



Identification of two species of *Binema* Travassos 1925 (Oxyurida: Travassosinematidae) and *Cameronia arecoensis* Marchissio and Miralles 1987 (Oxyurida: Thelastomatidae) based on morphological and 18S rRNA partial sequence

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Abstract: Oxyurid nematodes parasitizing the mole cricket *Neoscapteriscus vicinus* were isolated in the framework of sampling fields of mole-crickets from the pampean region, in Argentina. In this work, molecular characterization of the 18S rRNA partial sequence of nematodes belonging to the families Thelastomatidae (*Cameronia arecoensis* Marchissio and Miralles 1987) and Travassosinematidae (*Binema korsakowi* Sergiev 1923 and *Binema klossae*, Marchissio and Miralles 1993) were carried out. This is the first world report of sequences belonging to *B. klossae* and *C. arecoensis* and first Argentinian report of *B. korsakowi* sequence. Also, morphological and morphometric features of *B. klossae*, *B. korsakowi* and *C. arecoensis* from Argentinian populations are reported.

Key words: Parasitism, mole-crickets, nematodes, gene markers.

INTRODUCTION

Thelastomatoidea is one of the superfamilies of the Oxyurida Order that mostly infects arthropods and is taxonomically separate from the vertebrate parasitizing Oxyuroidea superfamily (Basir 1956).

Adamson and van Waerebeke (1992a, b, c) recognize five families within this superfamily: Protrelloidae, found only in cockroaches; Hystrignathidae, exclusive of passalid beetles; Travassosinematidae, mainly cited in mole-crickets; Pseudonymidae, parasites of hydrophilic beetles and Thelastomatidae, with 31 genera parasitizing a great diversity of insects. These are exclusively

intestinal parasites and their infective stages are the eggs which are ingested by the insect host (passive penetration), reaching the stomodeum where J2 hatch and the juveniles undergo successive molts reaching adulthood. Male dies after copulation and females reproduce by oviposition. Eggs are eliminated with faeces and wait to be ingested by a new host (Camino and Achinelly 2008).

In this work, oxyurid nematodes belonging to the families Thelastomatidae (*Cameronia arecoensis* Marchissio and Miralles 1987) and Travassosinematidae (*Binema korsakowi* Marchissio and Miralles 1993 and *Binema klossae* Sergiev 1923) were isolated in the framework of sampling fields of mole-crickets from the pampean region, in Argentina. A morphological description and a 18S rRNA partial sequence characterization is also presented.

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MATERIALS AND METHODS

Nymphs and adults of the mole-cricket *Neoscapteriscus vicinus* found in Hudson (34° 47' 25" S; 58° 08' 55" W), Buenos Aires state, Argentina. The Pampean region is characterized by its warm climate and relatively high humidity, with an annual average temperature of approximately 14-15°C (Baldi et al. 2006). Insects were collected using a garden shovel and placed in individual recipients during spring and summer seasons of 2016.

The Poinar's (1975) technique was used to isolate the nematodes. Isolated insects were dissected in Petri dishes with distilled water under a stereomicroscope. Nematodes were isolated from the hind gut, killed in distilled water at 60°C for 2 minutes and then fixed in T.A.F. (2% triethanolamine, 7.5% formaldehyde in distilled water). Specimens for molecular studies were fixed in absolute ethanol. Nematodes were measured using a camera lucida and an ocular micrometer in a Zeiss compound microscope. All measurements were given in micrometers unless otherwise stated. Photographs were taken with an Olympus DP-71 camera. Voucher specimens were deposited in the Museo de Ciencias Naturales de La Plata, Buenos Aires, Argentina.

To confirm the nematodes identification, a molecular approach was performed. Genomic DNA was extracted using 100 µl of a 5% suspension of Chelex in deionized water and 2 µl of proteinase K, followed by overnight incubation at 56°C, boiling at 90°C for 8 minutes and centrifugation at 14,000 rpm for 10 minutes. An aliquot of 1 µl of the supernatant was utilized as template for PCR. The 18S rRNA partial sequences were amplified using the primers Nem 18SF (5'-CGCGAATRGTCTATTACAACAGC-3') and Nem 18SR (5'-GGGCGGTATCTGATCGCC-3') according to Singh et al. (2013) with the Go Taq Master Mix (Promega). The thermocycler conditions were: 94°C for 15 min; 35 cycles of

94°C denaturation for 30 s, annealing 52°C for 40 s and extension 72°C for 60 s; a single final extension period of 72°C for 10 min. PCR products were analyzed by electrophoresis on 1% agarose gels and visualized by staining with ethidium bromide. The amplicons were sequenced in Macrogen Inc. (Korea), and edited with the platform GENEIOUS (<http://www.geneious.com>) (Kearse et al. 2012). The consensus sequences obtained were compared with sequences in the BLAST tool available in the NCBI database (<http://www.ncbi.nlm.nih.gov>). The 18S rRNA partial sequence generated from this study were submitted to the National Center for Biotechnology Information (NCBI) GenBank database (<http://www.ncbi.nlm.nih.gov>) and can be accessed using the GenBank accession numbers: MH055748, MH151853, MH151854 for *B. korsakowi*, *B. klossae* and *C. arecoensis*, respectively.

RESULTS

Family Travassosinematidae *Binema korsakowi* (Sergiev 1923)

DESCRIPTION

Morphology

Female: body spindle shaped with its maximum width at the center, narrowing towards the extremities (Fig. 1a). Cuticle with few striations near the cephalic extremity. Oral opening with eight labiopapillae. Amphids with circular openings. A very short buccal cavity, almost absent. Oesophagus 366.3 µ long, with a corpus, a short isthmus and a posterior valvular bulb. Nerve ring situated in the middle of the corpus. Excretory pore posterior to the basal bulb (Fig. 1b). Intestine dilated anteriorly, but this enlargement remains less in diameter than the oesophageal bulb. Vulva near the middle of the body, slightly posterior; vagina directed anteriorly (Fig. 1c). Two ovaries, one lying

anteriorly and the other posteriorly, both reflexed. Each set of reproductive organs forming four loops; uteri divergent. Tail with conical form (Fig. 1d). Eggs bearing a bunch of filaments at each pole, laid in capsules, each capsule usually containing two or three eggs (Fig. 1e, 1f). The size of the capsule depends on the number of eggs contained in it. Individual eggs measure 36 μ to 54 μ long by 36 μ to 45 μ wide.

Morphometry

Female (n=7): total length: 2815.2 (2112-3024), cephalic diameter: 21 (18-36), distance from anterior end to the nerve ring: 162.9 (99-207), width at

level of the nerve ring: 144 (126-207), oesophagus length: 366.3 (360-387), anterior distance to the basal bulb: 272.7 (243-279), distance from anterior end to the excretory pore: 643.5 (549-738), greatest width: 391.5 (279-441), width at level of vulva: 344.2 (261-387), vulval length: 31.5 (9-45), vulval width: 9, V= 53.8% (52% - 60%), posterior end width: 173.7 (135-270), tail length: 327.6 (297-351), egg length: 45 (36-54), egg width: 39 (36-45).

Morphology

Male: 1190 μ long by 90 μ in maximum width of the body. Buccal cavity short. Body transversely striated throughout its whole length (Fig. 2a).

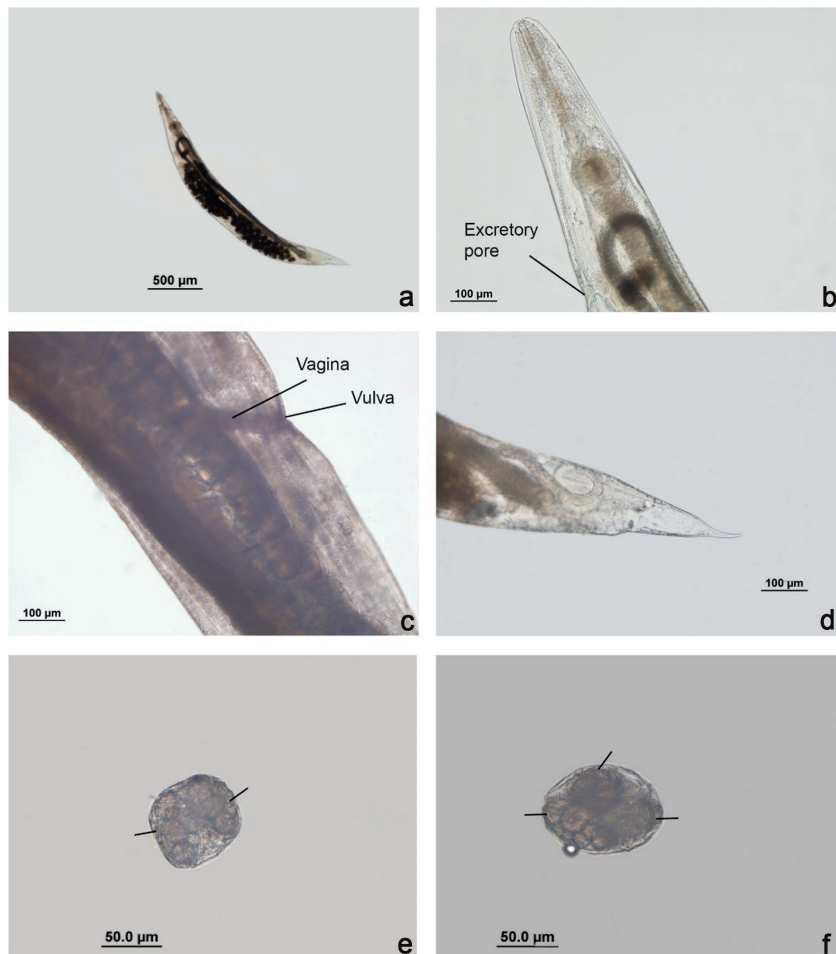


Figure 1 - *Binema korsakowi*. **a.** Female, entire. **b.** Cephalic region. **c.** Vulval region (lines showing the vulva and the vagina). **d.** Tail. **e.** Capsule containing 2 eggs (lines). **f.** Capsule containing 3 eggs (lines).

Oesophagus 126 μ long with a cylindrical corpus, a short isthmus and a basal bulb (Fig. 2b). The nerve ring is located approximately in the middle of the corpus. Excretory pore posterior to the base of the oesophagus. Nine pairs of caudal papillae, of which four pairs are pre-cloacal, one pair ad-cloacal and four pairs post-cloacal. A single median papilla between the last pair of caudal papillae at the base of the caudal spike is seen. Spicule single (Fig. 2c). Tail filiform.

Morphometry

Male (n=2): total length: 1015 (840-1190), cephalic diameter: 13.5 (9-18), distance from anterior end to the nerve ring: 67.5 (63-72), width at level of the nerve ring: 54 (45-63), oesophagus length: 131 (126-136), anterior distance to the basal bulb: 99, distance from anterior end to the excretory pore: 162, greatest width: 90, width at level of the anus: 31.5 (27-36), spicule length: 36, spicule width: 4.5, tail length: 54.

TAXONOMIC SUMMARY

Country: Argentina
 State: Buenos Aires
 Locality: Hudson (34° 47' 25" S 58° 08' 55" W)
 Number of specimens: 9 (7 ♀♀, 2 ♂♂)
 Host: *Neoscapteriscus vicinus* (Orthoptera: Gryllotalpidae)
 Localization: hindgut
 Collection number: Museo de Ciencias Naturales de La Plata (MLP-He 7309)

Binema klossae (Marchissio and Miralles 1993)

DESCRIPTION

Male: not found

Morphology

Female: fusiform body (Fig. 3a). Mouth surrounded by eight cephalic papillae and a pair of amphids.



Figure 2 - *Binema korsakowi*. a. Male, cephalic region. b. Posterior region (line showing the spicule).

Annulated cuticle in the cephalic region, smooth in the rest of the body. Buccal ornamentation. Stoma with chitinous walls. Oesophagus with a chitinous wall-well differentiated corpus, a highly developed isthmus and a basal bulb (Fig. 3b). Nerve ring situated in the middle of the corpus. Excretory pore posterior to the base of the oesophagus. Rectilinear intestine. Reproductive system didelphic amphidelphic. Vulva located at the middle of the body (Fig. 3c). Large ovoid eggs, with polar filaments surrounded or not by a

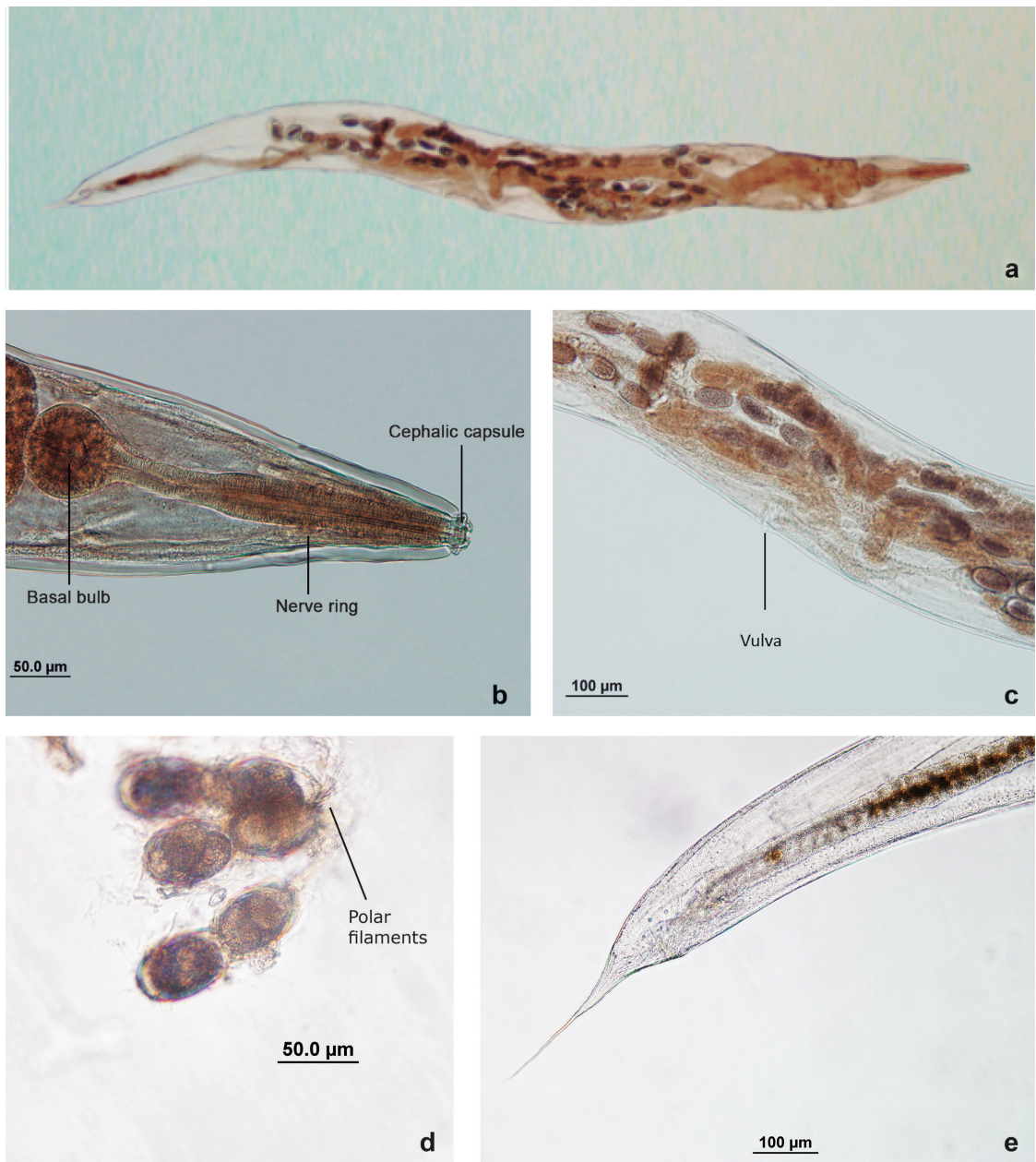


Figure 3 - *Binema klossae*. **a.** Female, entire. **b.** Cephalic region (lines showing the cephalic capsule, nerve ring and basal bulb). **c.** Vulval region (line showing the vulva). **d.** Eggs with polar filaments. **e.** Posterior region.

membranous capsule (Fig. 3d). Phasmids present. Conical tail, provided with a flagellum as a caudal appendix (Fig. 3e).

Morphometry

Female (n=3): total length: 3952 (2640-4848), cephalic diameter: 18, distance from anterior end

to the nerve ring: 117 (108-126), width at level of the nerve ring: 93 (81-99), oesophagus length: 375 (315-405), anterior distance to basal bulb: 294 (252-315), distance from anterior end to the excretory pore: 552 (441-630), greatest width: 222 (153-261), width at level of vulva: 222 (135-270), vulval length: 36 (18-45), vulval width: 24 (18-

36), V= 49.3% (49% - 49.5%), posterior end width: 93 (81-108), tail length : 297 (288-306), egg length: 58.5 (54-63), egg width: 31.5 (27-36).

TAXONOMIC SUMMARY

Country: Argentina

State: Buenos Aires

Locality: Hudson (34° 47' 25" S 58° 08' 55" W)

Number of specimens: 3 ♀♀

Host: *Neoscapteriscus vicinus* (Orthoptera: Gryllotalpidae)

Localization: hindgut

Collection number: Museo de Ciencias Naturales de La Plata (MLP-He 7309)

Family Thelastomatidae

Cameronia arecoensis (Marchissio and Miralles 1987)

DESCRIPTION

Morphology

Female: fusiform body (Fig. 4a). Buccal cavity surrounded by eight cephalic papillae and a pair of amphids. Annulated cuticle evident in the cephalic region and barely perceptible in the caudal region. Short stoma. Oesophagus with a well differentiated corpus, a small isthmus and a basal bulb (Fig. 4b). Excretory pore posterior to the base of the oesophagus. Rectilinear intestine. Reproductive system didelphic amphidelphic. Vulva in the posterior third of the body (Fig. 4c). Elliptical large eggs, flattened on one of their sides and fused in pairs along their flattened faces (Fig. 4d). Conical tail (Fig. 4e).

Morphometry

Female (n=7): total length: 2516.6 (2400-2688), cephalic diameter: 18, distance from anterior end to the nerve ring: width at level of the nerve ring: oesophagus length: 363 (396-450), anterior

distance to the basal bulb: 316.5 (297-369), distance from anterior end to excretory pore: 922.4 (882-963), greatest width: 263.6 (252-279), width at the level of vulva: 239.1 (207-261), vulval length: 42.4 (36-45), vulval width: 24.4 (18-36), V= 68.8% (65.1% - 76%), posterior end width: 84.8 (63-99), tail length: 113.1 (81-136), egg length: 210 (201-216), egg width: 60 (54-63).

Morphology

Male: smaller than the female. Body transversely striated throughout its whole length (Fig. 5a). Oesophagus with a cylindrical corpus, a short isthmus and a basal bulb (Fig. 5b). Excretory pore posterior to the base of the oesophagus. Testicle single, reflexed approximately in the middle of the body. Spicule single. A pair of pre-anal papillae, a pair of ad-anal papillae, and two pairs of post-anal (being the first one small), proximal to the anus and the second one more evident and situated in the base of the tail. Tail conical, slightly curved (Fig. 5c).

Morphometry

Male (n=1): total length: 828, cephalic diameter: 9, distance from anterior end to the nerve ring: width at level of the nerve ring: oesophagus length: 144, anterior distance to the basal bulb: 117, distance from anterior end to excretory pore: 171, greatest width: 72, width at level of the anus: 36, spicule length: 31.5, spicule width: 4.5, tail length: 54.

TAXONOMIC SUMMARY

Country: Argentina

State: Buenos Aires

Locality: Hudson (34° 47' 25" S 58° 08' 55" W)

Number of specimens: 8 (7 ♀♀, 1 ♂)

Host: *Neoscapteriscus vicinus* (Orthoptera: Gryllotalpidae)

Localization: hindgut



Figure 4 - *Cameronia arecoensis*. **a.** Female, entire. **b.** Cephalic region. **c.** Elliptical large eggs. **d.** Vulval region (line showing the vulva). **e.** Tail.

Collection number: Museo de Ciencias Naturales de La Plata (MLP-He 7309)

MOLECULAR ANALYSIS

DNA characterization

The 18S rRNA fragments sequenced obtained were 889 bp for *B. korsakowi*, 880 bp for *B. klossae*,

and 899 for *C. arecoensis*. The BLAST tools grouped the two new sequences of *Binema* with the sequences JX852712, JX852711, KC763368 belonging to *B. korsakowi* (99%), *Binema ornata* Travassos, 1925 (94%) and *Binema mirzaia* (Basir 1942) Basir 1956 (86%), respectively; and *C. arecoensis* with the sequence KC763369 belonging

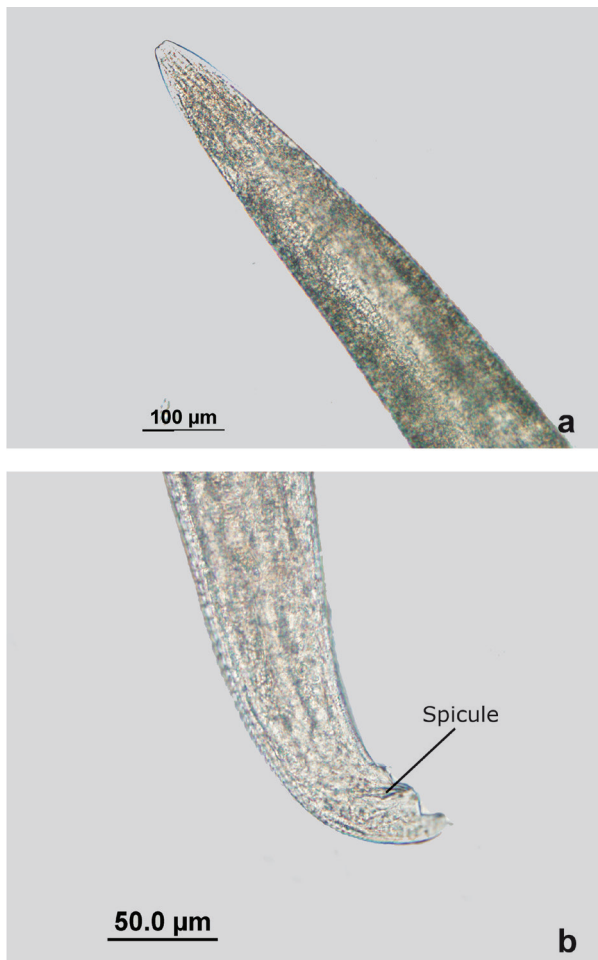


Figure 5 - *Cameronia arecoensis*. a. Male, cephalic region. b. Posterior region (line showing the spicule).

to *Cameronia nisari* Parveen and Jairajpuri, 1985 (97%).

DISCUSSION

The family Travassosinematidae gathers mainly nematodes of mole-crickets (*Binema* and *Pulchrocephala*), whereas only one genera (*Travassosinema*) was found in diplopods (Adamson and van Waerebeke 1992b).

The genus *Binema* is characterized by having eggs broadly oval with polar filaments deposited in capsules containing 2-3 eggs or non-encapsulated and laid in pairs, conical tail or rounded with short or long caudal appendage or flagella-like, with

or without fine striations near its tips and caudal extremity in male, conical, filiform or spike-like (Shah et al. 2012). Eleven species have been reported worldwide: Tewarson and Gupta (1978) described *B. thapari*, Parveen and Jairajpuri (1985) described *B. parva*, Singh and Singh (1990) did the same with *B. chauhani* and Adamson and van Waerebeke (1992b) cited 4 species in their review of the Travassosinematidae family. Singh (2003) described the species *B. atrophicaudata* in India, and a year later Shah and Rizvi (2004) described *B. annulinervus*.

In South-America 4 species were isolated: *Binema ornata* (Travassos 1925) in Brazil from *Gryllotalpa africana* (Beauvois 1805) and *G. europaeus* L., *Binema korsakowi* in Brazil and Argentina from *G. africana*, *G. europaeus*, *G. vulgaris* Latreille 1804 and *Neocurtilla claraziana* Saussure 1874, while *B. bonaerensis* (Camino and Reboredo 1999) and *B. klossae* in Argentina from *N. claraziana*.

Marchissio and Miralles (1993) did not provide a description morphometric values for *B. korsakowi*, so this kind of information for an Argentinian isolate of this species was lacking. Our investigation contributes with the first description and morphometric data for a *B. korsakowi* isolate in Argentina. We also compared the *B. korsakowi* population reported in the present study with that of Farooqui's (1968): In our isolate the body of the male was longer (1190 μ vs 810 μ), and wider (90 μ vs 60 μ) respect to Farooqui's population, whilst the position of the nerve ring from the head end (62 μ vs 60 μ), the spicule length (4.5 μ vs 4 μ) and the tail length (54 μ vs 60 μ) presented similar values. The prominent lateral alae which runs from the caudal appendage up to the level of the nerve ring seen in Farooqui's worm was absent in ours. Regarding the female, the body was longer (2800 μ vs 2100 μ) and wider (441 μ vs 280 μ). The nerve ring position from the head end was almost identical (162 μ vs 160 μ) but the excretory pore

was located further away (640 μ vs 530 μ). The oesophagus was shorter (360 μ vs 420 μ) and the tail was longer (320 μ vs 280 μ). The eggs were shorter (45 μ vs 59 μ), but wider (39 μ vs 34 μ).

Binema klossae was first described by Marchissio and Miralles in 1993. When we compare our population (females) with the one described by Marchissio and Miralles, we noticed that in our isolate the body was longer (4848 μ vs 4200 μ), the width was quite similar (261 μ vs 270 μ), the oesophagus (405 μ vs 420 μ), tail (306 μ vs 313 μ), the nerve ring position from the head end (126 μ vs 165 μ) and the position of the excretory pore (630 μ vs 810 μ) were shorter. Eggs were almost identical in length (63 μ vs 64 μ) and width (36 μ vs 34.8 μ).

Respect to the genus *Cameronia*; 12 species have been cited so far. In 1984, Parveen and Jairajpuri described *C. klossi*. Marchissio and Miralles (1987) described *C. arecoensis*. Adamson and van Waerebeke (1992 a) cited 6 species in their review of the Thelastomatidae family. Reboredo and Camino (2001) described *C. laplatae* and Rizvi and Jairajpuri (2002) described *C. basiri*. Finally, Shah (2007) described *C. manipurensis* and *C. triovata*, the latter based only in female morphology. When we compared our *C. arecoensis* populations with that of Marchissio and Miralles (1987), the female body length of our isolate (2688 μ vs 4605 μ) and the body width (279 μ vs 465 μ) were shorter respect to the population described by these authors, the oesophagus (369 μ vs 600 μ) and tail length were also shorter (136 μ vs 297 μ), and the excretory pore was closer to the head (963 μ vs 1050 μ). The eggs were longer (216 μ vs 194 μ) and wider (64 μ vs 58 μ). With regard to the male, the body length was larger (828 μ vs 803 μ), the body width smaller (72 μ vs 89 μ) and the oesophagus longer (144 μ vs 139 μ). The excretory pore was closer to the head end (171 μ vs 197 μ). The spicule (31.5 μ vs 29 μ) and the tail length were almost identical (54 μ vs 55 μ).

The Blast analysis confirmed the identity of *Binema korsakowi* with 1% difference with the sequence of the same species deposited in the GenBank, and also the identity of *B. klossae* as a member of the genus *Binema*. *Cameronia arecoensis* was 97% similar to *C. nisari*, supporting genetically the position of our specimen as a species in *Cameronia*.

This paper contributes with a morphological redescription of these three species, the first molecular characterization of an Argentinian isolate of *B. korsakowi*, and the first molecular characterization of *B. klossae* and *C. arecoensis* in the world. The results supported the validity of these three nematode species based on morphological and molecular observations.

AUTHOR CONTRIBUTIONS

JMR and MFA did the samplings, JMR, MFA and MM wrote the manuscript; JMR identified and characterized morphologically the nematodes and WF and MM performed the molecular analysis.

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