

First record of *Diploscapter coronata* (Rhabditida), a possible health significance nematode associated with tomato crops in Argentina

Primer registro de *Diploscapter coronata* (Rhabditida), un posible nematodo de importancia sanitaria asociado a cultivos de tomate en Argentina

Augusto Salas ¹, José Matías Rusconi ¹, Nora Camino ^{1,2}, Daiana Eliceche ¹,
María Fernanda Achinelly ^{1,3}

Originales: Recepción: 21/03/2016 - Aceptación: 08/10/2016

Nota científica

ABSTRACT

Diploscapter coronata is a free-living soil bacterial-feeding nematode found in compost, sewage or agricultural soil and as a facultative parasite of insects and vertebrates, even humans. The clinical symptoms include epigastric tenderness, diarrhea, crampy abdominal pain, weakness and nausea. Also, they have been considered as potential carriers of bacteria pathogenic to the surface of preharvest fruits and vegetables in contact with soil. In this note, we reported the presence of *D. coronata* in the framework of diverse soil nematodes samplings in orchards of Abasto town, Buenos Aires province, Argentina. Soil samples taken from tomato growing (*Lycopersicon esculentum*) were processed in the laboratory by the centrifugation method, while collected roots were observed directly under stereomicroscope in order to isolate nematodes. Specimens were identified by morphological and morphometric characteristics. Results showed the presence of *D. coronata* in agricultural soil and in association with root galls, caused by the plant-parasitic nematode, *Nacobbus aberrans*. Females were the only isolated stage. The detection of this nematode in greenhouses where dogs, cats and poultry live together without any health control highlights the importance of applying proper hygiene measures during agricultural practices to avoid contamination of fruits and vegetables and prevent infections in domestic animals and humans. This report constitutes the first record of the *Diploscapter* genus with the species *D. coronata* in Argentina.

Keywords

Diploscapter coronata • *Lycopersicon esculentum* • soil nematodes • facultative parasite • root galls • *Nacobbus aberrans*

- 1 Centro de Estudios Parasitológicos y de Vectores, (CEPAVE). CONICET, Universidad Nacional de la Plata, Argentina. augustokan@gmail.com
- 2 CEPAVE Miembro de la carrera de Investigación de la Comisión de Investigaciones Científicas (CIC) de Buenos Aires. (CCT, La Plata, CONICET).
- 3 CEPAVE Miembro de la carrera de Investigación de CONICET

RESUMEN

Diploscapter coronata es un nematodo de vida libre que habita el suelo y se alimenta de bacterias. Se ha encontrado en compost, alcantarillas o suelos agrícolas y como parásito facultativo de insectos y vertebrados, incluyendo al hombre. Los síntomas clínicos incluyen: sensibilidad epigástrica, diarrea, dolor abdominal y náuseas. Incluso, han sido considerados potenciales portadores de bacterias patogénicas asociadas con la superficie de frutas y vegetales pre-cosechadas en contacto con el suelo. En esta nota, se reporta la presencia de *D. coronata* en el marco de diversos muestreos de nematodos de suelo, en huertas de la localidad de Abasto, provincia de Buenos Aires, Argentina. Las muestras de suelo tomadas de cultivo de tomate (*Lycopersicon esculentum*) se procesaron en el laboratorio mediante el método de centrifugación, mientras que las raíces recolectadas fueron observadas directamente bajo microscopio estereoscópico con el fin de aislar los nematodos. Los especímenes fueron identificados por características morfológicas y morfométricas. Los resultados mostraron la presencia de *D. coronata* en suelos agrícolas y en agallas radicales causadas por el nematodo parásito de plantas *Nacobbus aberrans*. Las hembras fueron el único estadio aislado. La detección de este nematodo en invernaderos donde gatos, perros y aves de corral viven juntos sin ningún control sanitario, realza la importancia de adoptar medidas apropiadas de higiene durante las prácticas agrícolas para evitar la contaminación de frutas y verduras y prevenir infecciones en animales domésticos y el hombre. Este informe constituye el primer registro del género *Diploscapter* con la especie *D. coronata* en Argentina.

Palabras claves

Diploscapter coronata • *Lycopersicon esculentum* • nematodos de suelo • parásito facultativo • agallas radicales • *Nacobbus aberrans*

INTRODUCTION

Rhabditid nematodes are very abundant in all types of soil and sediments of fresh-water bodies and play important ecological roles mainly as primary consumers, but also as animal parasites (1).

The genus *Diploscapter* Cobb, 1913, includes free-living bacterivorous nematodes characterized by an unusual head with hook-like appendages and membrane-like lateral lips (4). Among them *Diploscapter coronata* (Cobb, 1893) Cobb, 1913 is a cosmopolitan rhabditid nematode obtained from decaying banana roots in Fiji Island (7), and later from agricultural soils, compost, sewage and as a facultative parasite of vertebrates (cats, dogs, snakes, tigers), even humans,

with clinical signs of the gastrointestinal or genitourinary systems (5, 6, 10, 12, 13). In addition, their potential as vectors of food-borne pathogenic bacteria, to the surface of preharvest fruits and vegetables in contact with soil has been determined (9).

In South-America, the presence of this nematode has been reported for Venezuela, Brazil, Peru and Paraguay (4).

In this work, we extend this distribution, with the report of *Diploscapter coronata* in the framework of a nematode soil study of different crop areas including tomato samplings in Argentina, constituting the first report of the genus and species for this country.

MATERIALS AND METHODS

Specimens were isolated during 2015, in a survey of *Phytophagous nematodes* associated with tomato crops, from four orchards located in the Abasto town (34°56'35" S 58°5'30" W), Buenos Aires province, Argentina. Twenty sub-samples of 20 cm deep were taken to form a composed sample from each site. Roots of tomato plants with signs of damage by phytonematodes were also collected.

In the laboratory, each composed sample was homogenized and sieved. Nematodes were isolated from soil by sugar centrifugation method (100-cm³ soil by each site) for 5 minutes at 2700 rpm, and from roots by examination and dissection under stereoscopic microscope (10). Characterization was carried out by a morphometric and morphological analysis, using a light microscope, following specific bibliography (4). Measurements were given in micrometers. Photographs were taken using an Olympus DP-71 microscope camera.

RESULTS AND DISCUSSION

Adults of the rhabditid nematode *Diploscapter coronata* were observed in two of the four sampled orchards. A total of nine females were obtained: six from the agricultural soil for tomato growing and three from roots. Nematodes were characterized by annulated cuticle, two pairs of lips in the mouth region; lips transformed into a pair of medial, outwardly acting, distally bifurcate fossorae and a pair of lateral lamellae; rhabditoid type of oral cavity and absence of glottoid apparatus, vulva median with a crosswise slit (figure 1 A-C, page XXX). Measures are presented in table 1 (page XXX).

Diploscapter coronata has been collected previously from soil and decaying plant material in association with forest and horticultural crops, and as a facultative parasite of animals, even humans (1, 12). However, morphometric data of these nematode populations are scarce in the world (table 1, page XXX).

In this work, females were isolated from agricultural soil and in association with root galls, caused by the plant-parasitic nematode, *Nacobbus aberrans* (Thorne, 1935) Thorne & Allen, 1944. Specimens were observed inside and over the surface of galls. *Phytophagous nematodes* cause wounds in the roots favoring the penetration of other pathogens, and are mainly responsible for major damage to plants (2, 11).

Bacterial-feeding of *Diploscapter coronata* could be one of the reasons of the presence of this nematode in association with root galls of tomato crops, which may be attracted by bacteria of the rhizosphere being able to penetrate plant tissues through wounds produced by plant parasitic nematodes.

Males of *D. coronata* were not observed in our study. The causes are unknown, although may be due to the low or nule occurrence of these nematodes in soil. According to this, the only description of males for this species was presented by Ali Asghar Shah and Shavish Vaid, 2015.

Cats, dogs, and human infections by *D. coronata* were also reported (5, 12). In this case, the life cycle is characterized by an adult worm, generally inhabiting the stomach wall. Mature female produces fertilized ova that are excreted with the host's feces; eggs are spread by rain into ponds, canals and rivers, where they hatch into first stage larvae; the infective larvae are then ingested accidentally by a host. Therefore, potentially serious consequences could be produced when people are exposed to contaminated environments with infected feces.

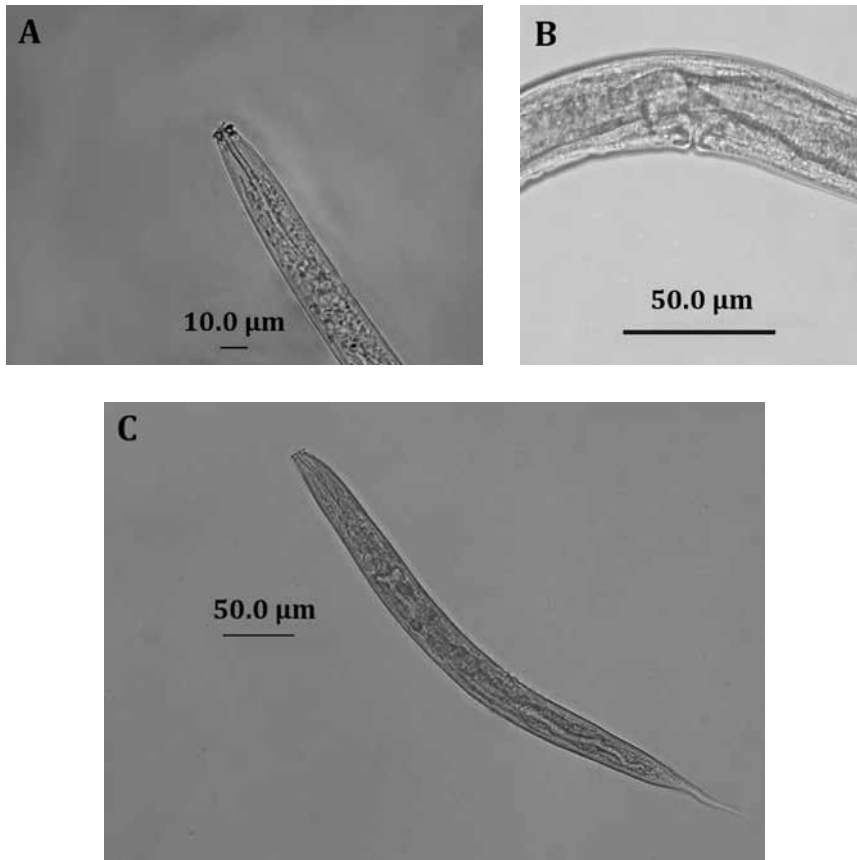


Figure 1. Light micrographs of *Diploscapter coronata* female: A. Anterior end. Note the two fossae and the pair of lamellae in the head region. B. Vulval region. C. Entire worm.

Figura 1. Fotos al microscopio óptico de hembras de *Diploscapter coronata*. A. Extremo anterior. Nótese los dos fosores y el par de lamellas en la región cefálica. B. Región vulvar. C. Nematodo vista general.

The clinical symptoms include epigastric tenderness, diarrhea, crampy abdominal pain, weakness and nausea (5).

Studies showed that *Diploscapter coronata* was found in great abundance and in all stages of development in human stomachs containing little or no hydrochloric acid (6, 12).

Worms may establish themselves and multiply in diseased female urino-genital systems as well as in hypohydrochloric stomachs, and live as juvenile in the skin, particularly in cases of scabies. Athari and Mahmoudi (2008), described the report of a man who developed a mild gastrointestinal discomfort including diarrhea associated with feces containing many larvae of *Diploscapter coronata*.

Table 1. Morphometry of *Diploscapter coronata* females, isolated in present work and those provided by the bibliography. All measurements: mean \pm s.d (range) are in μm except V%, (NA: not available).

Tabla 1. Morfometría de *Diploscapter coronata* (hembras) determinada en el presente trabajo y aquellas proporcionadas por la literatura. Todas las medidas: media \pm desvío (rango) se expresan en μm excepto V% (N/A: no disponible).

Characters	Populations of <i>D. coronata</i> mean \pm s.d (range)				Present work
	Abolafia, J.; Peña-Santiago, 2007	Eyualem <i>et al.</i> , 1998 (Ethiopian population)	Eyualem <i>et al.</i> , 1998 (Iranian population)		
Total length	437.6 \pm 39.5 (358-504)	427.5 \pm 25 (395-480)	350.9 \pm 23.4 (317-403)		357.04 \pm 75.99 (270-464.3)
Stoma width	N/A	3 \pm 0.2 (2-3)	2.3 \pm 0.1 (2-2.5)		2.32 \pm 0.00 (2.32)
Stoma length	18.3 \pm 1.4 (16-22)	21.8 \pm 0.9 (20-23)	20.9 \pm 1.2 (19-23)		19.47 \pm 1.24 (18.6-20.8)
Esophagous length	95.3 \pm 5.0 (90-109)	108.5 \pm 5.2 (95-114)	67.5 \pm 2.7 (64-73)		83.96 \pm 2.99 (81.2-88.1)
Beginning of basal bulb from anterior end	N/A	N/A	N/A		67.72 \pm 2.57 (64.9-69.6)
Nerve ring from anterior end	70.5 \pm 4.0 (66-85)	70 \pm 3 (65-77)	66.5 \pm 3.1 (60-71)		54.28 \pm 5.59 (48.7-60.3)
Excretory pore from anterior end	78.0 \pm 9.0 (69-100)	79 \pm 4.9 (70-89)	75.1 \pm 3.9 (67-82)		73.74 \pm 8.72 (60.32-83.5)
Maximum body width	26.3 \pm 3.1 (20-32)	24.3 \pm 1.8 (21-28)	21.7 \pm 1.5 (20-25)		24.11 \pm 3.86 (18.6-27.8)
Vulva body width	N/A	N/A	N/A		23.88 \pm 4.73 (16.2-27.8)
Posterior end width	12.0 \pm 1.7 (9-17)	11.3 \pm 1 (10-14)	9.6 \pm 0.8 (9-12)		19.92 \pm 4.22 (16.2-25.5)
Tail length	68.8 \pm 5.9 (55-80)	55.5 \pm 6.3 (45-71)	51 \pm 9 (36-67)		54.26 \pm 12.23 (41.7-69.6)
Vagina length	7.3 \pm 0.8 (7-9)	N/A	N/A		6.03 \pm 1.27 (4.6-6.9)
V %	N/A	53.2 \pm 1.5 (50-55.7)	53.7 \pm 2.1 (50.1-58.4)		53 \pm 4.00 (48-58%)

Nematodes were first diagnosed as the rhabditiform larvae of *Strongyloides stercoralis*, a soil-transmitted helminth, causal agent of strongyloidiasis, commonly found in tropical and subtropical areas.

Authors considered that while there are few reports of these worms as a real parasite, the presence of *Diploscapter* larvae in feces, should be considered in the differential diagnosis of Strongyloidiasis in endemic areas, because of the similarity between larvae of *D. coronata*, and *Strongyloides stercoralis* under microscopic examination (5).

The health significance of *D. coronata* is also based on their potential as vectors of food-borne pathogenic bacteria to the surface of fruits and vegetables in contact with soil (9).

In Argentina and other regions of South-America, is common to find in

agricultural production models, subsistence farming, in which much of crop production is used to maintain the farmer, and the farmer's family, leaving the rest for sale or trade. In some of them, dogs, cats and poultry live together in contact with crops, without any health control.

For these reasons, the isolation of *D. coronata* in this study in greenhouses under this type of agricultural management highlights the divulgation of hygiene and prevention measures in activities involving soil management, to avoid contamination of fruits and vegetables and infections in domestic animals and humans.

The detection of this nematode in the present work, constitutes the first report of the *Diploscapter* genus with the species *D. coronata* in Argentina.

REFERENCES

1. Abolafia, J.; Peña-Santiago, R. 2007. Nematodes of the Order Rhabditida from Andalucía Oriental, Spain. The Genera *Protorhabditis* (Osche, 1952) Dougherty, 1953 and *Diploscapter* Cobb, 1913, with Description of *P. spiculocrestata* sp. n. and a Species *Protorhabditis* Key. *Journal of Nematology*. 39: 263-274.
2. Agrios, G. N. 2005. *Plant pathology*. New York. Ed. Elsevier Academic. 922 p.
3. Ali Asghar, S.; Shavish, V. 2015. Description of *Sclerorhabditis miniata* n. sp. and First Description of Male of *Diploscapter coronatus* Cobb, 1913 (Nematoda: Rhabditidae). *Journal of Nematology*. 47(2): 153-158.
4. Andrassy, I. 1983. A taxonomic review of the suborder Rhabditina (Nematoda: Secernentea). Paris. ORSTOM. 241 p.
5. Athari, A.; Mahmoudi, M. R. 2008. *Diploscapter coronata* Infection in Iran: Report of the first case and review of Literature. *Iranian Journal of Parasitology*. 3: 42-47.
6. Chandler, A. C. 1938. *D. coronata* as a facultative parasite of man, with a general review of vertebrate parasitism by rhabditoid worms. In the *Journal of Parasitology*. 30: 44-55.
7. Cobb, N. A. 1913. New nematode genera found inhabiting fresh water and non-brackish soils. *Journal of Washington Academy of Sciences*. 3: 432-433.
8. Eyuaem, A.; Karegar, A.; Nabil, H.; De Ley, P. 1998. A redescription and ultrastructural study of *Diploscapter coronatus* (Cobb, 1893) Cobb, 1913 from Ethiopia and Iran. *Russian Journal of Nematology*. 6(1): 17-22.
9. Gibbs, D. S.; Anderson, G. L.; Beuchat, L. R.; Carta, L. K.; Williams, P. L. 2005. Potential role of *Diploscapter* sp. strain LKC25, a bacterivorous nematode from soil, as a vector of food-borne pathogenic bacteria to preharvest fruits and vegetables. *Journal of Applied Environmental Microbiology*. 71(5): 2433-2437.

-
10. Manzanilla-López, R. H.; Marbán-Mendoza, N. 2012. Practical Plant Nematology. México. Ed. Colegio de Post-graduados. 876 p.
 11. Martinotti, M.D.; Castellanos, S. J.; González, R.; Camargo, A.; Fanzone, M. 2016. Efecto nematocida de extractos de ajo, orujo de uva y alperujo de aceituna; sobre *Meloidogyne incognita*, en vid, cv Chardonnay. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 48(1): 211-224.11.
 12. Morimoto, N.; Korenaga, M.; Yagyu, K. 2006. Morphological observations and the effects of artificial digestive fluids on the survival of *Diploscapter coronata* from a Japanese patient. *Journal of Helminthology*. 80: 341-348.
 13. Thorne, G. 1961. *Principles of Nematology*. New York. Ed. McGraw Hill Book Co. 553 p.

ACKNOWLEDGEMENTS

We would like to thank Dr. Eliseo Chaves for his knowledge, cooperation and predisposition during this research.