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## A NEW SPECIES OF *BLATTICOLA* SCHWENK, 1926 (OXYURIDA, THELASTOMATIDAE) A PARASITE OF *ANUROGRYLLUS MUTICUS* (DE GEER, 1773) (ORTHOPTERA, GRYLLIDAE) FROM ARGENTINA

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### ABSTRACT

*Blatticola cristovata* n. sp. (Oxyurida, Thelastomatidae) a parasite of the cricket *Anurogryllus muticus* (De Geer, 1773) (Orthoptera, Gryllidae) from Argentina, is described and illustrated. This is the first species of the genus *Blatticola* found parasitizing cricket. This new species is characterized in females by having the cuticle annulated through the body length, the mouth opening is subtriangular and surrounded by eight cephalic papillae, the stoma is short with three pairs placed in two rows of cuticular sclerotised plates, amphids in small pore shaped, oesophagus is divided into three parts, anterior cylindrical corpus, isthmus distinct, and basal bulb valved, the nerve ring is situated around the middle of corpus, the intestine is wide broad anteriorly, oval eggs, smooth shell, with a straight side, and the other side with a band running longitudinally. Males with one pair of preanal, one pair of adanal and two pairs of postanal papillae, and the tail appendage short, conical and pointed.

KEYWORDS: Oxyurida, Thelastomatidae, *Blatticola cristovata* n. sp., Nematoda, taxonomy.

### INTRODUCTION

The genus *Blatticola* was proposed by Swenk (1926), who designated *Blatticola blatticola* (Galeb, 1877) as its type species. In 1932, Chitwood considered this species a synonym of *Blatticola blattae* (Graeffe, 1860). The taxonomic history of the genus and the type species was clarified by Dale in 1966. The emended diagnosis of the genus was modified (Adamson & Van Waerebeke, 1992) to accommodate the species described by Chitwood (1932) in a revision of the thelastomatid group. The genera

*Blattellicola* Basir (1940) and *Blattellicoloides* Farooqui (1966) match *Blatticola* in all essential respects and they are here considered synonyms of the latter. All species described until the present date are parasites of cockroaches from several regions of the world. While conducting field surveys on agricultural pests in the area of La Plata, province of Buenos Aires, Argentina, we found nymphs of crickets which were parasitized by a species of thelastomatid. In this contribution we report a new species, *Blatticola cristovata* n. sp., a parasite of the cricket *Anurogryllus muticus* (De Geer, 1773) (Orthoptera, Gryllidae).

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## MATERIAL AND METHODS

Adults and nymphs of *Anurogryllus muticus* (n=50) were found in a recreation park, located in Tolosa (34°55'S; 57°57'W), La Plata, Buenos Aires, Argentina. They were collected during 2005-2006, in plastic containers by hand and then placed in individual vials. The insects were kept at 5°C for 10 min, and then dissected in Petri dishes, filled with distilled water, under a stereoscope microscope. A transverse incision was made along the posterior end of the abdomen and the digestive tract was removed to obtain the parasites. The nematodes were killed by placing them in distilled water at 60°C for 2 min. They were removed to a fixative, 50% TAF solution, water + triethanolamine formalin (1:1), for 48 h, and then placed into pure TAF (Poinar, 1975). The nematodes were transferred to glycerol for slow evaporation and to clear the parasites (Seinhorst, 1959). Fixed specimens were used for drawings and measurements using a lucida camera mounted on a Zeiss compound microscope. They were photographed by a Nikon digital camera. All measurements are in micrometers ( $\mu\text{m}$ ), with ranges in parenthesis.

Micrographs were obtained with SEM and used to develop a description of the external ultrastructure of the nematodes cuticle. Males and females were fixed in 1.5% glutaraldehyde/1.5% formaldehyde buffered with 0.1M cacodylate buffer (pH 7.35) for 12 h at 8°C, post fixed with 1% osmium tetroxide solution for 12 h 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<sub>2</sub>, mounted on SEM stubs, and coated with gold (Kaya & Stock, 1997). Observations of ultrastructure were realized by SEM JEOL JSM-100.

## RESULTS

### *Blatticola cristovata* n. sp.\*

(Figs. 1A-F, 2A-F, 3A-C)

*Description:* Small nematodes. Females have the cuticle annulated along the body. The second annule from anterior end is longer than the others (Fig. 2A). The first annule has small amphids, pore-shaped, small and circular amphidial apertures. Mouth hexagonal surrounded by eight papillae (Fig. 3A). The stoma is

longer than broad, without tooth, with thick sclerotised walls forming three pairs of plates aligned in two rows (Figs. 1C, 2A). Oesophagus tripartite, indistinctly sclerotised at anterior end, with a corpus subparallel-sided, expanded posteriorly, the isthmus is short, and basal bulb valved (Fig. 1A). The nerve ring is situated anterior to corpus and isthmus junction. The excretory pore is posterior to the anterior end of the intestine. The intestine is broad, tapering posteriorly. Vulva located posteriorly, protruding, with one lip more developed, vagina long and narrow (Figs. 1F, 2B,C, 3B). Uterus single, directed forward from the vagina, and near the anterior end of the intestine, in a loop, then goes towards the posterior end of the intestine where it makes another loop, and ascends -until the level of the vulva. Ovary single directed anteriorly. eggs oval, smooth shell, with a straight side, and the other side with a band like a ridge, running longitudinally (Figs. 1E, 2E). Tail appendage short, conical and pointed (Figs. 1F, 2C, 3B).

*Female* (n = 38): Total length:  $4,211 \pm 95.7 \mu\text{m}$  (4,110-4,300); diameter of head at cephalic papillae level:  $23.5 \pm 2.42 \mu\text{m}$  (21.15-26); stoma length:  $15.7 \pm 3.8 \mu\text{m}$  (12.9-20); stoma width:  $9.08 \pm 2.6 \mu\text{m}$  (7.05-12); width of body at nerve ring level:  $203.5 \pm 4.13 \mu\text{m}$  (198.65-208); width of body at excretory pore level:  $402.7 \pm 33.5 \mu\text{m}$  (364-424); maximum body diameter:  $453.3 \pm 25.16 \mu\text{m}$  (420-480); width of body at posterior end level (anus):  $114.3 \pm 15.05 \mu\text{m}$  (100-130); width of body at vulva level:  $270 \pm 20 \mu\text{m}$  (250-290); distance from anterior end to nerve ring:  $285.3 \pm 47.6 \mu\text{m}$  (244-352); oesophagus length:  $525.3 \pm 24.4 \mu\text{m}$  (504-552); distance from anterior end to excretory pore:  $875.3 \pm 68.3 \mu\text{m}$  (816-950); vagina length:  $320 \pm 1.37 \mu\text{m}$  (240-350); vagina width:  $46.6 \pm 1.88 \mu\text{m}$  (43-48); V\*\*:  $83.08\% \pm 1.37$  (81.5-84.02); eggs length and width:  $84 \pm 4 \mu\text{m}$  (80-88) x  $34.6 \pm 4 \mu\text{m}$  (32-36); tail appendage length:  $25.3 \pm 2.3 \mu\text{m}$  (22-25).

Males are smaller in size than females. Cuticle is slightly annulated along the body. Amphids are small and pore-shaped. Cephalic papillae are not visible at optical microscope. Stoma is longer than broad, with sclerotised walls and two rows with three pairs of plates, without tooth. The oesophagus and intestine are similar to female (Fig. 1B). nerve ring located anterior to corpus and isthmus junction. Excretory pore is posterior to the anterior end of the intestine. Testis single (monorchic), extended anteriorly, reflexed

\* The species epithet is derived from the presence of longitudinal bands on the eggs.

\*\*V: distance from anterior end to vulva/body length x 100.

posteriorly at midbody. *Vas deferens* evident. Spicule single, straight, forming an arrow, without sculpture, with pointed tip (Fig. 1D, 2D). Capitulum and gubernaculum absent. Genital papillae arranged ventrolaterally in one pair of preanal, one pair of adanal and two pairs of postanal papillae (Fig. 1D, 2F, 3C). Tail appendage is short, conical and pointed (Fig. 1D, 2F, 3C).

terally in one pair of preanal, one pair of adanal and two pairs of postanal papillae (Fig. 1D, 2F, 3C). Tail appendage is short, conical and pointed (Fig. 1D, 2F, 3C).

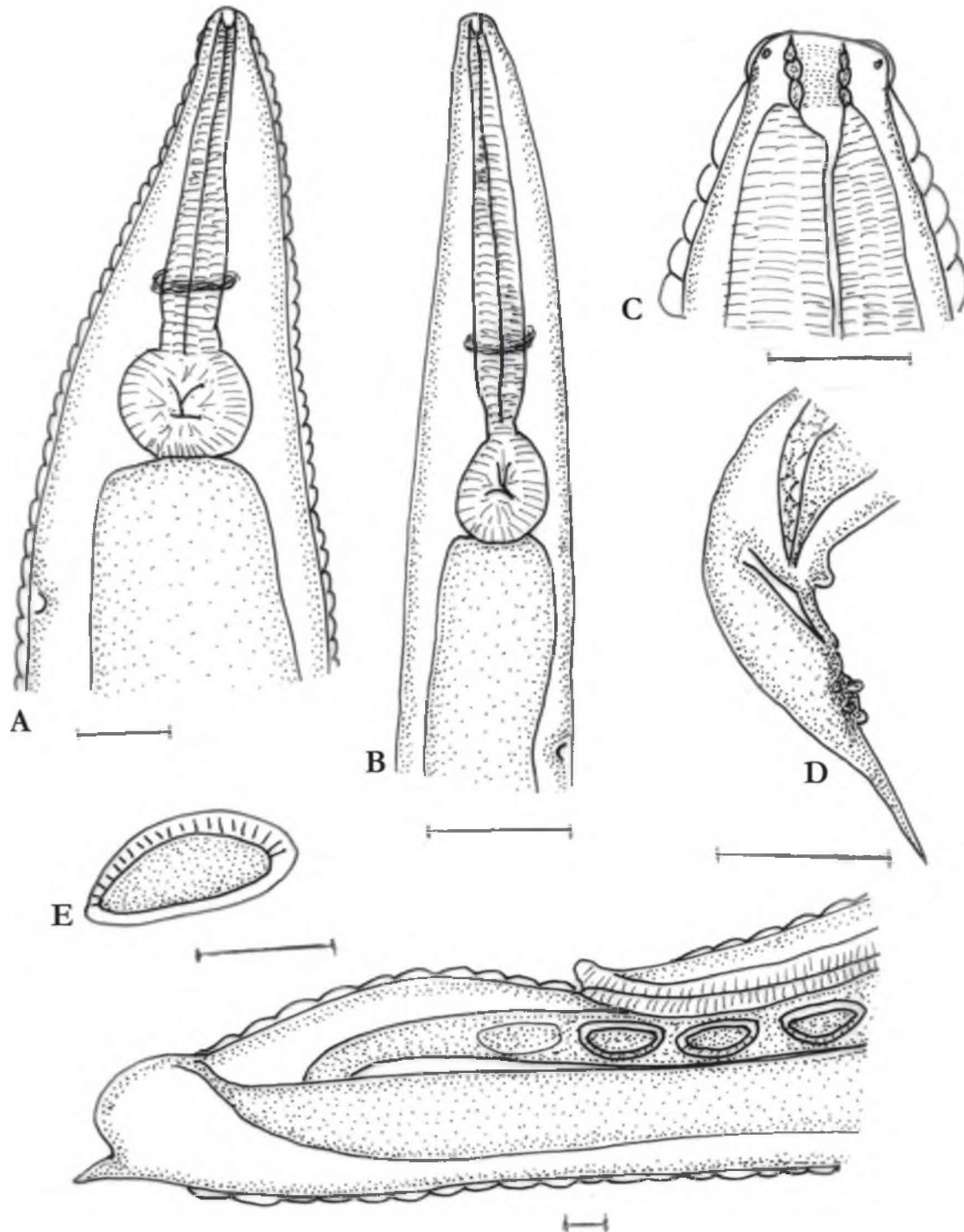


FIGURE 1. *Blatticola cristovata* n. sp. A. female anterior end. B. male anterior end. C. female stoma. D. male posterior end, lateral view. E. Egg F. female posterior end and vagina and uterus with eggs. Bars: A, B, D, E = 50  $\mu$ m; C, F = 25  $\mu$ m.

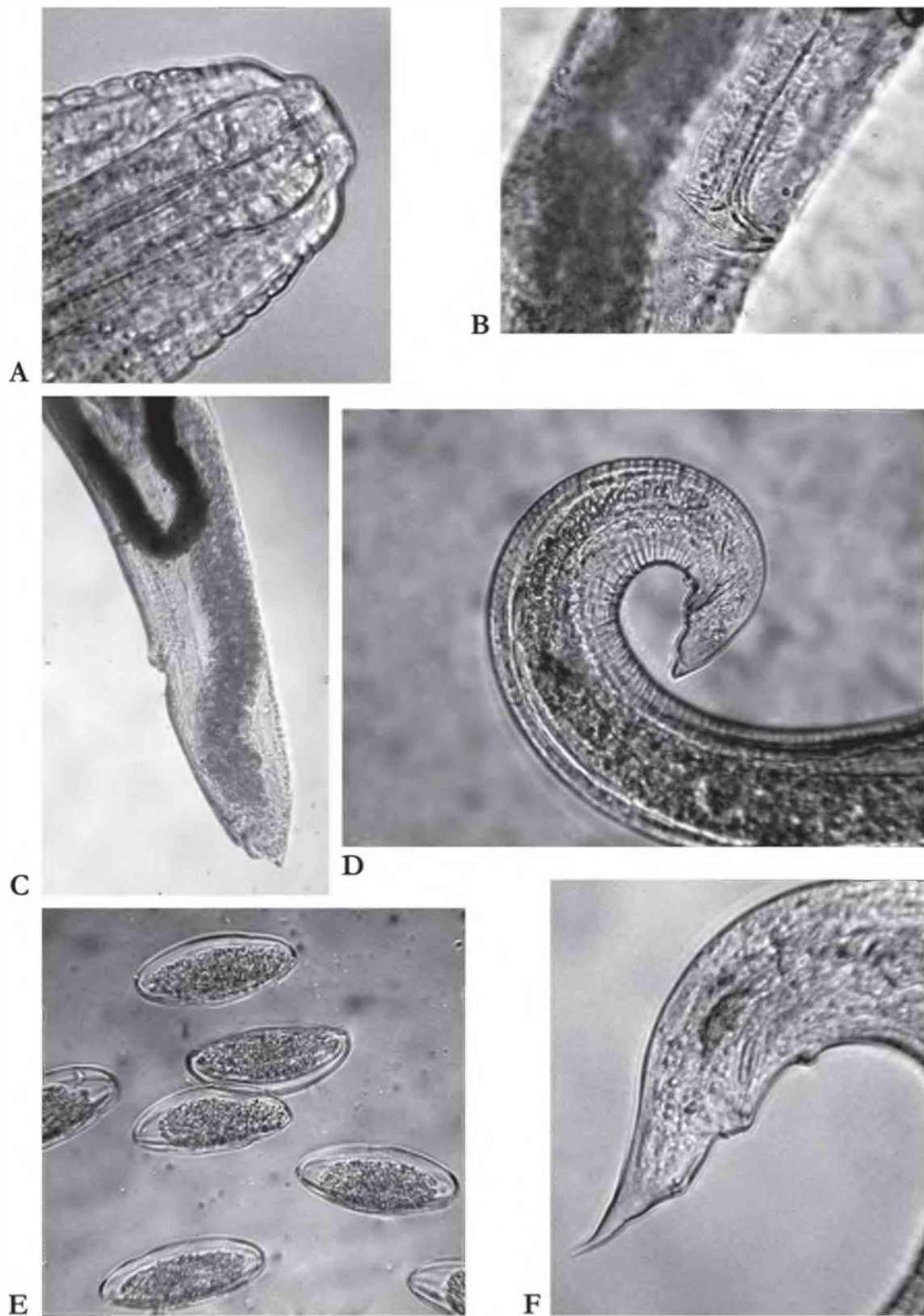


FIGURE 2. *Blatticola cristovata* n. sp. A. female stoma. B. Vulva. C. female posterior end, vulva and vagina. D. male posterior end (arrow = spicule). E. Eggs (arrow = longitudinal band). F. male posterior end (arrow = genital papillae). Bars = 50 µm.

*Male* (n = 30): Total length:  $2,113 \pm 221.8 \mu\text{m}$  (1,930-2,360); diameter of head at cephalic papillae level:  $13.7 \pm 0.68 \mu\text{m}$  (12.92-14.1); stoma length:  $10.02 \pm 2.35 \mu\text{m}$  (9.7-10.57); stoma width:  $4.7 \pm 0.15 \mu\text{m}$  (4.5-4.8); width of body at nerve ring level:  $54.05 \pm 2.35 \mu\text{m}$  (51.7-56.4); width of body at excretory pore level:  $82.3 \pm 6.23 \mu\text{m}$  (77.5-89.3); maximum body diameter:  $103.4 \pm 6.21 \mu\text{m}$  (98.7-110.45); width of body at anus level:  $53.26 \pm 9.78 \mu\text{m}$  (42.3-61.1); distance from anterior end to nerve ring:

$117.5 \pm 10.24 \mu\text{m}$  (112.8-129.25); oesophagus length:  $235.78 \pm 78.2 \mu\text{m}$  (223.25-244.4); distance from anterior end to excretory pore:  $465.3 \mu\text{m}$  (319.6-465.3); spicule length:  $354.46 \pm 1.35 \mu\text{m}$  (32.9-35.25); spicule width:  $4.28 \pm 0.72 \mu\text{m}$  (3.45-4.7); tail appendage length:  $22.5 \pm 3.58 \mu\text{m}$  (18.8-25.85).

*Type host:* nymphs of *Anurogryllus muticus* (De Geer) (Orthoptera, Gryllidae).

*Type locality:* Tolosa (34°55'S; 57°57'W), La Plata, Buenos Aires, Argentina.

*Type material:* Holotype, allotype and paratypes deposited in the Helminthological collection, Museo de La Plata, N° 5485, paseo del Bosque s/n, 1900 La Plata, Argentina.

*Site of infection:* intestine, midgut.

*Prevalence:* 27%

*Number of nematodes per larva:* 3.93

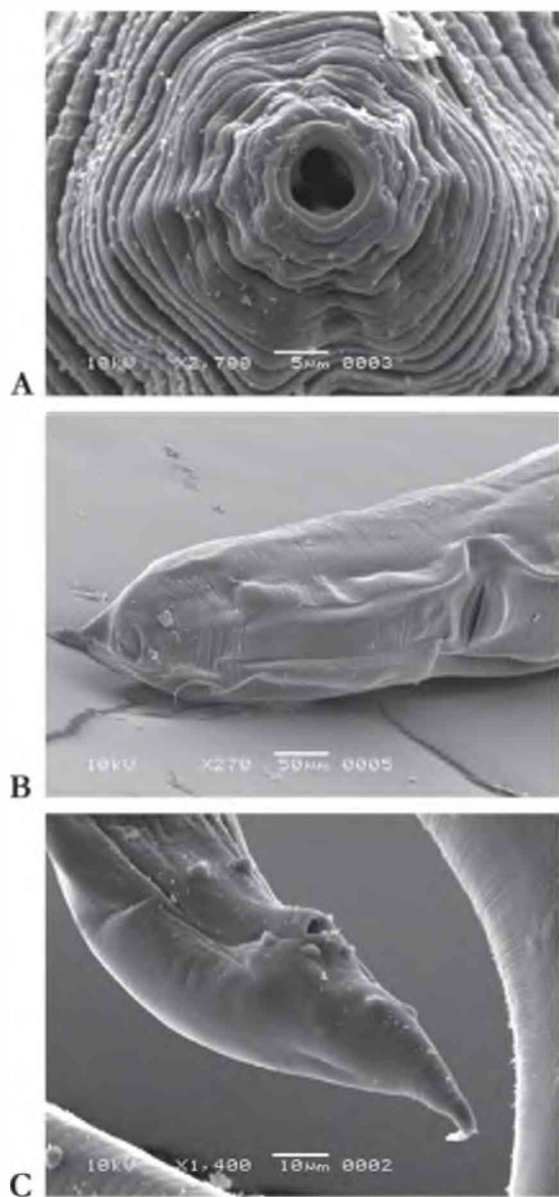


FIGURE 3. *Blatticola cristovata* n. sp. SEM photographs. A. Female stoma showing mouth opening. B. Vulva posterior lip (arrow). C. Male posterior end, genital papillae (arrow).

## DISCUSSION

*Blatticola cristovata* n. sp. is close to five species of the genus: *B. barryi* Zervos, 1987; *B. caucasica* Skrjabin, 1923; *B. monandros* Zervos, 1983; *B. supellaïmae* Rao & Rao, 1965; *B. tuapakae* Dale, 1966. Females have the intestine tapering posteriorly, and the nerve ring located around the corpus.

*Blatticola barryi* can be distinguished from *B. cristovata* by vulva position (17% of the body length), cuticle annulated only anteriorly, distinct rectal glands, eggs with operculum, male with three pairs of genital papillae, and two shallow constrictions in the tail. *Blatticola caucasica* is distinguished by having male with four pairs of genital papillae, from which two pairs are preanal and two pairs are postanal. *Blatticola monandros* can be characterized by the distance from vulva to anus being about 7-17% of body length, three pairs of tail genital papillae, cuticle annulated only anteriorly, tail without sharply linear point, corpus not broad medially, flask shaped, egg with operculum, and spicule short (less than 15 μm). *Blatticola supellaïmae* is separated by having the distance from vulva to anus about 5% of body length, four pairs of tail papillae, conical tail, sharply linear point, and nerve ring located around half corpus. *Blatticola tuapakae* is different by the distance from vulva to anus being about 7% of body length, presence of three pairs of tail papil-

lae, cuticle annulated only anterior, convex and conoid tail, tending subulate near tip. *Blatticola cristovata* n. sp. can be distinguished from the type species of the genus, *B. blattae*. This one possesses the diagnostic characteristics of the genus, but differs from the new species by female posterior part of the intestine broader than medial intestine, outstretched testis in male and nerve ring situated around the isthmus, near base of the corpus.

*Blatticola cristovata* n. sp. is characterized by *i*) female with annulated cuticle through the body, *ii*) mouth opening subtriangular and surrounded by 8 cephalic papillae, *iii*) stoma short with three pairs of cuticular sclerotised plates placed in two rows, *iv*) amphids aperture pore shaped, *v*) oesophagus divided into three parts, anterior cylindrical corpus, isthmus distinct, and basal bulb valved, *vi*) nerve ring located around the middle of corpus, *vii*) intestine broad anteriorly, *viii*) oval eggs, smooth shell, with a straight side, and the other side with a band running longitudinally, *ix*) male with one pair of preanal, one pair of adanal and two pairs of postanal papillae, and *x*) tail appendage short, conical and pointed.

## RESUMEN

Una nueva especie *Blatticola cristovata* n. sp. (Oxyurida, Thelastomatidae) parásita del grillo *Anurogryllus muticus* (De Geer, 1773) (Orthoptera, Gryllidae) en Argentina, se describe e ilustra. Esta es la primera especie del género *Blatticola* que se encontró parasitando a grillos. Esta nueva especie se caracteriza por tener las hembras la cutícula anillada a lo largo de todo el cuerpo, la abertura bucal triangular rodeada por ocho papilas cefálicas, el estoma corto con tres pares de placas esclerotizadas dispuestas en dos líneas, anfídios pequeños en forma de poro, esófago dividido en tres partes, una anterior con un corpus cilíndrico, un istmo distintivo, y un bulbo basal con valvas, el anillo nervioso situado rodeando el corpus, el intestino ensanchado anteriormente, huevos ovales, de cáscara lisa, con un lado recto y el otro con una cresta longitudinal. Macho con un par de papilas preanal, uno adanal y dos pares de papilas postanales, y apéndice caudal corto, cónico y punteagudo.

PALABRAS-CLAVE: *Blatticola cristovata* sp. n., Nematoda, taxonomía.

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