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A description of *Blatticola ancoracauda* (Nematoda, Thelastomatidae) a parasite of cricket (Orthoptera, Gryllidae) with notes of cytogentics from Argentina

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Abstract

Blatticola ancoracauda n. sp. (Oxyurida, Thelastomatidae) a parasite of the cricket *Anurogryllus muticus* (De Geer) (Orthoptera, Gryllidae) from Argentina, is described and illustrated. This is the second species of the genus *Blatticola* found parasitizing crickets. Females of this new species are characterized by the cuticle that is annulated from the anterior end up to the middle of the body, the mouth opening being subtriangular in shape surrounded by 8 cephalic papillae, the short stoma and the telostoma with two movable teeth, small pore shaped amphids, an oesophagus divided into three parts, anterior cylindrical corpus, isthmus distinct, and basal bulb valvated, the nerve ring situated a rounded the isthmus, the intestine broadest anteriorly, eggs oval, smooth shell, in apical view show a triangular section, males with one pair of preanal and two pairs of postanal papillae, and the tail appendage short, conical and pointed, females have a structure of anchorage on the top, like structure of grasp. Cytogenetically presents 2n with 8 chromosomes in female and 4 chromosomes in male.

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Introduction

The genus *Blatticola* was proposed by Schwenk (1926), who designated *B. blatticola* as its type species. In 1932 Chitwood considered this species a synonym of *B. blattae* (Graeffe, 1860). The confused taxonomicy history of the genus and of type species was clarified by Dale in 1966. Earlier 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 genus *Blattellicola* Basir (1940) and *Blatellicoloides* Farooqui (1966) agree with *Blatticola* in all essential respects and they are considered synonymous. All the species described up to the present are parasites of cockroaches from all major regions of the world. While conducting field surveys on agricultural pests in Argentina in areas of La Plata, Buenos Aires province, we found nymphs of crickets parasitized by a species of this group of thelastomatids. Achinelly and Camino (2007) described a new species, *Blatticola cristovata,* and then Camino and Schargorodsky (2009) described *B. biannulata*. These records represent the first and second reports of a cricket in Argentina. We recognized our new species belonging to the genus *Blatticola* which is characterized by the female with cuticle annulated up to the middle of the body, the mouth opening subtriangular surrounded by 8 cephalic papillae, the stoma short and telostoma with two movable teeth, the amphid small pore shaped, the oesophagus divided into three parts, anterior cylindrical corpus, isthmus distinct, and basal bulb valvated, the nerve ring situated arround the isthmus, the intestine broadest anteriorly, the egg oval, smooth shell, in apical view show a triangular section.

The cytogenetic study of nematodes shows up to the present the description of 9 species belonging to 6 genera within the family Thelastomatidae (Table 2) (Rizvi, 1997, Adamson 1981, Adamson 1984, Cutillas *et al.* 1985, Pham Van Luc and Spiridonov 1990, Zervos 1988). In all cases the diploid female and the haploid male were recorded. The distribution of chromosome numbers within the family for female, 2n = 8 and 2n = 10; in male n= 4 and n=5 (Table 2). In two species, *Protrellus dixoni* Zervos, 1986 and *Blaticola blatae* Graeffe, 1860 only the presence of arrhenotoky (species where the male is haploid and the female is diploid) has been described; however, data on the species cytogenetic is still unknown (Pham Van Luc and Spiridonov 1990 y Zervos 1988). The arrhenotoky has been described for 18 species of Oxyuridae, all parasites of cockroaches, diplopods, lizards, tortoises, lagomorphs and rodents. Representatives of all major groups of Oxyurida (Thelastomatoidea, Pharyngodonidae, Oxyuridae and Heteroxynematidae) have been shown to be haplodiploids, which suggests that it is a characteristic of order (Adamson, 1989).

In this contribution we described morphologically another new species of the genus and we carried out a cytogenetic study on *Blatticola ancoracauda* n. sp., a parasite of the cricket *Anurogryllus muticus* (De Geer) (Orthoptera, Gryllidae) found in Argentina.

Material and methods

Insects

Adults and nymphs of the cricket *Anurogryllus muticus* (De Geer) (n=50) were collected using a tensioactive solution: detergent + tap water (1:3) during spring 2015 from a farm in Villa Elisa (S 34°52´ 10´´; W 58°08´ 45´´), La Plata, Buenos Aires, Argentina. The insects were put in plastic containers by hand, transferred to individual vials, kept at 5°C for 10min and 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.

Nematodes

The nematodes were killed by placing them in distilled water at 60°C for 2min. They were removed to 50% TAF solution in water for 48 h, then placed into pure TAF. Fixed specimens were photographed on camera mounted on a Zeiss compound microscope. All measurements are in µm, and presented as the mean and standard deviation, with ranges in parenthesis. Scanning electron micrographs

were obtained using a JEOL JSM-100 scanning electron microscope, and used to describe the external ultrastructure of the cuticle of the nematodes. Specimens prepared for scanning electron microscopy were fixed in a cold solution of 1.5% glutaraldehyde/1.5% formaldehyde in 0.1M acidulate buffer (pH 7.35) overnight, postfixes in cold aqueous solution of 1% osmium peroxide overnight, transferred to 70% ethanol and hydrated in ethanol. The nematodes were dehydrated in a graded series of ethanol washes from 10% to 90%, to finish the dehydration in absolute alcohol (100%), and then critical point dried with liquid CO2, mounted on SEM stubs, and coated with gold for 1 hour.

Cytogenetic

For cytogenetic studies 3 adults were placed in distilled water for 30 minutes and then fixed in Carnoy's solution (3 parts pure ethyl alcohol and 1 part acetic acid). The cytogenetic preparations were made by squash and stained with 45% acetic orcein (Rodríguez Gil, 2009). The representative cells of each stage were photographed with an Olympus microscope with digital camera DP 71, with the program DP controller 3.3.1.292 and the images were processed with the programs GIMP version 2.8.16 and INKs cape GNU GENERAL PUBLIC LICENSE, Version 2.

Results

Taxonomy description

Blatticola ancoracauda n. sp.

Small nematodes. Cuticule of the female annulated from anterior end up to middle of body. The first three rings bigger than the remaining rings. Mouth surrounded by six cephalic papillae (Fig. 1a). The first annul has the cephalic papillae. Small amphids with amphidial apertures small and circular positioned between second and third annulies. Stoma longer than broad, the telostoma with two movable teeth (Fig. 1f). Oesophagus tripartite, with corpus subparallel-sided, expanded posteriorly, short isthmus, and basal bulb valvated. Nerve ring situated around isthmus. Excretory pore is at the begin of intestine. Intestine broad decreasing in width posteriorly. Vulva posterior, not protruding, with one lip more developed; vagina long and narrow (Fig. 1 d). Uterus single, directed anteriorly from vagina, and looping near anterior end of intestine, then posteriorly to posterior end of intestine, looping again and ascends anteriorly to level of vagina. Ovary single directed anteriorly to anterior end of intestine, near excretory pore. Eggs oval, with smooth shell, in apical view showing triangular section. Tail appendage short, conical and pointed, with anchorage-like structure at tip for grasping and sticking to the gut wall of the host (Figs. 1 b, c). Males smaller in size than females. Cuticle slightly annulated along body. Amphids are small and pore-shaped. Cephalic papillae not visible with light microscopy. Stoma longer than broad, telostoma with two teeth, like in female. Oesophagus and intestine similar as in female (Fig. 1 e). Nerve ring surrounding isthmus. Excretory pore posterior to anterior end of intestine. Testis single, extending anteriorly, and then reflexed linearly towards posterior end of the body. Ejaculatory duct evident. Spicule single, small, linear, straight forming an arrow, without sculpture, tip pointed (Fig. 1 e). Gubernaculum absent. Genital papillae arranged ventrolaterally in one pair of preanal papillae, and two pairs of postanal papillae. Tail appendage short, wide, conical and pointed (Fig. 1 e).

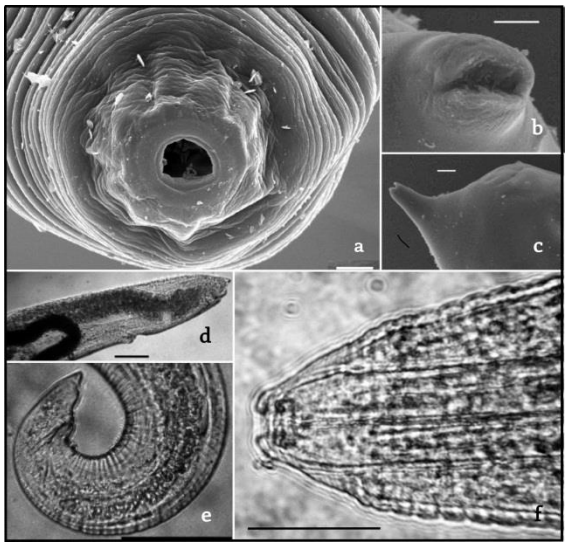

Fig.s 1. a-f. *Blatticola ancoracauda* n. sp. a. Anterior end of female. b. Detail of the structure of anchorage. C, d. Posterior end of female. e. Posterior end of male in lateral view. f. Stoma of the female. Bars = a, b: 5µm, c: 10µm, d, e: 100µm, f: 50µm.

Taxonomic summary

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

Type locality

A farm in Villa Elisa (S 34º52´ 10´´; W 58º08´ 45´´), La Plata, Buenos Aires, Argentina.

Etymology

The name refers to the anchoring structure at the tip of the caudal appendage

Type material

Holotype and Paratypes were deposited in the Helminthological collection of the Museo de La Plata, Paseo del Bosque s/n, 1900 La Plata, Argentina.

Site of infection

Intestine, midgut.

Prevalence: 22%

Number of nematodes per nymph: 1-8

Remarks

Blatticola ancoracauda n. sp. is characterized by *i)* females with cuticle annulated up to the middle of the body, *ii)* mouth opening subtriangular surrounded by 8 cephalic papillae, *iii)* stoma short and telostoma with two movable teeth, *iv)* amphids small pore shaped, *v)* oesophagus divided into three parts, anterior cylindrical corpus, isthmus distinct, and basal bulb valvated, *vi)* nerve ring situated arround the isthmus, *vii)* intestine broadest anteriorly, *viii)* eggs oval, smooth shell, in apical view show a triangular section, *ix)* males with one pair of preanal, and two pairs of postanal papillae, and the tail appendage short, conical and pointed, *x)* females with anchorage-like structure at tip for grasping and stick to the gut wall.

Blatticola ancoracauda n. sp. is close to seven species of the genus: *B. barryi* Zervos, 1987; *B. biannulata* Camino & Schargorodsky, 2009; *B. caucasica* Skrjabin, 1923; *B. cristovata* Achinelly & Camino, 2007; *B. monandros* Zervos, 1983; *B. supellaimae* Rao & Rao, 1965; *B. tuapakae* Dale, 1966; by having the intestine of female taper posteriorly and the nerve ring is located around the corpus. *B. barryi* can be distinguished from the new species by having the vulva located to a distance of 17% of the length of the body, cuticle annulated only anteriorly, distinct rectal glands, eggs with operculum, males with three pairs of genital papillae, and two shallow constrictions in the tail. *B. biannulata* is characterized by a short stoma with two sclerotised semicircles and the telostoma with one movable tooth, eggs have a triangular section, with three wings, the dorsal one and two lateroventrals, males with one pair of preanal, and two pairs of postanal papillae.

It shares with our new species the following characteristics: tail appendage with a structure of anchorage. *B. caucasica* is distinguished by having males with four pairs of genital papillae, two pairs are preanal and two pairs are postanal. *B. cristovata* can be distinguished by having the stoma without tooth, with thick sclerotised walls forming three pairs of plates aligned in two rows with three pairs of plates, and the genital papillae arranged ventrolaterally in one pair of preanal, one pair of adanal and two pairs of postanal papillae. *B. monandros* can be characterized by the distance from vulva to anus of about 7-17% of body length, three pairs of tail genital papillae, the cuticle annulated only anteriorly, tail without sharply linear point, corpus not broadest medially, flask shaped, egg with operculum, and spicule short (less than 15µm). *B. supellaimae* is separated by having the distance from vulva to anus about 5% of body length, four pairs of tail papillae, tail conical, sharply linear point, and nerve ring located around half corpus. *B. tuapakae* is different by the distance from vulva to anus about 7% of body length, three pairs of tail papillae, cuticle annulated only anteriorly, tail convex and conoid, tending subulated near tip. We can also compare our species with the type species, *B. blattae (*Chitwood, 1932) since this one possesses the diagnostic characteristics of the genus, but differs from our new species by females which have the posterior part of the intestine broader than medial intestine, outstretched testis in the male, and the nerve ring situated around the isthmus, near base the corpus.

Cytogenetics results

Analysis of the eggs present inside the adult cells showed cells with two chromosomal complements, cells with 8 chromosomes and other cells with 4 (Fig. 2 y 3). The chromosomes are very tiny; the length is about 2 µm. During meiosis, in paquitene, all chromosomes are equally stained (Fig. 2a). During the stages of prophase I, the bivalents are not clearly quantified (Fig. 2b). In prometaphase II and metaphase II it is observed that the chromosomes are all very small, but one of them can be differentiated one of larger size that is almost three times bigger than the other three bivalents (Fig. 2. c y d).

The other stages of meiosis did not present cells with individualizable chromosomes. Mitosis of 4 and 8 chromosomes is observed (Fig. 3). The mitosis of 8 chromosomes are all of equal size (Fig 3 a). Cells with 4 chromosomes have different sizes, which will generate sperm and those inside the egg that will generate male individuals (Fig. 3. b-d). In several cells a positive heteropynotic punctiform corpuscle was observed in the metaphases that could be correlated with a B chromosome due to its irregular behavior and its sporadic appearance. In a few mitoses a monovalent of irregular behavior was observed (Fig. 3. a y c).

Table 1. The measurements presented as the mean and standard deviation, with ranges minimun and maximun in parenthesis.

	Female (n = 10)	Male (n = 8)
Total length	4,120 ± 286.29 µm (4,010-4,320)	2,650 ± 187.42 µm (2,110-2,860)
Diameter of head at level of cephalic papillae	26 ± 4 µm (21-32)	15 ± 4.31 µm (12-19)
stoma length	11 ± 2.6 µm (7-15)	6.95 ± 3.28 µm (3.9-9.8)
stoma width	17 ± 2.3 µm (15-20)	4.92 ± 1.56 µm (3.8-6)
width of body at level of nerve ring	160 ± 27.6 µm (148-240)	145.32 ± 31.65 µm (125.1-172.4)
maximum body diameter	375 ± 46.6 µm (320-450)	198.26 ± 45.42 µm (140-232)
width of body at level of posterior end	118.6 ± 42 µm (98-180)	48.7 ± 12.45 µm (40.25-65.3)
width of body at level of vulva	224 ± 27.8 µm (190-250)	---
distance anterior end to nerve ring	420 ± 44.12 µm (362-454)	206.5 ± 25.8 µm (162-225)
oesophagus length	542.12 ± 45.14 µm (490-590)	243 ± 25.6 µm (225.12-286.24)
distance from anterior end to excretory pore	850 ± 182.23 µm (760-1210)	356 ± 185.4 µm (246.5-458)
vagina length	276.35 ± 19.56 µm (220-320)	---
vagina width	27.55 ± 14.24 µm (25-40)	---
V (distance anterior end to vulva/body length x 100)	82.14 ± 2.56 % (80.16-85.5)	---
spicule length	---	40 ± 5.6 µm (35.8-45.7)
spicule width	---	3.8 ± 1.9 µm (3.2-5.5)
length of eggs	85.18 ± 2,6 µm (83.5-87.12)	---
Width of eggs	36.7 ± 2,8 µm (32.5-40)	---
tail appendage length	90.15 ± 36.23 µm (68-132)	72 ± 4.6 µm (68.1-76.5)

Discussion

This paper represents the first cytogenetic study on cricket parasites in Argentina, the first study on the genus *Blatticola* and the second on parasites of crickets in the world (Table 2).

The only previous work involving *Blatticola* was made by Pham Van Luc and Spiridonov (1990) who described the presence of the arrhenotoky in nematodes; similar results were reported by Zervos (1988) in *Protrellus dixoni* (Thelastomatidae).

Table 2. List of species cytogenetically studied.

Species	2n male	2n female	Host	Country	Cited
Hammerschmidtiella diesingi (Hammerschmidt, 1838)	5	10	cockroach	Canada	Adamson & Nasher, 1987
				Spain	Cutillas *et al*., 1985
				India	Rizvi, 1997
H. andersoni Adamson, 1987	5	10	diplopod	Spain	Adamson, 1984
Thelastoma sp.	4	8	diplopod	Spain	Adamson, 1984
Thelastoma basiri Farooqui, 1967	unkown	8	cockroach	India	Rizvi, 1997
Cameronia aspiculata Parveen & Jairajpuri, 1985	4	8	mole cricket	India	Rizvi, 1997
C. nisari Adamson & Van Waerebeke, 1992	unkown	10	mole cricket	India	Rizvi, 1997
C. psilocephala Adamson & Van Waerebeke, 1992	unkown	10	mole cricket	India	Singh *et al*., 2013
Gryllophila basiri Parveen & Jairajpuri, 1981	unkown	10	cockroach	India	Rizvi, 1997
Leidynema appendiculata (Leidy,1850) Chitwood, 1932	5	10	cockroach	Spain	Cutillas *et al*., 1985
				India	Rizvi, 1997
Protellus dixoni Zervos, 1978	arrhenotoky		cockroach	Russia	Zervos, 1988
Blatticola blattae (Graeffe, 1860)	arrhenotoky		cockroach	Russia	van Luc & Spiridonov, 1990
Blatticola ancoracauda		8	cricket	Argentine	This study

The modal number of the family is 2n = 10 females and 5 males, present in six species, whereas 2n = 8 in females and 4 in males is present only in three species *Thelastoma* sp., *Cameronia aspiculata, Blaticola apiculata*. Our new species, *Blatticola ancoracauda*, is the fourth species that presents 2n chromosomes with 8 in female and 4 chromosomes in male.

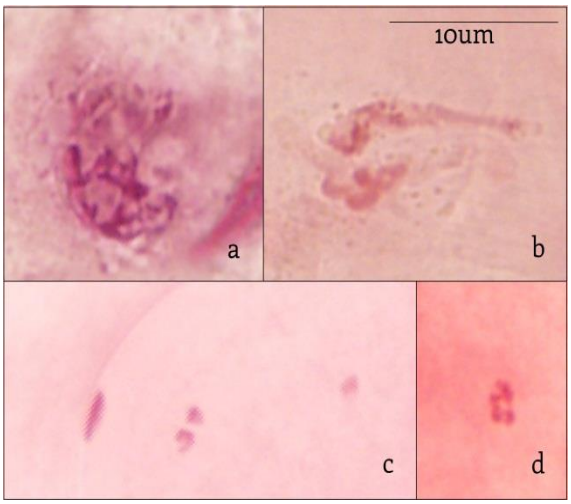

Fig. 2. a-d. Meiosis. a. Pachytene. b. Prophase I. c. Prometaphase II. d. Metaphase II. Bar 10µm.

The presence of a monovalent of irregular behavior in mitosis and the presence of the heteropynotic punctate body positive is described for the first time; however, in the work by Adamson and Nasher (1987) that corpuscle is observed in the images of Fig. 3, 9, 10 and 12 of their publication, without the author making reference to it.

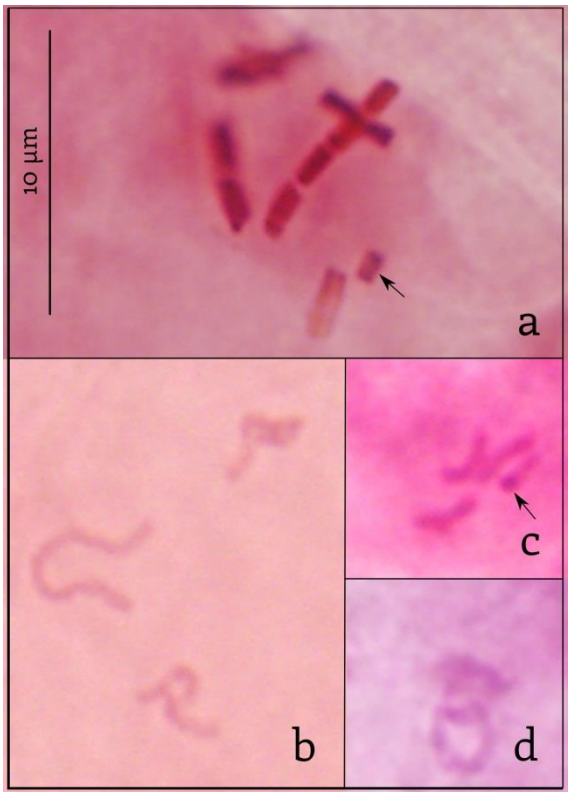

Fig. 3. a-d. Mitosis. a. Metaphase with 8 chromosomes. b. Prometaphase with 4 chromosomes. c. Metaphase with 4 chromosomes. d. Anaphase with 4 chromosomes each pole. The arrow mark the possible B-chromosome. Bar 10µm.

Cutillas (1985) described that cytogenetic analysis became almost impossible due to the small size of the specimens and the low rate of division found in the

specimens. In our case the limitation was not the size of the specimens but the very low rate of division found, which did not allow a detailed description of the different phases of cell division

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