



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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3 **Identification of two species of *Binema* Travassos, 1925 (Oxyurida:**
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5 **Travassosinematidae) and *Cameronia arecoensis* Marchissio and Miralles, 1987**
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7 **(Oxyurida: Thelastomatidae) based on morphological and 18S rRNA partial sequence**
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36 **Academy section:** Biological Sciences
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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 the 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.

INTRODUCTION

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

Adamson and van Waerebeke (1992 a, b, c) recognize five families within this superfamily: Protrelloididae, found only in cockroaches; Hystrignathidae, exclusive of passalid beetles; Travassosinematidae, mainly cited in mole-crickets; Pseudonymidae, parasites of

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3 hydrophilic beetles and Thelastomatidae, with 31 genera parasitizing a great diversity of insects.
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5 These are exclusively intestinal parasites and their infective stages are the eggs which are ingested
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7 by the insect host (passive penetration), reaching the stomodeum where J2 hatch and the
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9 juveniles undergo successive molts reaching adulthood. Male dies after copulation and females
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11 reproduce by oviposition. Eggs are eliminated with faeces and wait to be ingested by a new host
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13 (Camino and Achinelly 2008).
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17 In this work oxyurid nematodes belonging to the families Thelastomatidae (*Cameronia*
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19 *arecoensis* Marchissio and Miralles 1987) and Travassosinematidae (*Binema korsakowi* Marchissio
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21 and Miralles 1993 and *Binema klossae* Sergiev 1923) were isolated in the framework of sampling
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23 fields of mole-cricket from the pampean region, in Argentina. A morphological description and a
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25 18S rRNA partial sequence characterization is also presented.
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30 MATERIAL AND METHODS

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33 Nymphs and adults of the mole-cricket *Neoscapteriscus vicinus* were found in Hudson (34°
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35 47' 25" S; 58° 08' 55" W), Buenos Aires state, Argentina. The Pampean region is characterized by
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37 its warm climate and relatively high humidity, with an annual average temperature of
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39 approximately 14-15 °C (Baldi et al. 2006). Insects were collected using a garden shovel and placed
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41 in individual recipients during spring and summer seasons of 2016.
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44 The Poinar's (1975) technique was used to isolate the nematodes. Insects were dissected in Petri
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46 dishes with distilled water under a stereomicroscope. Nematodes were isolated from the hind gut,
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48 killed in distilled water at 60° C for 2 minutes and then fixed in T.A.F. (2% triethanolamine, 7.5%
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50 formaldehyde in distilled water). Specimens for molecular studies were fixed in absolute ethanol.
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52 Nematodes were measured using a camera lucida and an ocular micrometer in a Zeiss compound
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54 microscope. All measurements were given in micrometers unless otherwise stated. Photographs
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3 were taken with an Olympus DP-71 camera. Voucher specimens were deposited in the Museo de
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5 Ciencias Naturales de La Plata, Buenos Aires, Argentina.
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8 To confirm the nematodes identification, a molecular approach was performed. Genomic
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10 DNA was extracted using 100 µl of a 5% suspension of Chelex in deionized water and 2 µl of
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12 proteinase K, followed by overnight incubation at 56°C, boiling at 90°C for 8 minutes and
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14 centrifugation at 14,000 rpm for 10 minutes. An aliquot of 1 µl of the supernatant was utilized as
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16 template for PCR. The 18S rRNA partial sequences were amplified using the primers Nem 18SF (5'-
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18 CGCGAATRGCTCATTACAACAGC-3') and Nem 18SR (5'-GGGCGGTATCTGATCGCC-3') according to
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20 Singh et al. (2013) with the Go Taq Master Mix (Promega). The thermocycler conditions were:
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22 94°C for 15 min; 35 cycles of 94°C denaturation for 30 s, annealing 52°C for 40 s and extension
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24 72°C for 60 s; a single final extension period of 72°C for 10 min. PCR products were analyzed by
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26 electrophoresis on 1% agarose gels and visualized by staining with ethidium bromide. The
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28 amplicons were sequenced in Macrogen Inc. (Korea), and edited with the platform GENEIOUS
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30 (<http://www.geneious.com>) (Kearse et al., 2012). The consensus sequences obtained were
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32 compared with sequences in the BLAST tool available in the NCBI database
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34 (<http://www.ncbi.nlm.nih.gov>). The 18S rRNA partial sequence generated from this study were
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36 submitted to the National Center for Biotechnology Information (NCBI) GenBank database ([http://](http://www.ncbi.nlm.nih.gov)
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38 www.ncbi.nlm.nih.gov) and can be accessed using the GenBank accession numbers: MH055748,
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40 MH151853, MH151854 for *B. korsakowi*, *B. klossae* and *C. arecoensis*, respectively.
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49 RESULTS

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52 Family Travassosinematidae

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55 *Binema korsakowi* (Sergiev 1923)
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Description

Morphology

Female: spindle-shaped body, with its maximum width in the center of it, 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 very short isthmus and a posterior valvular bulb. Nerve ring situated at 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 the 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 the level of the 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). Oesophagus 126 μ long with a cylindrical corpus, a very short isthmus and a basal bulb (Fig. 2B). The nerve ring located approximately at 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 the 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 the 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

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3 Collection number: Museo de Ciencias Naturales de La Plata (MLP-He 7309)
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8 *Binema klossae* (Marchissio and Miralles 1993)
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11 **Description**
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14 Male: not found
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17 **Morphology**
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20 Female: fusiform body (Fig. 3A). Mouth surrounded by eight cephalic papillae and a pair of
21 amphids. Annulated cuticle in the cephalic region, smooth in the rest of the body. Buccal
22 ornamentation. Stoma with chitinous walls. Oesophagus with a chitinous wall-well differentiated
23 corpus, a highly developed isthmus and a basal bulb (Fig. 3B). Nerve ring situated at the middle of
24 the corpus. Excretory pore posterior to the base of the oesophagus. Rectilinear intestine.
25 Reproductive system didelphic amphidelphic. Vulva located at the middle of the body (Fig. 3C).
26 Large ovoid eggs, with polar filaments surrounded or not by a membranous capsule (Fig. 3D).
27 Phasmids present. Conical tail, provided with a flagellum as a caudal appendix (Fig. 3E).
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39 **Morphometry**
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42 Female (n=3): total length: 3952 (2640-4848), cephalic diameter: 18, distance from anterior end to
43 the nerve ring: 117 (108-126), width at the level of the nerve ring: 93 (81-99), oesophagus length:
44 375 (315-405), anterior distance to the basal bulb: 294 (252-315), distance from anterior end to
45 the excretory pore: 552 (441-630), greatest width: 222 (153-261), width at the level of the vulva:
46 222 (135-270), vulval length: 36 (18-45), vulval width: 24 (18-36), V= 49.3% (49%-49.5%), posterior
47 end width: 93 (81-108), tail length : 297 (288-306), egg length: 58.5 (54-63), egg width: 31.5 (27-
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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 at 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 the 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 the 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 the level of the nerve ring: -, oesophagus length: 144, anterior distance to the basal bulb: 117, distance from anterior end to the excretory pore: 171, greatest width: 72, width at the level of the anus: 36, spicule length: 31.5, spicule width: 4.5, tail length: 54.

TAXONOMIC SUMMARY

Country: Argentina

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3 State: Buenos Aires

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

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7 Number of specimens: 8 (7 ♀ ♀, 1 ♂)

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10 Host: *Neoscapteriscus vicinus* (Orthoptera: Gryllotalpidae)

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12 Localization: hindgut

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

19 20 Molecular analysis

21 22 23 DNA characterization

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26 The 18S rRNA fragments sequenced obtained were 889 bp for *B. korsakowi*, 880 bp for *B. klossae*,
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28 and 899 for *C. arecoensis*. The BLAST tools grouped the two new sequences of *Binema* with the
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30 sequences JX852712, JX852711, KC763368 belonging to *B. korsakowi* (99%), *Binema ornata*
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32 Travassos 1925 (94%) and *Binema mirzaia* (Basir 1942) Basir 1956 (86%), respectively; and *C.*
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34 *arecoensis* with the sequence KC763369 belonging to *Cameronia nisari* Parveen and Jairajpuri
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36 1985 (97%).
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43 44 DISCUSSION

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47 The family Travassosinematidae gathers mainly nematodes of mole-crickets (*Binema*
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49 and *Pulchrocephala*), whereas only one genera (*Travassosinema*) was found in diplopods
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51 (Adamson and van Waerebeke 1992 b).
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3 The genus *Binema* is characterized by having eggs broadly oval with polar filaments
4 deposited in capsules containing 2-3 eggs, or non-encapsulated and laid in pairs; conical tail or
5 rounded, with short or long caudal appendage or flagella-like, with or without fine striations near
6 its tips and caudal extremity in male, conical, filiform or spike-like (Shah et al., 2012). Eleven
7 species have been reported worldwide: Tewarson and Gupta (1978) described *B. thapari*, Parveen
8 and Jairajpuri (1985) described *B. parva*, Singh and Singh (1990) did the same with *B. chauhani* and
9 Adamson and van Waerebeke (1992b) cited 4 species in their review of the Travassosinematidae
10 family. Singh and Malti (2003) described the species *B. atrophicaudata* in India, and a year later
11 Shah and Rizvi (2004) described *B. annulinervus*.

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

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21 Marchissio and Miralles (1993) did not provide a description nor morphometric values for
22 *B. korsakowi*, so this kind of information for an Argentinian isolate of this species was lacking. Our
23 investigation contributes with the first description and morphometric data for a *B. korsakowi*
24 isolate in Argentina.

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26
27 We also compared our *B. korsakowi* population with that of Farooqui's (1968): In our
28 isolate the body of the male was longer (1190 μ vs 810 μ), and wider (90 μ vs 60 μ) respect to
29 Farooqui's population, whilst the position of the nerve ring from the head end (62 μ vs 60 μ), the
30 spicule length (4.5 μ vs 4 μ) and the tail length (54 μ vs 60 μ) presented similar values. The
31 prominent lateral alae which runs from the caudal appendage up to the level of the nerve ring
32 seen in Farooqui's worm was absent in ours. Regarding the female, the body was longer (2800 μ vs
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3 2100 μ) and wider (441 μ vs 280 μ). The nerve ring position from the head end was almost
4 identical (162 μ vs 160 μ) but the excretory pore was located further away (640 μ vs 530 μ). The
5 oesophagus was shorter (360 μ vs 420 μ) and the tail was longer (320 μ vs 280 μ). The eggs were
6 shorter (45 μ vs 59 μ), but wider (39 μ vs 34 μ).
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12 *Binema klossae* was first described by Marchissio and Miralles in 1993. When we
13 compared our population (females) with the one described by Marchissio and Miralles, we noticed
14 that in our isolate the body was longer (4848 μ vs 4200 μ), the width was quite similar (261 μ vs
15 270 μ), the oesophagus (405 μ vs 420 μ), tail (306 μ vs 313 μ), the nerve ring position from the
16 head end (126 μ vs 165 μ) and the position of the excretory pore (630 μ vs 810 μ) were shorter.
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23 Eggs were almost identical in length (63 μ vs 64 μ) and width (36 μ vs 34,8 μ).
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26 As in the work of Marchissio and Miralles (1993), we also find mixed infections. Two
27 females of *B. klossae* were observed together with two females and one male of *B. korsakowi* in
28 the same host sample. *Binema klossae* differed from *B. korsakowi* in that females had a longer
29 isthmus in the esophagus (74 vs 7 μ), a longer egg length (58.5 vs 45 μ) and a caudal flagellum. In
30 addition, the males of *B. klossae* differed from *B. korsakowi* in that the former had a shorter spike
31 length (17-18 vs 36 μ) and a caudal flagellum.
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40 Respect to the genus *Cameronia*; 12 species have been cited so far. In 1984, Parveen
41 and Jairajpuri described *C. klossi*. Marchissio and Miralles (1987) described *C. arecoensis*. Adamson
42 and van Waerebeke (1992 a) cited 6 species in their review of the Thelastomatidae family.
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3 oesophagus (369 μ vs 600 μ) and tail length were also shorter (136 μ vs 297 μ), and the excretory
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5 pore was closer to the head (963 μ vs 1050 μ). The eggs were longer (216 μ vs 194 μ) and wider
6
7 (64 μ vs 58 μ). With regard to the male, the body length was larger (828 μ vs 803 μ) but the body
8
9 width was smaller (72 μ vs 89 μ) and the oesophagus was longer (144 μ vs 139 μ). The excretory
10
11 pore was closer to the head end (171 μ vs 197 μ). The spicule (31.5 μ vs 29 μ) and the tail length
12
13 were almost identical (54 μ vs 55 μ).
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16
17 The Blast analysis confirm the identity of *Binema korsakowi* with 1% difference with the
18
19 sequence of the same species deposited in the GenBank, and also the identity of *B. klossae* as a
20
21 member of the genus *Binema*. *Cameronia arecoensis* is 97% similar to *C. nisari*, supporting
22
23 genetically the position of our specimen as a species in *Cameronia*.
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25
26 This paper contributes with a morphological redescription of these three species, the first
27
28 molecular characterization of an Argentinian isolate of *B. korsakowi*, and the first molecular
29
30 characterization of *B. klossae* and *C. arecoensis* in the world. The results supported the validity of
31
32 these three nematode species based on morphological and molecular observations.
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39 ACKNOWLEDGEMENTS

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43 The authors would like to thank english professor Antonela Capurro for the revision of the
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45 manuscript.
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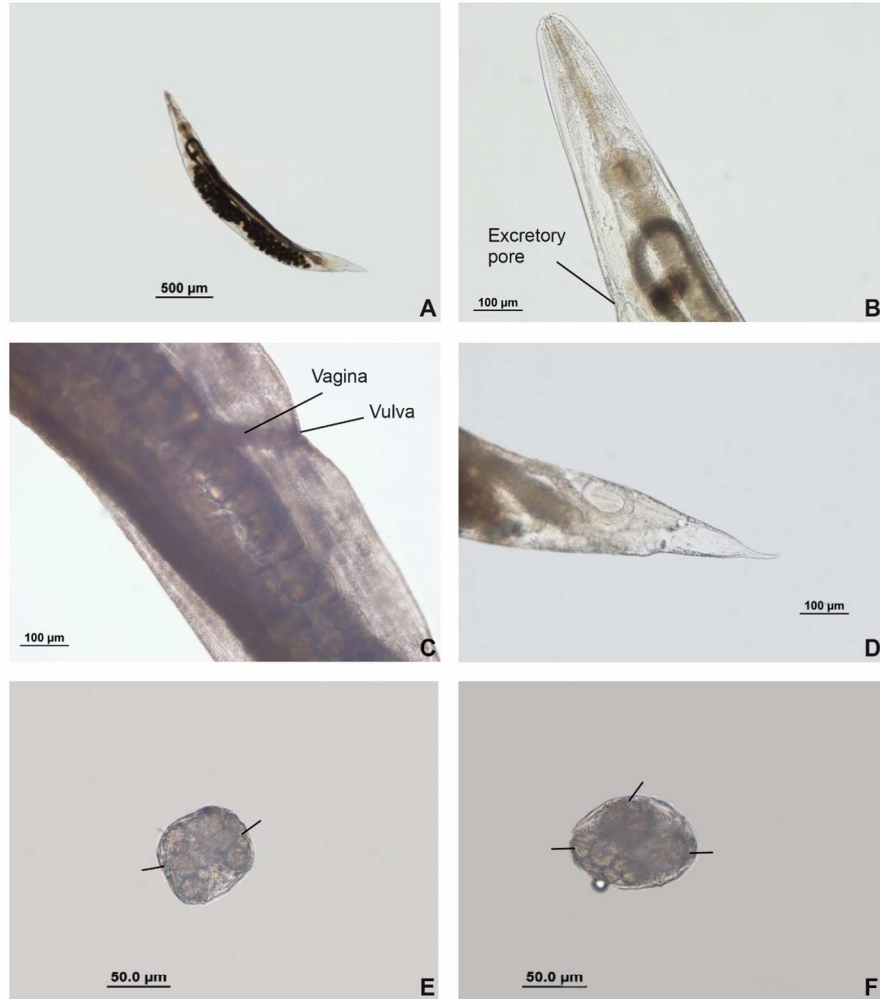


Fig. 1. *Binema korsakowi* A. Female, entire. B. Cephalic region. C. Vulval region (lines showing the vulva and the vagina). D. Tail. E. Capsule containing 2 eggs (lines). F. Capsule containing 3 eggs (lines).

170x193mm (300 x 300 DPI)



Fig. 2. *Binema korsakowi*. A. Male, cephalic region. B. Posterior region (line showing the spicule).

108x189mm (300 x 300 DPI)

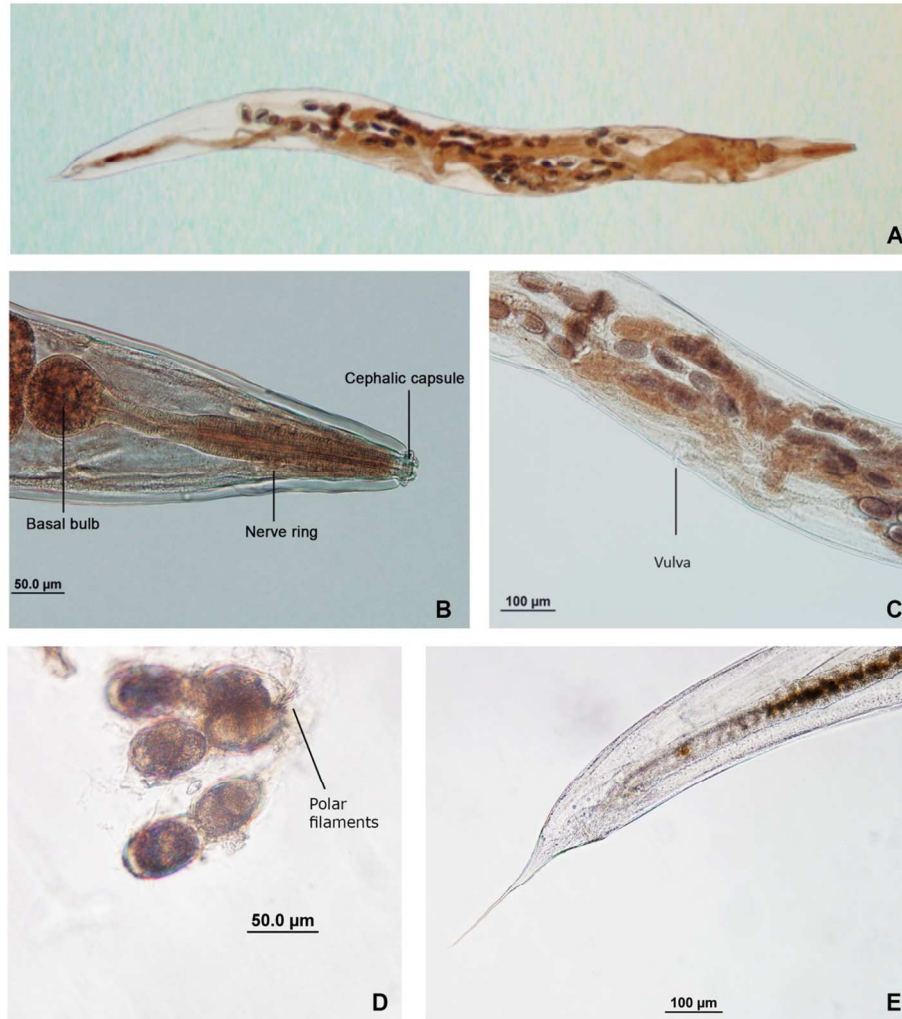


Fig. 3. *Binema klossae*. A. Female, entire. B. Cephalic region (lines showing the cephalic capsule, nerve ring and basal bulb). C. Vulval region (line showing the vulva). D. Eggs with polar filaments. E. Posterior region.

155x176mm (300 x 300 DPI)



Fig. 4. *Cameronia arecoensis*. A. Female, entire. B. Cephalic region. C. Elliptical large eggs. D. Vulval region (line showing the vulva). E. Tail

155x176mm (300 x 300 DPI)

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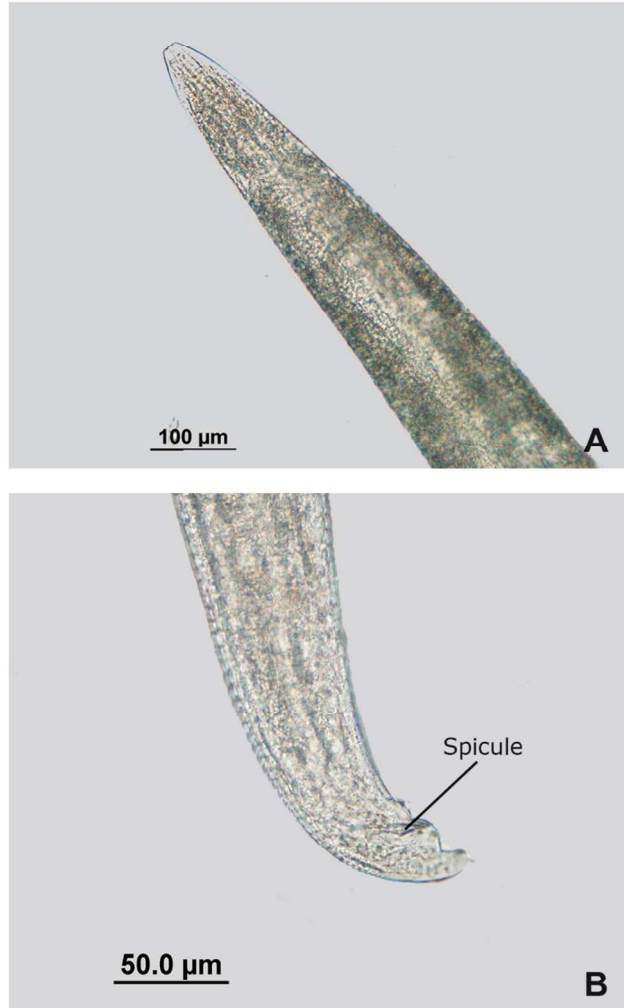


Fig 5. *Cameronia arecoensis*. A. Male, cephalic region. B Posterior region (line showing the spicule).

127x196mm (300 x 300 DPI)

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3 **Fig. 1.** *Binema korsakowi* **A.** Female, entire. **B.** Cephalic region. **C.** Vulval region (lines showing the
4 vulva and the vagina). **D.** Tail. **E.** Capsule containing 2 eggs (lines). **F.** Capsule containing 3 eggs
5 (lines).
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10 **Fig. 2.** *Binema korsakowi*. **A.** Male, cephalic region. **B** Posterior region (line showing the spicule).
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13 **Fig. 3.** *Binema klossae*. **A.** Female, entire. **B.** Cephalic region (lines showing the cephalic capsule,
14 nerve ring and basal bulb). **C.** Vulval region (line showing the vulva). **D.** Eggs with polar filaments.
15 **E.** Posterior region.
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20 **Fig. 4.** *Cameronia arecoensis*. **A.** Female, entire. **B.** Cephalic region. **C.** Elliptical large eggs. **D.** Vulval
21 region (line showing the vulva). **E.** Tail.
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27 **Fig 5.** *Cameronia arecoensis*. **A.** Male, cephalic region. **B** Posterior region (line showing the spicule).
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