eggs of the proconiine sharpshooter *Tapajosa rubromarginata* (Signoret) (Jones 2001; Jones, Logarzo, Virla *et al.* 2005; Logarzo *et al.* 2005; Pilkington *et al.* 2005; Triapitsyn *et al.* 2006, 2007; Logarzo, Triapitsyn & Virla unpublished data). Among them were specimens very similar morphologically to *G. tuberculifemur* (Ogloblin), which was originally described from a single specimen collected in Pucará, Neuquén Province, Argentina (Ogloblin 1957). The species, determined during 2001–2006 as *G. tuberculifemur* [and named below as *G.* sp. near *tuberculifemur* "Clade 1", based on a mitochondrial cyto-

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chrome oxidase subunit I (COI) phylogeographic analysis (de León, Logarzo *et al*. 2008)], was first reared in 1995 in Tucumán Province from eggs of *T. rubromarginata* on corn plants by E.G. Virla and then cultured on the same host in his laboratory at CIRPON, San Miguel de Tucumán. The same species was also recently found at high altitude in northern Chile, where it parasitizes eggs of the proconiine sharpshooter *Anacuerna centrolinea* (Melichar) (Logarzo *et al.* 2006). Because of its useful biological traits (Virla *et al.* 2005) and strong host preference for the members of Proconiini (Jones, Logarzo, Triapitsyn *et al.* 2005; Logarzo *et al.* 2008), *G.* sp. near *tuberculifemur* "Clade 1" was considered a promising candidate for introduction into California for neoclassical biological control of the glassy-winged sharpshooter (Jones, Logarzo, Virla *et al.* 2005). Hoddle & Irvin (2007) demonstrated that under quarantine laboratory conditions in California, its effectiveness was inferior to that of a local species, *G. ashmeadi* Girault (the only species compared). However, under natural conditions *G.* sp. near *tuberculifemur* "Clade 1" may potentially fill an important niche that *G. ashmeadi* cannot fill; that, however, would be impossible to demonstrate without releasing this species from quarantine and conducting field trials. Colonies of the species have been successfully maintained on eggs of a factitious host, *H. vitripennis*, at the USDA, APHIS Mission quarantine laboratory in Edinburg, Texas, USA, since 2001 (Virla *et al*. 2005) and also at the Department of Entomology, University of California in Riverside, California, USA, quarantine laboratory since 2002 (Hoddle & Triapitsyn 2005).

Differences in the results among laboratory and open field studies conducted in different sites within the distribution range of what was believed to be *G. tuberculifemur*, suggested the presence of cryptic species (Logarzo *et al.* 2008). Recent molecular evidence and preliminary results of reciprocal crosses among members of the different populations within the *G. tuberculifemur* complex revealed several different species and molecular clades (de León, Logarzo *et al.* 2006b, 2008; de León *et al.* 2007). During the genetic study of de León *et al.* (2007), five molecular clades were identified within the *G. tuberculifemur* species complex: clades "1", "2", "Y", "X", and "Z". One of these is *G.* sp. near *tuberculifemur* "Clade 1", which is common and widespread in central and northern Argentina and also occurs in northeastern Chile. The species reared in January 2006 from sentinel eggs of *T. rubromarginata* on citrus plants in San Rafael, Mendoza Province (called initially "Clade 2" of *G. tuberculifemur*) is described here as a new species, *G. deleoni*. *Gonatocerus* sp. 3, another common egg parasitoid of *T. rubromarginata* in northern Argentina (de León, Logarzo *et al.* 2006c), corresponds to the molecular clade "Z". Specimens from Tucumán Province, that emerged from Cicadellini (subfamily Cicadellinae) such as *Hortensia similis* (Walker) and *Scopogonalia subolivacea* (Stål) correspond to a separate molecular clade "Y". The specimens collected using sentinel eggs of *T. rubromarginata* in Pucará, belong to the molecular clade "X" (de León *et al.* 2007). All these molecular clades can be separated from each other using inter-simple sequence repeat-polymerase chain reaction (ISSR-PCR) DNA fingerprinting method, whereas sequencing of both the COI partial gene and the ribosomal internal transcribed spacer region 2 (ITS2) were able to discriminate only some of the five above-mentioned molecular clades (de León, Logarzo *et al.* 2006c; de León *et al.* 2007).

Following these studies, a more thorough investigation of the morphology of Clades 1 and 2 was conducted. Some inconspicuous but consistent differences in their male anatomy, particularly the shape of the submedian carinae on the propodeum and of the genitalia, were found. Host associations of both clades were also studied. The combination of molecular, biological, and morphological evidence allows us to describe here "Clade 2" from the San Rafael area of Mendoza Province as a new species, *G. deleoni*. The female of *G. tuberculifemur* is redescribed and illustrated based on the holotype and additional specimens from the type locality, and the male is newly described. Preliminary evidence shows that *G. tuberculifemur* collected at the type locality corresponds to a separate molecular clade "X" that is different from *G.* sp. near *tuberculifemur* "Clade 1" (de León *et al.* 2007). To demonstrate possible conspecificity of *G. tuberculifemur* from Neuquén with the specimens of *G. deleoni* **sp. n.** from Mendoza and *G.* sp. near *tuberculifemur* "Clade 1" from Mendoza and other states in Argentina and also from northeastern Chile, three of us (G.A. Logarzo, S.V. Triapitsyn, and E.G. Virla) collected at the type locality of *G. tuberculifemur* in Pucará, at the shore of Lago Lácar in

Parque Nacional Lanín, in late February 2007 to obtain fresh specimens of this species for molecular and taxonomic verification, and also to attempt to initiate insectary and quarantine colonies. The results are presented here.

Material and methods

Collecting of material. Most of the material examined was collected using sentinel eggs of *T. rubromarginata* on leaves of citrus and corn plants (Logarzo *et al.* 2008). Specimens were also reared from wild-laid egg masses of some Proconiini natural hosts of *G. tuberculifemur* (in the broad sense) on various plants. Other collecting methods (Malaise traps, yellow pan traps, and sweeping with a net) were also used. Previously collected specimens were also examined and identified.

In the type locality of *G. tuberculifemur*, we collected material in Pucará, within Parque Nacional Lanín. All the collecting methods described above were employed, including placing sentinel eggs of *T. rubromarginata* in citrus leaves and *Dechacona missionum* (Berg) on Johnson grass (both leafhoppers belong to the Proconiini), as well as sentinel eggs of eight species from other leafhopper subfamilies and tribes on various plants. The sentinel eggs were exposed for parasitization from 23.ii–2.iii.2007. Parasitized egg masses (only a few eggs of *T. rubromarginata* were parasitized) were then taken to the USDA, ARS South American Biological Control Laboratory (USDA, ARS SABCL) in Hurlingham, Buenos Aires, where 13 female parasitoids emerged on 15.iii.2007 and some were then exposed in the laboratory to fresh *T. rubromarginata* eggs. From them, numerous males of the next generation were obtained in early April, 2007. Some of these males then were set up to mate with the other, original females (i.e., their aunts), which had been in cold storage. Their progeny, obtained in late April 2007, included a few wasps of both sexes. Four original females (that emerged on 15.iii.2007) as well as one female and five males of their progeny were sent to the first author for taxonomic identification. Several original females were also sent to the third author's laboratory in Weslaco, Texas, USA, for molecular analyses. To our surprise, they were found to belong to a separate clade "X", that is genetically different from both *G. deleoni* **sp. n.** and *G.* sp. near *tuberculifemur* "Clade 1" (de León *et al.* 2007).

A Malaise trap was installed from 24 February 2007 till the end of April 2007 at the van Heden Nursery in Pucará (formerly Estación Experimental [Forestal] de Pucará), about 400 m off the shore of Lago Lácar (40°09'59.3''S 71°37'50.4''W, 664 m), the likely collecting locality of the holotype of *G. tuberculifemur*. According to Ogloblin (1957) and P. Fidalgo (personal communication), A.A. Ogloblin used to stay there on vacation at the house of his compatriot friend, forest engineer and former Russian prince (kniaz´) Sergio (Sergey Sergeyevich) Schajovskoi who worked, lived, and was buried at this former forest experimental station. Unfortunately, no specimens of *G. tuberculifemur* were captured either in that trap or in our total sweep and yellow (and also a few blue) pan trap samples collected in Pucará during 23–24.ii.2007 or in the second Malaise trap, installed during the same period in Hua Hum near the park ranger's house (40°07'23.9''S 71°39'36.2''W, 689 m). Following our departure, both Malaise traps were maintained, and samples were collected through April 2007, by Juan Parra, manager of the van Heden Nursery in Pucará.

Taxonomy

Terms for morphological features are those of Gibson (1997). Acronyms for depositories of specimens are as follows: BMNH, Natural History Museum, London, England, UK; CNCI, Canadian National Collection of Insects, Ottawa, Ontario, Canada; IMLA, Fundación e Instituto Miguel Lillo, San Miguel de Tucumán, Tucumán, Argentina; MLPA, Museo de La Plata, La Plata, Buenos Aires, Argentina; UCRC, Entomology

Research Museum, University of California, Riverside, California, USA; USDA, ARS SABCL, United States Department of Agriculture, Agricultural Research Service South American Biological Control Laboratory, Hurlingham, Buenos Aires, Argentina; USNM, National Museum of Natural History, Washington, District of Columbia, USA. An abbreviation used in the text is: F = antennal funicular segment (female) or antennal flagellar segment (male).

Host association studies

Jones, Logarzo, Triapitsyn *et al.* (2005) and Jones, Logarzo, Virla *et al.* (2005) reported results of the no-choice host specificity studies with *G.* sp. near *tuberculifemur* "Clade 1" under quarantine laboratory conditions in the USA; Logarzo *et al.* (2008) studied both laboratory no-choice host specificity of *G.* sp. near *tuberculifemur* "Clade 1" and its field host ranges in Argentina. Similar studies were conducted during 2006 by Logarzo and Virla (unpublished data) with *G. deleoni* **sp. n.** in San Rafael, Mendoza Province.

Genomic DNA isolation and ISSR-PCR DNA fingerprinting

Total genomic DNA extraction from individual specimens was performed as described in de León *et al.* (2004), de León, Jones *et al.* (2006), de León, Logarzo *et al.* (2008), and de León, Triapitsyn *et al.* (2008). ISSR-PCR assays were performed with the 5'-anchored primer HVH$(TG)_7$T (Zietkiewicz *et al*. 1994), where H = A/T/C, and V = G/C/A, as previously described (de León *et al.* 2004; de León, Jones *et al.* 2006; de León, Logarzo *et al.* 2008; de León, Triapitsyn *et al.* 2008). The assays were performed in a final volume of 20 µl with the following components: 1X PCR buffer [50 mM KCl, 20 mM Tris-HCl (pH 8.4), 1.5 mM $MgCl_2$, and 0.01% gelatin], 0.25 mM deoxynucleotide triphosphates, 0.25 µM ISSR primer, 1.0 µl of stock genomic DNA and 0.05 U/µl *Taq* DNA Polymerase (New England Biolabs, Beverly, MA). The cycling parameters were as follows: 1 cycle at 94°C for 2 min followed by 45 cycles at 94°C for 1 min, 56°C for 1 min, and 72°C for 2 min. Negative control reactions were performed in the absence of genomic DNA.

Amplification and sequencing of the partial mitochondrial cytochrome oxidase subunit I gene (COI) and the internal transcribed spacer regions 1 and 2 (ITS1 and ITS2)

The following primers C1-J-1718 (forward: 5'-GGAGGATTTGGAAATTGATTAGTTCC-3') and C1-N-2191 (reverse: 5'-CCCGGTAAAATTAAAATATAAACTTC-3') of Simon *et al*. (1994) were utilized (Tm 58°C; 2.0 mM $MgCl_2$; 2 U *Taq* DNA Polymerase; 40 cycles) to amplify the COI partial gene as described by de León, Jones *et al.* (2006), de León, Logarzo *et al.* (2008), and de León, Triapitsyn *et al.* (2008). For ITS-1 amplification, the following primers were used: ITS5, (forward: 5'-GGAAGTAAAAGTCGTAACAAGG-3' (White *et al*. 1990) and RNA2 (reverse: 5'-CACGAGCCGAGTGATCCACCGCTAAGAGT-3' (Chang *et al*. 2001) (Tm 55°C; 2.0 mM $MgCl_2$; 2 U *Taq* DNA Polymerase; 45 cycles), as described in de León *et al*. (2004). The ITS2 rDNA fragment was amplified (Tm 45°C; 1.5 mM $MgCl_2$; 2 U *Taq* DNA Polymerase; 45 cycles) with the following primers, 5.8S-F (forward), 5'-TGTGAACTGCAGGACACATGAAC-3' and 28S-R (reverse), 5'-ATGCTTAAATTTAGGGGGTA-3' (Porter & Collins 1991) as described in de León *et al.* (2004), de León, Jones *et al.* (2006), and de León, Triapitsyn *et al.* (2008). Amplification products were subcloned with the TOPO Cloning Kit (Invitrogen Life Technologies, Carlsbad, CA), plasmid minipreps were prepared by the QIAprep Spin Miniprep Kit (Qiagen Inc., Valencia, CA), and sequencing was performed by Davis Sequencing (Davis, California USA) as previously described (de León, Jones *et al.* 2006; de León, Logarzo *et al.* 2008; de León, Triapitsyn *et al.* 2008). Table 1 shows a summary of all the *Gonatocerus* species included in the phylogenetic analyses inferred from both COI and ITS2 sequenced data, including GenBank accession numbers.

DNA sequence analyses

The DNA sequencing software program Sequencher (Gene Codes Corp., Ann Arbor, MI) was utilized to process the raw sequences. The alignment program ClustalX (Thompson *et al*. 1997) and the phylogenetic

program PAUP version 4.0b10 for Macintosh (PPC) (Swofford 2002) were utilized for alignment, bootstrapping (as percentage of 1000 replications) (Felsenstein 1985), and reconstruction of trees, as described in de León, Jones *et al*. (2006), de León, Logarzo *et al.* (2008), and de León, Triapitsyn *et al.* (2008). Phylogenetic trees were constructed using the neighbor-joining algorithmic method utilizing the uncorrected 'p' genetic distance parameter (Saitou & Nei 1987). Mitochondrial COI sequences were translated into amino acid sequences by using the invertebrate mitochondrial code with the computer program EMBOSS Transeq (http://www.ebi.ac.uk/emboss/transeq/index.html).

TABLE 1. Summary of *Gonatocerus* species used for phylogenetic studies inferred from COI, ITS1, and ITS2 sequence data. SMT, San Miguel de Tucumán, Argentina, South America. Numbers in parenthesis indicate the number of individuals included. Assignment of clades is based on COI sequence data. np, not performed.

Species	Location	GenBank Accession Nos.		
North America[a]		**COI**	**ITS2**	**ITS1**
G. morrilli (Howard) (3)	Hidalgo County, TX USA	AY971851-53[b]	DQ002409-13[b]	np
G. walkerjonesi S. Triapitsyn (3)	San Diego County, CA USA	AY971858-60[b]	DQ002428-30[b]	np
G. morgani S. Triapitsyn (2)	San Diego County, CA USA	DQ922711-12[c]	EU684106-07	np
South America[d]				
G. sp. near *tuberculifemur*				
(Ogloblin) "Clade 1"[e] (1)	Río Colorado (Río Negro Province)	DQ922684[c]	EU684127	EU686723
	SMT (Tucumán Province)	DQ922686[c]	EU684128	EU686724
	Tunuyán (Mendoza Province)	DQ922689[c]	EU684129	EU686725
	Jalsuri, Chile (Región I)	DQ922681[c]	EU684126	EU686726
G. deleoni sp. n.[f] "Clade 2" (3)	San Rafael (Mendoza Province)	DQ922692-94[c]	EU684108-10	EU686727-29
G. annulicornis (Ogloblin) (3)	SMT (Tucumán Province) (M04005)[g]	AY971866-868[b]	DQ002420-22[b]	np
G. virlai S. Triapitsyn *et al.*[h] (3)	Argentina (M02013)[g]	DQ922733-35[c]	EU684111-13	np
G. tuberculifemur (3)				
(Ogloblin) "clade X"	Pucará (Neuquén Province); type locality	EU682930-32	EU684114-16	EU686730-32
G. sp. near *tuberculifemur*				
(Ogloblin) "Clade Y"[i] (3)	SMT (Tucumán Province)	EU682933-35	EU684117-19	EU686733-35
Gonatocerus sp. 3 "Clade Z"[j] (6)	SMT (Tucumán Province)	EU682936-41	EU684120-25	EU686736-41
Outgroup				
Anagrus ustulatus Haliday (2)	Arezzo, Toscana, Italy	EU015035-36[k]	EU015051-52[k]	EU686742-43

[a]Emerged or were reared on *Homalodisca vitripennis* (Germar) eggs (Triapitsyn 2006b).
[b]de León, Jones *et al*. (2006).
[c]de León, Logarzo *et al*. (2008); Logarzo *et al*. (2008).
[d]Unless otherwise stated, specimens emerged from *Tapajosa rubromarginata* (Signoret) eggs. Unless otherwise stated, specimens are from Argentina, South America.
[e]Previously referred to as *G. tuberculifemur* (Ogloblin) Clade 1 (de León, Logarzo *et al*. 2008; Logarzo *et al*. 2008). Clade assignment is based on COI sequence data.
[f]Previously referred to as *G. tuberculifemur* (Ogloblin) Clade 2 (de León, Logarzo *et al*. 2008; Logarzo *et al*. 2008).
[g]Unique codes associated with imported colonies at the USDA, APHIS, Mission Quarantine Facility, Edinburg, TX USA. In quarantine were reared on *H. vitripenni*s eggs.
[h]Previously identified as *Gonatocerus* sp. 6 (Triapitsyn *et al*. 2007; de León, Logarzo *et al.* 2008; Logarzo *et al*. 2008).
[i]*G. tuberculifemur*-like individuals emerged from *Hortensia similis* (Walker) (G. tub *H. similis*), referred to as *Gonatocerus* sp. near *tuberculifemur* Clade Y in this communication. Clade assignment is based on both COI and ITS2 sequence data.
[j]*G.* sp. 3 are from two different collection dates, January 2005 [emerged from *T. rubromarginata* (Proconiini)] and April 2006 [emerged from *Plesiommata mollicella* (Fowler) (Cicadellini)] (de León, Logarzo *et al*. 2006c). Clade assignment is based on ITS2 sequence data.
[k]de León, Logarzo *et al*. (2008); de León, Triapitsyn *et al*. (2008).

Second-round PCR (2nd-round PCR) of the *Wolbachia* 16S ribosomal RNA partial gene from several populations of *G. tuberculifemur* complex

2nd-round PCR of the *Wolbachia* 16S gene fragment was performed as previously described by de León,

Jones *et al.* (2006). For the first-round of PCR, the 16S rRNA primers (foward: 5'-TTGTAGCCTGCTATGG-TATAACT-3' and reverse: 5'-GAATAGGTATGATTTTCATGT-3') from O'Neill *et al.* (1992) were utilized (Tm 52ºC; 1.5 mM MgCl2; 45 cycles) to amplify an 896-bp gene fragment. The primers used for the second round of PCR in the previous study were replaced by new primers (WGaB-F/R: Tm 47C; 1.5 mM MgCl2; 45 cycles; 287-bp) because they were more sensitive. The sequences of the new primers are as follows - forward: 5'-GCTCTTTTAGTGAGGAAGA-3' and reverse: 5'-CTGGTGTTCCTCCTAATAT-3'.

Cross-breeding tests

To determine if reproductive compatibility occurs among the members of the *G. tuberculifemur* complex (i.e., clades 1, 2, X, Y, and Z), reciprocal crosses were conducted during 2005–2007 by Logarzo (unpublished data) at the USDA, ARS SABCL (Table 3). Unfortunately, specimens of *G. tuberculifemur* from Pucará ("Clade X") were obtained too late in the season of 2007, when the host material (i.e., eggs of *T. rubromarginata*) was scarce and later unavailable, and therefore they could not be cross-bred with the specimens belonging to any other clades of the *G. tuberculifemur* complex including *G. deleoni* **sp. n.** and *G.* sp. near *tuberculifemur* "Clade 1". Each experiment consisted of two reciprocal crosses and two controls made in the following way (for instance, for the clades 1 and 2): a clade "1" male was crossed with a clade "2" female; a clade "2" male was crossed with a clade "1" female; a clade "1" male with a clade "1" female (control), and a clade "2" male with a clade "2" female (control). Ten replications for each type of cross were made.

Taxonomy

Gonatocerus deleoni S. Triapitsyn, Logarzo & Virla, sp. n.

(Figs 1–9)

Gonatocerus tuberculifemur (Ogloblin) "Clade 2": de León, Logarzo *et al.* 2006a: 40–42; de León, Logarzo *et al.* 2006b: 44–46; de León *et al.* 2007: 73–75; de León, Logarzo *et al.* 2008: 97–106.

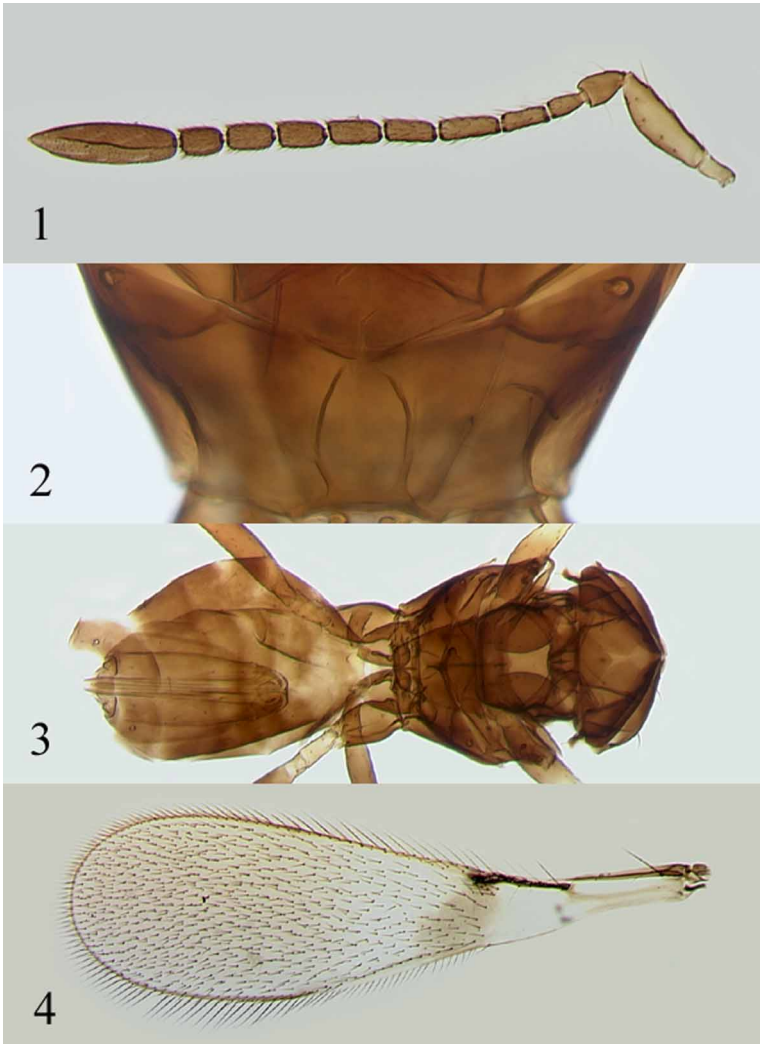
Type material. Holotype female on slide [MLPA]: ARGENTINA: Buenos Aires, Hurlingham, F1 [i.e., first generation] progeny reared on eggs of *Tapajosa rubromarginata* (Signoret) at USDA, ARS SABCL, emerged 22.iii.2006 (collection code SRc2); originally from: Mendoza, San Rafael, G. Logarzo, E. Virla, ex. *T. rubromarginata* sentinel eggs on citrus exposed 27–31.i.2006. Paratypes: ARGENTINA. Buenos Aires, Hurlingham, F1 progeny reared on eggs of *Tapajosa rubromarginata* (Signoret) at USDA, ARS SABCL (originally from: Mendoza, San Rafael, G. Logarzo, E. Virla, ex. *T. rubromarginata* sentinel eggs exposed 27–31.i.2006): emerged 8.iii.2006 [1 female on point, UCRC]; emerged 22.iii.2006 [1 male on point, UCRC, and 2 males on slides, MLPA, UCRC]; emerged 23–25.iii.2006 [1 female on slide, UCRC, 3 females on points, CNCI, UCRC, USNM, and 9 males on points: CNCI (1), IMLA (2), MLPA (2), UCRC (3), USNM (1)]; emerged 25.iii.2006 [2 females on points, IMLA, UCRC]; emerged 3.iv.2006 [1 female on point, UCRC]; emerged 4.iv.2006 [1 female on point, UCRC]. Mendoza, San Rafael, G. Logarzo, E. Virla, 10.ii.2006, 13.ii.2006, and 15.ii.2006, ex. eggs of *T. rubromarginata* on lemon (field host range study) [1 female on slide and 3 females on points, UCRC].

Additional material examined (all in alcohol, USDA, ARS SABCL)

ARGENTINA: Mendoza, San Rafael, 34°30'36.3''S 68°22'57.6''W, 714 m, emerged 24–30.i.2004 from sentinel eggs of *T. rubromarginata* on citrus exposed 10–18.i.2004, G. Logarzo, L. Varone [32 females, 6 males]. Originally from: Mendoza, General Alvear, 34°58'35.1''S 67°40'21.3''W, 506 m, emerged 1–3.ii.2007 from *T. rubromarginata* sentinel eggs exposed 19–23.i.2007, G. Logarzo, F. Palottini; F1 progeny reared on *T. rubromarginata* eggs at USDA, ARS SABCL in Hurlingham, Buenos Aires, exposed 1–3.ii.2007, emerged 11–15.ii.2007 [3 females, 2 males]. Originally from: Mendoza, Rama Caída**,** 34°41'20.0''S

68°21’27.6’’W, 681 m, emerged 1.ii.2007 from *T. rubromarginata* sentinel eggs exposed 21–23.i.2007, G. Logarzo, F. Palottini; F1 progeny reared on eggs of *T. rubromarginata* at USDA, ARS SABCL, exposed 1–3.ii.2007, emerged 15–16.ii.2007 [2 males]. Originally from: Mendoza, San Rafael, emerged 1–3.ii.2007 from *T. rubromarginata* sentinel eggs exposed 19–23.i.2007, G. Logarzo, F. Palottini; F1 progeny reared on eggs of *T. rubromarginata* at USDA, ARS SABCL, exposed 7–9.ii.2007, emerged 16–21.ii.2007 [7 females, 1 male].

Description. FEMALE (holotype and paratypes). Body length 0.9–1.1 mm. Head and mesosoma (Fig. 3) dark brown, gaster brown to dark brown (basal terga a little lighter than apical ones); appendages light brown to brown.

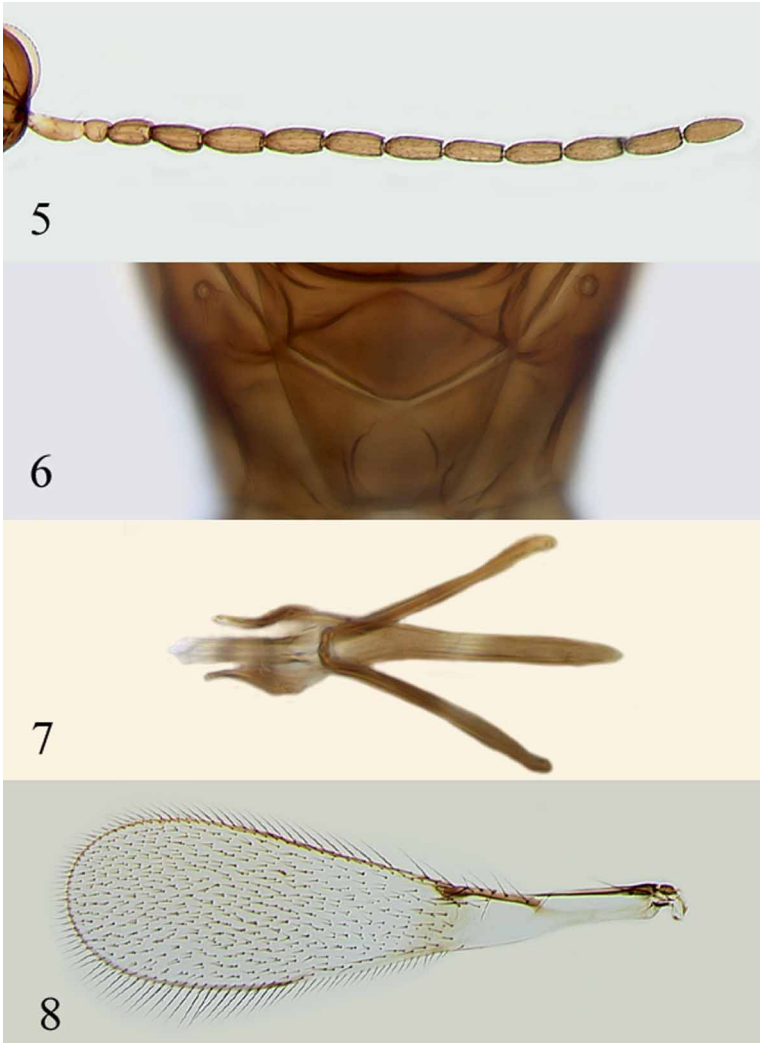


FIGURES 1–4. *Gonatocerus deleoni* (female, holotype). 1. Antenna. 2. Propodeum. 3. Mesosoma and metasoma. 4. Forewing.

Antenna (Fig. 1) with radicle 2.1–2.2 x as long as wide, rest of scape 2.8–3.0 x as long as wide, with strong setae; pedicel longer than F1; all funicular segments longer than wide and densely setose (setae short); F2 longer than F1 and shorter than F3 (usually the longest funicular segment, particularly when a longitudinal sensillum is present), F4 and F5 subequal in length, F6 as long as F7 and each a little shorter than F5; F8 shorter than F7; F1 and F2 without longitudinal sensilla, longitudinal sensilla on F3 (0 or 1), F4 (1), F5 (2), F6 (2), F7 (2), and F8 (2); clava with 8 longitudinal sensilla, 3.6–3.7 x as long as wide, its ventral surface covered with numerous minute, short setae and placoid sensilla, its dorsal surface densely covered with longer setae.

Pronotum divided medially, each lobe with 2 strong dorsal and 2 weak lateral setae. Mesoscutum much

wider than long, shorter than scutellum; midlobe of mesoscutum with a pair of strong setae. Dorsellum of metanotum with posterior margin widely angulate medially. Propodeum (Fig. 2) with lateral carinae and slightly curved submedian carinae (not meeting near anterior and meeting at posterior margins of propodeum, almost extending to its anterior margin); the propodeum smooth between submedian carinae but elsewhere with a faint cellulate sculpture (as in Fig. 9). Protibia without conical sensilla.



FIGURES 5–8. *Gonatocerus deleoni* (male, paratypes). 5. Antenna. 6. Dorsellum and propodeum. 7. Genitalia. 8. Forewing.

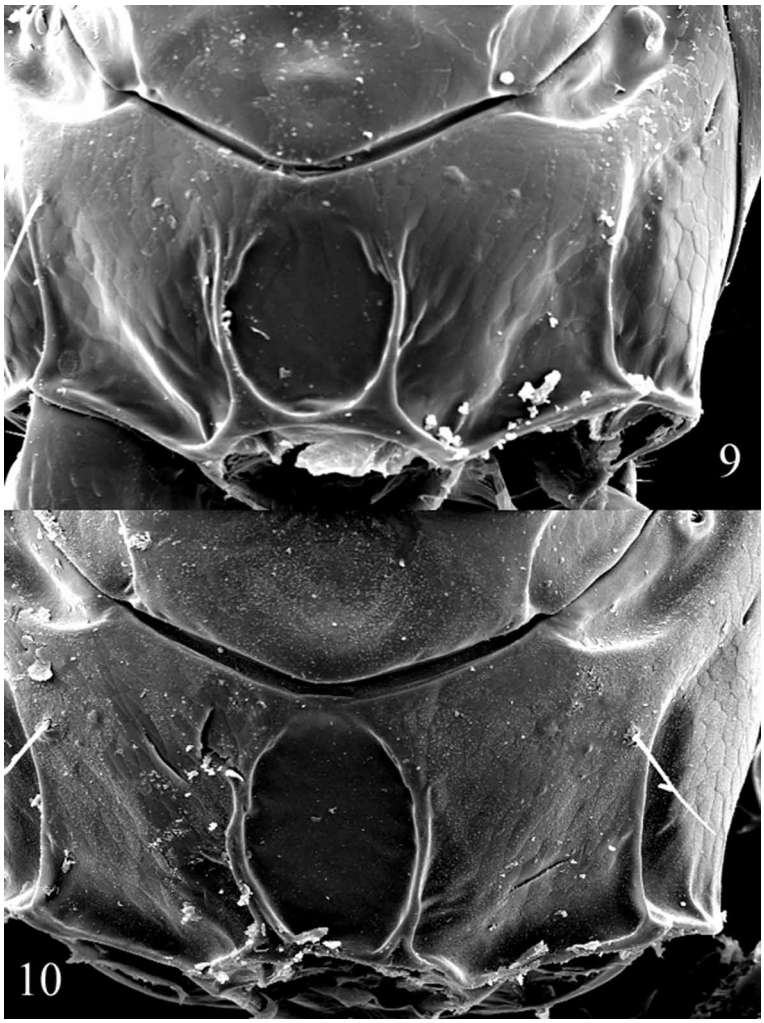
Forewing (Fig. 4) 3.4–3.5 x as long as wide; marginal setae short, the longest marginal seta 1/5–1/4 greatest wing width. Forewing disc slightly infuscate throughout, with a distinct darker spot behind stigmal vein, bare behind submarginal and marginal veins except for a few setae at apex of marginal vein, remainder of the disc densely setose. Submarginal vein with 1 macrochaeta and 2 smaller setae, marginal vein with 4 setae between proximal and distal macrochaetae. Hind wing 17–18 x as long as wide, the disc slightly infumate and mostly bare except for the usual two complete rows of setae along margins and several scattered setae at apex and behind apex of venation.

Gaster a little longer than mesosoma. Petiole about 1.6 x as wide as long, subtrapezoidal. Ovipositor about 7/10 length of gaster, not exserted beyond its apex. Ovipositor length: mesotibia length ratio about 0.9. Outer plates of ovipositor each with 1 distal seta.

Measurements of the holotype (in µm, as length or length:width). Mesosoma 535; petiole 43; gaster 548; ovipositor 373. Antenna: radicle 52; rest of scape 158; pedicel 70; F1 48; F2 66; F3 85; F4 72; F5 72; F6 67; F7 67; F8 60; clava 206. Forewing 1285:375; longest marginal seta 91. Hind wing 953:57; longest marginal

seta 124.

MALE (paratypes). Body length 0.9–1.1 mm. Similar to female in coloration. Antenna (Fig. 5) with scape and radicle fused, scape (excluding radicle) 2.9–3.0 x as long as wide; pedicel very small, all flagellomeres much longer than wide and with numerous longitudinal sensilla. Propodeum with submedian carinae not extending to its anterior margin (Figs 6, 9). Forewing (Fig. 8) 3.4–3.5 x as long as wide. Apex of apodeme of genital sternite more or less acute (Fig. 7).



FIGURES 9, 10. *Gonatocerus* spp. (male propodeum, scanning electron micrographs). 9. *Gonatocerus deleoni*. 10. *Gonatocerus* sp. near *tuberculifemur* "Clade 1".

Diagnosis. Member of the *ater* species group of *Gonatocerus* as defined by Huber (1988); its subgroup placement, however, is unclear: morphologically, it fits better the *ater* subgroup but molecularly, it clusters with the *morrilli* subgroup species based on both COI and ITS2 sequence data (de León, Logarzo *et al.* 2008). The following morphological features of the male distinguish this new species from *G. tuberculifemur* (i.e., "Clade X" from the type locality in Pucará) and *G.* sp. near *tuberculifemur* "Clade 1": submedian carinae on the propodeum (Figs 6, 9) relatively less prominent anteriorly, not extending to the anterior margin of the propodeum [almost extending to the anterior margin of the propodeum in *G.* sp. near *tuberculifemur* "Clade 1" (Fig. 10) and also in *G. tuberculifemur* from Pucará]; apex of the apodeme of the genital sternite (Fig. 7) more or less acute [blunt in *G.* sp. near *tuberculifemur* "Clade 1" (Fig. 16) and in *G. tuberculifemur* from Pucará]. *Gonatocerus deleoni* does not match the descriptions and available types of any of the numerous species of *Gonatocerus* from Argentina and elsewhere in South America described by A.A. Ogloblin and others [the first

author examined all of them except for one lost type of an unrelated species (from another species group) from Ecuador for a forthcoming revision of the described Neotropical species of *Gonatocerus* (Triapitsyn 2006b, 2007)].

Etymology. This species is named in honor of our colleague and friend (and co-author of this communication) Jesse H. de León, who first identified it as a separate entity from *G. tuberculifemur* and *G.* sp. near *tuberculifemur* "Clade 1" using molecular methods.

Natural hosts. Unknown ?Proconiini (Cicadellidae).

Factitious hosts. *Tapajosa rubromarginata* (Signoret) (from sentinel eggs of which this species was reared in Argentina) and also *Homalodisca vitripennis* (Germar) in the USA (both Proconiini). Adult females and males of *G. deleoni* **sp. n.** were sent by G.A. Logarzo under a permit from Buenos Aires, Argentina on 7.iii.2007 to the University of California, Riverside (UCR), California, USA quarantine facility, where a colony was then originated by S.V. Triapitsyn and V.V. Berezovskiy on eggs of a factitious host, *H. vitripennis*, in *Euonymus japonica* leaves. This material originated from the cross of the females (F1 progeny, reared at USDA, ARS SABCL on *T. rubromarginata* eggs, G. Logarzo, exposed 1–3.ii.2007**,** emerged 13–19.ii.2007; originally from the sentinel eggs of *T. rubromarginata* collected in San Rafael, Mendoza, exposed 19–23.i.2007, G. Logarzo, F. Palottini) and the males (F1 progeny of the cross between a male from the above-mentioned collection in San Rafael and a female collected in Rama Caída, Mendoza, exposed 19–23.i.2007, G. Logarzo, F. Palottini), and then reared (in the second generation) on eggs of *T. rubromarginata* at USDA, ARS SABCL (exposed 16–19.ii.2007**,** emerged 28.ii–1.iii.2007). Upon arrival to UCR quarantine, the colony originating females were exposed to *H. vitripennis* eggs on 9.iii.2007, and the next generation (progeny of both sexes) wasps emerged 28–20.iii.2007. Of the colony originators, we preserved as vouchers and slide-mounted 1 female and 1 male specimens [UCRC] (19.iii.2007, V. Berezovskiy); also preserved (4.v.2007, V. Berezovskiy) as vouchers and slide-mounted were 1 female and 1 male specimens [UCRC] of the second generation progeny from the established UCR quarantine colony on *H. vitripennis* eggs. These four specimens are not included in the type series of *G. deleoni* **sp. n.**

Biology. Our field host range studies revealed that *G. deleoni* **sp. n.** parasitizes only eggs of *T. rubromarginata* and it would not attack eggs of Cicadellini (Logarzo unpublished data). This biological trait, combined with the limited natural range of *G. deleoni* **sp. n.** that is confined to a few desert oases in Mendoza Province, Argentina, make this species potentially a more suitable and promising candidate agent for the neoclassical biological control against *H. vitripennis* in California than *G.* sp. near *tuberculifemur* "Clade 1". Otherwise, its biology is similar to that of *G.* sp. near *tuberculifemur* "Clade 1", which is well known (Virla *et al.* 2005). Another important factor that may make *G. deleoni* **sp. n.** a promising biological control candidate is the unique climate match of its very limited native range (CLIMEX software) to California, but not to the southeastern USA. It would not be predicted to migrate to the latter region where it might attack non-target native leafhoppers. This restriction is very important because it may reduce the risk factors of releasing this egg parasitoid in California (de León, Logarzo *et al.* 2008; Logarzo *et al.* 2008).

Gonatocerus sp. near *tuberculifemur* (Ogloblin) ("Clade 1")

(Figs 10–17)

Gonatocerus tuberculifemur (Ogloblin): Virla *et al.* 2005: 68–71 (biology); Logarzo *et al.* 2006: 880–882 (distribution and host records); Hoddle & Irvin 2007: 89–92 (comparison with *Gonatocerus ashmeadi* Girault under quarantine laboratory conditions in California).

Gonatocerus tuberculifemur (Ogloblin) "Clade 1": de León, Logarzo *et al.* 2006a: 40–42; de León, Logarzo *et al.* 2006b: 44–46; de León *et al.* 2007: 73–75; de León, Logarzo *et al*. 2008: 97-106.

Material examined. ARGENTINA. Buenos Aires: Hurlingham: progeny reared on eggs of *T. rubromargin-*

ata at USDA, ARS SABCL, emerged 24–25.iv.2006 [3 females, UCRC]; originally from: Mendoza, Tunuyán, xi.2005, G. Logarzo, E. Virla, F. Palottini (ex. sentinel eggs of *Hortensia similis* (Walker) on corn). F1 progeny reared on eggs of *T. rubromarginata* at USDA, ARS SABCL, emerged 17.iv.2006 [3 males, UCRC]; originally from: Mendoza, Tunuyán, 7.xi.2005, G. Logarzo, E. Virla, E. Frias (ex. field-collected eggs of *T. rubromarginata* on Johnson grass, *Sorghum halepense*). Contaminant of the laboratory colony at USDA, ARS SABCL, xi.2006, G. Logarzo (ex. eggs of *T. rubromarginata*) [4 females, 1 male, UCRC]. José C. Paz: 15.x.1938, A.A. Ogloblin [1 female, MLPA]; 2.x.1942, A.A. Ogloblin [5 males, MLPA, misidentified by A.A. Ogloblin as *G. brachyurus* (Ogloblin)]. Luján, Universidad Nacional de Luján, 34°35'07"S 59°04'45"W, 32 m, 31.iii.2006, C. Coviella [1 female, UCRC]. Otamendi, 34°13’17.7’’S 58°53’46.2’’W, 9 m, 23.i.2003, S. Triapitsyn, C. Hernández [1 male, UCRC]. Tigre, 5.xi.1938, A.A. Ogloblin [1 female, MLPA]. Córdoba: Las Tapias, 31°57’45.6’’S 65°05’25.9’’W, 650 m, 16.i.2003, M. Virla (ex. eggs of *T. rubromarginata* on grape leaf) [1 female, UCRC]. Los Túneles, 18.xi.2000, C. Porter, P. Fidalgo [17 females, 4 males, IMLA]. San Esteban, 17.xi.2000, C. Porter, P. Fidalgo [12 females, 2 males, IMLA]. Near Tanti, 31°20’47.1’’S 64°32’03.4’’W, 727 m, 17xii.2007-10.i.2008, G.A. Logarzo [11 females, UCRC]. Villa de Soto, 30°50’53’’S 65°00’18’’W, 540 m, 24.i.2003 (ex. sentinel eggs of *T. rubromarginata* on citrus left 17.i.2003 by G. Logarzo, L. Varone, E. & M. Virla, W. Jones, and S. Triapitsyn, emerged 7.ii.2003 in UCR quarantine, Riverside, California, USA) [1 female, 1 male, UCRC]. Formosa, S of Formosa, 26.27°S 58.27°W, 60 m, 26.iii.2003, J. Munro [1 female, UCRC]. Jujuy, Puente Río Yala (near Yala), 30.xi.1999, L. Williams, III, G.A. Logarzo [1 female, CNCI]. La Rioja: Alpasinche, 12.ii.2002, P. Fidalgo, C. Porter [3 males, IMLA]. Anillaco: 1–28.ii.2001, P. Fidalgo, J. Torréns, G. Fidalgo [2 females, IMLA, UCRC]; 1–31.iii.2001, P. Fidalgo, J. Torréns, G. Fidalgo [1 female, UCRC]; 1–30.iv.2001, P. Fidalgo, G. Fidago [1 female, IMLA]. Castro Barros, La Calera: 8.i.2001, P. Fidalgo [20 females, IMLA]; 10.ii.2002, P. Fidalgo [1 female, IMLA]. Chañarmuyo, 17.ii.2002, P. Fidalgo, C. Porter [3 females, IMLA]. Chuquis, 19.xii.2001, P. Fidalgo [2 females, IMLA, UCRC]. El Duraznillo (near El Cantadero and La Rioja): 26.xi.2001, P. Fidalgo [1 female, IMLA]; 1–15.i.2003, P. Fidalgo [1 female, IMLA]. Santa Vera Cruz, 28°40’42.7’’S 66°57’50.4’’W, 1660 m, 31.xii.2002, P. Fidalgo [2 females, IMLA, UCRC]. Mendoza: La Consulta, 3344’S 69°07’W, INTA - Estación Experimental Agropecuaria La Consulta, 22-26.i.2007, S. Lanati [5 females, UCRC]. Tunuyán, 8-9.ii.2006, G. Logarzo, E. Virla (ex. eggs of *H. similis* on corn) [2 females, UCRC]. Salta: Aguas Blancas, 22.72°S 64.40°W, 447 m, 23.iii.2003, J. Munro [1 female, 1 male, UCRC]. Campo Quijano (30 km E of Salta), 18-28.ii.1992, S.A. Marshall [1 female, CNCI]. Canyada La Gotera, 19.ii.1992, S.A. Marshall [1 female, CNCI]. San Ramón de la Nueva Orán: 21.x.1935, A.A. Ogloblin [1 female, MLPA]; 23.13°S 63.48°W, 633 m, 22.iii.2003, J. Munro [1 female, UCRC]. Tucumán: Cochuna, 11.xii.2002, P. Fidalgo [4 females, 1 male, IMLA]. El Cadillal, 23.i.1995, E. Virla (ex. eggs of *T. rubromarginata* on corn) [1 female, 3 males, CNCI]. Horco Molle (near San Miguel de Tucumán), 26°46’54.1’’S 65°19’42.1’’W, 750 m, 20.i.2003, S. Triapitsyn [1 male, UCRC]. La Ramadita, 27°05’31.5’’S 65°39’34.0’’W, 720 m, 19.i.2003, S. Triapitsyn, G. Logarzo [1 female, UCRC]. Las Mesadas, 27°05’33.1’’S 65°37’43.3’’W, 600 m, 19.i.2003, S. Triapitsyn, G. Logarzo [2 males, UCRC]. San Miguel de Tucumán: laboratory reared (F2) on eggs of *T. rubromarginata* by E.G. Virla at CIRPON [2 females, 3 males, CNCI] (originally from Tapia, i–ii.1995, E.G. Virla); 15.i.1996, M.J. Sharkey [4 females, CNCI]; 26°48’35.6’’S 65°14’24.6’’W, 500 m, xi.2002, E.G. Virla (laboratory culture at PROIMI on *T. rubromarginata* eggs on citrus) [6 females, UCRC]. Tafí del Valle, 19.i.1996, M.J. Sharkey [1 female, CNCI]. Tafí Viejo: 15.i.2001, E. Virla (ex. eggs of *T. rubromarginata*) [1 female, 1 male, UCRC]; 19.i.2001, E. Virla (ex. eggs of *T. rubromarginata*) [4 females, 1 male, UCRC]; 18–20.ix.2002, G. Logarzo (ex. eggs of *T. rubromarginata* on Johnson grass) [9 females, 4 males, UCRC]. Tapia, 14.i.1995, E. Virla [1 male, CNCI]. Yerba Buena, 10.x.1935, A.A. Ogloblin [1 male, MLPA]. CHILE. Región I (Tarapacá), Jalsuri (near Colchane), 19°13’58”S 68°43’34”W, ca. 4000 m, i.2005, G. Logarzo, V. Varni, ex. eggs of *Anacuerna centrolinea* (Melichar) on *Vicia faba* [3 females, 2 males, UCRC]. USA. California, Riverside Co., Riverside, laboratory colony at UCR quarantine: ca. F30, emerged v.2005 from eggs of *H. vitripennis* on *Euonymus japonica*

leaves, coll. V. Berezovskiy [4 females, 2 males, UCRC]; originally from: Argentina, Tucumán, Tafí Viejo, 18–20.ix.2002, G. Logarzo, L. Varone (ex. eggs of *T. rubromarginata* on Johnson grass). Texas, Hidalgo Co., Edinburg, USDA, APHIS Mission quarantine: laboratory colony MO3001 [1 female, 1 male, UCRC]; originally from: Argentina, Catamarca, Chumbicha, 30.xi.2002, G. Logarzo, S. Degese (ex. eggs of *T. rubromarginata* on citrus exposed 27–30.xi.2002). F1 progeny of the single female emerged 30.i.2003 [16 males, UCRC]; originally from: Argentina, Córdoba, Las Tapias, 31°57'45.6''S 65°05'29.9''W, 24.i.2003 (ex. sentinel eggs of *T. rubromarginata* on citrus left 16.i.2003 by G. Logarzo, L. Varone, E. & M. Virla, W. Jones and S. Triapitsyn).

Additional material examined (all in alcohol, USDA, ARS SABCL). ARGENTINA: Buenos Aires, Junín, 34º36'52.2''S 60º58'29.3''W, 83 m, emerged 25–27.x.2004 from sentinel eggs of *T. rubromarginata* on citrus [3 females, 4 males]. Catamarca: Chumbicha, 28°50'58.6''S 66°13'42.4''W, 429 m, emerged 14–26.xii.2001 from sentinel eggs of *T. rubromarginata* on citrus exposed 30.xi–4.xii.2001, G. Logarzo, L. Varone [13 females, 1 male]. Colpes, 28°03'37.3''S 66°12'10.1''W, 889 m, emerged 11–13.xii.2002 from sentinel eggs of *T. rubromarginata* on citrus exposed 26–30.xi.2002, G. Logarzo, S. Degese [6 females, 1 male]. San Isidro, 28°09'44.6''S 65°29'16.3''W, 525 m, emerged 12–17.xii.2001 from sentinel eggs of *T. rubromarginata* on citrus exposed 29.xi–4.xii.2001, G. Logarzo, L. Varone [6 females, 2 males]. Córdoba: Laboulaye, 34°08'04.6''S 63°23'06.6''W, 176 m, emerged 15.xii.2003 from sentinel eggs of *T. rubromarginata* exposed 26.xi–4.xii.2003, G. Logarzo, L. Varone [3 females, 1 male]. Santa Catalina, 33°12'04.3''S 64°25'52.4''W, 437 m, emerged 6–9.xii.2003 from sentinel eggs of *T. rubromarginata* exposed 25.xi–1.xii.2003, G. Logarzo, L. Varone [10 females, 4 males]. Villa Maria, 32º25'53.9''S 63º12'27''W, 199 m, G. Logarzo, L. Varone (emerged 21-28.x.2004 from sentinel eggs of *T. rubromarginata* on citrus) [2 females]. Jujuy, Purmamarca, 23°44'54.6''S 65°29'44.1''W, 2330 m, emerged 12.xii.2001 from sentinel eggs of *T. rubromarginata* on citrus exposed 25–28.xi.2001, G. Logarzo, L. Varone [1 female, 2 males]. La Rioja: Anillaco, 28°48'40.5''S 66°56'33.6''W, 1400 m, emerged 12.xii.2002 from sentinel eggs of *T. rubromarginata* on citrus exposed 27–30.xi.2002, G. Logarzo, S. Degese [7 females, 8 males]. Patquia, 30°02'40.3''S 66°53'00.2''W, 430 m, emerged 11–13.xii.2001 from sentinel eggs of *T. rubromarginata* on citrus exposed 30.xi–3.xii.2001, G. Logarzo, L. Varone [4 females, 1 male]. Mendoza: La Consulta, INTA – Estación Experimental Agropecuaria La Consulta, 33º42'31.3''S 69º04'22.4''W, 940 m, G. Logarzo, E. Virla, V. Varni (from sentinel eggs of eggs of *T. rubromarginata* on citrus) [1 male]. Luján de Cuyo, 33°02'01.3''S 68°51'59.5''W, 936 m, emerged 17–26.xii.2001 from sentinel eggs of *T. rubromarginata* on citrus exposed 1–3.xii.2001, G. Logarzo, L. Varone [24 females, 5 males]. San Carlos, 33°37'31.6''S 69°01'14.5''W, 897 m, emerged 2.ii.2004 from sentinel eggs of *T. rubromarginata* on citrus exposed 10–19.i.2004, G. Logarzo, L. Varone [1 female]. Tunuyán, 33°37'31.3''S 69°01'14.9''W, 885 m, emerged 10–11.xii.2001 from sentinel eggs of *T. rubromarginata* on citrus exposed 1–3.xii.2001, G. Logarzo, L. Varone [2 females, 2 males]. Río Negro, Río Colorado: 38°59'47''S 64°06'24''W, 90 m, emerged 7–9.iii.2007 from sentinel eggs of *T. rubromarginata* on citrus exposed 21.ii–2.iii.2007, G. Logarzo, E. Virla [4 females, 3 males]; 39°00'21.1''S 64°05'54.7''W, 66 m, emerged 23–25.xii.2004 from sentinel eggs of *T. rubromarginata* on citrus exposed 6–15.xii.2004, G. Logarzo, L. Varone [14 females, 3 males]. San Juan: Caucete, 31°39'10.2''S 68°17'19.1''W, 581 m, 12.xii.2001, ex. sentinel eggs of *T. rubromarginata* on citrus exposed 1–3.xii.2001, G. Logarzo [1 male]. Villa Media Agua, 31°57'47.6''S 68°26'56.7''W, 565 m, emerged 12–13.xii.2001 from sentinel eggs of *T. rubromarginata* on citrus exposed 1–3.xii.2001, G. Logarzo [14 females, 3 male]. San Luis, Villa Mercedes, 33°08'04.6''S 63°23'06.6''W, 176 m, emerged 9–10.xii.2003 from sentinel eggs of *T. rubromarginata* exposed 16.xi–2.xii.2003, G. Logarzo, L. Varone [8 females, 2 males]. Santiago del Estero: Añatuya, 28°27'21.2''S 62°50'34.8''W, 112 m, emerged 1–3.xii.2003 from sentinel eggs of *T. rubromarginata* exposed 15–20.xi.2003, G. Logarzo, L. Varone [6 females, 2 males]. El Zanjón, 27°52'22.7''S 64°14'31.1''W, 181 m, emerged 14.x.2003 from sentinel eggs of *T. rubromarginata* exposed 1–8.x.2003, G. Logarzo, L. Varone [7 females, 5 males].

Descriptive notes. FEMALE. Body length 0.8–1.6 mm. Head and mesosoma (Fig. 13) dark brown, gaster brown to dark brown (basal terga a little lighter than apical ones); appendages light brown to brown.

Antenna (Fig. 11) with radicle 2.0–2.5 x as long as wide, rest of scape 3.1–3.7 x as long as wide, with strong setae; pedicel longer than F1; all funicular segments longer than wide and densely setose (setae short); F2 longer than F1 and shorter than F3, F4 and F5 subequal in length, F6 as long as F7 and each a little shorter than F5; F8 shorter than F7; F1 and F2 without longitudinal sensilla, longitudinal sensilla on F3 (1), F4 (usually 2, sometimes 1), F5 (2), F6 (2), F7 (2), and F8 (2); clava with 8 longitudinal sensilla, 3.1–3.8 x as long as wide, its ventral surface covered with numerous minute, short setae and placoid sensilla, its dorsal surface densely covered with longer setae.

Pronotum divided medially, each lobe with 2 strong dorsal and 2 weak lateral setae. Mesoscutum much wider than long, shorter than scutellum; midlobe of mesoscutum with a pair of strong setae. Dorsellum of metanotum with posterior margin broadly rounded or widely angulate medially. Propodeum (Fig. 12) with lateral carinae and slightly curved submedian carinae (not meeting near anterior and meeting at posterior margins of propodeum, almost extending to its anterior margin); the propodeum smooth between submedian carinae but elsewhere with a faint cellulate sculpture (Fig. 10). Protibia without conical sensilla.

Forewing (Fig. 14) 3.1–3.7 x as long as wide; marginal setae short, the longest marginal seta about 1/5 greatest wing width. Forewing disc usually slightly infuscate throughout, with a distinct darker spot behind stigmal vein, bare behind submarginal and marginal veins except for a few setae at apex of marginal vein, remainder of the disc densely setose. Submarginal vein with 1 macrochaeta and 2 smaller setae, marginal vein with 4 or 5 setae between proximal and distal macrochaetae. Hind wing 17–20 x as long as wide, the disc slightly infumate and mostly bare except for the usual two complete rows of setae along margins and several scattered setae at apex and behind apex of venation.

Gaster almost as long as mesosoma. Petiole about 1.5 x as wide as long, subtrapezoidal. Ovipositor 3/4–4/5 length of gaster, not at all or barely exserted beyond its apex. Ovipositor length: mesotibia length ratio 0.9–1.0. Outer plates of ovipositor each with 1 distal seta.

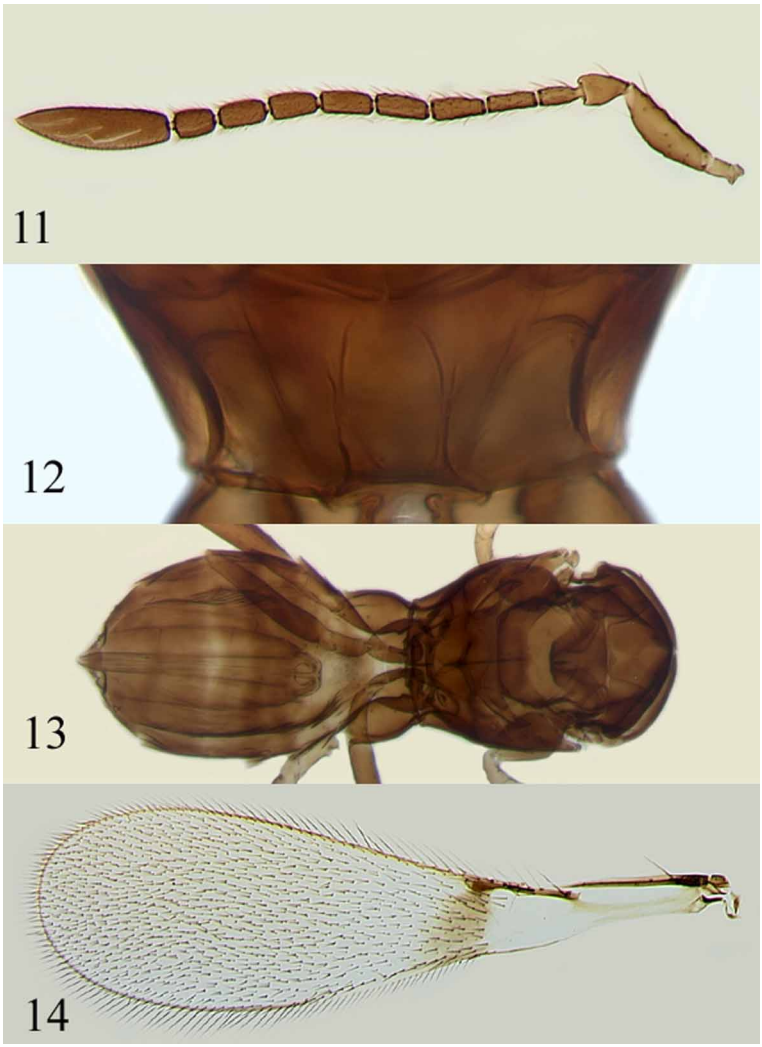
MALE. Body length 0.8–1.5 mm. Similar to female in coloration. Antenna (Fig. 15) with scape and radicle fused, scape (excluding radicle) about 3.3 x as long as wide; pedicel very small, all flagellomeres much longer than wide and with numerous longitudinal sensilla. Propodeum (Fig. 10) with submedian carinae extending almost to its anterior margin. Forewing (Fig. 17) 3.3–3.6 x as long as wide. Apex of apodeme of genital sternite blunt (Fig. 16).

Diagnosis. Member of the *ater* species group, where it clusters with the *morrilli* subgroup species based on both COI and ITS2 sequence data (de León, Logarzo *et al.* 2006d, 2008; de León *et al.* 2007). Morphologically, that placement is less evident (and rather falls in the *ater* subgroup) unless sculpture on the propodeum is observed under a scanning electron microscope (Fig. 10) or a compound light microscope (Fig. 12); in the latter case a good quality microscopic slide of a well-cleared, dorsoventrally mounted specimen is needed. *Gonatocerus* sp. near *tuberculifemur* "Clade 1" is most similar to *G. tuberculifemur* ("Clade X") from Pucará, from which it practically cannot be distinguished morphologically. It is also almost indistinguishable from *G. deleoni* **sp. n.**, as discussed above in its diagnosis, and also from *G.* sp. 3 from Argentina (also a primary egg parasitoid of *T. rubromarginata*). *Gonatocerus* sp. 3 has a more or less distinct brown, round spot in the widest part of the forewing disc (the widest part of the forewing disc is usually uniformly infumate in *G.* sp. near *tuberculifemur* "Clade 1", *G. tuberculifemur* ("Clade X"), and *G. deleoni* **sp. n.**, or sometimes almost hyaline in *G.* sp. near *tuberculifemur* "Clade 1").

Distribution. Argentina and Chile (Tarapacá). The following specimens may also belong to this form: PERU. Cuzco (Cusco), Picol, 13°29'S, 71°52, 3700 m: 6.vii.2003, W. Vargas [1 female, UCDC]; 27.xii.2004, W. Vargas [1 female, UCDC]. URUGUAY. Cerro Largo, 30 km SW of Melo, 32°32'S 54°14'W, 180 m, 9-25.xii.2002, S. & J. Peck [1 female, CNCI]. Rocha, Parque Nacional Lacustre, Reserva Laguna Rocha, 34°40'S 54°17'W, 3 m, 6-23.xii.2002, S. & J. Peck [2 females, CNCI].

Natural hosts. Proconiini: *Anacuerna centrolinea* (Melichar) (Logarzo *et al.* 2006), *Oncometopia tucumana* Schröder (Virla *et al.* 2008), and *Tapajosa rubromarginata* (Signoret).

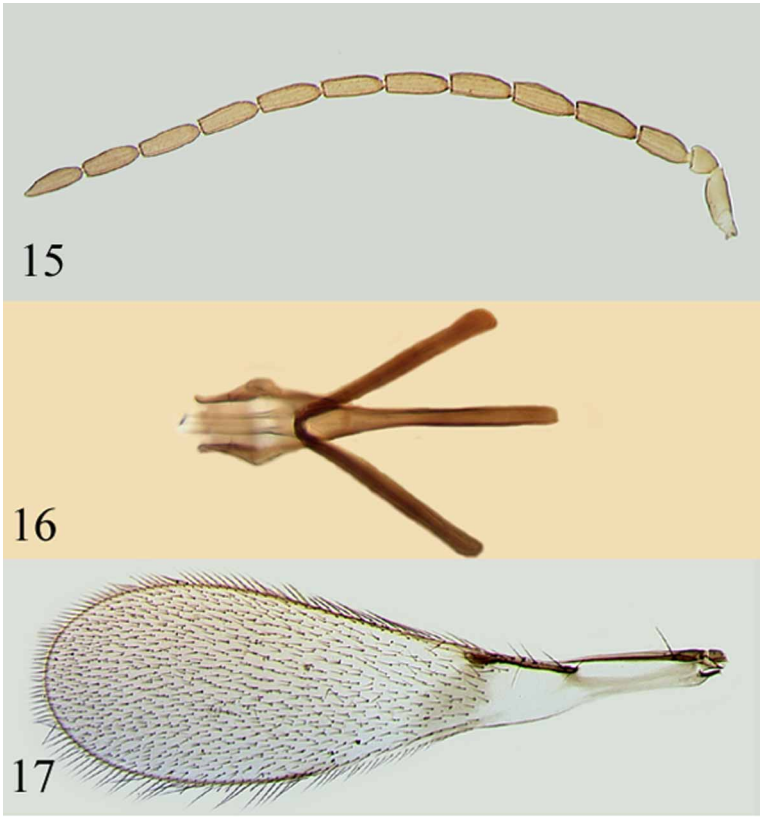
Factitious hosts. Proconiini: *Homalodisca liturata* Ball, *H. vitripennis* (Germar), and *Oncometopia* sp. (Jones, Logarzo, Triapitsyn *et al.* 2005; Jones, Logarzo, Virla *et al.* 2005), as well as *Dechacona missionum* (Berg), *Molomea consolida* Schröder, *Pseudometopia amblardii* (Signoret), *Tapajosa similis* (Melichar), and *Tretogonia notatifrons* Melichar (Logarzo *et al.* 2008). Some of the specimens in the colonies reared for many generations (more than 30) on *H. vitripennis* eggs in the quarantine facilities in California and Texas have fused or missing funicular (in females, particularly F4–F7) and flagellar (in males, particularly F3–F7) segments.



FIGURES 11–14. *Gonatocerus* sp. near *tuberculifemur* "Clade 1" (female from culture at USDA, ARS SABCL, Hurlingham, Buenos Aires, originally from Tunuyán, Mendoza, Argentina). 11. Antenna. 12. Propodeum. 13. Mesosoma and metasoma. 14. Forewing.

Biology. Biological traits of *G.* sp. near *tuberculifemur* "Clade 1" were reported by Virla *et al.* (2003), Jones, Logarzo, Triapitsyn *et al.* (2005), and Virla *et al.* (2005) (as *G. tuberculifemur*). In particular, no-choice laboratory host specificity tests in Argentina and United States confirmed exclusiveness of proconiine sharpshooters as hosts for *G.* sp. near *tuberculifemur* "Clade 1" (Jones, Logarzo, Triapitsyn *et al.* 2005; Jones, Logarzo, Virla *et al.* 2005; Logarzo *et al.* 2008), whereas field host range studies in Mendoza and Tucumán Provinces of Argentina revealed just strong preference of the members of Proconiini as its hosts (Logarzo *et al.* 2008). There, a few *G.* sp. near *tuberculifemur* "Clade 1"–like parasitoids were reared from sentinel eggs of

the leafhoppers *Ciminius platensis* (Berg), *Hortensia similis* (Walker), *Plesiommata mollicella* (Fowler), *Scopogonalia subolivacea* (Stål), and *Syncharina punctatissima* (Signoret) (Cicadellini). However, we are not 100% sure about the correctness of their identifications as *G.* sp. near *tuberculifemur* "Clade 1" as they may belong to another (cryptic) species (de León, Logarzo *et al*. 2008; Logarzo *et al.* 2008) or perhaps even might be specimens of *G.* sp. 3 that lack a round brown spot in the middle of the forewing disc. The specimens reared from eggs of *H. similis* in Tucumán Province were indeed found to belong to a separate molecular clade "Y", based on both COI and ITS2 sequence analyses (de León *et al.* 2007).



FIGURES 15–17. *Gonatocerus* sp. near *tuberculifemur* "Clade 1" (male from culture at USDA, ARS SABCL, Hurlingham, Buenos Aires, originally from Tunuyán, Mendoza, Argentina). 15. Antenna. 16. Genitalia. 17. Forewing.

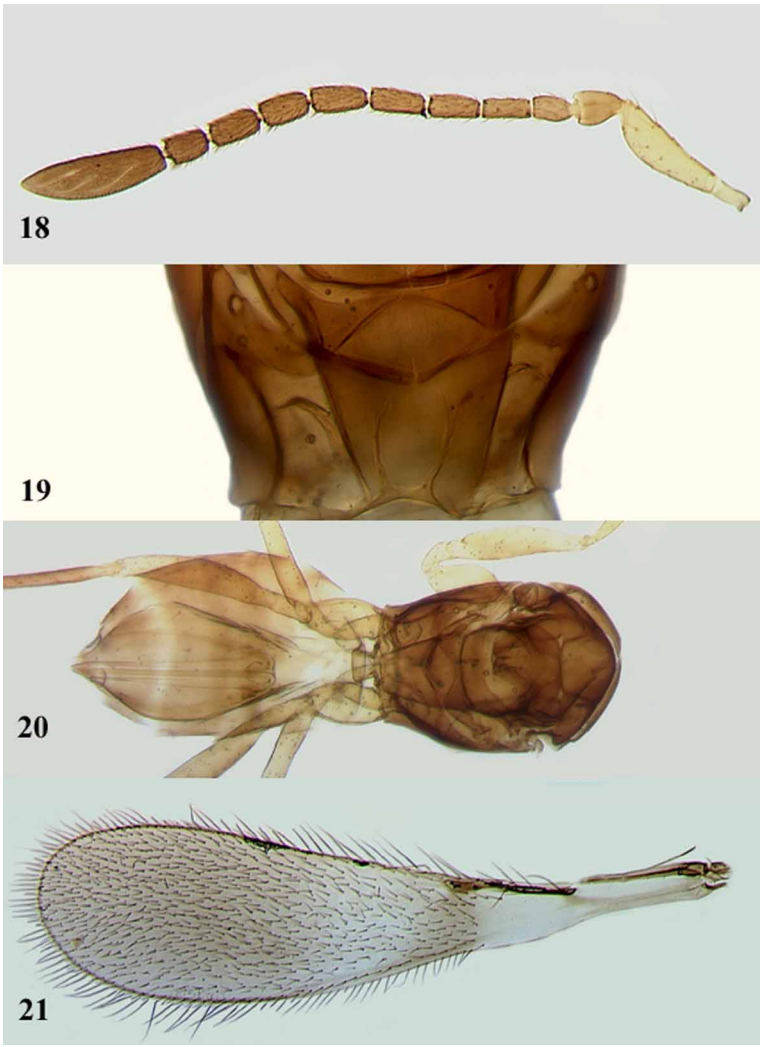
***Gonatocerus* sp. 3 [= *Gonatocerus* sp. near *tuberculifemur* "Clade Z"]**
(Figs 18–25)

Gonatocerus sp. 3: de León, Logarzo *et al.* 2006c: 48–50; de León *et al.* 2007: 73–75 (as "Clade Z").

Material examined. ARGENTINA. Buenos Aires: Castelar, INTA, 34°36'20''S 58°40'33''W, 5.i–6.ii.2007, G. Logarzo [1 female, UCRC]. Hurlingham, USDA, ARS SABCL: 7-8.xii.1999, L. Williams, III (near water hyacinth) [5 females, CNCI]; 23.i.2003, C. Hernández, S. Triapitsyn [1 female, UCRC]. José C. Paz, 3.xii.1967, A.A. Ogloblin [1 female, MLPA]. Luján, Universidad Nacional de Luján, 34°35'07"S 59°04'45"W, 32 m, 17.iii.2005, C. Coviella [2 females, UCRC]. Moreno, 34°08'57''S 58°46'57''W: 21.i.2005, C. Coviella [1 female, UCRC]; 31.i.2005, C. Coviella [1 female, UCRC]; 9.iii.2005, C. Coviella [1 female, UCRC]; 30.v.2005, C. Coviella [2 females, UCRC]; 22.vii.2005, C. Coviella [1 female, UCRC]; 6.viii.2005, C. Coviella [1 male, UCRC]; 16.x.2005, C. Coviella [1 female, UCRC]. Otamendi, 34°13'17.7''S 58°53'46.2''W, 9 m, 23.i.2003, S. Triapitsyn, C. Hernández [1 female, UCRC]. Tigre, 34°23'50''S 58°34'32''W, 5 m: 9–16.xi.2005, G. Logarzo [5 females, UCRC]; 23–28.xi.2005, G. Logarzo [3 females,

UCRC]; 27.xii.2005–3.i.2006, G. Logarzo [1 female, UCRC]; 3–10.i.2006, G. Logarzo [4 females, UCRC]; 10–17.i.2006, G. Logarzo [1 male, UCRC]; 2–11.ii.2006, G. Logarzo [3 females, UCRC]; 1–14.iv.2006, G. Logarzo [3 females, UCRC]. Chaco: Parque Nacional Chaco, 100 km NW of Resistencia, 12-17.xii.1990, S. & J. Peck [1 female, CNCI]. Puerto Tirol, xii.2003, G. Logarzo, L. Varone (ex. eggs of *T. rubromarginata* on citrus) [1 female, UCRC]. Córdoba: Las Tapias, 31°57'46''S 65°05'25''W, 643 m, 16.i.2003, W. Jones, S. Triapitsyn [1 female, UCRC]. Villa de Soto, 30°50'53''S 65°10'18''W, 540 m, 17.i.2003, S. Triapitsyn [1 female, UCRC]. Corrientes: Santo Tomé, 23-25.x.2006, G. Logarzo, V. Varni (ex. sentinel eggs of *T. rubromarginata* on citrus) [3 females, 1 male, UCRC]. Yapeyú, x.2006, G. Logarzo, V. Varni (ex. sentinel eggs of *T. rubromarginata* on citrus) [2 females, 1 male, UCRC]. Formosa: S of Formosa, 26.27°S 58.27°W, 60 m, 26.iii.2003, J. Munro [1 male, UCRC]. Herradura, 16.x.2003, G. Logarzo, L. Varone, ex. sentinel eggs of *Scoposcartula limitata* (Signoret) [1 male, UCRC]. Jujuy, Caimancito, 23.67°S 64.57°W, 423 m, 22.iii.2003, J. Munro [1 male, UCRC]. La Rioja, Anillaco, 1–31.iii.2001, P. Fidalgo, J. Torréns, G. Fidalgo [1 female, UCRC]. Misiones: Loreto: 10.ii.1932, A.A. Ogloblin [1 female, MLPA]; 20.iv.1932, A.A. Ogloblin [1 female, MLPA]; 27.v.1932, A.A. Ogloblin [1 female, MLPA]; 2.vi.1932, A.A. Ogloblin [1 female, MLPA]; 5.vi.1932, A.A. Ogloblin [1 female, MLPA]; 21.vi.1932, A.A. Ogloblin [1 male, MLPA]; 2.vii.1932, A.A. Ogloblin [1 female, MLPA]; 27.vii.1932, A.A. Ogloblin [1 female, MLPA]; 2.viii.1932, A.A. Ogloblin [1 female, MLPA]; 15.iii.1933, A.A. Ogloblin [1 male, MLPA]; 19.iv.1933, A.A. Ogloblin [1 female, MLPA]; 1.vi.1933, A.A. Ogloblin [1 female, MLPA]; 15.x.1933, A.A. Ogloblin [1 female, MLPA]; 18.x.1933, A.A. Ogloblin [1 female, 1 male, MLPA]; 5.i.1934, A.A. Ogloblin [1 female, MLPA]; 23.i.1934, A.A. Ogloblin [1 female, MLPA]; 27.i.1934, A.A. Ogloblin [1 male, MLPA]; 8.ii.1934, A.A. Ogloblin [1 female, MLPA]; 24.ii.1934, A.A. Ogloblin [1 female, MLPA]; 10.iii.1934, A.A. Ogloblin [1 female, MLPA]; 28.v.1934, A.A. Ogloblin [1 male, MLPA]; 7.vi.1934, A.A. Ogloblin [1 female, MLPA]; 10.vi.1934, A.A. Ogloblin [1 male, MLPA]; 27.vi.1934, A.A. Ogloblin [1 female, MLPA]; 1937, A.A. Ogloblin [1 female, MLPA]; 20.ii.1949, A.A. Ogloblin [1 female, MLPA]; 16.xi.1949, A.A. Ogloblin [1 male, MLPA]; Jesuit ruins, 21–26.viii.2000, P. Fidalgo [4 females, 2 males, UCRC]; 15.ii.2001, P. Fidalgo [1 female, UCRC]; 9.ix–5.x.2001, S.O. Martínez, P. Fidalgo [1 female, UCRC]; 15.x.2006, G. Logarzo (ex. sentinel eggs of *T. rubromarginata* on citrus) [1 female, UCRC]. 15 km SE of Puerto Iguazú, 27.xii.1990-6.i.1991, S. & J. Peck [1 female, CNCI]. Reserva de Vida Silvestre Urugua-í, 25°58.471'S 54°06.986'W, 400 m, 10–12.xii.2003, B. Brown, G. Kung [2 females, UCRC]. Santa Ana, 27.34°S 55.53°W, 77 m, 27.iii.2003, J. Munro [3 females, 1 male, UCRC]. S of Santa Ana, near Loreto, 27.42°S 55.53°W, 175 m, 28.iii.2003, J. Munro [3 females, UCRC]. Salta: Aguas Blancas, 22.72°S 64.40°W, 447 m, 23.iii.2003, J. Munro [2 females, UCRC]. Ampascachi, 25°20'S 65°32'W, 11-12.xii.2001, G. Logarzo (ex. *T. rubromarginata* eggs) [3 females, 2 males, UCRC]. La Cadera, 1500 m, 27.ii.1992, S.A. Marshall [1 male, CNCI]. Metán, 20.xi.1952, A.A. Ogloblin [1 female, MLPA]. San Ramón de la Nueva Orán: 21.x.1935, A.A. Ogloblin [1 female, MLPA]; 17.v.1955, A.A. Ogloblin [4 females, MLPA]; 23.11°S 64.52°W, 535 m, 23.iii.2003, J. Munro [1 female, UCRC]. Río Piedras, 22.vi.1940, A.A. Ogloblin [1 female, MLPA]. Rosario de la Frontera, 25.83°S 64.88°W, 745 m, 20.iii.2003, J. Munro [1 female, UCRC]. Tucumán: El Cadillal, 27.i.1993, E. Virla (ex. eggs of *Plesiommata mollicella* (Fowler) on maize) [1 female, CNCI]. Las Mesadas, 27°05'33.1''S 65°37'43.3''W, 600 m, 19.i.2003, S. Triapitsyn, G. Logarzo [15 females, 2 males, UCRC]. San Miguel de Tucumán: 15.i.1996, M.J. Sharkey [1 female, CNCI]; xii.2002, laboratory reared (F6–F7) on eggs of *T. rubromarginata* by E. Virla at PROIMI (originally from: soccer field near CIRPON and PROIMI, 26°48'35.6''S 65°14'24.6''W, 500 m, viii.2002, E. Virla, ex. *T. rubromarginata* eggs on Johnson grass) [44 females, 30 males, UCRC]; exposed 9–12.i.2003 by E. Virla, G. Logarzo, L. Varone, laboratory reared on eggs of *T. rubromarginata* by E. Virla at PROIMI (originally from: soccer field near CIRPON and PROIMI, 26°48'35.6''S 65°14'24.6''W, 500 m, viii.2002, E. Virla, ex. *T. rubromarginata* eggs on Johnson grass), emerged in UCR quaratine (Riverside, California, USA) 3.ii.2003, coll. V. Berezovskiy (UCR quarantine S&R # 03–02–04) [1 male, UCRC]. Tafí Viejo: 21.xi.2000, E. Virla [1 female, UCRC]; 18–21.xii.2000, E. Virla [6 females, UCRC]; 11.i.2001, E. Virla (ex. sentinel eggs of *T.*

rubromarginata) [1 male, UCRC]; i.2001, E. Virla (ex. eggs of *T. rubromarginata* in citrus orchard) [1 female, UCRC]. BOLIVIA. La Paz, Chulumani, Apa Apa Reserve, 16°37'S 67°51'W, 2000 m, 1–3.iv.2001, B. Brown [1 female, UCRC]. BRAZIL. Goiás: Campinaçu, 13°52.0'S 48°23.3'W, 21–22.ii.1996, Serra da Mesa Survey [1 female, UCRC]. Uruaçu, 14°17'S 48°55'W, 26.v.1996, Serra da Mesa Survey [1 female, UCRC]. Minas Gerais, Belo Horizonte, Universidade Federal de Minas Gerais campus, 19°52'S 43°58'W, 800 m, xi.1996, D. Yanega [1 female, UCRC]. Rio de Janeiro, near Desengano State Park, 21.87°S 41.80°W, 200 m, 9.v.1999, B. Brown [1 female, UCRC]. Santa Catarina, Nova Teutonia, 27°11'S 52°23'W, 14.x.1944, F. Plaumann [1 female, BMNH].



FIGURES 18–21. *Gonatocerus* sp. 3 [= *Gonatocerus* sp. near *tuberculifemur* "Clade Z"] (females from Argentina: Figs 18–20 from Tafí Viejo, Tucumán; Fig. 21 from Loreto, Misiones). 18. Antenna. 19. Propodeum. 20. Mesosoma and metasoma. 21. Forewing.

Descriptive notes. FEMALE. Similar to female of *G.* sp. near *tuberculifemur* "Clade 1", particularly the antenna (Fig. 18), propodeum (Fig. 19), and mesosoma and metasoma (Fig. 20). Forewing (Figs 21, 25) with a more (Fig. 25) or less (Fig. 21) distinct brown, round spot in the middle of the disc.

MALE. Similar to male of *G.* sp. near *tuberculifemur* "Clade 1", particularly the antenna (Fig. 22). Forewing (Fig. 23) occasionally more or less uniformly infumate, without a distinct brown, round spot in the middle of the disc. Apex of apodeme of genital sternite more or less blunt (Fig. 24).

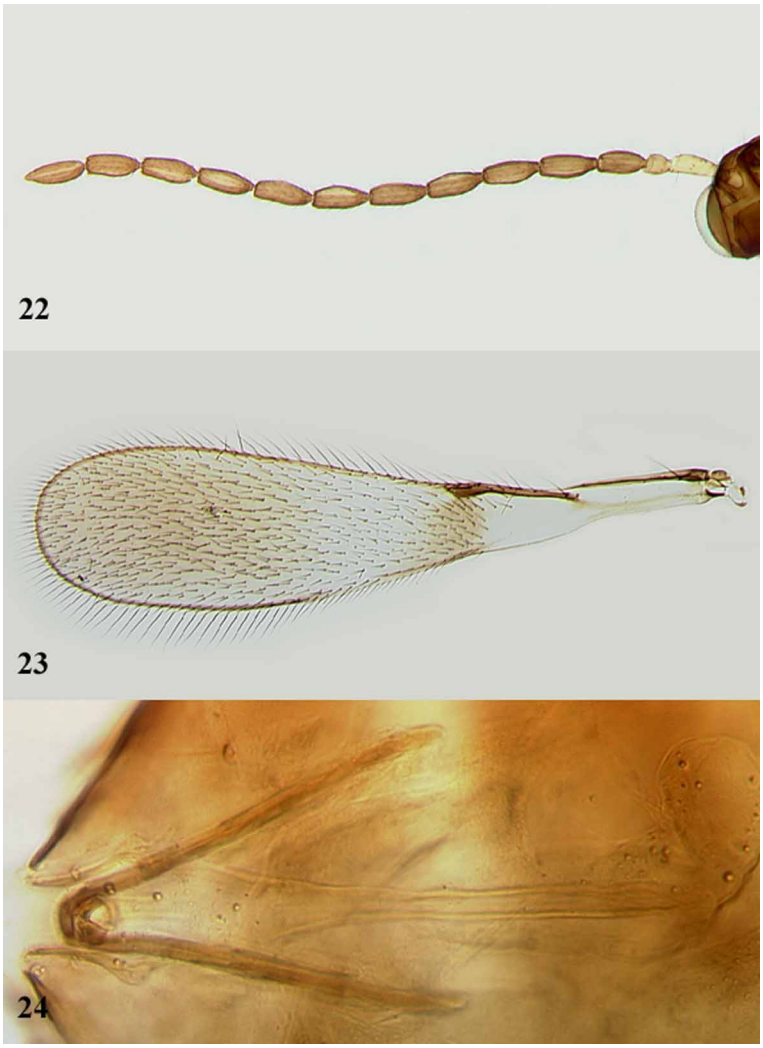
Diagnosis. Virtually identical to *G. tuberculifemur* from Pucará ("Clade X") and *G.* sp. near *tuberculifemur* "Clade 1" except for the usually distinct brown, round spot in the middle of the forewing disc (Figs 21,

25), although such a spot is occasionally indistinct in some males. The intensity of this brown spot on the forewing disc varies, and that puts the taxonomic value of this character in some doubt. In Mymaridae, maculations of the forewing disc mostly occur in the species from the generally warmer (usually subtropical and tropical) areas whereas the species from the generally colder (usually higher latitude or higher altitude areas with a temperate climate) tend to have the forewing disc hyaline or only slightly pigmented. That potentially may be true also for the individuals of the same species occurring in the different climatic zones. Therefore it is quite possible that at least some individuals identified as *Gonatocerus* sp. 3 (which occurs in the warmer parts of Argentina) based on the presence of a more or less distinct brown spot on the forewing disc, could rather belong to *G.* sp. near *tuberculifemur* "Clade 1". Thus, *Gonatocerus* sp. 3 itself may be a complex of several cryptic species or otherwise perhaps of different populations of the same species that can be recognized only using sensitive molecular methods such as ISSR-PCR fingerprinting (de León, Logarzo *et al.* 2006c).

Distribution. Argentina, Bolivia, and Brazil.

Natural hosts. *Tapajosa rubromarginata* (Signoret) (Proconiini) as well as *Plesiommata mollicella* (Fowler) and *Scoposcartula limitata* (Signoret) (Cicadellini).

Biology. Apparently similar to that of *G.* sp. near *tuberculifemur* "Clade 1"; the biology of the latter was reported by Virla *et al.* (2005).



FIGURES 22–24. *Gonatocerus* sp. 3 [= *Gonatocerus* sp. near *tuberculifemur* "Clade Z"] (male from San Miguel de Tucumán, Tucumán, Argentina). 22. Antenna. 23. Forewing. 24. Genitalia.

***Gonatocerus tuberculifemur* (Ogloblin)**
(Fig. 26)

Lymaenon tuberculifemur Ogloblin 1957: 38–39, 42 [illustrations: Figs 114–116, second unnumbered plate page] (holotype female [MLPA], labeled [in pencil]: 1. "*Lymaenon tuberculifemur* Ogl. female 18.III–55 PUCARÁ [the label is framed in purple pencil]"; 2. "Pucará *tuberculifemur* n. sp." [followed by several abbreviated, illegible words and a drawing in pencil; the label data are written on a piece of paper with an unrelated imprint "STABEL Botánica"] (examined).
Gonatocerus (Gonatocerus) tuberculifemur (Ogloblin): De Santis 1967: 105.
Gonatocerus tuberculifemur (Ogloblin): Yoshimoto 1990: 41; Triapitsyn 2007: 59; de León *et al.* 2007: 73–75 (as "Clade X").

Type locality. Pucará, at Lago Lácar, Neuquén, Argentina.

Material examined. ARGENTINA. Buenos Aires: Hurlingham: USDA, ARS SABCL, F1 progeny on *Tapajosa rubromarginata* (Signoret) eggs, iv.2007, G. Logarzo (progeny of virgin females, originally from: Argentina, Neuquén, Parque Nacional Lanín, Pucará, 40°09'59.3''S 71°37'50.4''W, 664 m, ca 400 m off shore of Lago Lácar, G. Logarzo, E. Virla, S. Triapitsyn, emerged 15.iii.2007 from sentinel eggs of *T. rubromarginata* on a citrus plant exposed near van Heden Nursery during 23.ii–2.iii.2007) [4 males, UCRC]. Neuquén, Parque Nacional Lanín, Pucará, 40°09'59.3''S 71°37'50.4''W, 664 m, ca 400 m off shore of Lago Lácar, G. Logarzo, E. Virla, S. Triapitsyn (emerged 15.iii.2007 from sentinel eggs of *T. rubromarginata* on a citrus plant exposed near van Heden Nursery during 23.ii–2.iii.2007) [4 females, UCRC].

Redescription. FEMALE [holotype (Fig. 26) and non-type specimens]. Body length 1.1–1.4 mm. Head and mesosoma dark brown, gaster brown to dark brown (basal terga a little lighter than apical ones); appendages light brown to brown.

FIGURES 25, 26. *Gonatocerus* spp. 25. *G.* sp. 3 [= *Gonatocerus* sp. near *tuberculifemur* "Clade Z"] (Loreto, Misiones,

Argentina), female forewing. 26. *G. tuberculifemur* (female, holotype).

Antenna with radicle 2.3–2.4 x as long as wide, rest of scape about 3.2 x as long as wide, with strong setae; pedicel longer than F1; all funicular segments longer than wide and densely setose (setae short); F2 longer than F1 and shorter than F3, F4 and F5 subequal in length, F6 as long as F7 and each a little shorter than F5; F8 shorter than F7; F1 and F2 without longitudinal sensilla, longitudinal sensilla on F3 (1 or 2) [unfortunately, poor (uncleared) condition of the specimen does not allow for verification of presence or absence of a longitudinal sensillum on F3 in the holotype although apparently there is one], F4 (2), F5 (2), F6 (2), F7 (2), and F8 (2); clava with 8 longitudinal sensilla, about 3.7 x as long as wide, its ventral surface covered with numerous minute, short setae and placoid sensilla, its dorsal surface densely covered with longer setae.

Pronotum divided medially, each lobe with 2 strong dorsal and 2 weak lateral setae. Mesoscutum much wider than long, shorter than scutellum; midlobe of mesoscutum with a pair of strong setae. Dorsellum of metanotum with posterior margin widely angulate medially. Propodeum with lateral carinae and slightly curved submedian carinae (not meeting near anterior and meeting at posterior margins of propodeum, almost extending to its anterior margin); the propodeum smooth between submedian carinae but elsewhere with a faint cellulate sculpture. Protibia without conical sensilla.

Forewing 3.3–3.8 x as long as wide; marginal setae short, the longest marginal seta about 1/5 greatest wing width. Forewing disc slightly infuscate throughout, with an indistinct, slightly darker spot behind stigmal vein, bare behind submarginal and marginal veins except for a few setae at apex of marginal vein, remainder of the disc densely setose. Submarginal vein with 1 macrochaeta and 2 smaller setae, marginal vein with 3 or 4 setae between proximal and distal macrochaetae. Hind wing about 18 x as long as wide, the disc almost hyaline or slightly infumate and mostly bare except for the usual two complete rows of setae along margins and several scattered setae at apex and behind apex of venation.

Gaster almost as long as mesosoma. Petiole about 1.5 x as wide as long, subtrapezoidal. Ovipositor 7/10–4/5 length of gaster, not at all or barely exserted beyond its apex. Ovipositor length: mesotibia length ratio 0.9–1.1. Outer plates of ovipositor each with 1 distal seta.

Measurements of the holotype (in µm, as length or length:width). Body 1058; head 160; mesosoma 445; petiole 42; gaster 440; ovipositor 355. Antenna: radicle 40; rest of scape 136; pedicel 66; F1 49; F2 64; F3 76; F4 73; F5 73; F6 67; F7 67; F8 58; clava 191. Forewing 1335:403; longest marginal seta 73. Hind wing 984:55; longest marginal seta 107.

Description. MALE. Body length 1.3–1.4 mm. Similar to female in coloration. Antenna with scape and radicle fused, scape (excluding radicle) about 3.2 x as long as wide; pedicel very small, all flagellomeres much longer than wide and with numerous longitudinal sensilla. Propodeum with submedian carinae almost extending to its anterior margin. Forewing about 3.7 x as long as wide. Apex of apodeme of genital sternite blunt.

Diagnosis. Member of the *ater* species group; its subgroup placement, however, is unclear: morphologically, it fits better the *ater* subgroup but molecularly, it clusters with the *morrilli* subgroup species as other members of the *G. tuberculifemur* complex do (de León, Logarzo *et al.* 2007). *Gonatocerus tuberculifemur* is most similar to *G. deleoni* **sp. n.** and particularly (the male genitalia) to *G.* sp. near *tuberculifemur* "Clade 1", as discussed above in their diagnoses, and also to *G.* sp. 3 from Argentina, which has a more or less distinct brown, round spot in the widest part of the forewing disc (the widest part of the forewing disc is uniformly infumate in *G. tuberculifemur*).

Distribution. Argentina (Neuquén).

Natural hosts. Unknown.

Factitious host. Proconiini: *Tapajosa rubromarginata* (Signoret).

Biology. Similar to that of *G.* sp. near *tuberculifemur* "Clade 1", as reported by Virla *et al.* (2005).

Gonatocerus sp. near *tuberculifemur* ("Clade Y")

Gonatocerus sp. (*tuberculifemur*-like): de León *et al*. 2007: 73–75 (as "Clade Y").

Material examined. ARGENTINA. Buenos Aires, Hurlingham, USDA, ARS SABCL, F8 progeny reared on eggs of *T. rubromarginata*, G. Logarzo**,** exposed 19–22.ii. 2007, emerged 3.iii.2007 (originally from: Tucumán, San Miguel de Tucumán, E. Virla, E. Luft Albarracin, from sentinel eggs of *Hortensia similis* (Walker), exposed 14–15.xii.2006); colony originators: USA, California, Riverside Co., Riverside, UCR quarantine, sent 7.iii.2007 by G. Logarzo, arrived 9.iii.2007 (UCR quarantine S&R # 07–13–03), set up with eggs of *Homalodisca vitripennis* (Germar) on *Euonymus japonica* leaves 9.iii.2007 by S. Triapitsyn & V. Berezovskiy and preserved 19.iii.2007 by V. Berezovskiy [1 female, UCRC]. USA. California, Riverside Co., Riverside, UCR quarantine, F1 progeny, emerged 26–28.iii.2007, preserved 4.iv.2007 by V. Berezovskiy (from colony on eggs of *H. vitripennis* on *Euonymus japonica* leaves that was discontinued at F1, originally from Argentina, as listed above).

Diagnosis. Morphologically, both sexes of this form are virtually identical to *G. tuberculifemur* ("Clade X") and *G.* sp. near *tuberculifemur* "Clade 1", as described above.

Distribution. Argentina (Tucumán).

Natural host. Cicadellini: *Hortensia similis* (Walker).

Factitious hosts. Proconiini: *Homalodisca vitripennis* (Germar) and *Tapajosa rubromarginata* (Signoret).

Biology. Similar to that of *G.* sp. near *tuberculifemur* "Clade 1", as reported by Virla *et al.* (2005).

Comments. The following slide-mounted specimen may also belong *G.* sp. near *tuberculifemur* "Clade Y": Argentina, Tucumán, El Manantial, 30.xii.2004–3.i.2005, E. Luft Albarracin, ex. eggs of *Plesiommata mollicella* (Fowler) [1 female, UCRC].

Molecular results

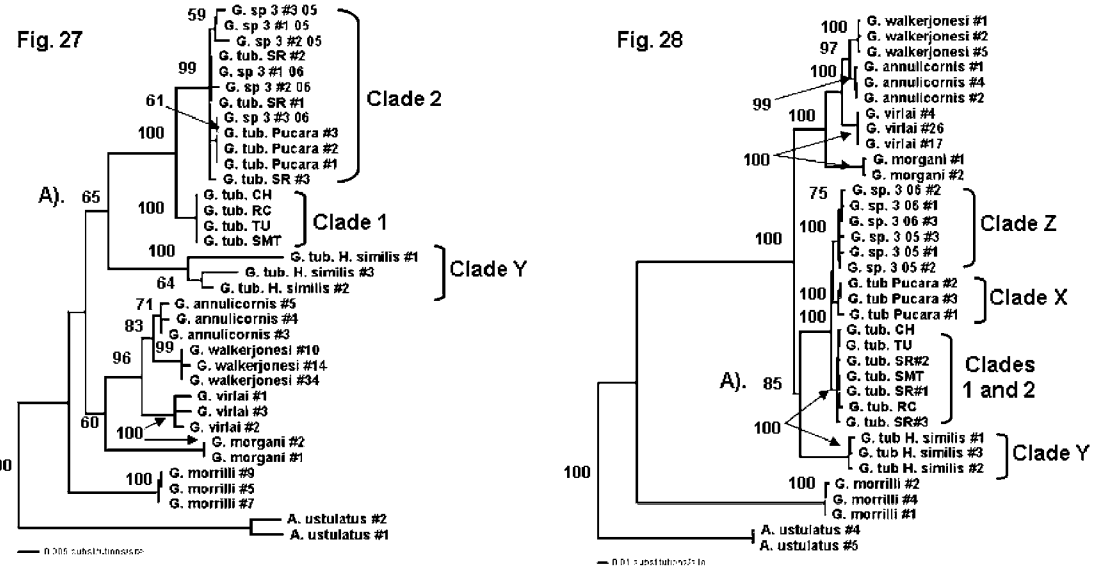
Phylogenetic analysis of individuals belonging to the *G. tuberculifemur* complex inferred from COI sequence data

Ten *Gonatocerus* species (or strains) were included in this study. A total of 33 ingroup specimens were analyzed and two specimens of *Anagrus ustulatus* Haliday (Mymaridae) were included as an outgroup. Many species formed their own taxonomic unit or distinct clade, confirming the species boundaries shown recently, including the two previous clades ["1" (*G.* sp. near *tuberculifemur* "Clade 1") and "2" (*G. deleoni* **sp. n.**)] of the *G. tuberculifemur* complex (de León, Logarzo *et al*. 2006d, 2008; Triapitsyn 2006a; de León *et al.* 2007) (Fig. 27). In addition, a new clade ("Y") was observed in *G.* sp. near *tuberculifemur* individuals (*G.* sp. near *tuberculifemur* "Clade Y") emerging from the host *Hortensia similis*, suggesting that "Clade Y" individuals could be a separate species. However, COI sequence variation was unable to discriminate *G. deleoni* **sp. n.** individuals from those of *G.* sp. 3, as shown previously (de León, Logarzo *et al*. 2006c), and *G. tuberculifemur* (G. tub Pucará) individuals collected from the type locality (Pucará). The current data also suggests that individuals from the *G. tuberculifemur* complex (main clade "A") are related and belong to the *morrilli* subgroup of the *ater* species group of *Gonatocerus*. Very strong bootstrap support (99-100%) was seen within the clades ("1", "2," and "Y") of the *G. tuberculifemur* complex.

Phylogenetic analysis of individuals belonging to the *G. tuberculifemur* complex inferred from ITS2 sequence data

The results of this analysis are shown on Fig. 28. Again, the species boundaries of many of these species

utilizing the ITS2 rDNA fragment were confirmed (de León, Logarzo *et al*. 2006d), with very strong support (99-100%). Clade "Y" (G. tub *H. similis* = *G.* sp. near *tuberculifemur* "Clade Y") was again observed based on the ITS2 phylogenetic analysis, confirming the results of the COI analysis. In this case, ITS2 sequence variation was able to discriminate individuals from both *G.* sp. 3 (*G.* sp. near *tuberculifemur* "Clade Z") and G. tub Pucará [*G. tuberculifemur* "Clade X"]. However, ITS2 was unable to discriminate individuals from the previous *G. tuberculifemur* complex [clades "1" (*G.* sp. near *tuberculifemur* "Clade 1") and "2" (*G. deleoni* **sp. n.**)] that was based on COI sequence data (de León, Logarzo *et al.* 2008). This is an interesting observation because COI sequence data and ISSR-PCR DNA fingerprinting were both able to discriminate these two clades (de León, Logarzo *et al*. 2008).



FIGURES 27 (COI) and 28 (ITS2). Bootstrap 50% majority-rule consensus trees constructed with the neighbor-joining algorithm of egg parasitoid species belonging to the *G. tuberculifemur* complex inferred from COI and ITS2 sequence data, respectively. The trees display bootstrap values, as percentage of 1000 replications. Collections of the *G. tuberculifemur* (G. tub) complex were from: RC, Río Colorado (Río Negro Province, Argentina); SMT, San Miguel de Tucumán (Tucumán); TU, Tunuyán (Mendoza); and CH, Chile; these individuals belong to "clade 1" as identified by de León, Logarzo *et al*. (2008), and in this communication they referred to as *G.* sp. near *tuberculifemur* "Clade 1". G. tub. SR, *G. deleoni* **sp. n.** specimens are from San Rafael (Mendoza), previously identified as "clade 2" (de León, Logarzo *et al*. 2008); G. tub Pucará, are *G. tuberculifemur* from the type locality (Pucará, Lago Lácar, Neuquén); G. tub *H. similis*, *G.* sp. near *tuberculifemur* ("Clade Y") are specimens that emerged from *Hortensia similis* (Walker); and *G.* sp. 3, are *Gonatocerus* sp. 3 [= *G.* sp. near *tuberculifemur* ("Clade Z")]. Main clade "A" are individuals from the *G. tuberculifemur* complex.

Phylogenetic analysis of individuals belonging to the *G. tuberculifemur* complex inferred from ITS1 sequence data

Analysis of the ITS1 gene fragment produced similar results to those seen with the ITS2 fragment (Fig. 29). Twenty-eight individuals from the *G. tuberculifemur* complex were included as ingroups and two individuals (*A. ustulatus*) were included as an outgroup. Four clades were identified with strong bootstrap support: clade Y (100%), clade X (96%), clade Z (94%), and clades 1 and 2 (81%). In a similar fashion to ITS2, ITS1 was also unable to discriminate clades 1 and 2.

ISSR-PCR DNA fingerprinting of individuals belonging to the *G. tuberculifemur* complex

The results of this experiment are shown on Fig. 30. ISSR-PCR uncovered fixed banding pattern differences in all of the species or strains belonging to the *G. tuberculifemur* complex: *G. tuberculifemur* from the type locality (Pucará) ("Clade X"); *G.* sp. near *tuberculifemur* "Clade Y" emerging from *H. similis* (G. tub *H.*

similis); results for *G.* sp. 3 (*Gonatocerus* sp. near *tuberculifemur* "Clade Z") are shown elsewhere (de León, Logarzo *et al*. 2006c); *G.* sp. near *tuberculifemur* "Clade 1"; and *G. deleoni* ("Clade 2"), as shown previously (de León, Logarzo *et al.* 2006a, 2008). Fixed DNA banding patterns differences among species is usually an indication of reproductive isolation (Hoy *et al*. 2000; de León *et al*. 2004; de León, Jones *et al.* 2006; de León, Logarzo *et al.* 2006b, 2008). The separation of all of the species or strains within the *G. tuberculifemur* complex was accomplished by the ISSR-PCR method, whereas no single gene (COI, ITS2, or ITS1) sequenced was able to discriminate all of the species within the complex.

Furthermore, we demonstrated the utility of the ISSR-PCR method by analyzing newly collected specimens from the *G. tuberculifemur* complex from various geographic locations. Versus the "Clade 1" control, it was determined that the three isofemale lines created from field-collected material (El Manantial, Tucumán Province) for cross-mating studies and specimens from Tunuyán (Mendoza Province) belong to "Clade 1" (*G.* sp. near *tuberculifemur* "Clade 1"). Likewise, versus the "Clade 2" control, it was determined that the new specimens collected in San Rafael, Rama Caída, and General Alvear (all from Mendoza Province) belong to "Clade 2" (*G. deleoni* **sp. n.**).

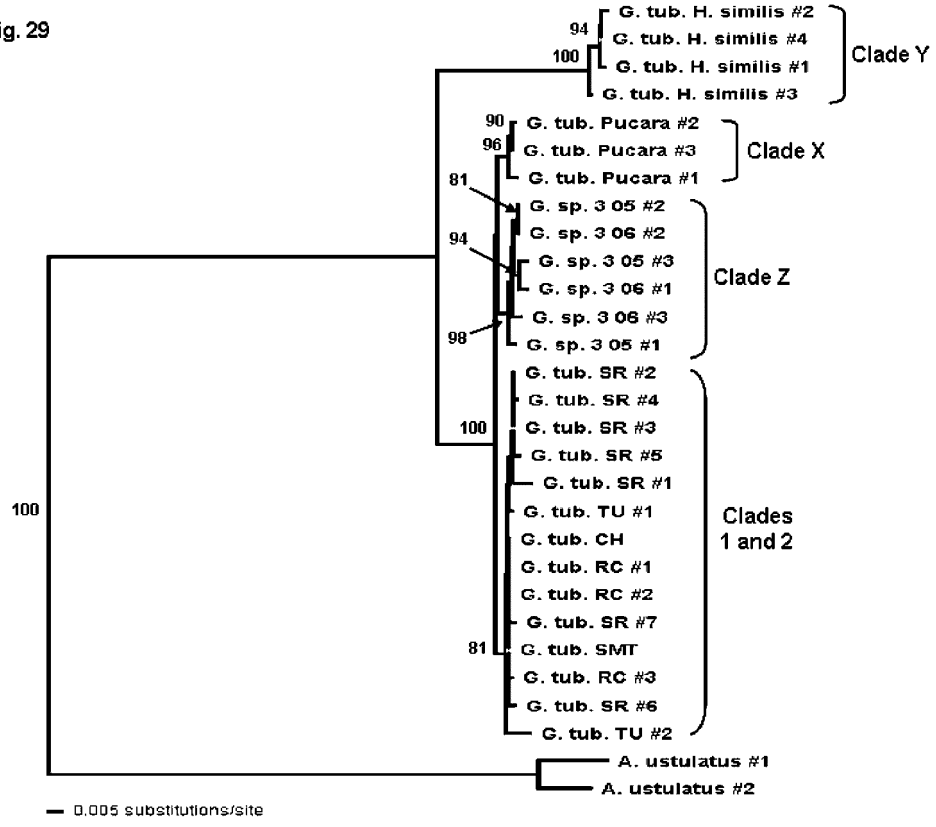


FIGURE 29. Bootstrap 50% majority-rule consensus trees constructed with the neighbor-joining algorithm of egg parasitoid species belonging to the *G. tuberculifemur* complex inferred from ITS1 sequence data. Refer to the figure legend on Figs 27 and 28 for labels.

Detection of *Wolbachia* in randomly selected *G. tuberculifemur* species complex specimens by 2nd-round PCR of the 16S rRNA partial gene

To determine whether the presence of *Wolbachia* could explain the reproductive incompatibility seen in the various cross-mating studies (Table 3), we used a sensitive 2nd-round PCR method (de León, Jones *et al.* 2006) to detect *Wolbachia* in various field collected populations, including isofemale lines used for the crossing studies. These results are shown on Table 4. Out of 110 individuals screened, only 9 (9%) were found infected with *Wolbachia*. This low number does not appear to be causing *Wolbachia*-induced ctyoplasmic incompatibility (Werren 1997). These results lend support to the fact that we are actually seeing multiple sep-

arate species within the *G. tuberculifemur* complex.

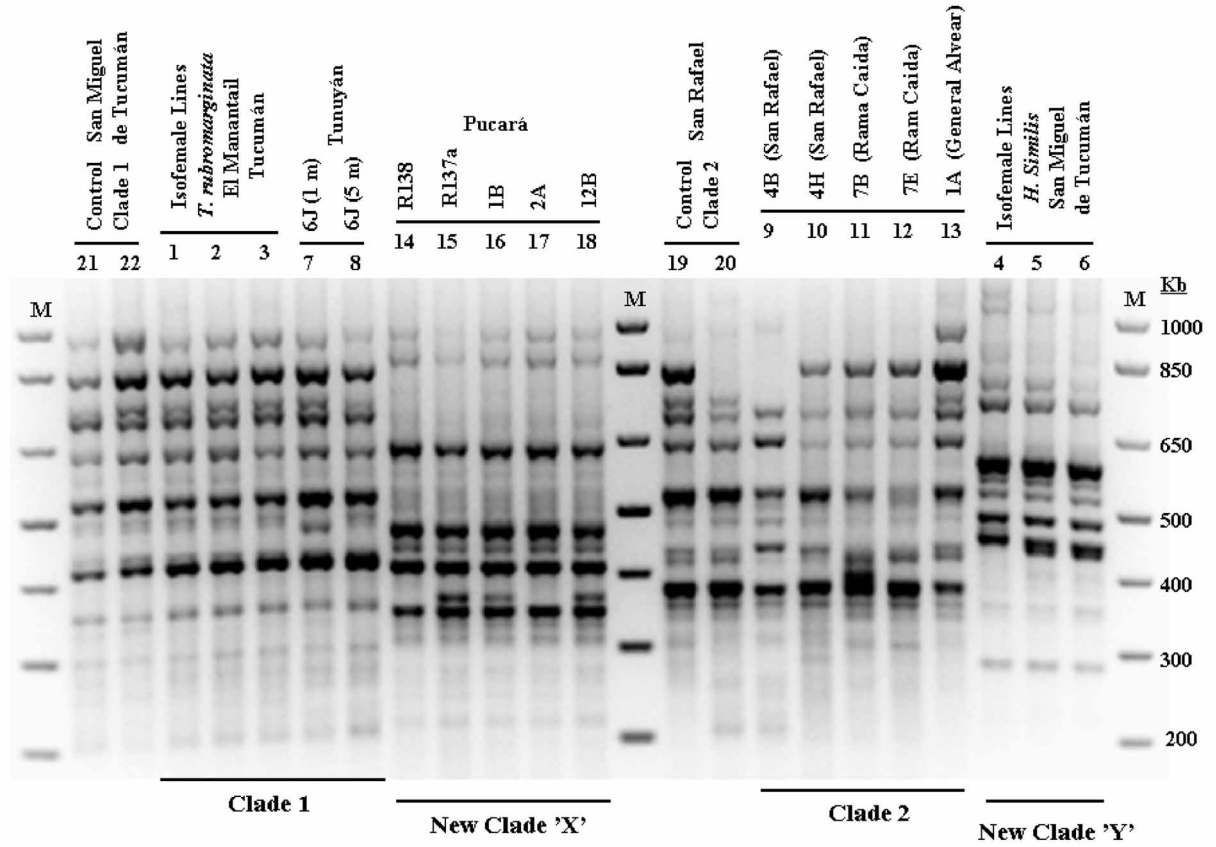


FIGURE 30. ISSR-PCR DNA fingerprinting of various species within the *G. tuberculifemur* complex. Reactions were performed with total genomic DNA from 2-5 separate individuals and a 5'-anchored ISSR primer as described in the Material and Methods. Specimens (*G.* sp. near *tuberculifemur* "Clade 1") from San Miguel de Tucumán and El Manantial are from Tucumán Province; Pucará individuals (*G. tuberculifemur*) are from Neuquén Province (type locality); San Rafael, Rama Caída, and General Alvear individuals (*G. deleoni* **sp. n.**) are from Mendoza Province; *G.* sp. 3 specimens [= *Gonatocerus* sp. near *tuberculifemur* "Clade Z"] are not included [see de León, Logarzo *et al*. (2006c)]; and *G. tuberculifemur*-like individuals (*G.* sp. near *tuberculifemur* "Clade Y") emerged from *Hortensia similis* (Walker) (Cicadellini); the rest emerged from *Tapajosa rubromarginata* (Signoret) (Proconiini). M, 1.0-Kb Plus DNA Ladder.

TABLE 2. Summary of the variations (Yes) or lack of (No) seen in COI and ITS2 gene regions among the five members of the *Gonatocerus tuberculifemur* complex. Note: ITS1 and ITS2 sequence data give the same results.

Species/ Molecular Clades	*G.* sp. near *tuberculifemur* /Clade 1	*G. deleoni* / Clade 2	*G. tuberculifemur* (from Pucará) /Clade X	*G.* sp. near *tuberculifemur* (ex. *Hortensia similis*) /Clade Y	*G.* sp. 3 /Clade Z
Clade 1	-	COI Yes ITS2 No	COI No ITS2 Yes	COI Yes ITS2 Yes	COI No ITS2 Yes
Clade 2	COI Yes ITS2 No	-	COI No ITS2 Yes	COI Yes ITS2 Yes	COI No ITS2 Yes
Clade X	COI No ITS2 Yes	CO1 No ITS2 Yes	-	COI No ITS2 Yes	COI No ITS2 Yes
Clade Y	COI Yes ITS2 Yes	COI Yes ITS2 Yes	COI No ITS2 Yes	-	COI No ITS2 Yes
Clade Z	COI No ITS2 Yes	COI No ITS2 Yes	ITS2 Yes COI No	COI No ITS2 Yes	-

TABLE 3. Summary of the results of the reciprocal cross-breeding tests among the four members of the *Gonatocerus tuberculifemur* complex (clade "X" has not been tested yet): Yes = complete reproductive compatibility (i.e., progeny consisted of both sexes); No = complete reproductive incompatibility (i.e., progeny consisted of the males only).

Species/ Molecular Clades	*G.* sp. near *tuberculifemur* /Clade 1	*G. deleoni* /Clade 2	*G.* sp. near *tuberculifemur* (ex. *Hortensia similis*)/Clade Y	*G.* sp. #3 /Clade Z
Clade 1	Yes	No	No	No
Clade 2	No	Yes	No	Not conducted
Clade Y	No	No	Yes	Not conducted
Clade Z	No	Not conducted	Not conducted	Yes

TABLE 4. Random detection of *Wolbachia* in the *Gonatocerus tuberculifemur* species complex from South America. All specimens are from Argentina unless otherwise stated. Assignment of clades are based on COI sequence data. SMT, San Miguel de Tucumán.

Species	Location (Province)	# Ind.	# Positive
G. sp. nr. *tuberculifemur*/clade 1	Río Colorado (Mendoza)	4	0
G. sp. nr. *tuberculifemur*/clade 1	SMT (Tucumán)	19	1
G. sp. nr. *tuberculifemur*/clade 1[a,b]	El Manantial (Tucumán)	7	1
G. sp. nr. *tuberculifemur*/clade 1	Jalsuri, Chile (Región I)	9	1
G. sp. nr. *tuberculifemur*/clade 1	Tunuyán (Mendoza)	6	0
G. sp. nr. *tuberculifemur*/clade 1[a]	Tunuyán (Mendoza)	8	0
G. sp. nr. *tuberculifemur*/clade 1[a,b]	Tunuyán (Mendoza)	2	0
G. sp. nr. *tuberculifemur*/clade 1[a,c]	Tunuyán (Mendoza)	5	0
G. sp. nr. *tuberculifemur*/clade 1[a]	Argentina	11	5
G. sp. nr. *tuberculifemur*/clade 1[a]	Riverside, CA USA	5	0
G. sp. nr. *tuberculifemur*/clade 1[a]	Edinburg, TX USA	4	0
G. deleoni/clade 2	San Rafael (Mendoza)	8	1
G. deleoni/clade 2[a]	San Rafael (Mendoza)	4	0
G. tuberculifemur/clade X	Pucará (Neuquén)	9	0
G. sp. nr. *tuberculifemur*/clade Y[a,d]	SMT (Tucumán)	9	0
G. sp. 3/clade Z[e]	SMT (Tucumán)	6	0

[a]Isofemale line. Some of these lines were used in the cross-mating studies.

[b]Emerged from *Tapajosa rubromarginata*.

[c]Emerged from *Hortensia* sp.

[d]Emerged from *Hortensia similis*.

[e]From two collection dates, January 2005 and April 2006.

Synopsis of molecular data, summary of results of cross-breeding experiments, and discussion

As shown above, there are only minor morphological differences that separate some of the members of the *G. tuberculifemur* complex from the others, particularly from the only previously described species, *G. tuberculifemur*. The documented differences in the male anatomy between *G. tuberculifemur* ("Clade X" from Pucará) [and also *G.* sp. near *tuberculifemur* "Clade 1" whose male genitalia are identical to those of *G. tuberculifemur*] and *G.* sp. near *tuberculifemur* "Clade 2" allow us to describe the latter as a new taxon, *G. deleoni*. This is corroborated by the molecular data on all the members of the *G. tuberculifemur* complex that are reported here and also by de León *et al.* (2007). Because of the lack of any meaningful morphological differences, none of other molecular clades discussed here, including the common and widespread in Argentina *G.* sp. near *tuberculifemur* "Clade 1", are described as new species, as we are reluctant to describe new taxa based solely on molecular and biological evidence.

To facilitate presentation of the molecular data on the members of the *G. tuberculifemur* complex, these are summarized in Table 2, in which both differences and similarities in the different gene regions among the five molecular clades are shown, following de León *et al.* (2007). So far, cross-breeding tests were completed among the four different clades (1, 2, Y, and Z) that comprise the *G. tuberculifemur* complex (excluding *G. tuberculifemur* "Clade X" from Pucará). The results of these crosses are summarized in Table 3 (Logarzo unpublished data). None of the crosses among the four tested clades produced females in the progeny, indicating reproductive incompatibility (all members of the *G. tuberculifemur* complex reproduce by arrhenotoky), while all the controls produced both females and males in the progeny. These results fully corroborate the results of the molecular analyses indicating that all these molecular clades are different entities.

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