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Morphological and cytogenetic description of a new species of *Parasitylenchus* Micoletzky, 1922 (Tylenchida, Allantonematidae) parasitizing *Harmonia axyridis* (Pallas, 1773) (Coleopera, Coccinellidae) in Argentina

Descripción morfológica y citogenética de una nueva especie de *Parasitylenchus* Micoletzky, 1922 (Tylenchida, Allantonematidae) parasitando *Harmonia axyridis* (Pallas, 1773) (Coleopera, Coccinellidae) en Argentina

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Resumen

Se cita y describe por primera vez para Argentina *Parasitylenchus pseusobifurcatus* sp.n. (Tylenchida, Allantonematidae), parásito de adultos de *Harmonia axyridis* (Pallas, 1773) (Coleopera, Coccinellidae). Esta nueva especie está muy cercana a *P. bifurcatus* Poinar Jr. y Steenberg, 2012, por la característica de tener todas las hembras vermiformes y los machos inmaduros la punta del apéndice caudal bifurcada, pero se diferencian entre otros por el tamaño del gubernáculo. Se describe su fórmula cariotípica.

Summary

Parasitylenchus pseusobifurcatus sp.n. (Tylenchida, Allantonematidae) is described for the first time for Argentina as a parasite of adults of *Harmonia axyridis* (Pallas, 1773) (Coleopera, Coccinellidae). The new species is closed to *P. bifurcatus* Poinar Jr. & Steenberg, 2012, by the characteristic of having the tail tip of all vermiform females and immature males bifurcated, but they differ by the size of the gubernaculum. Its karyotype formula is described.

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Introduction

The genus *Parasitylenchus* was erected by Micoletzky in 1922 and was originally proposed as a subgenus of *Tylenchus*. The type species *P. dispar* was described by Fuchs in 1915 and placed in the genus *Tylenchus*. Fuchs later described several species that are now included in the genus *Parasitylenchus*. They are, namely: *P. ligniperdi* (Fuchs 1929), *P. morosus* (Fuchs 1929), *P. sulphurous* (Fuchs 1929), *P. chalcographi* (Fuchs 1938), *P. poligraphi* (Fuchs 1938), *P. pusilli* (Fuchs 1938). The latter three species were described as subspecies of *P. dispar*. In addition to the species described by Fuchs, Wuelker (1923-1929) described *P. hylastis* and *P. cossoni*, the former species within the genus *Tylenchus*. *P. scolyti* was described by Oldham in 1930. Ruhm described *P. grossmannae* in 1954. For a more complete listing of the species in this genus the reader is referred to Wachek (1955) and Ruhm (1956). Later, in 2012 Poinar & Steenberg described another species *P. bifurcates* in Denmark.

In this study we found another species of the genus *P. pseusobifurcatus* sp.n., from Gran La Plata, Buenos Aires, Argentina. In the order Tylenchida, cytogenetically 40 genera have been analyzed, belonging to eight families, all of them parasitic species of agricultural plants. The order presents a wide diploid number range that varies between 2n = 10 and 2n = 56. All genera have a fairly stable number, mostly 2n = 16 or 2n = 18, except the genus *Ditylenchus* Filipjev, 1936, whose only species studied *D. dipsaci* (Kuhn, 1857) Filipjev, 1936, has a wide distribution of values of 2n that has a minimum of 24 and a maximum of 56 (Siddiqi 2000). In the genus *Meloidogyne* Göldi, 1892, the chromosomes are of the holocinetic type, and they do not present a chromosome with a localized centromere (Márquez-Corro et al. 2017, Paliulis et al. 2012). From the order Tylenchida, no species has been cytogenetically analyzed, being the first study of the chromosomes of tylenchid nematodes parasite of insects.

Material and methods

Insect.- Adult coccinellid insects were collected by hand from September 2016 to April 2017 on plant species *Cucurbita maxima* var. *zapallito* (Carrière) Millán, 1947 (round green zucchini), at a locality near Gran La Plata, Colonia Urquiza (34°96'72"S, 58°04'96"W). The beetles were distributed in individual plastic containers. In total, 260 adults of *Harmonia axyridis* (Pallas, 1773) (Coleopera, Coccinellidae), were sampled and dissected. The coccinellids adults were dissected in Petri dishes filled with distilled water under a stereomicroscope.

Nematodes.- Living nematodes were removed from the hemocel of adult host, and then they were killed by placing them in distilled water at 60 °C for 2 minutes. They were fixed in 50% TAF solution in water for 48 hours and then into pure TAF (Poinar 1975). All the specimens were used for photographing in Olympus BX51 microscope with Olympus DP71 camera. Measurements of common nematode body features were performed on 16 fixed nematode specimens of each nematode stage of life cycle.

Cytogenetic.- For cytogenetic studies the females were placed in distilled water for 30 minutes and then fixed in Carnoy's solution (3 parts of pure ethyl alcohol and 1 part of acetic acid). The cytogenetic preparations were made by squash and stained with 45% acetic orcein (Rodríguez Gil et al. 2009). The representative cells of each stage were photographed with an Olympus microscope with digital camera DP 71, with the program DP controller 3.3.1.292 and the images were processed with the programs GIMP version 2.8.16 and inks-cape gnu general public license, version 2.

Results

Tylenchida Thorne, 1949
Sphaerularioidea Lubbock, 1861
Allantonematidae Pereira, 1931
Genus *Parasitylenchus* Micoletzky, 1922

Parasitylenchus pseusobifurcatus sp.n.

Figures 1 – 12

Description: Primary heterosexual female: develops from preadult fertilized female that enter into the host, in its body cavity, obese sausage-shaped curving ventrally, with round, wrinkled ends. Cephalic region usually overgrowns by body expansion. Stylet with minute basal knobs. Vulva subterminal. Uterus filling most of body, with ovary convoluted in anterior region. Ovoviviparus. Juveniles develop into bisexual forms in host´s body cavity. Secondary small heterosexual female, less obese and in larger numbers than primary females. Yellowish-brown body. Stylet similar to that in primary female. Excretory pore near stylet base. Ovary with two flexures. Tail short and conoid. Male in host´s body cavity smaller than secondary female but not obese. Cephalic region continuous. Excretory pore at the level of nerve ring. Testis outstretched. Tail tapering to a rounded tip. Bursa absent. Spicules cephalated, in bowling bottle shape or triangular. Gubernaculum present, the same size as the spicule and polymorphic. The tip of the caudal appendage of all vermiform females (Fig. 8) and immature females is bifurcated (Fig. 4).

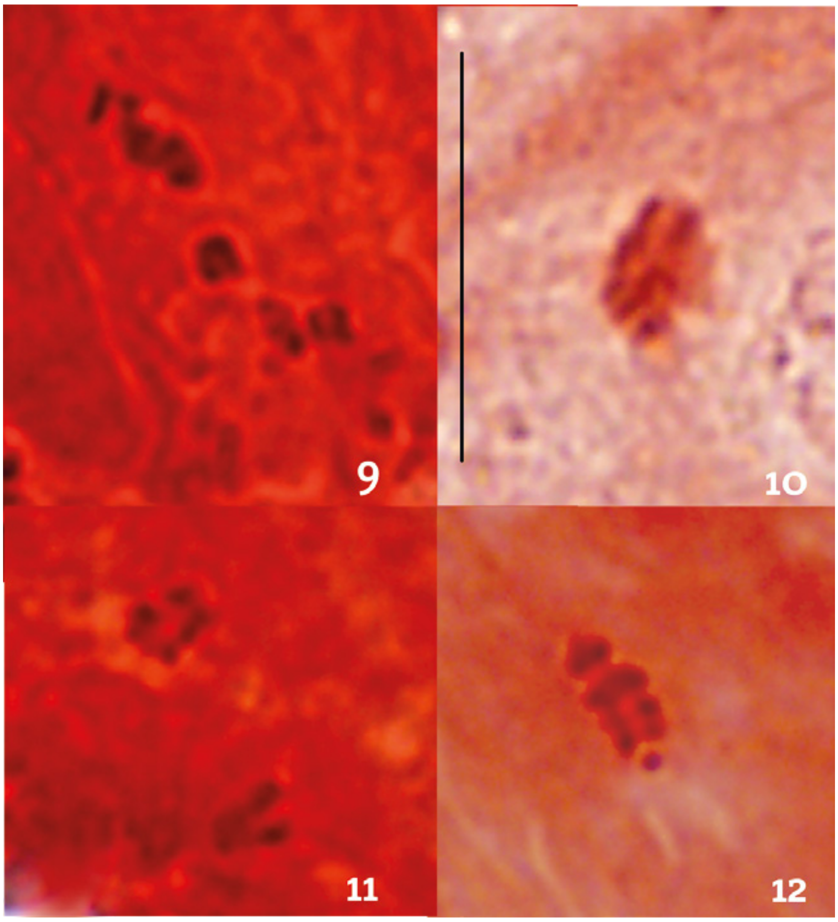
First generation entomoparasitic females (Fig. 1): develops from preadult fertilized female that invades host´s, obese, sausage-shaped curving ventrally, with round, wrinkled ends. Cuticle annulated. Vulva subterminal, uterus filling most of body cavity, with ovary convoluted in anterior region, ovoviviparous.

Second generation parasitic females (Fig. 2): ovoviviparous. The subsequent generations of parasitic (swollen, gravid) females tend to be shorter than the first generation forms. Young parasitic females of all generations have a relative long tail and long vulva-tail measurement. As they mature, the tail shortens, the vulva becomes more posterior in position, the stylet becomes faint, the pharyngeal glands become atrophied and the excretory pore, nerve ring, anus and vulva are more difficult to locate. The ovary is usually reflexed 2-3 times in the parasitic females.

Figure 1 – 8. *Parasitylenchus pseudobifurcatus* sp.n. (1) First generation entomoparasitic female. (2). Second generation parasitic female. (3) Anterior end of vermiform infective female. (4) Posterior end of vermiform infective female, showing tail bifurcated. (5) Posterior end of male showing the spicules triangular shaped and gubernaculum laminar. (6) Posterior end of male showing the spicules in bowling bottle shape. (7) Posterior end of male showing the spicules and gubernaculum long and wide. (8) Posterior end of female showing the tail bifurcated. Bars = (1) 100 µm; (2) 500 µm; (3, 6) 20 µm; (4, 5, 7, 8) 50 µm.

Males (Figs. 5, 6, 7): pharyngeal glands atrophied; tail tip angular-truncate, showing bifurcation in juveniles; spicules straight, in bowling bottle shape or triangular, with wide base. The gubernaculum long can assume several shapes, laminate and in bowling bottle shape. Usually it is straight, however it may be bent upward, or rarely appear double. Unfortunately the small size of this structure hinders further analysis. The bursa is absent

Vermiform (infective) female (Figs. 3, 4): the cuticle is very thick, and ridged both transversely and longitudinally. It has an obtuse head with the lip region somehow constricted. The straight stylet lacks basal knobs or thickenings and the dorsal and subventral gland openings are located within three stylet lengths from the base of the stylet. The excretory pore is fairly posterior and opens opposite to or posterior to the nerve ring. The



Figures 9 – 12. Cytogenetics of *Parasitylenchus pseudobifurcatus* sp.n. (9) Mitotic cell with 9 chromosomes. It is possible distinguish both sister chromatids. (10) Mitotic cell with 8 chromosomes. (11) Meiotic cell with 4 pairs of chromosomes. Two poles of Anaphase I. (12) Meiotic cell with 4 pair of chromosomes and plus one. Metaphase I. Bar: 9, 10, 11, 12: 10 µm.

hemizonid is located just posterior to the nerve ring. The pharyngeal glands may extend to the tip of the ovary. The uterus and ovary are fairly short until the female mates, at which time the uterus expands with circular sperm cells. The great majority of vermiform females inside the host have mated. The tail tip of all vermiform females and immature males is notoriously bifurcated.

Type host: Adults of *Harmonia axyridis* (Pallas, 1773) (Coleoptera, Coccinellidae). The collection was made at midday in a farm near La Plata city, during the summer of 2016/2017. The insect was found over round green zucchini´s flowers.

Type locality: Colonia Urquiza (34°96'72"S, 58°04'96"W), Gran La Plata, Buenos Aires province, Argentina.

Type material: deposited in Helminthological Collection of the Museo de Ciencias Naturales de La Plata. Holotype: NT20058. Paratypes deposited in Nematological Collection of CEPAVE nº 00378

The prevalence observed was 25%. The number of nematodes per *H. axyridis* was ranged from five to eighty-two

Cytogenetics results.- In the three types of females analyzed, stages of mitotic metaphases and meiotic anaphases were observed with two chromosomal complements. In cells with mitosis we observed 8 and 9 chromosomes (Figs. 9, 10); cells with meiosis presented four bivalents chromosome and others cells with four bivalents plus a univalent chromosome. (Figs. 11, 12). The chromosomes of this species are very tiny: the largest is 1.60 microns in metaphase. In meiotic metaphase, both homologous chromosomes are paired along their longitudinal axis, pretending not to have a localized centromere. No figures that denote the presence of chiasmas were observed. In mitotic metaphase, the largest chromosome usually has a break at one end that gives it an "L" appearance; both arms are separated at an approximate 45 degree angle.

Discussion

Current species described only two of the genus *P. bifurcatus* Poinar Jr. & Steenberg, 2012, from Denmark, and *P. coccinellinae* Iperti & van Waerebeke, 1968, from France, are parasitizing Coccinellidae, coincidentally to *Harmonia* spp. Most of the species of the genus are parasites of Curculionidae and a few of Drosophila. *P. pseudobifurcatus* sp. n. is very similar to *P. bifurcatus* by having the bifurcated vermiform infective female and immature male tail. The excretory pore opening at the level of the nerve ring, but it differs from our new species in the presence of the bursa. The gubernaculum is shorter. The male is smaller than our new species. And to *P. coccinellinae* seems to be our new species by the absence of the bursa, but it can be separated by the excretory pore near stylet base. The tail is wide, short and pointed. Iperti and van Waerebeke (1968) described the young female that it would seem to be the infective female, as having a short stylet (4–5 µm) with basal thickenings that are lacking in *P. bifurcates* and in *P. pseudobifurcatus* sp. n. They also show the spicule in lateral view as curved with the base separated, having our new species spicule in bowling bottle shape. They did not give a complete description of the vermiform infective female. They described two females, the young female world seems to be the infective and the other one is a gravid female, and a male. Also the measurements are incomplete so we do not incorporate *P. coccinellinae* into table 1.

The cytogenetic study of the Tylenchida order is very scarce and fragmentary: of the approximately 30,000 species taxonomically described as plant parasites (Siddiqi 2000), less than 100 are the species from which something of their chromosomes is known. This is the first cytogenetic contribution since there are no previous data of tylenchid nematodes parasites of insects (Eisenback & Triantaphyllou 1991; Triantaphyllou & Hirschmann 1980). The morphology of the chromosomes shows that they would not present a localized centromere, ie holokinetic chromosomes (Márquez-Corro et al. 2017). We describe for the first time two karyotypes compatible with the presence of a sexual chromosome (Adamson 1989). The haploid chromosome number described for *P. pseudobifurcatus* sp. n. would be the lowest of the tyilenchid nematodes and would be close to the plant parasitic nematodes, with genus *Helicotylenchus* Steiner, 1945, that presents n = 5 chromosomes and to *Meloidodera* (Chitwood, Hannon & Esser, 1956), which has description of species that also have n = 5 chromosomes (Triantaphyllou & Hirschmann 1980).

Conclusions

The genus *Parasitylenchus* is characterized by having three types of adults inside the host: a first generation parasitic female, a second generation parasitic female and a male. The eggs produced by the first generation females develop into juveniles that mature to adults, mate and keep eggs and juveniles inside the host. The juveniles from the second generation females exit the host, killing it, and the final molt and mating occurs in the environment where the infective females find new hosts. As parasitism results in the reduction of the fat body and partial or complete atrophy of the reproductive organs of the host that compromises its fecundity, it can therefore be considered as an agent of biological control to management abundant populations of this invasive species. Although *P. pseudobifurcatus* sp. n. is an insect parasite that kills its host, it does not belong to entomopathogenic nematodes, which kill its host with the help of an associated bacterium, relatively quickly (between 24 and 48 hours after infection). Thus, we consider our new species as a parasite that castrates and slowly kills its host, co-existing both a time necessary for the development of the nematode. Future genomic studies will help to determine how closely related these populations are.

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