

RESEARCH NOTE

First report of two *Mesocriconema* (Nematoda: Criconematidae) species in mulberry trees in Costa Rica¹

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RESUMO

Primeiro registro de duas espécies de *Mesocriconema* (Nematoda: Criconematidae) em amoreira na Costa Rica

Amostras de solo foram coletadas da rizosfera de plantas de amora (*Morus alba* L.), em uma parcela de 1.000 m², na Fazenda Experimental Santa Lucia, em Barva, Heredia, Costa Rica, em agosto de 2013. As plantas apresentaram sintomas de amarelecimento, declínio e pouco desenvolvimento. Espécimes de dois nematoides anelados foram coletados do solo e identificados como *Mesocriconema sphaerocephalum* (Taylor 1936) Loof 1989 e *M. anastomoides* (Maqbool & Shahina 1985) Loof & De Grisse 1989, com base na análise morfológica e morfométrica de fêmeas. Ambos os nematoides foram previamente encontrados na Costa Rica. Porém, este é o primeiro relato de nematoides da família Criconematidae associados à amoreira, o qual fornece informações adicionais sobre este fitoparasita.

PALAVRAS-CHAVE: *Mesocriconema sphaerocephalum* (Taylor 1936) Loof 1989; *Mesocriconema anastomoides* (Maqbool & Shahina 1985) Loof & De Grisse 1989; morfometria; nematoides anelados.

Mulberry is a native species from temperate zones of Asia, which has spread worldwide (Benavides et al. 1994). In Central America, particularly in Costa Rica, it is propagated by cutting plants directly.

Its foliage is used as fodder for cattle and goats (Rodríguez et al. 1994, González 1996) and it can reach 12 meters high, being adapted as a fast growing shrub, with vigorous leaf shoots (Ting-Zing et al. 1988). It also presents an excellent nutritional value, due to its high levels of protein (20-24%) and digestibility (75-85%) (Benavides 1995).

Costa Rica is endowed with a suitable climate for the cultivation of different crops, but there are constraints affecting its agriculture, such as diseases and plant pests, mainly nematodes.

The Criconematidae family is a taxonomy commonly known as ring nematodes, due to the

ABSTRACT

Soil samples were collected from the rhizosphere of mulberry (*Morus alba* L.) trees, in a 1,000 m² plot, at the Experimental Farm Santa Lucia, in Barva, Heredia, Costa Rica, in August 2013. The plants showed symptoms of yellowing, declining and poor development. Specimens of two ring nematode were collected from the soil and identified as *Mesocriconema sphaerocephalum* (Taylor 1936) Loof 1989 and *M. anastomoides* (Maqbool & Shahina 1985) Loof & De Grisse 1989, based on the morphological and morphometrical analysis of females. Both nematodes have been previously found in Costa Rica. However, this is the first report of nematodes from the Criconematidae family associated to mulberry trees and it provides additional information on the distribution of this phytoparasite.

KEY-WORDS: *Mesocriconema sphaerocephalum* (Taylor 1936) Loof 1989; *Mesocriconema anastomoides* (Maqbool & Shahina 1985) Loof & De Grisse 1989; morphometry; ring nematodes.

transversal annulation of the cuticle. All species are plant parasites that can attack vegetable crops and fruit trees, with a cosmopolitan distribution.

This notable group of nematodes has been reported in coffee plantations in El Salvador (Raski & Golden 1966); *Citrus* sp. in Morocco; *Zea mays* L. in Kenya; *Brasica oleracea* L., *Allium porrum* L. and *Lactuca sativa* L. in Madagascar (Luc 1970); apricot in the United States (Mckenry et al. 1990); peach and apple in Spain (Escuer & Palomo 1991); vineyards in Italy (Coiro et al. 1991); avocado, mango and tamarind in Spain (Castillo & Gómez 1993); *Mussa* sp. in Jordan (Yousef & S'Jacob 1994); peach in Mexico (Luna-Guerrero et al. 2011); barley, wheat and sugar beet in the Slovak Republic (Šály 1976), among others.

In Costa Rica, there is evidence of its presence in various crops, forests and different types of

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vegetation (Tarjan 1971, López-Chaves 1980 and 1988, López-Chaves & Azofeifa-Chacón 1980, López-Chaves & Salazar-Figueroa 1987a, Arroyo et al. 2004, Wingching-Jones et al. 2008, Powers et al. 2011, Guzmán-Hernández et al. 2011).

This study aimed to report the presence of ring nematodes (*Mesocriconema sphaerocephalum* and *M. anastomoides*) in mulberry trees, in Costa Rica.

The isolation and identification of nematodes were performed in the Laboratory of Nematology of the Universidad Nacional de Costa Rica, in Heredia, Costa Rica. Nine soil samples, which were placed in sealed plastic bags and stored at 4°C, before analysis, were collected from a 1,000 m² plot, at the Experimental Farm Santa Lucia, in Barva (10°01'34.40"N, 84°06'49.60"W, altitude of 1,220 m, average rainfall of 2,693 mm per year and average temperature of 23°C), Heredia, which belongs to the School of Agricultural Sciences of the Universidad Nacional de Costa Rica, in August 2013. The area had been previously planted with sorghum, which had showed symptoms of yellowing and poor development.

Nematodes were extracted from soil samples by flotation-centrifugation (Jenkins 1964), killed and fixed in hot 4% formaldehyde (70°C). Subsequently, fifteen females from each species were infiltrated with glycerin, by using the Seinhorst's modified slow method (Seinhorst 1959 and 1962), and mounted on slides, for observation and preservation. All specimens were deposited in the Criconematid Collection, in the Laboratory of Nematology of the Universidad Nacional de Costa Rica.

Both species were morphologically and morphometrically identified by using data collected by Geraert (2010). All measurements were taken in micrometers (µm) and in the format mean ± S.D. (standard deviation range), using an Olympus BX50 microscope and a stage micrometer. Images of key morphological features were taken with a Nikon DS-Fi1 camera and edited with the aid of Photoshop CS4. The classification of nematodes was based on Siddiqi (2000).

Five years after planting, mulberry trees showed symptoms of yellowing, declining and poor development. These general symptoms are often difficult to distinguish, because they are associated to other root pathogens and soil fungi. So, it is important to make a nematode analysis to detect phytoparasites, as proved in this investigation.

The nematode isolation and identification revealed the presence of *M. sphaerocephalum*

(Taylor 1936) Loof 1989 and *M. anastomoides* (Maqbool & Shahina 1985) Loof & De Grisse 1989, which belong to the Criconematidae (Taylor 1936) family, associated to mulberry.

The classification of the two nematode species was based on Siddiqi (2000), as it follows:

Tylenchina Chitwood in Chitwood & Chitwood 1950
Criconematina Siddiqi 1980

Criconematiodea Taylor 1936 (1914) (Geraert 1966)

Criconematidae Taylor 1936 (1914)

Criconematinae Taylor 1936 (1914)

Mesocriconema sphaerocephalum (Taylor 1936) Loof 1989

Mesocriconema anastomoides (Maqbool & Shahina 1985) Loof & De Grisse 1989

Mesocriconema species presents a cuticle with retrorse annuli, mean annular thickness of 4.7 µm, smooth uneven or crenated margins and no posterior appendages. Lateral field is often present, usually by anastomoses. The first head annulus is seldom separated and the second head annulus is usually wider than the first. Submedian lobes are present, rarely absent. Labial plates are present or absent. Open vulva and anterior vulva lip are ornamented in several species. The post-vulval body is usually short, with round or truncate terminus, seldom acute. Males have a lateral field with 4 lines. The bursa is usually well developed, with enclosing terminus, rarely narrow and short. The tail terminus is round to acute. Juveniles present smooth uneven or crenate annuli posterior margins, never with appendages, and with annuli in profile never drawn out posteriad (Geraert 2010).

The genus *Mesocriconema* is closely related to *Criconemoides*, but it differs by having an open vulva and a difference in the structure of the submedian lobes (Loof & De Grisse 1989). It is not always easy to evaluate these differences and both genera are synonymized by Hunt et al. (2005). On the other hand, Geraert (2010) uses the genus *Mesocriconema* as proposed by Brzeski et al. (1998).

M. sphaerocephalum (Figure 1) has been documented in crops in Europe, America, Africa and Asia. It was reported from Hawaii (Jensen et al. 1959); Mauritius (Willians 1960); Guatemala, Hawaii, Austria, India, Kenya, Nigeria, USA (South Carolina), Netherlands and Venezuela (Raski & Golden 1966,

Crozzoli & Lamberti 2001, Lugo et al. 2010); Congo (Coomans 1966); Iran (Kheiri 1972); South Africa (Van den Berg 1980); Japan (Toida & Momota 1981); Tanzania (Bridge 1984); India (Muthukrishnan 1987); Iran (Barooti 1987); Costa Rica (López-Chaves & Salazar-Figueroa 1987b); Eastern Sudan (Zeidan & Geraert 1989); Spain (Sierra de Cazorla) (Gómez-Barcina et al. 1989); Italy (Bello et al. 1988, Coiro et al. 1991); Spain (Gómez-Barcina et al. 1991, Escuer & Bello 1994); Cameroon (Sakwe & Geraert 1993); Belgium (Bert et al. 2003); and Brazil (Rossi & Camargo 2005), among others.

M. sphaerocephalum has a cuticular retrorse annuli with irregular margins. It's a small nematode, ventrally arcuate and characterized by the presence of a high number of anastomoses throughout the body, forming a zig-zag pattern. The anterior body end appears truncated, labial disc is low, submedian lobes and labial plates are small and not protruding. Stylet

is robust (about 51.0 μm), knobs 8.0-12.0 present a concave or anchor shape (about 10 μm). Criconematid oesophagus is typical. The vulva is open, simple, with rounded lips, devoid of projections. Gonad is often recurved, sometimes even twice. The post-vulval part is rounded. The vagina is straight and the post-vulval body part is short, with a rounded tail. Males were not found. All the principal morphometric values of the specimens are in agreement with the ranges described by Geraert (2010). The population of *M. sphaerocephalum* reported here has a similar morphometrics to the description made by Cordero et al. (2012).

Females were fixed in formaldehyde and mounted in dehydrated glycerine, and all measurements were expressed in μm and in the format mean \pm S.D. (standard deviation range), as it follows: n = 15; L = 342.5 \pm 33.7 (288.0-415.0); stylet length = 51.2 \pm 10.1 (31.0-70.0); a = 9.3 \pm 1.6 (6.4-12.6);



Figure 1. Micrographs of *Mesocriconema sphaerocephalum* (Heredia, Costa Rica, 2013). A: entire female body; B: anterior body portion showing stylet and submedian lobes; C and D: posterior portion showing vulva and tail shape. Scale bars = 20 μm .

$b = 3.6 \pm 0.3$ (3.1-4.1); $V\% = 92.0 \pm 2.4$ (84.5-94.2); first cephalic annulus diameter = 15.8 ± 1.1 (13.0-17.0); body annule width = 5.1 ± 0.8 (3.5-6.5); stylet knob width = 10.0 ± 1.3 (8.0-12.0); oesophagus length = 96.6 ± 10.9 (78.0-110.0); diameter at mid-body (MBW) = 37.4 ± 4.7 (32.0-45.0); diameter at vulva (VBW) = 27.6 ± 2.8 (22.0-32.0); DEGO from stylet base = 4.7 ± 1.3 (3.0-9.0); $R = 66.1 \pm 2.9$ (61.0-71.0); $Rex = 22.8 \pm 2.2$ (20.0-26.0); $RV = 4.9 \pm 0.8$ (4.0-7.0); $St\%L = 14.9 \pm 2.5$ (9.8-19.1); $St\%Oes = 52.7 \pm 7.5$ (39.7-69.0); vulva length (VL) = 27.4 ± 9.0 (19.0-57.0); $VL/VBW = 0.9 \pm 0.2$ (0.7-1.2).

M. anastomoides (Figure 2) was first described in the Sargodha surroundings, in roots of *Citrus* sp. and pears (*Pyrus comrnunis* L.) from Naushera, Pakistan (Maqbool & Shahina 1985, Loof & De Grisse 1989). It belongs to the species of a separate *Pakcriconemoides* (Shahina & Maqbool 1993) genus characterized by a small stylet with rounded knobs.

The species can be compared to *M. microdorum* and *M. sphaerocephala*.

M. anastomoides female nematodes are ventrally arcuate, bluntly rounded at both extremities, assuming a “C” shape. Body annuli is rounded anteriorly and more retrorse posteriorly, with irregular margins, slightly crenate towards the posterior region. Anastomoses of annuli are very prominent, forming a thick regular zig-zag structure distributed longitudinally over the body. Its head has two annuli, with the first one slightly anteriorly directed (10.0-11.0 μ m wide) and the second one slightly thicker, retrorse and larger than the first annulus. The labial region is hemispherical, with four small but distinct submedian lobes. Pseudolips are irregular, with hexagonal labial disc, offset. Oral opening and amphid apertures are oval, on both lateral sides of the disc. Stylet is short and robust (29.5 μ m). Basal knobs are rounded (5 μ m wide), not projected forward, but distinctly sloping backward,

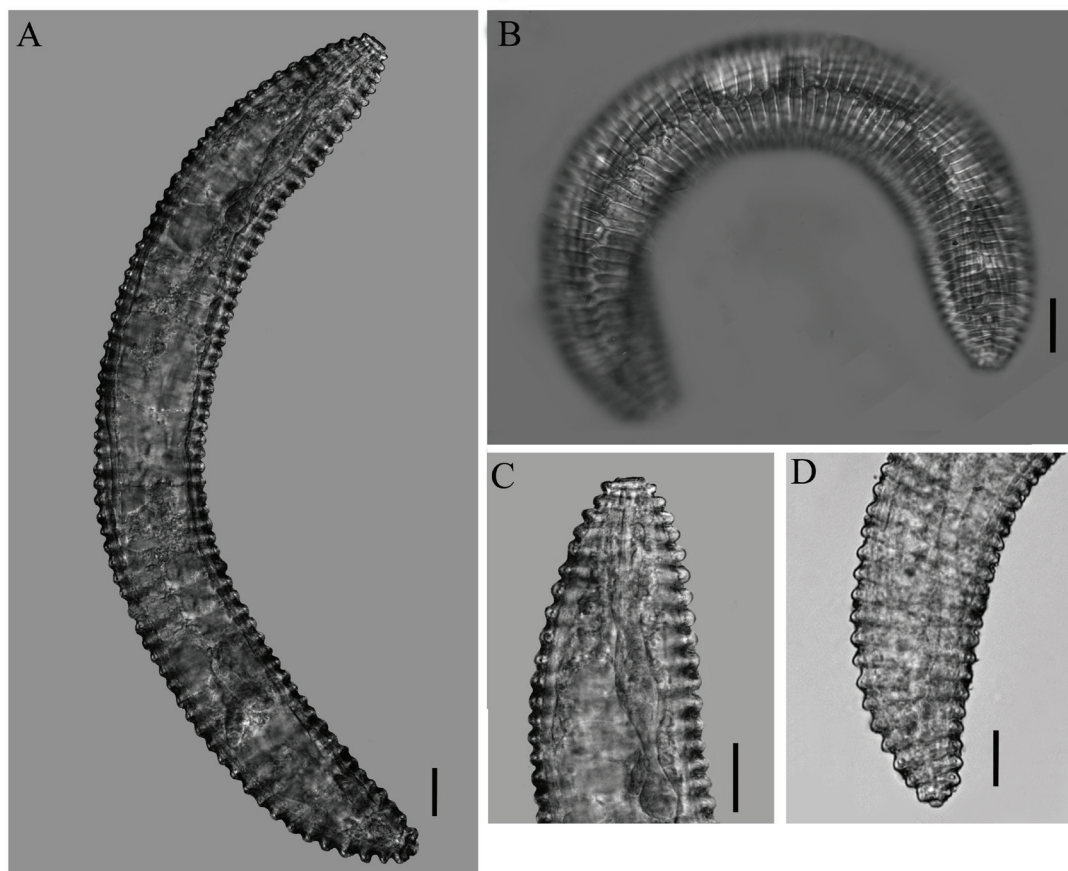


Figure 2. Micrographs of *Mesocriconema anastomoides* (Heredia, Costa Rica, 2013). A: entire female body; B: anastomosis of annuli very prominent, forming a thick zig-zag structure across all the body; C: anterior body portion; D: posterior body portion. Scale bars = 20 μ m.

and somewhat flattened at the base. Esophagus length is 76-79 μm and the vulva is closed. The vagina is straight and the tail rounded, usually with 2-3 lobes on terminus. Males were not found. The population of *M. anastomoides* reported here has a similar morphometrics to the original description made by Maqbolli & Shahina (1985) and Loof & De Grisse (1989).

Females were fixed in formaldehyde and mounted in dehydrated glycerine, and all measurements were expressed in μm and in the format mean \pm S.D. (standard deviation range), as it follows: $n = 15$; $L = 335.5 \pm 9.8$ (328.0-373.0); stylet length = 29.5 ± 1.0 (28.0-30.0); $a = 8.7 \pm 1.1$ (7.3-9.6); $b = 4.5 \pm 0.2$ (4.0-4.7); $V\% = 94.6 \pm 1.3$ (92.2-96.4); first cephalic annulus diameter = 10.3 ± 1.0 (10.0-11.0); body annule width = 4.2 ± 0.7 (4.0-5.5); stylet knob width = 5.0 ± 0.0 (5.0); oesophagus length = 77.0 ± 1.4 (76.0-79.0); diameter at mid-body (MBW) = 40.0 ± 3.6 (37.0-44.0); diameter at vulva (VBW) = 22.0 ± 1.4 (20.0-24.0); DEGO from stylet base = 3.5 ± 0.6 (3.0-4.0); $R = 67.5 \pm 1.3$ (66.0-69.0); $Rex = 20.3 \pm 0.5$ (20.0-22.0); $RV = 4.0 \pm 0.0$ (4.0); $St\%L = 8.5 \pm 0.5$ (7.8-9.0); $St\%Oes = 38.3 \pm 1.2$ (36.8-39.5); vulva length (VL) = 23.3 ± 4.6 (20.0-30.0); $VL/VBW = 0.9 \pm 0.1$ (0.8-1.0).

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