Morphological and molecular characterization of Labronema montanum sp. n. (Dorylaimida, Dorylaimidae) from Spain

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Abstract
A new species of the genus Labronema, collected in a natural mountain habitat of the southern Iberian Peninsula, in Spain, is studied, including its morphological and morphometric characterization, SEM observations, and D2-D3 28S-rRNA sequences. Labronema montanum sp. n. is distinguishable by its 1.56 to 2.08 mm long body, with lip region being offset by constriction and 19 to 24 µm broad, odontostyle 21 to 29 µm long, neck 417 to 551 µm long, pharyngeal expansion 205 to 272 µm long, the presence of three cardiac lobes at the pharyngo-intestinal junction, a long and tripartite uterus, longitudinal vulva (V = 57-60), a short and rounded caudal region (19–35 µm, c = 56-86, c = 0.5-0.8), spicules 65 to 76 µm long, and 20 to 25 nearly contiguous ventromedian supplements with hiatus.

Key words
Description, Dorylaims, Labronema, LSU, Morphology, New species, SEM, Taxonomy.

Material and methods
Nematode extraction and processing
Nematodes were collected from a grassy area with stony soil at 1,800 elevation in Pandera mountain, Jaén province, southern Iberian Peninsula, Spain. They were extracted using Flegg’s (1967) sieving method and a modified Baermann’s (1917) funnel technique; they were killed with heat, fixed into 4% formalin, transferred to pure glycerine following Siddiqi’s (1964) method, and mounted on permanent glass slides.

Light microscopy (LM)
Observations, measurements, line illustrations, and LM photomicrographs were made using a Nikon Eclipse 80i (Nikon, Tokio, Japan) microscope with differential interference contrast (DIC) optics, a drawing
tube \textit{(camera lucida)} and a Nikon Digital Sight DS-U1 camera. Photographs were edited using Adobe® Photoshop® CS software.

**Scanning electron microscopy (SEM)**

Specimens preserved in glycerine were selected for observation under SEM according to Abolafia (2015). They were hydrated in distilled water, dehydrated in a graded ethanol–acetone series, critical point dried, coated with gold, and observed with a Zeiss Merlin microscope (5 kV) (Zeiss®, Oberkochen, Germany).

**DNA extraction, PCR, and sequencing**

Nematode DNA was extracted from single fresh individuals using the proteinase K protocol and PCR assays, as described in the study of Castillo et al. (2003). The specimen was cut in small pieces using a sterilized needle on a clean slide, with 18 ml of AE buffer (10 mM Tris-Cl + 0.5 mM EDTA; pH 9.0), it was transferred to a microtube and 2 μl proteinase K (700 μg/ml−1) (Roche®, Basel, Switzerland) was added, and it was stored at −80°C for 15 min (for several days). The microtubes were incubated at 65°C for 1 hr, followed by 95°C for 15 min. The microtube was centrifuged at 13,000 r.p.m. or 15,900× g for 3 min, and 2 μl of the supernatant extracted DNA was transferred to a microtube containing 2.5 μl 10× PCR reaction buffer 5 μl Q-solution 5×, 0.5 μl dNTPs mixture (10 mM each), 1 μl of each primer (10 mM), 0.2 μl Taq DNA Polymerase (Qiagen®, Venlo, the Netherlands), and ddH2O with a final volume of 25 μl. The primers used for amplification of the D2-D3 region of 28S rRNA gene were the D2A (5′-ACAAGTACCGTGAGGGAAAGTTG-3′) and the D3B (5′-TCGGAAGGAACGAGCTACTA-3′) primers (De Ley et al., 1999). PCR cycle conditions were as follows: one cycle of 94°C for 3 min, followed by 35 cycles of 94°C for 1 min + annealing temperature of 55°C for 45 s + 72°C for 2 min, and finally one cycle of 72°C for 10 min. After DNA amplification, 5 μl of product was loaded on a 1% agarose gel in 0.5% Tris-acetate-EDTA (40 mM Tris, 20 mM glacial acetic acid and 2 mM EDTA; pH = 8) to verify the amplification using an electrophoresis system (Labnet Gel XL Ultra V-2, Progen Scientific®, London, UK). The bands were stained with RedSafe (20,000×), which was previously added to the agarose gel solution. The sequencing reactions were performed at Sistemas Genómicos (Valencia, Spain). The sequences obtained (with 769, 772 and 783 pb) were submitted to the GenBank database under accession numbers MK894244, MK894245 and MK894246.

**Phylogenetic analyses**

For phylogenetic relationships, analyses were based on 28S tRNA. The newly obtained sequences were manually edited using BioEdit 7.2.6 (Hall, 1999) and aligned with another D2–D3 expansion segments of 28S rRNA gene sequences available in GenBank, using MUSCLE alignment tool implemented in the MEGA7 (Kumar et al., 2016). The ambiguously aligned parts and divergent regions were known using the online version of Gblocks 0.91b (Castresana, 2000) (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) and were removed from the alignments using MEGA7. The best-fit model of nucleotide substitution used for the phylogenetic analysis was statistically selected using jModelTest 2.1.10 (Darriba et al., 2012). Phylogenetic tree was generated with Bayesian inference method using MrBayes 3.2.6 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Mononchus truncatus Bastian, 1865 (AY593064) was chosen as an outgroup. The analysis under GTR+I+G model was initiated with a random starting tree and run with the Markov Chain Monte Carlo (MCMC) for 1 x 106 generations. The tree was visualized and saved with FigTree 1.4.3 (Rambaut, 2014).

**Results**

**Labronema montanum** sp. n. (Figs. 1–4)

**Material examined**

In total, 17 females and 11 males, from one location, were examined.

**Morphometrics**

See Table 1.

**Description**

**Adult**

Nematodes of medium size are stout to moderately slender (a=18–26), with length 1.56–2.08 mm. The body is cylindrical, tapering toward both ends, but more so toward the anterior end, as the tail is short and rounded. Upon fixation, habitus visibly curved ventrad, more regularly in females, to an open C shape, and more perceptibly at posterior body region in males, to a J shape. Cuticle is three layered, especially obvious at caudal region, 3.5 to 6.5 μm thick at anterior region,
5.0 to 8.5 µm in mid-body, and 10.5 to 13.5 µm on female tail and 9 to 12 µm on male tail; outer layer is thin, with a very fine transverse striation (only appreciable in SEM images, Fig. 4A,B) and there is a constant thickness throughout the body; intermediate layer is much thicker than the outer one, particularly at the caudal...
Morphological and molecular characterization of *Labronema montanum* sp. n. (Dorylaimida, Dorylaimidae) from Spain

**Table 1. Morphometrics of *Labronema montanum* sp. n. from Spain. Measurements in μm except L in mm, and in the form: average ± sd (range).**

| Character                      | Holotype | Paratypes | Paratypes |
|--------------------------------|----------|-----------|-----------|
| ♂                              |          | 17♀       | 11♂♂      |
| L                              | 1.74     | 1.83±0.14 (1.56–2.07) | 1.87±0.12 (1.68–2.08) |
| a                              | 17.8     | 21.3±2.0 (17.8–24.4) | 22.7±1.8 (20.3–25.9) |
| b                              | 3.7      | 3.8±0.3 (3.3–4.3) | 3.7±0.2 (3.3–4.1) |
| c                              | 62       | 68.1±9.4 (56–86) | 72.2±5.5 (65–84) |
| V                              | 58       | 58.4±1.2 (56.5–60.2) | – |
| c’                             | 0.6      | 0.6±0.1 (0.5–0.8) | 0.6±0.0 (0.6–0.7) |
| Lip region diameter            | 23       | 22.0±1.1 (19–23) | 22.2±1.3 (19–24) |
| Odontostyle length ventral side| 27       | 25.0±1.9 (21–28) | 26.0±2.0 (22–29) |
| Odontostyle length dorsal side  | 28       | 26.5±2.1 (23–30) | 27.3±2.0 (23–30) |
| Odontophore length             | 43       | 42.0±1.9 (37–44) | 42.7±2.2 (38–45) |
| Neck length                    | 474      | 476±27 (417–514) | 500±31 (450–551) |
| Pharyngeal expansion length    | 238      | 242±19 (205–272) | 247±16 (212–264) |
| Body diam. at neck base        | 92       | 80.6±8.3 (65–98) | 78.9±6.1 (72–91) |
| mid-body                       | 98       | 85.9±7.6 (72–99) | 82.8±8.9 (71–101) |
| anus/cloaca                    | 44       | 43.9±3.3 (39–51) | 42.3±3.7 (36–47) |
| Distance vulva – anterior end  | 1015     | 1068±88 (889–1232) | – |
| Prerectum length               | 93       | 125±23 (84–160) | 202±34 (150–268) |
| Rectum/cloaca length           | 61       | 60.0±4.1 (52–68) | 71.6±5.6 (61–80) |
| Tail length                    | 28       | 27.4±4.5 (19–35) | 26.1±2.9 (20–30) |
| Spicules length                | –        | –           | 71.3±3.6 (65–76) |
| Ventromedian supplements       | –        | –           | (20–25) |

region; inner layer is much thinner than the intermediate layer, especially distinct at the caudal region. The lateral chord is 10 to 22μm broad, occupying 12 to 26% of mid-body diameter. Several ventral and dorsal pores are present at the cervical region. The lip region is offset by a deep constriction, 2.8 to 3.7 times broader than high and less than one-third (23–31%) of body diameter at the neck base. SEM observations are as follows: lips are mostly amalgamated, the lateral ones are barely differentiated from their adjacent subdorsal and subventral lips; papillae are button like with a pore at their center; labial papillae are surrounded by a circular striation that does not exist around the cephalic papillae; oral field is relatively wide, as the inner labial papillae are situated far from the oral aperture, bearing coarse radial incisures reaching the outer margin of the lip region, perioral area is differentiated in distinct but weakly protruding liplets. Amphid with funnel-shaped fovea is present; its aperture is 8 to 11μm, occupying two-fifths to one-half (41–50%) of the lip region diameter. Cheilostom is nearly cylindrical, with thick walls, but it is lacking any other differentiation. Odontostyle is typical of the genus, strong, 5.3 to 7.1 times as long as broad, somewhat longer (1.1–1.3 times) than the lip region diameter, and 1.15 to 1.86% of total body length; aperture is 7 to 11μm long, occupying one-third to two-fifths (33–42%) of the odontostyle length. Odontophore is rod-like; its length is 1.4 to 1.8 times the odontostyle. Pharynx is conspicuously muscular, with its slender portion enlarging very gradually and basal expansion being 5.1 to 7.9 times as long as wide, 2.5 to 3.6 times longer than body diameter at neck base, and occupying ca one-half (47–53%) of the neck length; pharyngeal gland nuclei are located as follows: DO = 46
to 55, DN = 49 to 58, S₁N₁ = 61 to 68, S₂N₂ = 69 to 78, S₂N = 82 to 87, DN nucleolus 5µm in diameter, S₁N, 2µm and very close to its outlet, S₂N, 4µm and at 3µm from its outlet, and S₂N 4.5µm and at 9µm from its outlet. The nerve ring is situated at 142 to 185µm or 30 to 38% of the total neck length from the anterior end. Pharyngo-intestinal junction is well developed; it consists of conoid to conical, 25–52 × 16–26 µm, cardia, enveloped by the intestinal wall and a distinct ring-like structure protruding in three lobes that in some specimens show gland-like bodies (see remarks). Intestine is present with no relevant differentiation. The tail is short, convex conoid to rounded, often showing some kind of indentations, mainly on its dorsal side, probably as result of fixation process affecting the very thick cuticle at this region.

Female

Genital system is amphidelphic, with both branches well and equally developed; the anterior is 207 to 399µm or occupying 13 to 22% of the total body
Morphological and molecular characterization of *Labronema montanum* sp. n. (Dorylaimida, Dorylaimidae) from Spain

Figure 3: *Labronema montanum* sp. n. from Spain (male, LM). (A): Entire. (B–D): Pharyngo-intestinal junction showing the three lobes. (E): Posterior body region. (F): Spicule. (G, H): Male. (I): Lateral guiding piece. (J): Sperm cells. (Scale bars: A = 500 µm; D–B = 20 µm; E = 50 µm; F–H = 10 µm; I = 5 µm; J = 10 µm).
length; the posterior is 226 to 380 µm or 13 to 23% of the total body length. Ovaries are reflexed, variably sized; the anterior is 57 to 183 µm long, whereas the posterior is 72 to 158 µm long, usually reaching the oviduct–uterus junction, with oocytes first in two or more rows and then in a single row. Oviduct is 78 to 180 µm long or 1.0–2.3 times longer than the body diameter, consisting of a distal, slender section made of prismatic cells and a moderately developed proximal pars dilatata with visible lumen inside. A narrowing surrounded by a muscular ring (sphincter) separates oviduct and uterus. Uterus is 148 to 264 µm long or 1.7 to 3.5 times longer than the body diameter; it is tripartite, as it consists of distal, nearly spheroidal section with a visible lumen inside, a much slender intermediate, often convoluted, section with narrow lumen, and a proximal part with thin walls and a very wide lumen, nearly always containing abundant sperm cells inside. Vagina is extending inwards, 29 to 41 µm, to 36 to 46% of the body diameter; pars proximalis is

Figure 4: Labronema montanum sp. n. from Spain (SEM). (A): Lip region in ventral view. (B): Same in frontal view. (C): Vulva, ventral view. (D, E): Male, posterior body region showing ad-cloacal pair (D) and ventromedian series (E) of genital papillae. (F): Female tail, ventral view. (G, H): Female tail, lateral view, showing indentations of body cuticle.
Morphological and molecular characterization of Labronema montanum sp. n. (Dorylaimida, Dorylaimidae) from Spain

19–33 × 11–20 µm in size and it is surrounded by moderately developed circular musculature; pars refringens consists of (lateral view) either two small triangular, 4.5 to 5 µm long, sclerotized pieces, separated by a slightly less sclerotized intermediate area, or two large trapezoidal pieces, 5 to 7 µm long, in both cases with a combined width of 16 to 22 µm; pars distalis is 3.5 to 7.5 µm long. Vulva is a very short, ca 3 µm long, longitudinal slit or elliptical opening (Fig. 4C). Prerectum is 1.9 to 4.0 and rectum is 1.2 to 1.7 times longer than the anal body diameter.

**Male**

Prerectum is 3.2 to 7.4 and cloaca is 1.5 to 1.9 times longer than the body diameter at cloacal aperture.
Genital system is diorichic, with opposed testes, and spindle-shaped sperm cells. In addition to the ad-cloacal pair, located at 8 to 11 µm from the cloacal aperture, there is a series of 20 to 25 contiguous, 5.5 to 11 µm apart, ventromedian supplements, ending in front of the anterior end of spicules, at 59 to 86 µm from the adcloacal pair; therefore, a distinct hiatus exists. Spicules are 4.6 to 5.8 times as long as wide, 1.4 to 1.9 times longer than body diameter at the cloacal aperture; head is 1.8 to 3.2 times longer than wide, occupying 31 to 36% of the total spicule length, and with slender walls, the dorsal one is longer than ventral and it is slightly curved; median piece is narrow, occupying hardly one-third (19–36%) of the maximum width of spicule, and reaching the posterior end, which is 4.5 to 6 µm broad; curvature is 130 to 140°; ventral hump and hollow are poorly demarcated but appreciable; the former is situated at 36 to 58% of the spicule length from its anterior end. Lateral guiding piece is curved ventrad, 19 to 25 µm long, 5.4 to 6.3 times longer than broad, and with somewhat bifid terminus.

**Molecular characterization**

Three D2-D3 28S-rRNA sequences were obtained, one with 769, 772 and 783 pb long. These three fragments agree in 729 pb long, all of them were 100% identical. Their analysis has facilitated a study of the evolutionary relationships of the new species. The results are presented in Figure 5.

**Diagnosis**

The new species is characterized by its 1.56 to 2.08 mm long body, with lip region being offset by constriction and 19 to 24 µm broad, and with pericloai liplets, odontostyle 21 to 29 µm long at its ventral side, with aperture occupying 35 to 42% of its length, neck 417 to 551 µm long, pharyngeal expansion 205 to 272 µm long or occupying 47 to 53% of the total neck length, the presence of three cardiac lobes at the pharyngo-intestinal junction, didelphic–amphidelphic female genital system, a long, pharyngeal expansion 205–272 µm long (142 µm), the presence (vs absence) of three large lobes at pharyngo-intestinal junction, and a higher number of ventromedian supplements (20–25 vs 15–17). It differs from *L. carusoii* in its much thicker body cuticle (for instance, 10.5–13.5 vs 4–8.5 µm on female tail), broader lip region (19–23 vs 15–20 µm), longer neck (417–551 vs 386–402 µm), and pharyngeal expansion (205–272 vs 150–191 µm), the presence (vs absence) of three large lobes at pharyngo-intestinal junction, and a higher number of ventromedian supplements (20–25 vs 15–17). Evolutionary relationships, as derived from the analysis of D2-D3 28S-rRNA gene sequences, are presented in molecular tree of Figure 5. The new species forms part of a highly supported clade, predominantly constituted by representatives of the family Dorylaimidae, namely *Crassolabium, Labronema, Mesodorylaimus*, and *Nevadanema* sequences, and also by sequences of the genera *Ecumenicus* (Qudsi nematidae) and *Opisthodorylaimus* (Thornenematidae). These results totally match recent previous findings (Varela-Benavides and Peña-Santiago, 2018), and confirm the present difficulties to elucidate the phylogeny of Dorylaimina. The internal relationships of the clade are not satisfactorily resolved, as the sequences corresponding to members of the subfamily Labronematinae (Crassolabium, Labronema, and Nevadanema) appear separated in two subclades, with both of them even including sequences of different species of the same genus, *Labronema*.

**Type habitat and locality**

The new species was collected from a grassy area with stony soil at 1,800 elevation in Pandera mountain, Jaén province, southern Iberian Peninsula, Spain.

**Type material**

Female holotype, 14 female paratypes and 7 male paratypes were deposited in the nematode collection of the University of Jaén, Spain. Two female paratypes
and two male paratypes were deposited in the US-DANC, Beltsville, Maryland, USA.

**Etymology**

The specific epithet is a Latin term that means belonging to the mountain, and it refers to type habitat where the new species was collected from.

**Remarks**

As mentioned above, the new species is similar to the three originally described in Italy, and together, they probably form a quite homogeneous group within the genus *Labronema* (cf. Peña-Santiago and Vinciguerra, 2019). They have been mainly reported in Mediterranean countries (Albania, Italy, Spain), and also in other neighboring territories (Romania and Switzerland).

The existence of three large lobes at the pharyngo-intestinal junction is a remarkable feature of the new species, already mentioned (as cardiac glands), in *L. pulchrum* (cf. Peña-Santiago and Vinciguerra, 2019). The present observations suggest that these lobes/glands are not comparable to those recognizable in nygolaims (suborder Nygolaimina). Actually, a more or less developed ring-like structure is easily perceptible in many Dorylaimina species, including *Labronema* representatives (for instance, *L. ferox* Thorne, 1930; see Peña-Santiago, 2019), which, in particular taxa, shows additional differentiation resulting in a large dorsal lobe, as observed in members of the genus *Aporcelinus* Andrásy, 2009, or three large lobes that apparently display a tri-radial symmetry.

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**References**

Abolafia, J. 2015. A low-cost technique to manufacture a container to process meiofauna for scanning electron microscopy. Microscopy Research and Technique 78:771–776, http://dx.doi.org/10.1002/mrmt.22538.

Altherr, E. 1963. Contribution à la connaissance de la faune des sables submersés en Lorraine. Nematodes. Annales de Spéléologie 18:53–98.

Álvarez-Ortega, S. and Peña-Santiago, R. 2013. Taxonomy of the genus *Aporcelaimellus* Heyns, 1965 (Nematoda, Dorylaimida, Aporcelaimidae). Zootaxa 3669:243–260, doi: 10.11646/zootaxa.3669.3.3.

Andrásy, I. 2009. Free-living nematodes of Hungary. III, Pedozoologica Hungarica No. 5. Budapest: Hungarian Natural History Museum.

Baermann, G. 1917. Eine einfache Methode zur Auffindung von Ankylostomum (Nematoden) Larven in Erdproben. Geneeskundig Tijdschrift voor Nederlandsch-Indië 57:131–137.

Bastian, H. C. 1865. Monograph of the Anguillulidae, or free nematoids, marine, land and freshwater; with descriptions of 100 new species. Transactions of the Linnean Society of London-Zoology 25:73–184.

Castillo, P., Vovlas, N., Subbotin, S. A. and Troccoli, A. 2003. A new root-knot nematode, *Meloidogyne baetica* n. sp. (Nematoda: Heteroderidae), parasitizing wild olive in Southern Spain. Phytopathology 93:1093–1102, doi: 10.1094/PHYTO.2003.93.9.1093.

Castesana, J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molecular Biology and Evolution 17:540–552.

De Ley, P., Felix, A. M., Frisse, L. M., Nadler, S. A., Sternberg, P. W. and Thomas, W. K. 1999. Molecular and morphological characterization of two reproducibly isolated species with mirror-image anatomy (Nematode: Cephalobidae). Nematology 1:591–612, doi: 10.1163/156854199508559.

Flegg, J. J. M. 1967. Extraction of *Xiphinema* and *Longidorus* species from soil by a modification of Cobb’s decanting, and sieving, technique. Annals of Applied Biology 60:429–437.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95–98, doi: 10.1021/bk-1999-0734.ch008.

Huelsenbeck, J. P. and Ronquist, F. 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17:754–755, https://doi.org/10.1093/bioinformatics/17.8.754.

Jiménez-Guirado, D. 1989. Nematodos acuáticos del Parque Nacional de Doñana. Oxyura 5:83–91.

Kumar, S., Stecher, G. and Tamura, K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33:1870–1874, doi: 10.1093/molbev/msw054.

Loof, P. A. A. and Grootaert, P. 1981. Redescription of *Labronema vulvapapillatum* Meyl, 1954. nov. comb. (Dorylaimoidea). Nematologica 27:139–145, doi: 10.1163/1875298281X00197.

Meyl, A. H. 1954. Die bisher in Italien gefundenen freilebenden Erd und Süßwasser-Nematoden. Archivo Zoológico Italiano, Torino 39:161–264.

Murillo-Navarro, R. and Jiménez-Guirado, D. 2006. Some dorylaimid nematodes from coastal dunes in Cadiz Bay (SW Spain). Journal of Nematode Morphology and Systematics 8(2005):161–178.
Peña-Santiago, R. 2019. Taxonomy of the genus *Labronema* Thorne, 1939 (Nematoda: Dorylaimida: Dorylaimidae) with redescriptions of its type species, *L. ferox* Thorne, 1939. Nematology 21:23–34, doi: 10.1163/15685411-00003192.

Peña-Santiago, R. and Vinciguerra, M. T. 2019. Redescription of three Italian species of the genus *Labronema* Thorne, 1939 (Dorylaimida, Dorylaimidae). Nematology, (in press, already published online), doi: 10.1163/15685411-00003250.

Peña-Santiago, R., Abolafia, J., Liébanas, G., Peralta, M. and Guerrero, P. 2003. Dorylaimid species (Nematoda, Dorylaimida) recorded in the Iberian Peninsula and the Balearic Islands: a compendium. Monographic Papers on Nematology No. 1, Servicio de Publicaciones, Universidad de Jaén, Jaén, Spain.

Rambaut, A. 2014. Figtree, a graphical viewer of phylogenetic trees. available at: http://tree.bio.ed.ac.uk/software/figtree (Accessed on January 20, 2019).

Ronquist, F. and Huelsenbeck, J. P. 2003. Mr-BAYES3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574, doi: 10.1093/bioinformatics/btg180.

Siddiqi, M. R. 1964. Studies on *Discolaimus* spp. (Nematoda: Dorylaimidae) from India. Zeitschrift für Zoologische Systematik und Evolutionsforschung 2:174–184, doi: 10.1111/j.1439-0469.1964.tb00720.x.

Thorne, G. 1930. Predaceous nemas of the genus *Nygolaimus* and a new genus *Sectonema*. Journal of Agricultural Research 41:445–466.

Thorne, G. 1939. A monograph of the nematodes of the superfamily Dorylaimoidea. Capita Zoologica 8:1–261.

Thorne, G. 1974. Nematodes of the Northern Great Plains. Part II. Dorylaimoidea in part (Nematoda: Adenophorea). South Dakota State University Agricultural Experiment Station Technical Bulletin 41:1–120.

Varela-Benavides, I. and Peña-Santiago, R. 2018. Description of *Crassolabium costarricense* sp. n. (Nematoda, Dorylaimida, Dorylaimidae) from Costa Rica. Nematology 20:1007–1014, doi: 10.1163/15685411-00003191.

Vinciguerra, M. T. and Clausi, M. 1994. Nematodes of Salina. Three new and one rare species of *Qudsianelmatidae* (Dorylaimida). Animalia 21:97–112.

Vinciguerra, M. T. and Orselli, L. 1998. Nematodes from Italian sand dunes. 3. Four new species of *Qudsianelmatidae* (Dorylaimida, Nematoda). Nematologia Mediterranea 26:255–266.

Vinciguerra, M. T. and Zullini, A. 1980. New and rare species from Italian sand dunes. Animalia 7:29–44.