

Morphological and molecular characterisation of the entomoparasitic nematode *Hammerschmidtella diesingi* (Nematoda, Oxyurida, Thelastomatidae)

¹María V. Blanco¹, ^{1*}Paola Lax, ²Juan C. Rondan Dueñas, ³Cristina N. Gardenal and ¹Marcelo E. Doucet

¹Laboratorio de Nematología, Centro de Zoología Aplicada, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Casilla de Correo 122, 5000 Córdoba, Argentina; ²Laboratorio de Biología Molecular, Pabellón CEPROCOR, Santa María de Punilla, X5164 Córdoba, Argentina; ³Cátedra de Genética de Poblaciones y Evolución, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Velez Sarsfield 299, 5000, Córdoba, Argentina

Abstract

A population of *Hammerschmidtella diesingi* was detected in specimens of *Periplaneta americana* collected in Córdoba city (Argentina) was studied. Nematodes were characterized based on morphological, morphometric and genetic (D2-D3 expansion segment) analyses. New data are provided, especially on particular morphological characters that were a matter of controversy in previous studies. *H. diesingi* is reported in Argentina for the first time.

Keywords

Argentina, D2-D3 expansion segment, *Hammerschmidtella diesingi*, morphology, morphometry, *Periplaneta americana*

Introduction

Cockroaches occupy a wide variety of habitats, including tropical and temperate forests, deserts, grasslands, and salt marshes (Bell *et al.* 2007). To date, more than four thousand species have been identified worldwide, of which only 1% are related to human environment (Thyssen *et al.* 2004). In Argentina 33 genera and 84 species have been cited (Crespo and Valverde 2005), *Periplaneta americana* (family Blattellidae) being one of the most important domestic pests.

Nematodes belonging to the family Thelastomatidae (order Oxyurida) are parasitic or commensal in saprophytic terrestrial arthropods (Shah 2007). It is a large family, currently represented by about 31 genera. These nematodes live within the hindgut of the host and would feed on the bacterial microfauna found there (Adamson 1994). Several studies conducted to detect nematodes associated with *P. americana* (Rao 1958, Biswas and Chakravarty 1963, Singh and Kaur 1988, Shah 2007) found that some species of *Leidynema*, *Thelastoma*, and *Hammerschmidtella* are common in the hindgut of this insect. The latter nematode genus currently comprises 12 validated species, some

of which parasitize cockroaches as well as diplopods. Each species is associated with a given group of arthropods, such as *H. diesingi* and the order Blattaria (Kloss, 1966).

In Argentina, studies on nematodes associated with *P. americana* are scarce (Camino et Marino, 2002), *T. domesticus* Camino et Quelas, 2008 being the only species reported to date (Camino and Quelas 2008). Recently, *H. diesingi* was detected in the province of Córdoba in *P. americana* (Lax 2008, Lax *et al.* 2009). Although the species was first described about 174 years ago (Hammerschmidt 1838), its populations have received limited investigation. Some previous descriptions have incomplete and inaccurate information and are often poorly illustrated.

Molecular tools provides the prospect for the specific identification of members of the superfamily Thelastomatoidea, as a basis for systematic, ecological and/or population genetic investigations (Jex *et al.* 2006). The expansion segments or divergent (D) domains in the large subunit (LSU) of the ribosomal DNA have been suggested as genetic markers for taxonomic and/or phylogenetic studies of parasites (Zhang *et al.* 2007). The D3 domain has been indicated as useful in deter-

*Corresponding address: plax@efn.uncor.edu

mining genetic relationships of thelastomatoids and its use was proposed in systematic and ecological studies in this nematode group (Jex *et al.* 2006). Currently, a single sequence of D2-D3 expansion segment is known for *H. diesingi* (Spiridonov and Guzeeva 2009). The aim of the present work was to provide a morphological, morphometric and molecular characterization of the nematode population detected in Argentina and to contribute to the knowledge of the species.

Materials and methods

Insect collection

Live specimens of *P. americana* were collected in the Centro de Zoología Aplicada, located in the city of Córdoba (Capital department, province of Córdoba, Argentina) using baited traps placed in drains. Cockroaches were dissected and the hindgut was excised and placed in a Petri dish containing Ringer solution. The hindgut was then cut longitudinally to extract nematodes.

Morphological and morphometric analyses

Hammerschmidtella diesingi females and males were fixed in 70% alcohol at 60°C. After 7–10 days, the alcohol was replaced with a solution composed of 97.5 parts of 70% alcohol and 2.5 parts of pure anhydrous glycerine. After 4 days, the solution was replaced with another solution consisting of 95 parts of 70% alcohol and 5 parts of pure anhydrous glycerine. At 72 h, the specimens were transferred to a Petri dish containing the latter solution; the dish was left half covered at room temperature (15–25°C) to allow the alcohol to evaporate, leaving nematodes in glycerine. Specimens were individually mounted between slides. Observations were made using an optical microscope equipped with an ocular micrometer and a drawing tube.

The morphological and morphometric characters recorded were those usually considered of taxonomic importance for the species (Chitwood 1932, Lee 1958, Rao 1958, Leiber-sperger 1960, Kloss 1966, Singh and Agarwal 1998, Shah 2007). The specimens were photographed *in vivo* using a digital camera mounted on the microscope. To ensure that the information obtained was representative of the population, 30 specimens of each sex were considered. All measurements are in micrometres unless otherwise stated.

Molecular analysis

For molecular studies, two species besides *H. diesingi* were processed: *Thelastoma* sp. collected from *P. americana* (Córdoba city, Córdoba, Argentina) and *Leidynema appendiculata* obtained from *Blaptica* sp. (unknown locality, province of Buenos Aires, Argentina). Three individuals of each nematode species were transferred to a sterile 0.5 ml tube containing

5 µl of distilled water. DNA was extracted following the protocols of Lax *et al.* (2007). The D2-D3 domain was amplified using the primers D2A (5'-ACA AGT ACC GTG AGG GAA AGT TG-3'), and D3B (5'-TCG GAA GGA ACC AGC TAC TA-3') (Spiridonov and Guzeeva 2009).

PCR reaction was performed in a total volume of 25 µl containing: 2 µl of DNA, 2.5 µl 10x reaction buffer (500 mM KCl, 15 mM MgCl₂, 100 mM Tris-HCl, pH 9.0), 2.5 mM MgCl₂, 0.2 mM of each dNTP, 15 ng of each primer, and 0.5 U of Taq DNA Polymerase (Amersham Biosciences). The PCR conditions for amplification were: an initial denaturation at 94°C for 5 min, followed by 40 cycles of 30 sec at 94°C, 60 sec at 50°C, 80 sec at 72°C, and a final extension of 10 min at 72°C. Reactions were performed in a thermal cycler (Mastercycler Eppendorf). Amplified PCR products were separated in 1.5% agarose gel stained with ethidium bromide with 100 bp DNA ladder (Promega, USA) as size markers. Gels were photographed with a Kodak-DC digital camera using a UV transilluminator. PCR products were purified and sequenced in both directions.

All the sequences obtained were aligned using default settings in ClustalX 2.0 (Larkin *et al.* 2007) with sequences deposited in GenBank for *H. diesingi* (EU365628), *H. cristata* (EU365629), as well as for other related taxa belonging to the family Thelastomatidae, which inhabit the hindgut of several cockroach species: *Cranifera cranifera* (EU365632), *L. appendiculata* (EU365630), *L. portentosa* (GQ401114), *Severianoia* sp. (EU365631), and *Thelastoma* sp. (GQ368468). Sequences were submitted to the NCBI database.

The sequence data were subjected to phylogenetic analysis using maximum parsimony (MP) and neighbour joining (NJ) algorithms in MEGA v. 4 software (Tamura *et al.* 2007). The data were also subjected to analysis using Bayesian inference (BI). Sequences of *Singhiella* sp. (GQ368465) and *Ascaris lumbricoides* (AY210806) were included as an outgroup. MP analysis was performed using heuristic search settings with 100 replicates of random taxon addition. The Kimura 2-parameter model of base substitution (Kimura 1980) was used to obtain distance measures which were then displayed in a NJ tree. Bootstrap analysis with 1000 replicates was conducted to assess the degree of support for each branch on the MP and NJ trees. Bayesian inference analysis was performed using MRBAYES 3.1.2 (Ronquist and Huelsenbeck 2003). To determine the most appropriate model of nucleotide substitution for the data set, sequences were analysed with jMODEL-TEST 0.1.1 (Posada 2008). The GTR + G model was selected using the Akaike criterion. Two independent runs were performed simultaneously on the data set, each using one cold and three heated chains. After discarding burn-in samples and evaluating convergence, the remaining samples were retained for further analysis. After one million generations, the average standard deviation of split frequencies between the two independent runs was 0.0038. A 50% majority rule consensus tree was generated; it was displayed using the TreeView (Win32) program (Page 1996).

Results

Description (Tables I, II; Figs 1, 2)

Female. Body cylindrical, fusiform, greatest diameter at vulva. Cuticle annulated; the first six body annules wide and extending up to beginning of oesophageal pseudobulb. The

remaining body annules narrow posteriorly. Lip annule with triangular stoma opening. Buccal cavity as long as wide. Oesophageal corpus clearly differentiated into two parts, an anterior cylindrical part and a posterior part represented by a large pseudobulb. Nerve ring located at the cylindrical portion of corpus, near beginning of pseudobulb. Isthmus cylindrical, short, followed by a voluminous spheroid basal bulb

Table I. Morphometric characters of females and males of *Hammerschmidtella diesingi* detected in *Periplaneta americana* in Córdoba city of Argentina

Character	Females (n = 30)	Males (n = 30)
Body length	2666.7 (1717.6–3435.3)	701.4 (481.1–1018.9)
a (Body length/maximum body width)	11.2 (7.5–14.4)	12.4 (8.9–16.3)
b (Body length/oesophagus length)	11.4 (9.5–16.0)	5.6 (4.6–7.9)
c (Body length/tail length)	3.1 (2.6–4.0)	7.0 (4.8–9.6)
c' (Tail length/ width at anus)	11.5 (6.6–14.7)	4.4 (3.4–5.5)
V	23.2 (20.0–32.6)	–
Maximum body width	240.5 (166.2–312.5)	57.4 (39.0–88.0)
Buccal cavity width	10.9 (8.0–12.0)	4.8 (4.0–8.0)
Buccal cavity length	9.6 (7.0–12.0)	3.8 (3.0–6.0)
Labial ring width	22.0 (19.0–25.0)	–
First body annule length	14.0 (13.0–17.0)	–
Second body annule length	18.0 (15.0–20.0)	–
Third body annule length	19.0 (16.0–22.5)	–
Forth body annule length	21.0 (18.0–27.0)	–
Fifth body annule length	23.0 (19.0–28.0)	–
Six body annule length	24.0 (19.0–29.0)	–
Oesophagus length	297.8 (260.0–342.5)	124.7 (102.0–152.0)
Pseudobulb-anterior end	185.7 (162.5–218.7)	64.1 (52.0–79.0)
Corpus length	105.0 (87.5–127.5)	46.2 (38.0–58.0)
Corpus width	24.8 (20.0–30.0)	9.6 (8.0–11.0)
Pseudobulb length	89.4 (71.3–120.0)	18.2 (14.0–23.0)
Pseudobulb width	58.7 (45.0–71.2)	13.3 (11.0–16.0)
Isthmus length	38.5 (30.0–50.0)	33.9 (22.0–43.0)
Isthmus width	22.9 (17.5–28.7)	9.8 (7.5–13.0)
Basal bulb length	73.1 (57.5–83.7)	27.9 (22.0–54.0)
Basal bulb width	76.4 (62.5–90.0)	21.0 (13.0–26.0)
Nerve ring-anterior end	97.1 (81.0–115.0)	83.8 (66.0–103.0)
Excretory pore-anterior end	355.4 (287.5–445.0)	170.5 (122.5–219.0)
Vulva-anterior end	615.6 (438.6–836.4)	–
Spicule length	–	30.3 (25.0–35.0)
Gubernaculum length*	–	12 (12–14)
Width at anus	75.8 (60.0–95.0)	23.1 (20.0–30.0)
Tail length	871.6 (479.4–1132.2)	101.3 (78.0–115.0)

*n = 12. Measurements in μm .

Table II. Morphometric characters of eggs and second-stage larvae of *Hammerschmidtella diesingi* detected in *Periplaneta americana* in Córdoba city of Argentina

Character	Eggs (n = 30)	Second-stage larvae (n = 10)
Total length	76.9 (61.0–85.0)	52 (50–56)
Maximum width	31.1 (25.0–35.0)	20.5 (19–22)

Measurements in μm .

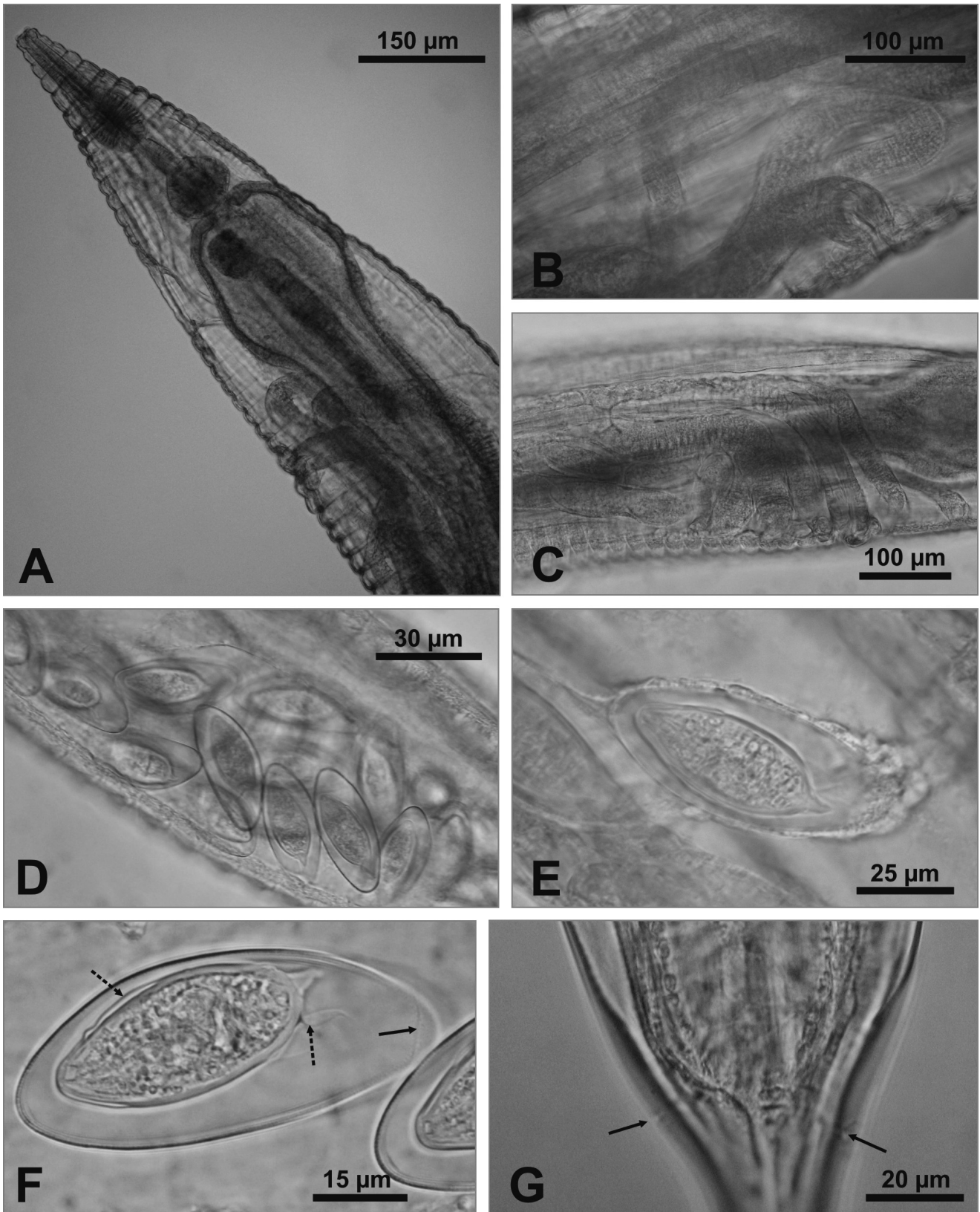


Fig. 1. *Hammerschmidtella diesingi* from Córdoba city, Argentina. Females. **A.** Anterior region, showing oesophagous, intestine and vulvar region. **B.** Detail of vulvar region, with vagina posteriorly directed. **C.** Detail of vulvar region, with vagina directed to midbody. **D.** Female with eggs containing second-stage larvae. **E.** Detail of an egg inside the uterus. **F.** Egg with operculum (arrow) and second-stage larvae with con molt cuticle (arrow with dashed line). **G.** Location of phasmids (arrows)

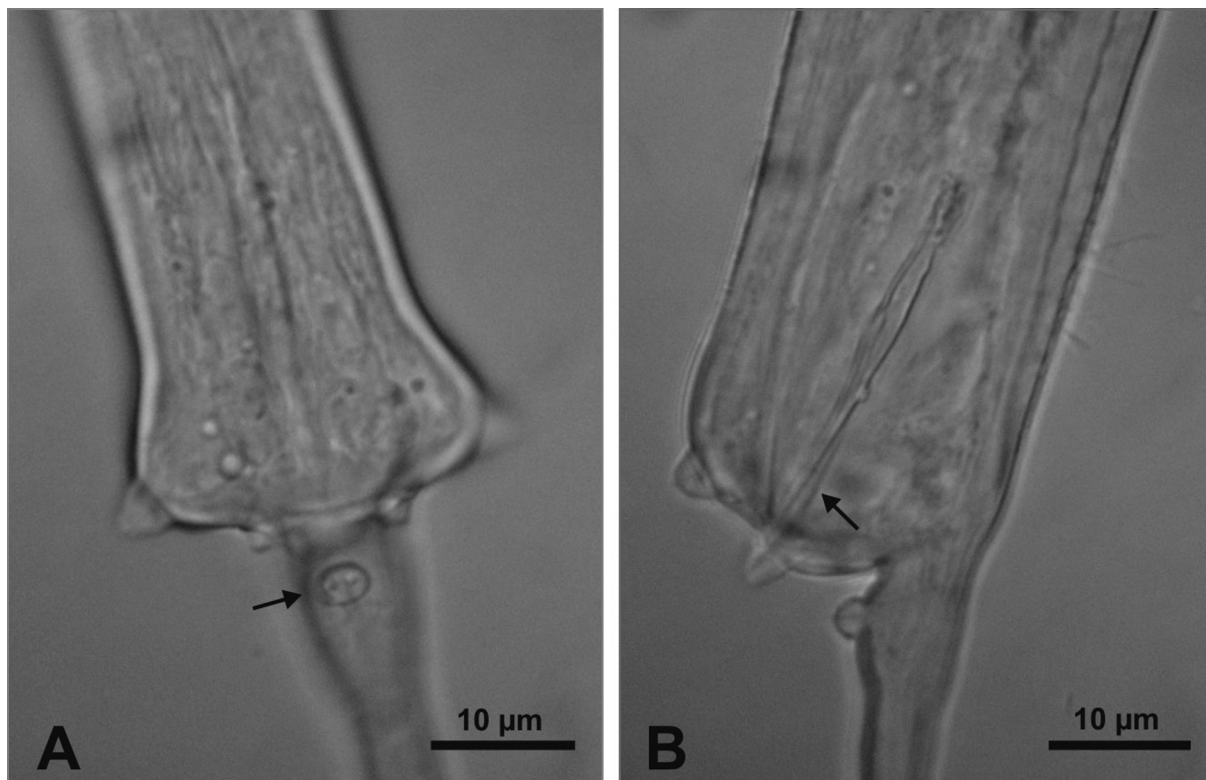


Fig. 2. *Hammerschmidtella diesingi* from Córdoba city, Argentina. Males. **A.** Double papillae at base of caudal filament. **B.** Spicule and gubernaculum (arrow)

with a prominent valve apparatus. Presence of narrow lateral alae originating at level of isthmus and extending near beginning of caudal filament. Diameter of anterior portion of intestine slightly smaller than body width. Excretory pore situated slightly behind of oesophagus-intestine junction, at approximately 13.5% (11–17) of body length. Reproductive system didelphic. Vulva located about 23% of body length; vulvar opening small (about 35 µm in length) and lips slightly protuberant. Vagina connected to common uterus. In some individuals, vagina may flex immediately to posterior or be directed to midbody. Common uterus directed posteriorly, flexed between 198–330 µm before the anus and then continues to anterior. Then it divides into two uteri; taking into account these uteri, the reproductive system is prodelphic. Each uterus is connected to an oviduct that usually contains sperm. Sperm with oval body (3 µm long and 2 µm wide) and a filament of about 7 µm in length. The long ovaries reflexed many times, extend anterior to the vulva and end anterior or posterior to it. Uterus containing numerous eggs. Intestine simple and tubular. Phasmids located laterally, behind the anus, not opposite each other. Tail filiform, forming 25–38% of body length.

Eggs. Ellipsoidal; egg shell with numerous pores irregularly distributed on the surface; presence of an operculum on one end. Operculum separated from the remainder of the shell by a groove. Eggs in the uterus usually in one cell stage, generally arranged in a single or double row. Three females had

eggs in the uterus containing second-stage larvae; body of this stage oval, with conical posterior end. Larva oriented with its tail to the operculated part of the egg. In the anterior region, the buccal capsule is differentiated; oesophagus slightly curved with a basal bulb. The first-stage cuticle loose, surrounding the larva; the loose cuticle is observed at the posterior end.

Male. Body fusiform, truncate in the posterior region at anus level, terminating in a caudal filament. Cuticle annulated; very narrow annules at anterior region, widening at isthmus level; annule size is maintained along the remainder body. Labial annule small; stoma opening triangular. Buccal cavity small, wider than long. Oesophageal corpus cylindrical, terminating in a slight widening. Isthmus short, followed by a conspicuous, slightly oval basal bulb; isthmus has a refringent valvular apparatus. Presence of a pair of lateral alae extending from isthmus level to proximity of anus. Nerve ring located at mid isthmus. Excretory pore located at about 24% (20–28%) of body length, behind the oesophago-intestinal junction. Anterior portion of intestine occupying almost the entire body width. One testis of large size, extending from the cloacal opening to mid body. Presence of three pairs of caudal papillae (one subventral, one adanal and one subventral postanal) and a conspicuous double papilla at base of caudal filament. Spicule straight; gubernaculum slightly curved, difficult to distinguish in cleared specimens. Tail with a caudal filament. No phasmids were observed.

Molecular analysis

A 736-bp fragment of the D2-D3 domain was amplified from the specimens of *H. diesingi*. Nucleotide frequencies (percent) were T = 25.5, C = 20.5, A = 24.0, and G = 29.9. Phylogenetic trees of LSU sequences showed similar grouping using the

different methods employed (Fig. 3); *H. diesingi* from Argentina grouped with the only sequence known, to date, for a population of the same species (99% similarity) from Russia, collected in the Oriental cockroach *Blatta orientalis*. Our sequence of *Thelastoma* sp. clustered with a sequence of an individual of the same genus (94% similarity), also extracted

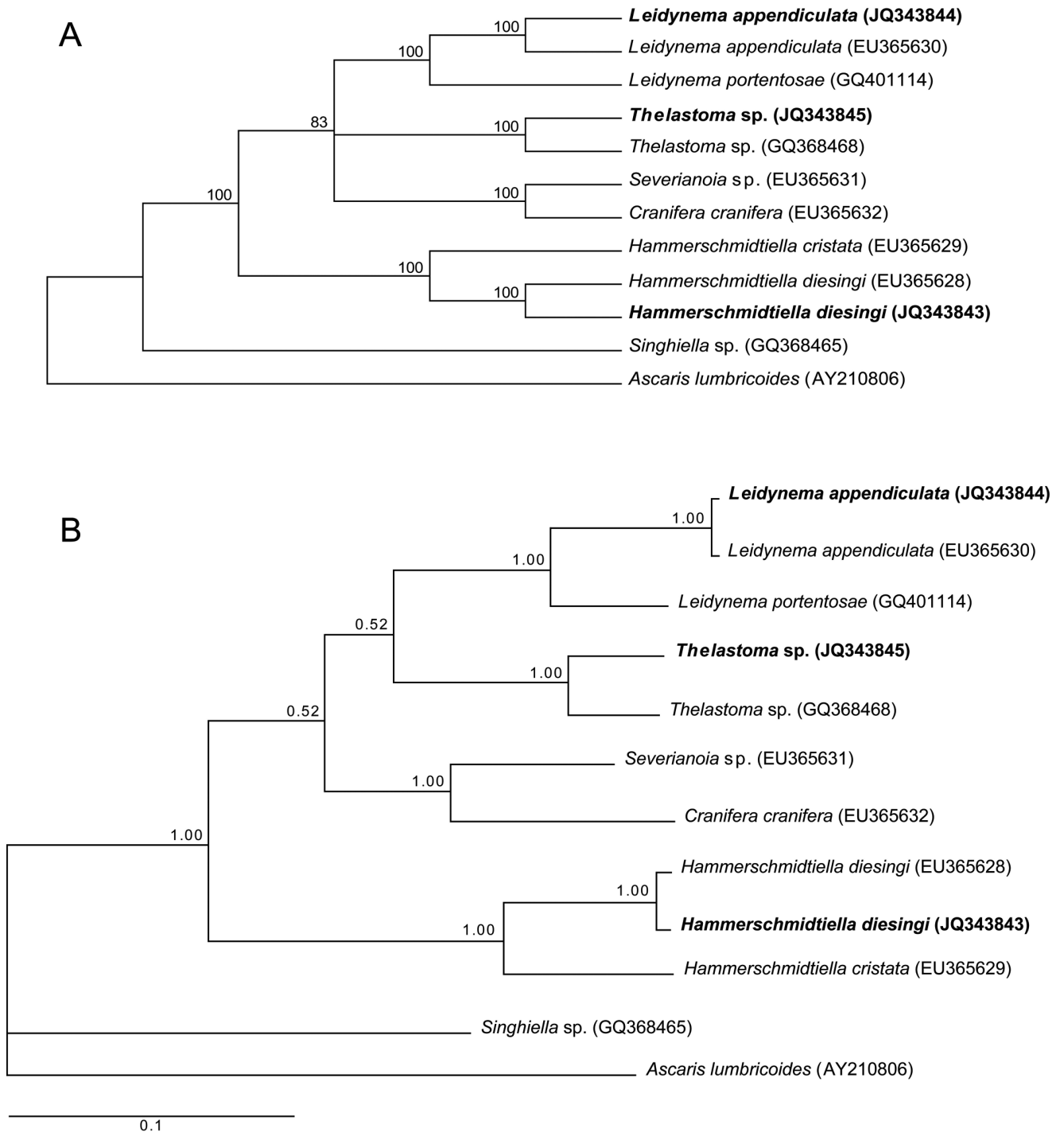


Fig. 3. Phylogenetic relationships between the *Hammerschmidtella diesingi* population from Argentina and other oxyurids. **A.** Maximum parsimony tree (1000 bootstrap replicates); bootstrap values are indicated in the nodes. **B.** Bayesian consensus tree obtained after one million generations under the GTR + G model; posterior probabilities are given in nodes. Newly sequenced samples are indicated in bold

from the gut of *P. americana* in Russia, whereas *L. appendiculata* was related to a sequence of the same nematode (99% similarity), obtained from *P. americana* according to Spiridonov and Guzeeva (2009). This nematode species was associated with *L. portentosae*, a parasite of *Gromphadorhina portentosae*.

Discussion

The close relationship between *H. diesingi* and *P. americana* suggests that the nematode would have been spread worldwide via the dispersal of its host (Adamson and van Waerebeke 1992). To date, *H. diesingi* has been found parasitizing the same insect in several countries, such as Germany (Leibersperger 1960), Brazil (Kloss 1966), India (Rao 1958, Jain and Kaur 2001, Shah 2007), Malaysia (Khairal and Paran 1977), Poland (Gabryelow and Lonc 1986), and USA (Dobrovolsky 1933). The present work reports the first record of the nematode in Argentina.

Some of the morphological characters of the population studied are consistent with records for the species (Chitwood 1932, Leibersperger 1960, Kloss 1966, Gupta and Kaur 1978, Yu and Crites 1986, Gupta 1997, Shah 2007); however, there are some differences from previous descriptions. In nematodes from the locality of Manipur, India, the presence of a terminal cap-like structure in the caudal filament of females was mentioned and illustrated (Shah 2007). In the present work, as in previous descriptions (Lee 1958, Leibersperger 1960, Kloss 1966, Gupta 1997), that characteristic was not observed.

According to Maggenti (1981), the position and direction of the uteri – and not of the ovary – are important for the nomenclature of the female reproductive system; these terms were usually mistakenly used as synonymous. This may explain the contradictions observed not only within *Hammer-schmidtella*, but also among descriptions of *H. diesingi* populations. When Chitwood (1932) created the genus, he indicated that the female reproductive system was amphidelphic. Later, Adamson and van Waerebeke (1992) provided the diagnosis of the genus, indicating that it was prodelphic. In populations of *H. diesingi*, the female reproductive system was described as prodelphic (Kloss 1966, Shah 2007), opisthodelphic (Wharton 1979) and amphidelphic (Leibersperger 1960), whereas in other populations, it was not described (Lee 1958, Gupta 1997). Kloss (1966) also indicated the presence of a single uterus for two ovaries; however, according to observations in the present work, the common uterus is divided into two uteri, each one connected with an oviduct and its corresponding ovary.

Another morphological character that has led to contradictory interpretations is the papilla present at the base of caudal filament in males. In the specimens analyzed in the present work, a double papilla was observed, in agreement with previous records for other populations of the species (Leibersperger 1960, Shah 2007). That characteristic, however, was de-

scribed as a simple papilla in other studies based on optical microscopy (Lee 1958, Rao 1958) and SEM (Yu and Crites 1986). In the latter work, the papilla was mentioned by the authors but not illustrated in apical view (only in dorsolateral view).

The influence of the environment on the development of thelastomatid eggs would be a rewarding area for study (Hominick 1972). Lee (1958) stated that eggs of *H. diesingi* are deposited in two- or four-cell stage, whereas Todd (1944) mentioned that at the time of extrusion, eggs are usually in the one-cell stage. This is in disagreement with some results of the present work, because females with the uterus containing eggs at the infective stage were observed; this aspect has never been indicated previously for this species. In *H. diesingi*, the first molt occurs outside the host and results in a “resting (infective) stage”; the second molt occurs after the ingestion of the egg by the cockroach and is followed by a second active stage and the egg hatches in the digestive tract of the host (Todd 1944). Length and width measurements as well as morphological aspects provided by Adamson and Clease (1989) for the “resting stage” (second-stage larva) are consistent with our results. Hominick (1972) also found females of *L. appendiculata* containing eggs at the infective stage. A similar situation was observed in dead females of this nematode, which were recovered when treating specimens of *P. americana* with an anthelmintic (Pawlik 1966). It should be noted that in the present work, during dissection of cockroaches, live and active nematodes were extracted and immediately fixed. The ecological role of the infective stage in thelastomatoids is to ensure the parasite persistence over time (Adamson and Clease 1989). For this reason, it would be important to evaluate the development of eggs retained in the uterus of *H. diesingi* females when the nematode (and even its host) is exposed to different environmental factors.

The gubernaculum is another character whose description was controversial in the different characterizations of *H. diesingi*. When describing the characteristics of this species, Kloss (1966) indicated the absence of this structure, whereas other authors did not mention it (Lee 1958, Rao 1958, Gupta 1997, Shah 2007). In the description of *H. andersoni*, Adamson and Nasher (1987) remarked that this was the only species with a gubernaculum within the genus. However, Leibersperger (1960) mentioned and even illustrated the gubernaculum in *H. diesingi*, which is consistent with what was observed in the present work. In addition, its length falls within the range provided by that author. These contradictions about such important character are possibly due to the difficulty in observing it in cleared specimens.

Phasmids have not been mentioned in some descriptions (Lee 1958, Kloss 1966, Gupta 1997, Shah 2007). In the present work, they were observed in females but were not visible in males, which is consistent with previous SEM observations (Yu and Crites 1986). According to the latter authors, phasmids in males may be internal without external openings.

Most of the morphometric characters were within the ranges known for the species (Lee 1958, Rao 1958, Leibers-

sperger 1960, Kloss 1966, Sharma and Gupta 1983, Shah 2007). Females identified as *H. diesingi* (Carreno and Tuhela 2011) were recently found in peppered cockroach, *Archimandrita tessellata* Rehn, 1903; however, some measurements, such as buccal cavity length, isthmus length and vulva-anterior end were much higher than the values known for the species. The range obtained for location of nerve ring in males agrees with previous descriptions (Chitwood 1932, Lee 1958, Leibersperger 1960, Kloss 1966, Shah 2007). However, illustrations provided in some of the mentioned works are confusing. Leibersperger (1960) and Chitwood (1932) did not include the nerve ring in the male drawings, whereas in the illustrations of Kloss (1966), Shah (2007) and Gupta (1997), the nerve ring was situated anteriorly to the pseudobulb. According to Lee (1958), it is located in the middle of the oesophageal isthmus, as it was observed in the present work. These considerations show that some descriptions of *H. diesingi* are neither complete nor fully accurate. Therefore, other populations of the nematode should be studied to evaluate the controversial characters mentioned above, some of which are of taxonomic importance for the species.

Jex *et al.* (2006) stated that the D3 domain provides sufficient information to resolve thelastomatoids at the genus level. *Hammerschmidtella diesingi* and *H. cristata*, the only two species of the genus for which there is information available on the D2-D3 segment, were clearly discriminated based on the comparison of the sequences obtained in the present work and in others already documented for the family Thelastomatidae. A similar situation was also observed between the two species of *Leidyndema*. Hence, further molecular studies conducted with other species of the genus *Hammerschmidtella* would be important to evaluate the validity of some of them, mainly of those whose descriptions are incomplete and which have also been detected in *P. americana* in several locations worldwide, such as: *H. bisiri*, *H. aspiculus*, *H. neyrai* and *H. poinari*. Because of the differences observed in the described populations of *H. diesingi*, the morphological characterization should be complemented with molecular studies using the D2-D3 domain data and/or data for other marker regions.

Acknowledgements. This work was financially supported by the Secretaría de Ciencia y Técnica (Universidad Nacional de Córdoba) and the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

References

Adamson M. 1994. Evolutionary patterns in life histories of Oxyurida. *International Journal of Parasitology*, 24, 1167–1177. DOI: 10.1016/0020-7519(94)90189-9.

Adamson M.L., Cleese D.F. 1989. Morphological changes during in ovo development in the Thelastomatoidea: description and functional considerations. *Journal of Parasitology*, 75, 728–734. DOI: 10.2307/3283057.

Adamson M.L., Nasher A.K. 1987. *Hammerschmidtella andersoni* sp. n. (Thelastomatidae: Oxyurida) from the diplopod, *Archispirostreptus tumuliporus*, in Saudi Arabia with comments on the karyotype of *Hammerschmidtella diesingi*. *Proceedings of the Helminthological Society of Washington*, 54, 220–224.

Adamson M.L., van Waerebeke D. 1992. Revision of the Thelastomatoidea, Oxyurida of invertebrate hosts I. Thelastomatidae. *Systematic Parasitology*, 21, 21–63.

Bell W.J., Roth L.M., Nalepa C.A. 2007. Cockroaches: ecology, behavior, and natural history. The Johns Hopkins University Press, Baltimore, Maryland, 248 pp.

Biswas P.K., Chakravarty G.K. 1963. The systematic studies of the zoo-parasitic oxyuroid nematodes. *Zeitschrift für Parasitenkunde*, 23, 411–428. DOI: 10.1007/BF00259929.

Camino N.B., Marino H.A. 2002. Blatarios domiciliarios parasitados por nematodos. Libro de resúmenes, V Congreso Argentino de Entomología. Buenos Aires. Marzo de 2002, 429 (in Spanish).

Camino N.B., Quelas M.A. 2008. Descripción de *Thelastoma domesticus* sp. nov. (Oxyurida, Thelastomatidae) parásita de ninfas *Periplaneta americana* (Blattodea, Blattidae) en Argentina. *Iheringia, Série Zoologia*, 98, 24–27. DOI:10.1590/S0073-47212008000100003.

Carreno R.A., Tuhela L. 2011. Thelastomatid nematodes (Oxyurida: Thelastomatoidea) from the peppered cockroach, *Archimandrita tessellata* (Insecta: Blattaria) in Costa Rica. *Comparative Parasitology*, 78, 39–55. DOI: 10.1654/4455.1.

Chitwood B.G. 1932. A synopsis of the nematodes parasitic in insects of the family Blattidae. *Zeitschrift für Parasitenkunde*, 5, 14–50. DOI: 10.1007/BF02120633.

Crespo F.A., Valverde A. del C. 2005. Blattaria-Cucarachas. In: (Ed. O.D. Salomón) *Artrópodos de interés médico en la Argentina*. 1ª Edición. Fundación Mundo Sano. Buenos Aires, pp. 107–113 (in Spanish).

Dobrovolsky G. 1933. Studies on the nematode parasites of the American cockroach, *Periplaneta americana*. *Transactions of the Kansas Academy of Science*, 36, 213. DOI: 10.2307/3625358.

Gabryelow K., Lonc E. 1986. Parasites of *Periplaneta americana* from laboratory rearing. *Wiadomości Parazytologiczne*, 32, 75–78.

Gupta N.K., Kaur J. 1978. On some nematodes from invertebrates in Northern India. Part I. *Revista Ibérica de Parasitología*, 38, 301–324.

Gupta R. 1997. Description of the two species of nematodes of cockroach (*Periplaneta americana*) reported from Nepal. *Tribhuvan University Journal*, 20, 25–30.

Hammerschmidt K.E. 1838. Helminthologische Beiträge. *Isis von Oken*, 5, 351–358.

Hominick W.M. 1972. Relationships among *Leidyndema appendiculata*, *Hammerschmidtella diesingi* (Nematoda: Thelastomatidae) and the American cockroach: the influence of host physiology on numbers of parasites. PhD Thesis, Institute of Parasitology, McGill University, Montreal, Canada.

Jain M., Kaur L. 2001. Qualitative and quantitative analysis of nematode parasites of cockroaches from Chandigarh. *Journal of Parasitology and Applied Animal Biology*, 10, 55–58.

Jex A.R., Hu M., Rose H.A., Schneider M., Cribb T.H., Gasser R.B. 2006. Molecular characterization of Thelastomatoidea (Nematoda: Oxyurida) from cockroaches in Australia. *Parasitology*, 133, 123–129. DOI: 10.1017/S0031182006009978.

Khairal A.A., Paran T.P. 1977. Parasites of *Periplaneta americana* Linn., in Penang, Malaysia. I. *Thelastoma malaysiense*, new species, with notes on *Hammerschmidtella diesingi* and *Leidyndema appendiculata*. *Malayan Nature Journal*, 30, 69–77.

Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nu-

- cleotide sequences. *Journal of Molecular Evolution*, 16, 111–120. DOI: 10.1007/BF01731581.
- Kloss G.R. 1966. Revisão dos nematóides de Blattaria do Brasil. *Papeis Avulsos do Departamento de Zoologia*, 18, 147–188.
- Larkin M.A., Blackshields G., Brown N.P., Chenna R., McGettigan P.A., McWilliam H., Valentin F., Wallace I.M., Wilm A., Lopez R., Thompson J.D., Gibson T.J., Higgins D.G. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics*, 23, 2947–2948. DOI: 10.1093/bioinformatics/btm404.
- Lax P., Rondan Dueñas J.C., Gardenal C.N., Doucet M.E. 2007. Assessment of genetic variability in *Nacobbus aberrans* (Thorne, 1935) Thorne & Allen, 1944 (Nematoda: Pratylenchidae) populations from Argentina. *Nematology*, 9, 261–270. DOI: 10.1163/156854107780739063.
- Lax P. 2008. Nematode parasites of the urban cockroach *Periplaneta americana* from Córdoba city, Argentina. *Fifth International Congress of Nematology, Brisbane, Australia, 13–18 July 2008*, 12 (Abstract).
- Lax P., Blanco M.V., Doucet M.E. 2009. Detección de un nematodo del género *Hammerschmidtella* en *Periplaneta americana* en la ciudad de Córdoba. *V Congreso Argentino de Parasitología, 25–28 March 2009*, 100 (Abstract, in Spanish).
- Lee D.L. 1958. On the morphology of the male, female and fourth-stage larva (female) of *Hammerschmidtella diesingi* (Hammerschmidt), a nematode parasitic in cockroaches. *Parasitology*, 48, 433–436. DOI: 10.1017/S0031182000021375.
- Leibersperger E. 1960. Die Oxyuroidea der euroäischen Arthropoden. *Parasitologische Schriftenreihe*, 2, 1–150.
- Maggenti A. 1981. *General Nematology*. Springer-Verlag, New York, Inc., 372 pp.
- Page R.D.M. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences*, 12, 357–358.
- Posada D. 2008. jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution*, 25, 1253–1256. DOI: 10.1093/molbev/msn083.
- Pawlik J. 1966. Control of the nematode *Leidynema appendiculata* (Leidy) (Nematoda: Rhabditida: Thelastomatidae) in laboratory cultures of the American cockroach. *Journal of Economic Entomology*, 59, 468–469.
- Rao P.N. 1958. Studies on the nematode parasites of insects and other arthropods. *Arquivos do Museu Nacional*, 46, 33–83.
- Ronquist F., Huelsenbeck J.P. 2003. MrBayes version 3.0: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.
- Shah M.M. 2007. Some studies on insect parasitic nematodes (Oxyurida, Thelastomatoidea, Thelastomatidae) from Manipur, North-East India. *Acta Parasitologica*, 52, 346–362. DOI: 10.2478/s11686-007-0051-y.
- Sharma R.K., Gupta L.N. 1983. A new entomogenous nematode *Hammerschmidtella bareillyi* from *Periplaneta americana*. *Revista Ibérica de Parasitología*, 43, 319–323.
- Singh H.S., Agarwal A. 1998. Further observation on *Hammerschmidtella diesingi* Chitwood, 1932, with a note on early embryonic development and validity of various species of the genus. *Uttar Pradesh Journal of Zoology*, 18, 27–36.
- Singh H.S., Kaur H. 1988. On a new nematode, *Hammerschmidtella bisiri* n. sp. from *Periplaneta americana* Linn. *Indian Journal of Parasitology*, 12, 187–189.
- Spiridonov S.E., Guzeeva E.A. 2009. Phylogeny of nematodes of the superfamily Thelastomatoidea (Oxyurida) inferred from LSU rDNA sequence. *Russian Journal of Nematology*, 17, 127–134.
- Tamura K., Dudley J., Nei M., Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, 24, 1596–1599. DOI: 10.1093/molbev/msm092.
- Thyssen P.J., de Carvalho Moretti T., Tiduko Ueta M., Ribeiro O.B. 2004. O papel de insetos (Blattodea, Diptera e Hymenoptera) como possíveis vetores mecânicos de helmintos em ambiente domiciliar e peridomiciliar. *Cadernos de Saúde Pública, Rio de Janeiro*, 20, 1096–1102. DOI: 10.1590/S0102-311X2004000400025.
- Todd A.C. 1944. On the development and hatching of the eggs of *Hammerschmidtella diesingi* and *Leidynema appendiculatum*, nematodes of roaches. *Transactions of the American Microscopical Society*, 63, 54–67. DOI: 10.2307/3223338.
- Wharton D.A. 1979. The structure and formation of the egg-shell of *Hammerschmidtella diesingi* Hammerschmidt (Nematoda: Oxyuroidea). *Parasitology*, 79, 1–12. DOI: 10.1017/S0031182000051933.
- Yu X., Crites J.L. 1986. Scanning electron microscope studies on *Hammerschmidtella diesingi* (Nematoda: Oxyuroidea). *Proceedings of the Helminthological Society of Washington*, 53, 117–120.
- Zhang L., Hu M., Chilton N.B., Huby-Chilton F., Beveridge I., Passer R.B. 2007. Nucleotide alterations in the D3 domain of the large subunit of ribosomal DNA among 21 species of equine strongyle. *Molecular and Cellular Probes*, 21, 111–115. DOI: 10.1016/j.mcp.2006.08.008.