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Research paper

Two new species of the genus *Metaxonchium* Coomans & Nair 1975 (Nematoda, Dorylaimida, Belondiridae) from Costa Rica, with new insights in the phylogeny of the family Belondiridae

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ABSTRACT

Two new species of the genus *Metaxonchium* are described from natural habitats in Costa Rica. *Metaxonchium parvisaccatum* sp. n. is characterized by its 1.62–1.71 mm long body, lip region offset by constriction and 8.5–10 μm broad, odontostyle 10–11 μm long, neck 632–674 μm long, pharyngeal expansion occupying 68–71% of neck length, anterior genital branch reduced to a short uterine sac, 22–27 μm long or 1–2% of body length, posterior uterus long and tripartite without apophyses, vulva transverse ($V = 54–55$), caudal region short and rounded (23–26 μm, $c = 62–76$, $c' = 0.7–0.8$), and male absent. *Metaxonchium stenospiculum* sp. n. is characterized by its 2.15–2.85 mm long body, lip region offset by constriction and 12.5–16 μm broad, odontostyle 13.5–16 μm long, neck 756–1011 μm long, pharyngeal expansion occupying 64–74% of neck length, anterior genital branch 8–13% of body length and consisting of a long uterine sac plus a terminal mass, posterior uterus long and tripartite without apophyses, vulva transverse ($V = 53–59$), caudal region short and rounded, nearly hemispheroid (20–28 μm, $c = 91–126$, $c' = 0.4–0.6$) in females, and more conoid (29–41 μm, $c = 80–88$, $c' = 0.6–0.8$) in males, spicules 96–102 μm long, and 11–14 spaced ventromedian supplements without hiatus. Molecular analysis of *M. stenospiculum* sp. n. D2-D3 LSU provides new insights in the phylogeny of Belondiridae as its rounded-tailed genera might constitute a monophyletic group.

1. Introduction

The nematode genus *Metaxonchium* Coomans & Nair, 1975 is a dorylaimid (order Dorylaimida) taxon, with 26 valid species, known to occur in all continents except Antarctica. Its representatives often dwell soils in both wild habitats (forests, meadows, etc.) and agroecosystems. Diversity of *Metaxonchium* in Tropical America has remained poorly explored as only Loof & Zullini (2000) had hitherto reported the presence of *Metaxonchium micans* (Thorne, 1939) Andrássy 1996 in rainforests of Costa Rica. A nematological survey conducted in 2016 in natural and seminatural areas of Costa Rica has however revealed that members of this genus are better represented than previously known. Actually, this is the second contribution devoted to Costa Rican representatives of *Metaxonchium*. The first one (Varela-Benavides & Peña-Santiago 2018) dealt with the description of the new species *Metaxonchium toroense*. The study of several specimens collected from four locations resulted in the discovery of other two unknown species, which are described in the following.

2. Material and methods

2.1. Sampling, extraction and morphological identification

Soil samples were collected in July and August 2016 at four locations in the boundaries of the Parque Nacional del Agua, located at Alajuela province, Costa Rica. Sampling, extraction and mounting processes were carried out as described in Varela-Benavides & Peña-Santiago (2018).

Specimens were examined and measured using a micrometric eyepiece and/or a drawing tube attached to an Olympus BHS light microscope. Morphometrics included de Man's indices and other

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measurements. Some of the best preserved specimens were photographed with a Nikon Eclipse 80i microscope and a Nikon DS digital camera. Raw photographs were edited using Adobe® Photoshop® CS. After examination and identification, a single specimen preserved in glycerine was re-processed for observation with Scanning Electron Microscopy (SEM) following the protocol by Abolafia & Peña-Santiago (2005). Thus, this nematode was hydrated in distilled water, dehydrated in a graded ethanol and acetone series, critical point dried, coated with gold, and observed with a Zeiss Merlin microscope.

2.2. Phylogenetic analysis

Three individuals from the Toro Amarillo Valverde Vega population, and that were preserved in DESS, were recovered, washed in water and separately used to DNA extractions as described by Castillo et al. (2003). The extracted DNA was used to amplify the D2-D3 expansion segments of LSU-rDNA using the D2A/D3B primers (De Ley et al. 1999). The PCR was performed with the addition of 2 μl of extracted DNA to PCR mix containing 1× PCR buffer (Dream TaqTM buffer), 200 μM of each dNTPs, 0.4 μM of each primer, 2 mM of $MgCl_2$ and 1.25 U of Dream taq DNA polymerase (Thermo Fisher Scientific) to a final volume of 25 μl. Amplification conditions consisted of an initial denaturation at 94 °C for 4 min, followed by 35 cycles at 94 °C for 1 min, 55 °C for 90 s and 72 °C for 2 min, and final extension at 72 °C for 5 min. PCR products were purified using the Wizard® SV Gel and PCR Clean-Up System kit (Promega) and used for direct sequencing in both directions using the primers referred above. The sequencing reactions were performed using the sequencing service from Macrogen Inc. (Korea). The obtained sequences were submitted to GenBank under accession numbers: MH167346, MH167347 and MH167348.

The obtained sequences were aligned with other dorylaimid LSU-rDNA sequences available from GenBank using MUSCLE (Edgar 2014). Outgroup taxa used for phylogenetic reconstruction were those used by Peña-Santiago et al. (2013). Sequence alignment were edited by Gblocks v0.91b (Castresana 2000) using options for a less stringent selection (minimum number of sequences for a conserved or a flanking position: 50% of the number of sequences + 1; maximum number of contiguous non-conserved positions: 8; minimum length of a block: 5; allowed gap positions: with half). The best fit model of DNA evolution for the analysis was obtained using jModelTest v2.1.10 (Darriba et al. 2012) with the Akaike information criterion (AIC). Bayesian inference (BI) was performed using MrBayes 3.2.6 (Ronquist et al. 2012) under the general time-reversible model with invariable sites and a gamma-shaped distribution (GTR + I + G). The analysis was initiated with a random starting tree and run with four Metropolis-coupled Markov chain Monte Carlo (MCMC) cycles for 1×10^6 generations. The MCMC were sampled at intervals of 100 generations. Two runs were performed for each analysis. After discarding burn-in samples and evaluating convergence, the remaining samples were retained for further analyses. The topologies were used to generate a 50% majority rule consensus tree. The tree was visualized with the program FigTree v 1.4.3 and drawn with Adobe Acrobat Pro DC.

3. Results and discussion

3.1. Metaxonchium parvisaccatum sp. n

(Figs. 1 and 2).

3.1.1. Material examined

Three females from one location, in excellent state of preservation.

3.1.2. Morphometrics

See Table 1.

Female: Slender nematodes ($a = 31–33$) of medium size, 1.62–1.71 mm long. Body cylindrical, visibly tapering towards the anterior end, less so towards the posterior one as the caudal region is short and rounded. Upon fixation, habitus slightly curved ventrad, to an open C shape. Cuticle two-layered, 2.0–2.5 μm thick at anterior region (at level of odontostyle), 2.5–3.0 μm in mid-body and 7.5–9.5 μm on tail; outer layer thin, with constant thickness throughout the body, nearly smooth under LM; inner layer much thicker than the outer one, especially obvious at caudal region where it bears visible radial striation. Lateral chord very narrow, nearly indistinguishable. Body pores obscure in general. Lip region cap-like, offset by constriction, 2.1–2.6 times as broad as high and up to one-fifth (17–20%) of body diameter at neck base; lips separate, with distinct but not protruding papillae. Amphid fovea cup-like, its aperture 6.5–7.5 μm wide, occupying up to three-fourths of lip region diameter. Cheilostom a truncate cone, lacking any differentiation. Odontostyle small, somewhat fusiform, 4.4–4.6 times as long as wide, hardly longer (1.0–1.2 times) than lip region diameter, and 0.59–0.63% of body length; aperture 3.5–4.0 μm long, occupying one-third to two-fifths of total length. Guiding ring thin, simple but visibly refractive, at 10.0–11.5 μm or 1.1–1.3 times the lip region diameter from the anterior end. Odontophore rod-like, bearing a very weak thickening at about its middle, 1.2–1.3 times the odontostyle. Pharynx consisting of a slender muscular anterior portion, separated from the basal expansion by a short isthmus-like narrowing, and lacking any other differentiation; basal expansion 13–16 times as long as broad, 8.5–8.9 times longer than body diameter at neck base, and occupying less than three-fourths (68–71%) of total neck length; a very distinct spiral muscular sheath, with nearly straight muscular bands, envelopes the whole basal expansion; gland nuclei obscure excepting DN = 34, with DO very close to it. Cardia tongue-like, 25–29 × 11–15 μm, surrounded by a thick cover of intestinal tissue. Genital system monodelphic-opisthodelphic. Anterior branch reduced to an uterine sac 22–27 μm long, up to one-half of body diameter or 1–2% of body length. Posterior branch 111–141 μm long or 7–8% of total body length, with reflexed ovary 56–58 μm long and oocytes arranged first in several rows and then in a single row; oviduct joining the ovary subterminally, 60–66 μm long or 1.1–1.3 body diameters, and consisting of a tubular part made of prismatic cells and a well-developed *pars dilatata* with perceptible lumen; a distinct sphincter separating oviduct and uterus; uterus 93–113 μm or 1.5–1.9 times the body diameter —these morphometrics, however, should be taken with caution as the uterus appears variably convoluted— and tripartite, *i.e.*, differentiated in relatively short and thick proximal region with visible lumen, a long and narrow intermediate region with very narrow lumen and without apophyses (non-echinophor), and a large spherical distal *pars dilatata*; vagina 24–30 μm long, extending inwards about one-half (47–57%) of the corresponding body diameter: *pars proximalis*, 16–19 × 14–17 μm, with nearly straight walls and surrounded by moderately developed, circular musculature, *pars refringens* (in lateral view) consisting of two trapezoidal pieces measuring 7–10 × 5.0–6.0 μm and with a combined width of 11–13 μm, and *pars distalis* 4.0–5.0 μm long; vulva a somewhat posterior, transverse slit; the female holotype bears abundant refractive granules on body cuticle around the vulva, but such differentiation is not appreciable in the other two females. Prerectum 3.1–4.4, rectum 0.9–1.0 anal body diameters long. Caudal region short and rounded; caudal pores two pairs at the middle of tail, one sublateral, another subdorsal.

Male: Unknown.