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First record of *Gryllophila skrjabini* Sergiev, 1923 and *Cephalobellus magalhaesi* Schwenk, 1926 (Nematoda: Thelastomatidae) parasites of *Neocurtilla claraziana* Saussure, 1874 (Orthoptera: Gryllotalpidae) in Argentina

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ABSTRACT

Thelastomatidae is one of the largest families parasitizing insects, within the order Oxyurida. In this work we reported parasitism in nymphs and adults of Neocurtilla claraziana *by two different thelastomatid species as a part of a field survey on agricultural pests. Nymphs and adults of this insect were isolated from grasslands of Buenos Aires State, Argentina using a tensio-active solution. The nematode species* Gryllophila skrjabini *Sergiev, 1923 and* Cephalobellus magalhaesi *Schwenk, 1926 are briefly described and measurements are given. Both nematodes are reported for the first time in Argentina with* C. magalhaesi *being the second isolation of this species in the world.* Neocurtilla clarziana *is a new host record for* G. skrjabini.

Key-Words: Entomonematodes; Oxyurida; Parasitism; Crickets; Orthoptera.

INTRODUCTION

Thelastomatidae is by far the largest family in the Thelastomatoidea (Adamson & van Waerebeke, 1992) and one of the main families parasitizing insects, within the order Oxyurida. These are exclusively intestinal parasites and their life cycle is as follows: the infective forms are eggs which are ingested by the insect host (passive penetration), reaching the stomodeum where J2 hatch; these juveniles undergo successive molts reaching adulthood. After copulation the male dies and oviposition occurs. Eggs are eliminated with feces outside, waiting to be ingested by a new host (Camino & Achinelly, 2008).

The study of this family is scarce in the world, unlike other members of the order Oxyurida. Contributions have been carried out by Travassos (1925, 1929) and Basir (1956), and more recently by Carreno (2007, 2014), Carreno & Tuhela (2011) and Shah *et al.* (2012).

In Argentina, five species of thelastomatid nematodes were described in the mole cricket *Neocurtilla claraziana* (Saussure, 1874): *Cephalobellus lobulata, Gryllophila cephalolobulata, Euryconema brevicauda,*

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Fontonema gracilis and *Schwenkiella tetradentatum* (Camino & Achinelly, 2011).

In this work we add to this list the report of parasitism by two more different thelastomatid species in nymphs and adults of *N. claraziana,* as part of a field survey on agricultural pests.

MATERIALS AND METHODS

Nymphs and adults of the mole cricket *Neocurtilla claraziana* were collected from grasslands of Gorina (34°54'29"S; 58°02'25"W) and Villa Elisa (34°51'12"S; 58°04'45"W), Buenos Aires State, Argentina using a tensio-active solution. The Poinar's (1975) technique was used in the laboratory to isolate nematodes: insects were dissected in Petri dishes with distilled water under a stereomicroscope. Nematodes were killed by being placed in distilled water at 60°C for 2 minutes. Then, submerged in a solution of distilled water plus a fixative T.A.F (7 ml formalin, 40% formaldehyde; 2 ml trietanolamine, 91 ml distilled water) in a ratio 1:1 for 48 hours, and finally fixed in pure T.A.F. Specimens were measured using a camera lucida and an ocular micrometer in a Zeiss compound microscope. All measurements are given in micrometers unless otherwise stated. Photographs were taken with an Olympus DP-71 camera. Micrographs were also obtained with SEM. Adults were fixed in 1.5% glutaraldehyde/1.5% formaldehyde buffered with 0.1 M cacodylate buffer (pH 7.35) for 12 hs at 8°C, post fixed 1% osmium tetroxide solution for 12 hs at 25°C. After post-fixation, nematodes were rinsed three times in water (5 min. each) and dehydrated using a series of ethanol washes (30, 50, 70, 90, 100%), then critical point were dried with liquid CO_2, mounted on SEM stubs, and coated with gold (Kaya & Stock, 1997). Observations of ultrastructure were realized by an SEM JEOL JSM-100. Specimens were determined using Basir's (1956) monographic study.

RESULTS

Gryllophila skrjabini (Sergiev, 1923)

Description

Morphology

Female: 3.4 mm long. Cuticule conspicuously striated; first annule 17 µm large, the following annules increases regularly until the ninth annule which is 50 µm wide; posterior to the ninth annule a width of 30 to 40 µm is maintained almost regularly; behind the vulva they may decrease to 10 µm only (Fig. 1A). Oral opening subtriangular, surrounded by a circumoral elevation and eight labiopapillae; amphids present. Buccal cavity cylindrical, 20 µm deep by 10 µm wide. Oesophagus 446 µm long, consisting of a corpus of 316 µm by 45 µm wide, an isthmus 30 µm long by 35 µm wide, and a valvular bulb 100 µm long by 110 µm wide. Nerve ring 184.5 µm from anterior end of body. Excretory pore very much posterior to base of oesophagus, at about one-third of body length from anterior end (Fig. 1C). Intestine dilated anteriorly to form a bulky cardia, which narrows suddenly into a rather thin intestine (Fig. 1A). Anus 337. 5 µm from the posterior end of body; tail conically attenuated (Fig. 1F). Vulva in the posterior third of the body. Two ovaries, both connected with their respective uteri at about the level of the excretory pore, the uteri running parallel in a posterior direction, uniting a little behind the level of the vulva and giving rise to a single uterus which runs anteriad, coming up to the level of the excretory pore where it is reflexed and runs backwards until it meets the vagina; vagina long (Fig. 1E). Ellipsoidal large eggs (144 to 155 µm long by 90 to 99 µm wide), with a thick shell bearing spine-like outgrowths (Fig. 1D), placed in a two-cell stage and chained by a tubular structure probably formed by a mucous secretion oviduct (Basir, 1956; Shah *et al.,* 2012).

Morphometry

Female (n = 3): total length = 3451.5 ± 27 (3432-3471), cephalic diameter = 18, distance from anterior end to nerve ring = 184.5 ± 6.36 (180-189), width at level of nerve ring = 202.5 ± 6.36 (198-207), oesophagus length = 446 ± 7.07 (441-451), anterior distance to basal bulb = 329 ± 7.07 (324-334), distance from anterior end to excretory pore = 948.5 ± 4.9 (945-952), greatest width = 445 ± 5.6 (441-449), width at level of vulva = 322 ± 9.8 (315-329), vulval length = 18, vulval width = 36 V = 74% ± 1 (73%-75%), posterior end width = 265.6 ± 63 (261-270), tail length = 337.5 ± 6.3 (333-342), egg length = 147.6 ± 7.7 (144-155), egg width = 96 ± 6.3 (90-99).

Male: not found.

Host: adults of the mole cricket *Neocurtilla claraziana* Saussure, 1874.

Locality: Villa Elisa (34°52'02"S; 58°04'28"W)

Site of infection: hindgut (last part of intestine).

Prevalence: 0.3%.

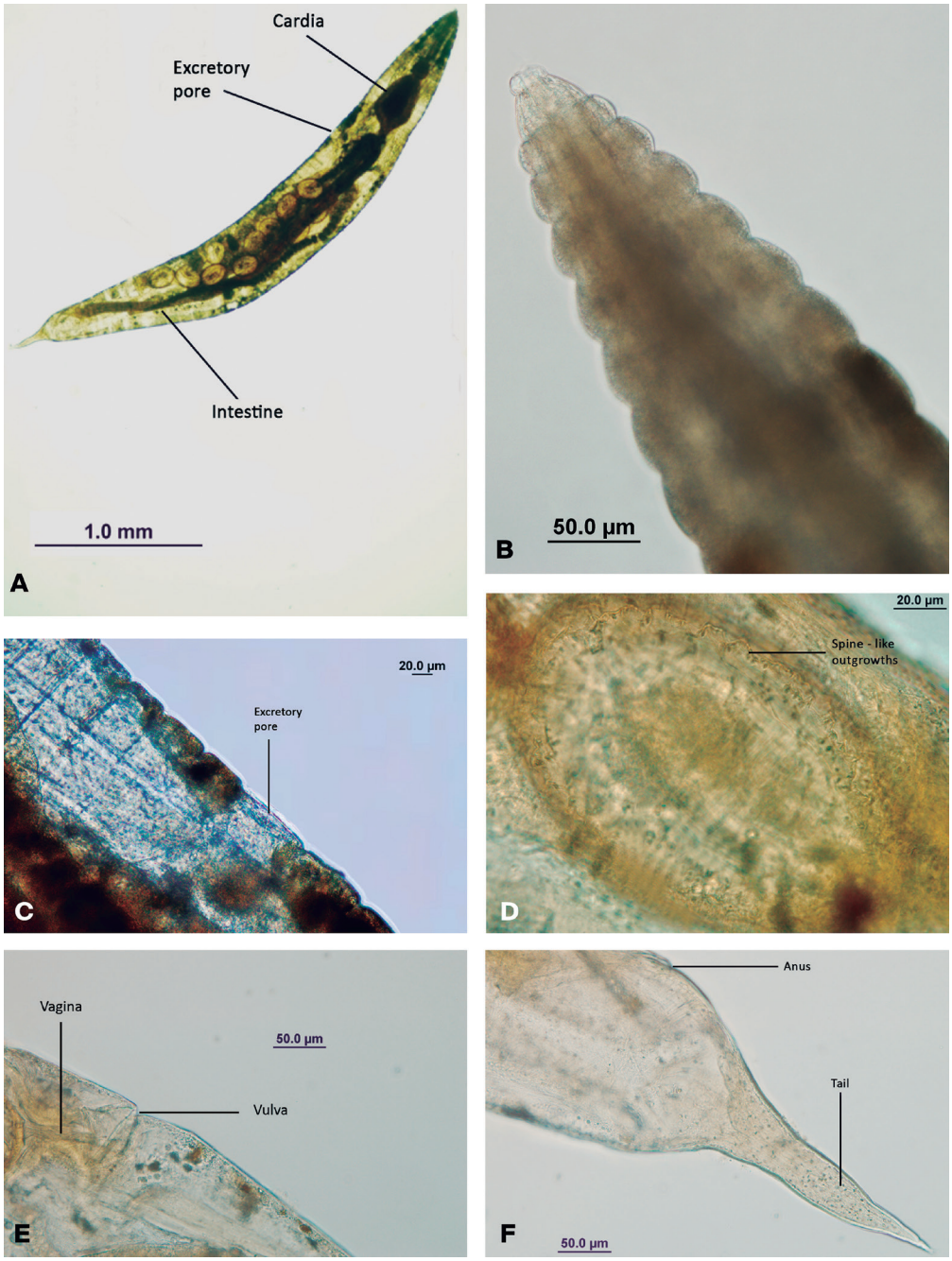

FIGURE 1: *Gryllophila skrjabini.* **(A)** Female, entire. **(B)** Cephalic region. **(C)** Excretory pore (maximized). **(D)** Egg showing spine-like outgrowths. **(E)** Vulval region. **(F)** Posterior region.

Cephalobellus magalhaesi (Schwenk, 1926)

Description

Morphology

Female: 5.5 to 6.4 mm long by 270 to 315 µm wide. Cuticle transversely striated up to the level of the oesophagus (Figs. 2B and C). Lips not salient. Buccal cavity about 7 µm deep. Oesophagus 569 µm long, consisting of an anterior cylindrical corpus, 432 µm long by 46 µm wide; an isthmus 36 µm long; and a posterior valvular bulb 102 µm in diameter. Intestine dilated anteriorly to form a cardia. Nerve ring 261 µm from the anterior end of body. Excretory pore posterior to base of oesophagus, 774 µm from the anterior extremity. Anus

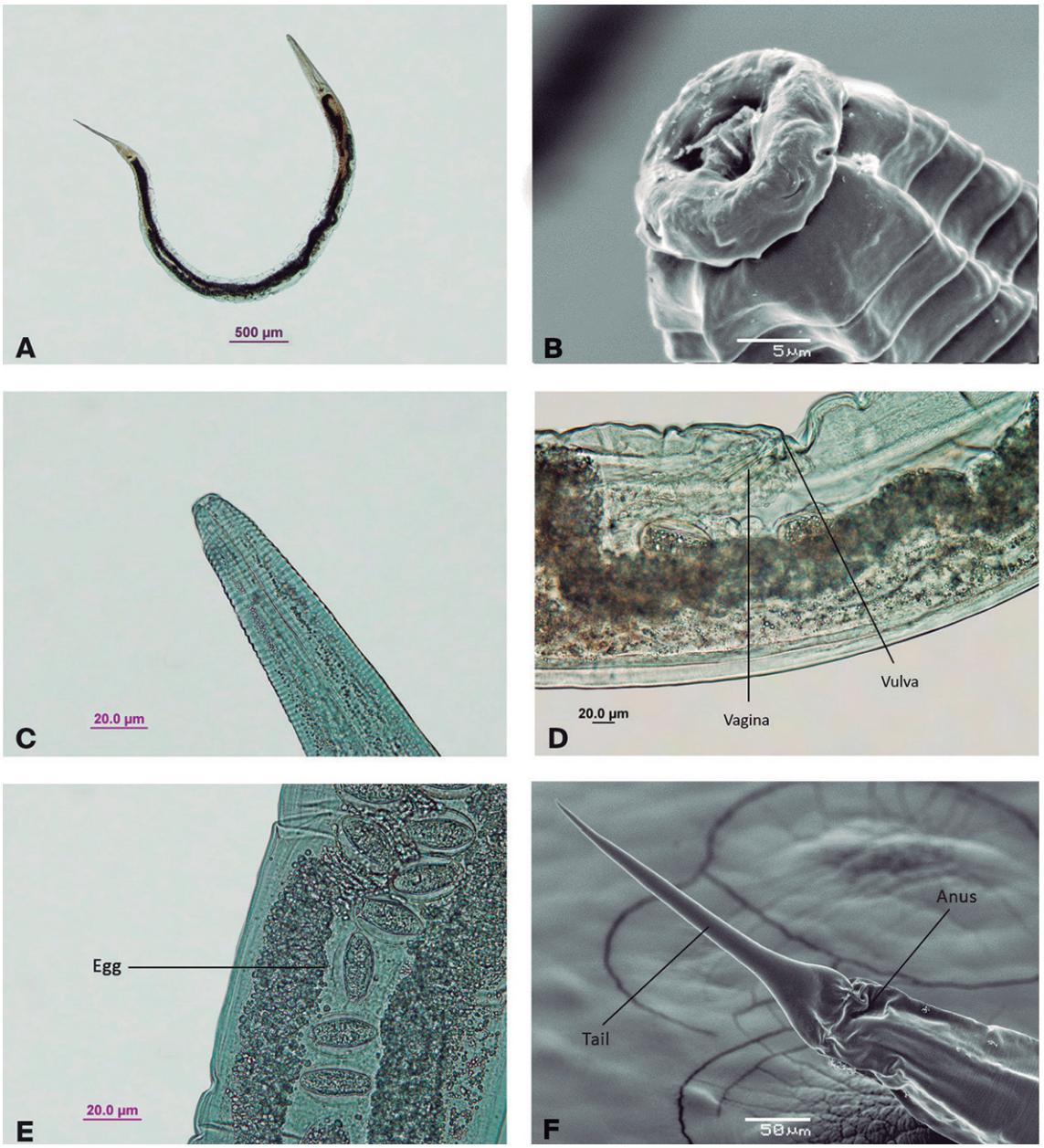

FIGURE 2: *Cephalobellus magalhaesi.* **(A)** Female, entire. **(B)** Cephalic region (SEM). **(C)** Cephalic region. **(D)** Vulva, lateral view. **(E)** Eggs. **(F)** Tail (SEM).

270 µm from posterior end of body; tail short, attenuated conical (Fig. 2F). Vulva just behind the middle of body (Fig. 2D). Ovaries lying in the opposite direction to their corresponding uteri and reflexed near their terminations; uteri divergent. Eggs ellipsoidal, almost spherical (Fig. 2E), 57 µm long by 42 µm wide.

Morphometry

Female (n = 130): total length = 5862 ± 413.08 (5520-6456), cephalic diameter = 22.5 ± 5.19 (18-27), distance from anterior end to nerve ring = 261.4 ± 12.7 (252-270), width at level of nerve ring = 126 ± 12.7 (108-135), oesophagus length = 569.2 ± 11.3 (333-639), anterior distance to basal bulb = 469.3 ± 11.3 (458-485), distance from anterior end to excretory pore = 774 ± 7 (767-781), greatest width = 292.5 ± 21.4 (270-315), width at level of vulva = 272.2 ± 4.5 (270-279), vulval length = 29.2 ± 4.5 (27-36), vulval width = 11.2 ± 4.5 (9-18), V = 50.8% ± 3.4 (46.5%-55%), posterior end width = 195.7 ± 13.5 (189-216), tail length = 270 ± 5.7

TABLE 1: Host range and isolation site of *Gryllophila* spp. from mole crickets (Adamson & van Waerebeke, 1992).

Nematode species	Author	Hosts	Location
G basiri	Parveen & Jairajpuri, 1981	*Gryllotalpa africana*	India
G cephalolobulata	Camino & Maiztegui, 2002	*Neocurtilla claraziana*	Argentina
G gryllotalpae	Farooqui, 1970	*G africana*	India
G nihali	Rizvi *et al.,* 2002	*G africana*	India
Gryllophila skrjabini	Sergiev, 1923	*Gryllotalpa* sp.	India
		G africana	Madagascar; India
		G europaeus	Spain
		G gryllotalpa	Germany
		G hexadactyla	Brazil
		G septemdecimochromosomica	France
		G vulgaris	Kazakhstan

(263-277), egg length = 57 ± 6.3 (54-63), egg width = 42 ± 6.3 (36-45).

Male: not found.

Host: nymphs and adults of the mole cricket *Neocurtilla claraziana* Saussure, 1874.

Locality: Gorina (34°54'29"S; 58°02'25"W)

Site of infection: hindgut (last part of intestine).

Prevalence: 33%.

DISCUSSION

The genus *Gryllophila* is characterized by having an oesophagus with a corpus, an isthmus and a valvular bulb, an intestine dilated anteriorly forming a bulky cardia, vulva 77% of body length from the anterior end and eggs unusually large, with or without spine-like outgrowths. Five species have been reported, all parasites of mole-crickets of which two were isolated in South-America: *G. skrjabini* in Brazil from *G. hexadactyla,* and *G. cephalolobulata* from *N. claraziana* in Argentina (Table 1).

In our study a population of *Gryllophila skrjabini* is reported for the first time in Argentina, constituting the second record of this species for South-America. *Gryllophila skrjabini* was characterized by ellipsoidal large eggs, (147.6 µm in length by 96 µm wide) with a thick shell with excrescences in the form of thorns, placed in a two-cell stage and chained by a tubular structure, probably formed by a mucous secretion oviduct. Morphometric differences were observed between Indian populations described by Basir (1956), and Argentinian specimens, presenting the last ones higher values for the length of the body (3.4 mm vs 3.1 mm), and oesophagus (446 µm vs 420 µm), and smaller eggs (144-155 µm length × 90-99 µm width vs 170-190 µm length × 100-110 µm width).

The genus *Cephalobellus* is characterized by a mouth that may or may not be armed with three cuticular teeth, excretory pore much behind the base of the oesophagus, vulva from the middle to almost the posterior third part of the body and eggs usually numerous, oval or elliptical. Specimens have been isolated from blattids, crickets, scarabeids, lucanids, tipulids, and Miriapoda (Adamson & van Waerebeke, 1992). Of the thirty-one species, only three were reported for South America: *C. lobulata* in the mole-cricket *N. claraziana* and *C. cyclocephalae* from the white grub *Cyclocephala signaticolis* respectively, both in Argentina (Camino & Reboredo, 2000, 2005), and *C. magalhaesi* from wild cockroaches from Brazil (Table 2). *Cephalobellus magalhaesi* is the first record of the species in Argentina and the second of this genus parasitizing *N. claraziana.*

Cephalobellus magalhaesi can be easily distinguished from other species of the genus by its comparatively longer tail and nearly spherical eggs; the male being unknown (Basir, 1956). Populations from Argentina also showed differences for some morphometric features with respect to specimens of *Cephalobellus magalhaesi* isolated by Basir (1956) from India. Argentinian females were longer for total body length (5.8 mm vs 4 mm), oesophagus length (569 µm vs 465 µm), distance of the nerve ring from the anterior end (261 µm vs 250 µm) and distance from the anterior end to the excretory pore (774 µm vs 710 µm), although the tail length was shorter (263-277 µm vs 500 µm).

Within the genus *Cephalobellus* the length of the tail shows variation. Female tails of *C. lobulata, C. nolani* and *C. cyclocephalae* do not exceed 120 µm (72 µm, 109 µm and 117 µm, respectively) and *C. unicoloris* has tails that range from 231 µm to

TABLE 2: Host range and isolation site of *Cephalobellus* spp. (Adamson & van Waerebeke, 1992).

Species	Author	Hosts	Country
C. annulobellus	Jairajpuri & Parveen, 1984	Unidentified larval Coleoptera	India
C. brevicaudatus	(Leidy, 1851) Christie, 1933	*Ligyrodes relictus* (Scarabaeidae: Coleoptera)	USA
C. cetonicola	(Theodories, 1955)	*Cetonia* sp. (Scarabaeidae: Coleoptera)	France
		Potosia cuprea and *Potosia* sp. (Scarabaeidae: Coleoptera)	France
C. costelytra	Dale, 1964	Larvae of *Costelytra zealandica* (Scarabaeidae: Coleoptera)	New Zealand
C. cuspidatum	(Rudolphi, 1814) Leibersperger, 1960	Larvae of *Oryctes nasicornis* (Scarabaeidae: Coleoptera)	Germany
C. cyclocephalae	Camino & Reboredo, 2005	*Cyclocephala signaticolis* (Scarabaeidae: Coleoptera)	Argentina
C. cylindricum	Christie, 1931	Scarabaeidae: Coleoptera	USA
C. fluxi	Dale, 1966	*Macromastix* sp. (Tipulidae: Diptera)	New Zealand
C. galliardi	(Dollfus, 1952) Basir, 1956	*Glomeris annulata* (Diplopoda)	France
		G marginata (Diplopoda)	France
		Anomala dubia (Scarabaeidae: Coloptera)	France
C. glomeridis	(Linstow, 1885) Jarry, 1964	*Glomeris conspersa* (Diplopoda)	Germany
		G undulata (Diplopoda)	Germany
		G hexasticha (Diplopoda)	Germany
C. granatensis	(Serrano Sanchez, 1955) Jarry, 1964	Diplopoda	Spain, Austria, Sweden, Germany, Switzerland and France
C. hexodontos	van Waerebeke, 1970	*Hexodon patella* (Scarabaeidae: Coleoptera)	Madagascar
		H. latissimum (Scarabaeidae: Coleoptera)	Madagascar
C. indiana	(Basir, 1940) Basir, 1956	*Leucophaea* sp. (Blattodea)	India
C. jewari	(Lal, 1968)	*Onthophagus bonasus* (Scarabaeidae: Coleoptera)	India
C. julicola	(Dollfus, 1952) Basir, 1956	*Julus terrestris* (Diplopoda)	France
C. leukarti	(Hammerschmidt, 1838) Christie, 1933	*Amphimallon assimilis* (Scarabaeidae: Coleoptera)	Germany
		Amphimallon solstitialis (Scarabaeidae: Coleoptera)	Germany
C. lobulata	Camino & Reboredo, 2000	*Neocurtilla claraziana* (Gryllotalpidae: Orthoptera)	Argentina
C. lohmanderi	(Leibersperger, 1960)	*Glomeris marginata* (Diplopoda)	Germany
C. lucani	(Leibersperger, 1960)	*Lucanus cervus* (Lucanidae: Coleoptera)	Germany
C. lloydi	Baylis, 1946	*Tipula* spp. (Diptera)	UK
C. magalhaesi	Schwenk, 1926	Unknown wild cockroach	Brazil
C. melolonthae	Leibersperger, 1960	*Melolontha* sp. (Scarabaeidae: Coleoptera)	Germany
C. nolani	Jex *et al.,* 2006	*Geoscapheus dilatatus* (Blaberidae: Blattodea)	Australia
C. orientalis	Singh *et al.,* 2014	*Blatta orientalis* (Blattodea)	India
C. osmodermae	Leibersperger, 1960	*Osmoderma eremita* (Scarabaeidae: Coleoptera)	Germany
		Potosia aeruginosa (Scarabaeidae: Coleoptera)	Germany
C. ovumglutinosus	van Waerebeke, 1978	*Blatella germanica* (Blattodea)	
	Sinnott *et al.,* 2015	*Py. Surinamensis* (Blaberidae: Blattodea)	Madagascar
		I. santacruzensis (Blattellidae: Blattodea)	Ecuador
		Pe. americana (Blattidae: Blattodea)	
C. papilliger	Cobb, 1920	Larvae of Scarabaeidae (Coleoptera)	Australia
C. potosiae	Leibersperger, 1960	Larvae of *Potosia cuprea* (Scarabaeidae: Coleoptera)	Germany
C. skaifei	Osche, 1960	*Cherastus* sp. (Spirobolida: Diplopoda)	South Africa
C. spicatus	Jairajpuri & Parveen, 1984	Larval Coleoptera from India	India
C. tipulae	(Leibersperger, 1960)	*Tipula* spp. and *Dictenidia bimaculata* (Tipulidae: Diptera)	Germany, France and Switzerland
Cephalobellus sp.	Sinnon *et al.* 2015	*Py. surinamensis* (Blaberidae: Blattodea)	Ecuador

418 µm. Basir (1956) described *C. galliardi* having a 292 µm tail but Gupta & Lamba (1980) redescribed this same species with a tail that reaches a measure of 848 µm. For that reason, we consider that the tail length would not be a reliable character even at species level.

CONCLUSIONS

Two new species of thelastomatid entomonematodes were isolated from the mole cricket *Neocurtilla claraziana* in Argentina, constituting the first report of *G. skrjabini* and *C. magalhaesi* for this country and the second isolation of *C. magalhaesi* from the world. *Neocurtilla clarziana* is a new host record for *G. skrjabini* and *C. magalhaesi.*

The report of these two new populations of Thelastomatidae in Argentina constitutes a significative contribution to the study of this family due to current limited information for South America.

RESUMEN

Thelastomatidae es una de las principales familias que parasitan insectos, dentro del orden Oxyurida. En este trabajo informamos parasitismo en ninfas y adultos de Neocurtilla claraziana, *por dos especies de thelastomatidos, como parte de un estudio de campo sobre plagas agrícolas. Las ninfas y los adultos de este insecto fueron aisladas de pastizales de la Provincia de Buenos Aires, Argentina, utilizando soluciones tensio-activas. Las especies de nematodos* Gryllophila skrjabini *Sergiev, 1923 y* Cephalobellus magalhaesi *Schwenk, 1926 son descriptas brevemente y sus medidas son dadas. Ambos nematodos son citados por primera vez para Argentina, constituyendo* Cephalobellus magalhaesi *el segundo aislamiento de esta especie en el mundo.* Neocurtilla clarziana *constituyó un nuevo registro de hospedador para* G. skrjabini.

Palabras-Clave: Entomonematodos; Oxyurida; Parasitismo; Grillos; Orthoptera.

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