MALENCHUS HERRERAI N. SP. (NEMATODA: TYLENCHIDAE) FROM THE RAINFOREST OF PERU WITH ADDITIONAL INSIGHTS ON THE MORPHOLOGY OF THE GENUS

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ABSTRACT

Mundo-Ocampo, M., O. Holovachov, and T. J. Pereira. 2015. *Malenchus herrerai* n. sp. (Nematoda: Tylenchidae) from the rainforest of Peru with additional insights on the morphology of the genus. Nematropica 45:158-169.

A new species of the genus *Malenchus* from the rainforest of Peru is described. *Malenchus herrerai* n. sp. was collected from an epiphytic moss associated with shade trees of coffee plantations (*Coffea arabica*) in the Chacra, Santa Fe, La Merced region, Peru. This new species is characterized by having a very well developed (robust and long) stylet. So far, it is the species with the largest stylet in the genus *Malenchus*. The robust stylet of *M. herrerai* n. sp. and its association with an epiphyte moss may indicate a host-parasite relationship between both organisms. In addition to the species description, morphological information of cuticular and sensory organs (amphids and pro-phasmids) is provided. These findings complement the existing scarce information on the morphology of the genus.

Key words: diagnosis, *Malenchus*, morphology, scanning electron microscope, taxonomy.

RESUMEN

Mundo-Ocampo, M., O. Holovachov, y T. J. Pereira. 2015. *Malenchus herrerai* n. sp. (Nematoda: Tylenchidae) procedente de la selva tropical de Perú con percepciones adicionales sobre la morfología del género. Nematropica 45:158-169.

Una nueva especie del género *Malenchus* recolectada en la selva tropical de la región de La Merced en Perú, Sur América es descrita. *Malenchus herrerai* n. sp. fue encontrada en una planta de musgo que se desarrolla en la parte foliar de los árboles que proveen sombra a las plantas de café (*Coffea arabica*) en la chacra de Santa Fe. Esta nueva especie se caracteriza por tener el estilete más robusto y largo que las especies actualmente incluidas en este género. La asociación de *M. herrerai* n. sp. con la planta epifita de musgo podría indicar una relación estrecha planta-parásito entre ambos organismos. Además de la descripción de *M. herrerai* n. sp., se provee nueva información morfológica de la región cuticular y de los órganos sensoriales (anfidios y pro-fasmidios). Esta nueva información complementa y contribuye al poco conocimiento existente sobre la morfología del género.

Palabras clave: diagnosis, *Malenchus*, morfología, SEM, taxonomía.

INTRODUCTION

During a survey to study the distribution of *Meloidogyne exigua* Goeldi, 1892 in a cultivated coffee (*Coffea arabica*) area, in the Santa Fe farm, La Merced region in the Peruvian rainforest, samples of an epiphyte moss were collected from trees commonly grown in the area to provide shade to the coffee plants. Several nematodes, including some representatives of the family Tylenchidae Örley, 1880, were recovered. Nematode specimens belonging to the genus *Malenchus* Andrássy, 1968 were later characterized as a new species.

The genus *Malenchus* was proposed by Andrássy (1968) and it is currently represented by approximately 28 nominal species (Siddiqi, 2000; Geraert, 2008). It occurs in all kinds of soil, dried and moist; it can also be associated with roots of grasses and trees, in forest

litter and moss. In addition, the genus is widespread occurring on all continents except Antarctica, with the greatest number of species described in North America (Andrássy, 1981); *M. bryophilus* Steiner, 1914 is considered the most common species in the genus, being reported in 22 countries (Andrássy, 1981).

Siddiqi (2000) proposed a subdivision of the genus *Malenchus* into three subgenera: *Malenchus* Andrássy, 1968; *Neomalenchus* Siddiqi, 1979; and *Telomalenchus* Siddiqi, 2000. According to Siddiqi (2000), these subgenera can be distinguished on the basis of their muscular medium bulb, absence of lateral membranes in the vulva, and the number of lines in the lateral field (LF), as observed under the light microscope (LM). On the other hand, Andrássy (1981), and more recently Geraert (2008), did not completely recognize this division and synonymized the subgenus *Neomalenchus* with *Malenchus*. In this work, we follow Geraert (2008) thus considering *Malenchus* under the subfamily Tylenchinae Örley, 1880 (Marcinowski, 1909), family Tylenchidae.

Under the LM, the LF of *Malenchus* appears as a plain band with smooth or crenate margins (i.e., the outer lines of the LF). However, the inner lines are, in most cases, imperceptible. Therefore, the correct number of lines in the LF of *Malenchus* can only be unambiguously resolved with the use of scanning electron microscopy (SEM). In *Malenchus*, the LF appears as a protruding band with about 12 lines; yet in *Telomalenchus*, the lateral field might have four to six lines.

Andrássy (1981) recognized the genus *Malenchus* as being morphologically close to *Tylenchus* Bastian, 1865 and *Aglenchus* Andrássy, 1954, thus as part of the subfamily Tylenchinae within Tylenchidae. However, the author pointed out that *Malenchus* differed from these genera mostly on the shape of the head, prominent annulations of the cuticle, and the number of lines in the LF (Andrássy, 1981). On the other hand, Siddiqi (1979) considered *Malenchus* as being closely related to the genera *Ottolenchus* Husain & Khan, 1965, *Miculenchus* Andrássy, 1959, and *Duosulcius* Siddiqi, 1979 under the subfamily Duosulciinae Siddiqi, 1979, although still within Tylenchidae. According to Andrássy (1981), the genus *Malenchus* is morphologically very homogenous, and therefore the use of SEM is crucial for detecting novel diagnostic characters in descriptions of new species. Herein, we describe a new *Malenchus* species named as *M. herrerai* n. sp. based on data provided from SEM and LM observations. Additionally, the morphology of *M. herrerai* n. sp., in particular sensory organs, is compared to other *Malenchus* species. Finally, the position of *Malenchus* within Tylenchidae as well as the need for further studies on this group is discussed.

MATERIALS AND METHODS

Several samples of hanging moss plants growing on the top of coffee shade trees were collected. Mixed subsamples of plant tissues were cut in small pieces and placed in a Baermann funnel for nematode extraction during a period of 24 hr. Recovered nematode specimens were concentrated in a 12.5 ml volume of distilled water, transferred to a 25 ml glass vial, and immediately fixed in a 5.0% buffered (7.0 pH) formalin solution at 55 to 60°C for several days. Subsequently, the specimens were processed following the Seinhorst's method (1959) and mounted for LM observations at UCR-Nematode Systematics laboratory.

For LM observations, fixed specimens were rinsed several times in distilled water to remove the excess of formalin and then processed to anhydrous glycerin following the glycerin-ethanol method (Seinhorst, 1959) as modified by De Grisse (1969). After the specimens were fully infiltrated in 100% glycerin, permanent mounts were prepared to continue with LM observations. Measurements and LM images were prepared manually with a camera lucida on a Zeiss Axioskop and a RT Color Spot digital camera (Diagnostics Inst. Inc., Sterling, MI, USA) coupled to a Nikon Eclipse E600, respectively. Drawings were made using Olympus BX61 microscope equipped with differential interference contrast (DIC) optics.

Additional specimens were prepared for Scanning Electron Microscope (SEM) observations according to Mundo-Ocampo *et al*. (2003). Nematodes were rinsed with several changes of 0.1 M phosphate buffer and post-fixed for 4 hr in a 2.0% osmium tetroxide solution. Post-fixed specimens were then rinsed with several changes of cold 0.1 M phosphate buffer, within a 15-min period and dehydrated through a series of aqueous solutions of ethanol ranging from 20% to 100% absolute ethanol. Dehydrated specimens were critical-point dried in a Tousimis Autosandri®-810 (Rockville. MD, USA). Specimens were mounted in several positions, on top of double sticking copper tape attached to aluminum stabs, coated for 1 to 3 min with a 25-nm layer of gold palladium in a Cressington® 108 Auto spotter coater and observed with a XL 30-FEG Phillips® 35 Scanning Electron Microscope at 10 kV.

RESULTS

SYSTEMATICS
Malenchus herrerai n. sp.
(Figs 1-5; Table 1)

Description

Female: Median size body, straight, or slightly ventrally arcuate or curved as an opened "C" shape; maximum body width 19 to 24 µm. Cuticle strongly annulated, annuli width at mid-body about 1.3 to 1.8 µm. Head elevated, with truncate appearance dorso-ventrally compressed; cephalic region continuous with body (narrower in ventral or dorsal view), cephalic framework weak, sclerotization not observed. Posteriorly to the cephalic framework a series of annuli (6 to 10) exhibit a distinct fine longitudinal striae, similar to the fine annuli in the tail posterior to the end of the lateral fields. Rectangular labial plate with an oral aperture well differentiated, labial papillae not visible, large and conspicuous amphidial apertures, "S" shaped, typical of *Malenchus* species, starting anteriorly within the labial plate (as an oval or round opening hole or slit) and continuing on the lateral side of the head as irregular longitudinal slits. Stylet robust, with a strong shaft including rounded oblong and strong knobs, directed backwards; stylet ranging from 15 to 18 µm in length. Esophagus thin or slender, metacorpus oval to spindle-shaped; basal bulb pyriform. Oesophageal gland opening close to the stylet base. Excretory pore visible at about half of the oesophageal basal bulb length; 73 to 86 µm from the anterior end. Hemizonid not observed. Under the LM, the lateral field appears as two smooth or slightly crenate, incisures; however, SEM observations show a range of 10 to 12 longitudinal lines, beginning shortly after the cephalic region, at about the level of the stylet shaft (between the 7th to 9th annuli from the anterior end) and ending around half of the tail length. Width of lateral field is about 3.0 µm at mid body and occupies between 12.0 to 13.5% of the total-body width. Latero-dorsal advulval papillae or prophasmids are located 8 annuli (about 5 to 12 µm) anterior to vulva, both papillae are opposite in their positions within an interval of 5 annuli of each other. Vagina thin-walled. Vulval opening a transversal slit (located in an oval depression) sunk into body, with epitigma or inconspicuous vulval flaps. No dikes around the vulva were observed. Posterior uterine sac slightly more than half of the corresponding body diameter at vulva level. Ovary single, outstretched; spermatheca ovoid, filled with spermatozoa. Tail tapering towards an acute terminus, sharply pointed (with needle-like tip), finely striated to tip. Anus not commonly visible. In some specimens the lateral field under SEM, appears to be broken or divided at the level of anus. Typical phasmids were not observed in the tail region. Vulva-anus distance about the same as tail length or about 6 to 7 times anal body width. Tail annuli become much finer posterior to ending of lateral field with longitudinal markings visible only with SEM.

Male: Common but less frequent and smaller than females. General morphology similar to female, no sexual dimorphism. Cloacal lips distinct and protruding without hypoptygmata. Spicules ranging from 16 to 23 µm long; gubernaculum length from 5.5 to 8 µm; bursa adanaI small but prominent (could be considered as an outgrowth of the lateral field) 27 to 40.9 µm in length. Spicules ventrally bent, with a slightly tapering open tip. Gubernaculum short and thin. Tail similar to female, tapering gradually to sharply pointed, needle-like tip, straight or only terminally curved, and varying in length from 57 to 113 µm, about 17 to 24% of entire body length.

Type material: Holotype female (Slide No. 31417) and Paratypes (Slide Nos. 31418, 31419, and 31420) were deposited at the University of California, Riverside, Nematode Collection (UCRNC).

Type habitat and locality: Epiphyte moss associated with coffee plants. Chacra: Santa Fe. In the region of La Merced, Peru.

Etymology: Malenchus herrerai n. sp. is named after one of the most prestigious Peruvian nematologists, Ing. Eleodoro Herrera Alvariño.

Diagnosis and relationships: Malenchus herrerai n. sp. is particularly characterized by the size and shape of the stylet. Most *Malenchus* species have a shorter and delicate stylet (less than 14 µm). The closest species to *M. herrerai* n. sp. are *M. macrodorus* Geraert & Raski, 1986 and *M. laccocephalus* Andrássy, 1981. Both species have a stylet ranging between 13 to 14 µm in length; however, the stylet in *M. herrerai* n. sp. is very well developed and also larger (15 to 18 µm). The stylet knobs are well developed, probably the most robust among all *Malenchus* species. In addition, the position of the excretory pore in *M. macrodorus* is at base of the esophagus bulb whereas in *M. herrerai* n. sp. is located about half of the bulb length. Observations based on SEM show protuberances or "dikes" on the inner surface of the annuli anterior and posterior to the vulva in *M. macrodorus* and *M. laccocephalus*; such structures are absent in *M. herrerai* n. sp. The number of annuli between the vulva and the anus in *M. herrerai* n. sp. is, on average, 55 annuli, whereas in *M. laccocephalus* it ranges from 70 to 86 annuli.

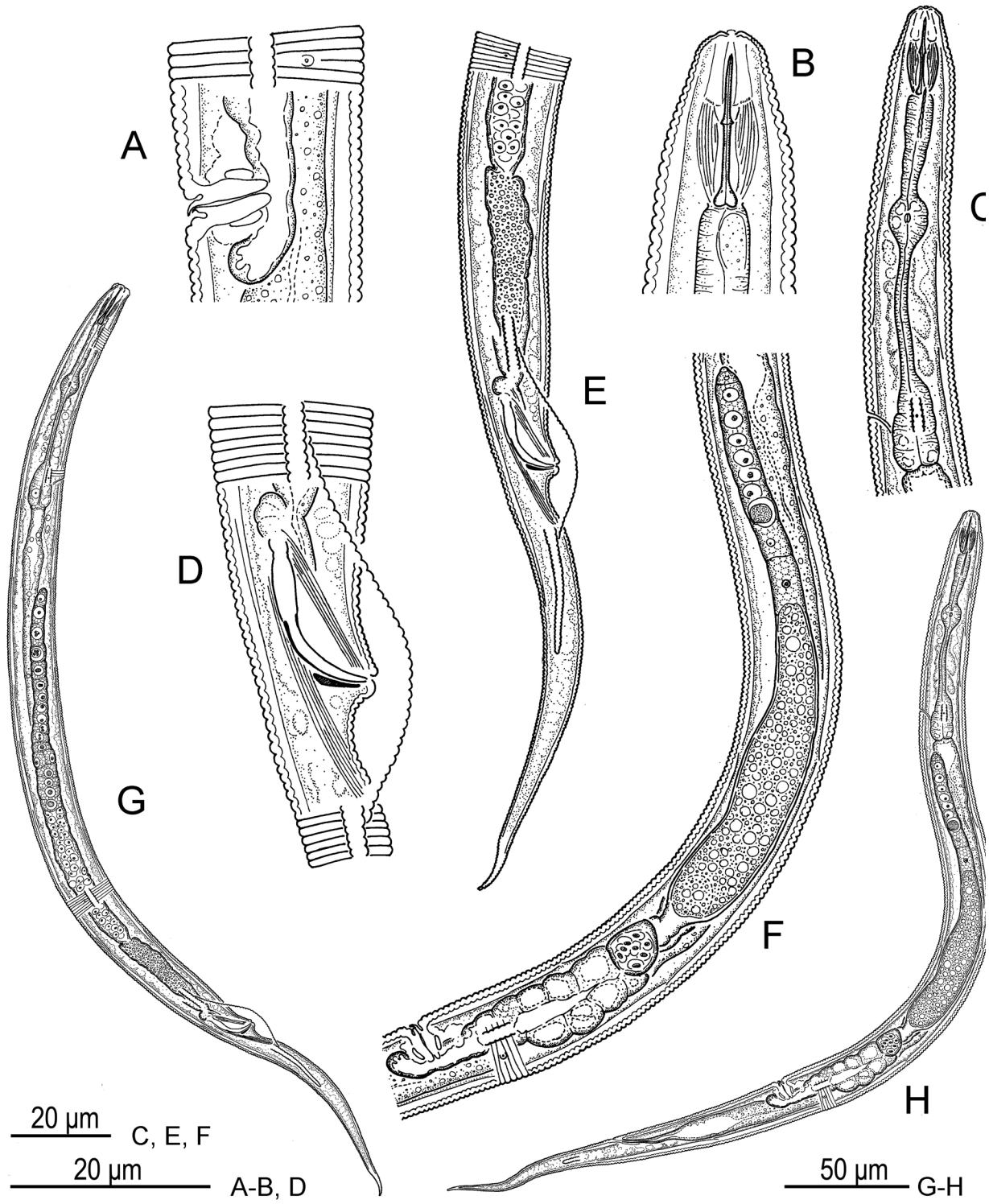

Fig. 1. Line drawings of *Malenchus herrerai* n. sp. A: Female vulval region, showing vulva and terminal portion of the reproductive system; B, C: Female anterior region, showing stylet and esophagus and position of the excretory pore; D: Male view of the cloacal region showing spicules and caudal allae; E: Posterior region of a male showing the reproductive system and lateral lines: F: Reproductive system of the female showing a single ovary, spermatheca and postuterine sac; F: Entire view of male; H: Entire view of female.

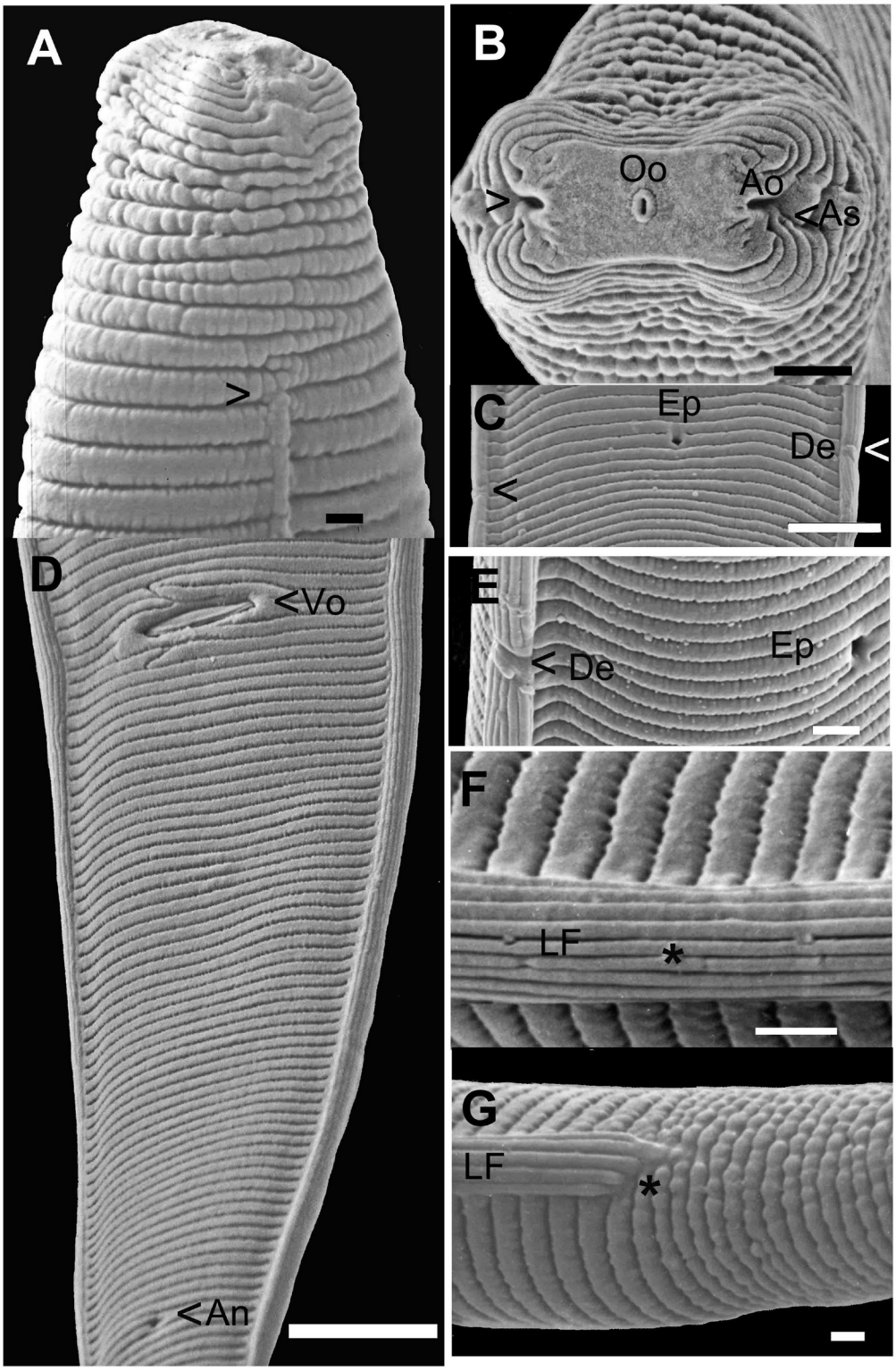

Fig. 2. Scanning electron microscope (SEM) photographs of a female of *Malenchus herrerai* n. sp. A: Lateral-dorsal view showing cephalic annulations and point of origin of LF (black arrow); B: Face view showing labial plate and amphidial openings; C: Ventral view, showing position of the excretory pore and the deirids on the lateral fields (black and white arrows); D: Ventral view showing the vulva opening, the crescent plates covering the vulva, and more posterior the anus opening; E: Close view of the deirid and excretory pore; F: Lateral view showing the lateral field and longitudinal lines (*); G: End of lateral field; ornamentation of annules in the posterior region of the tail (*). (Abbreviations: LF = lateral field; Oo = oral opening; Ao = amphidial opening. As = amphidial slit; Ep = excretory pore; De = deirids; Vo = vulva opening; An = anus opening. Scale bars: A = 1 µm, B, C, E = 5 µm; D = 10 µm; F = 2 µm; G = 3 µm).

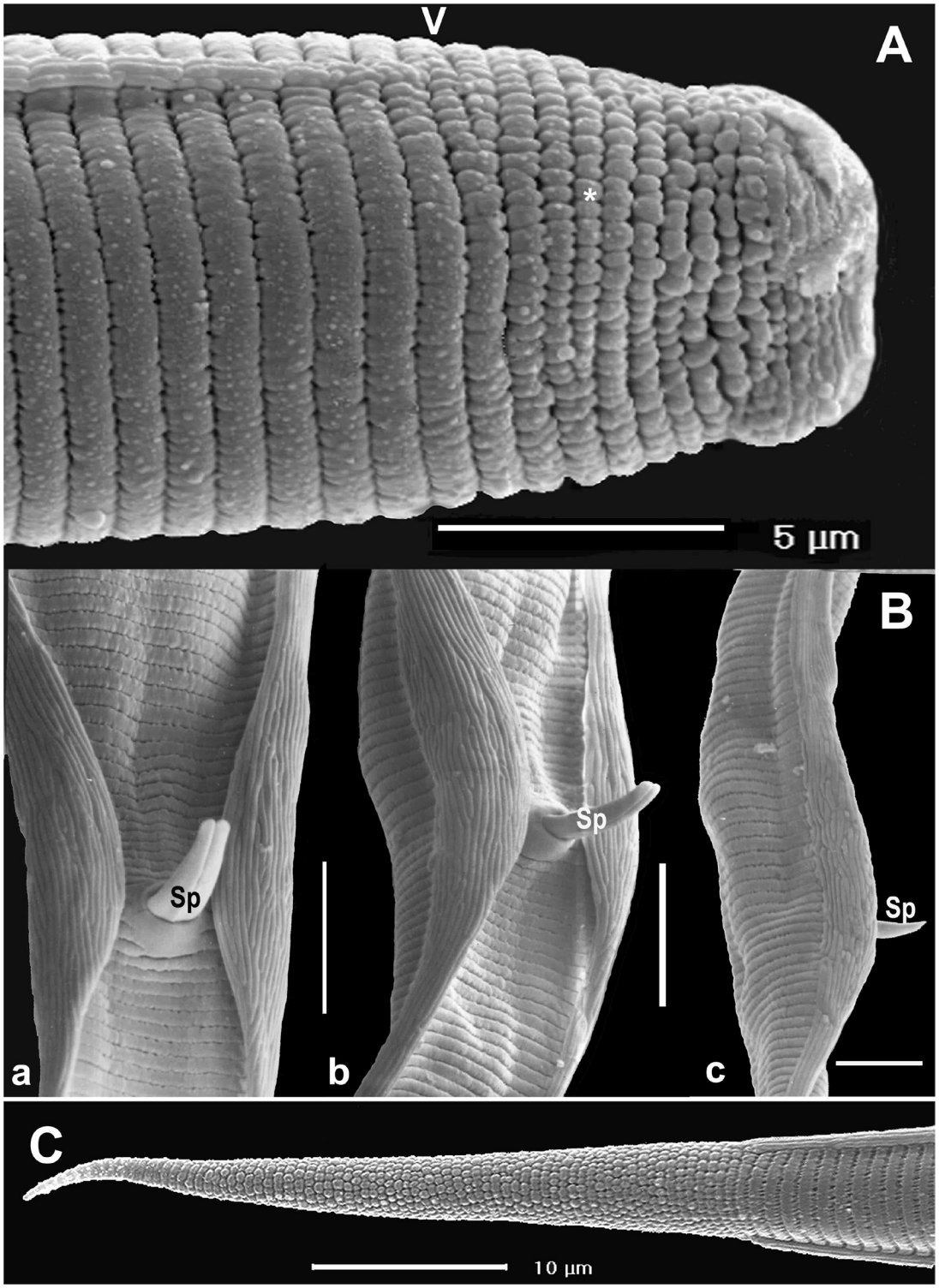

Fig. 3. SEM photographs of a male of *Malenchus herrerai* n. sp. A: Ventro-lateral view of a male showing the pattern of annulations on the cephalic region and the point where the lateral field begins (arrow). The most anterior annulations are characterized by a fine ornamentation (*); B: Serial views (a = ventral, b = latero-ventral, and c = lateral) of the cloacal region showing different positions of the caudal allae or bursa and the spicules. (Abbreviation. Sp = spicules. Scale bars: A = 5 µm; B, C = 10 µm); C: Terminal portion of tail showing ornamentation of annules after the terminal point of lateral fields.

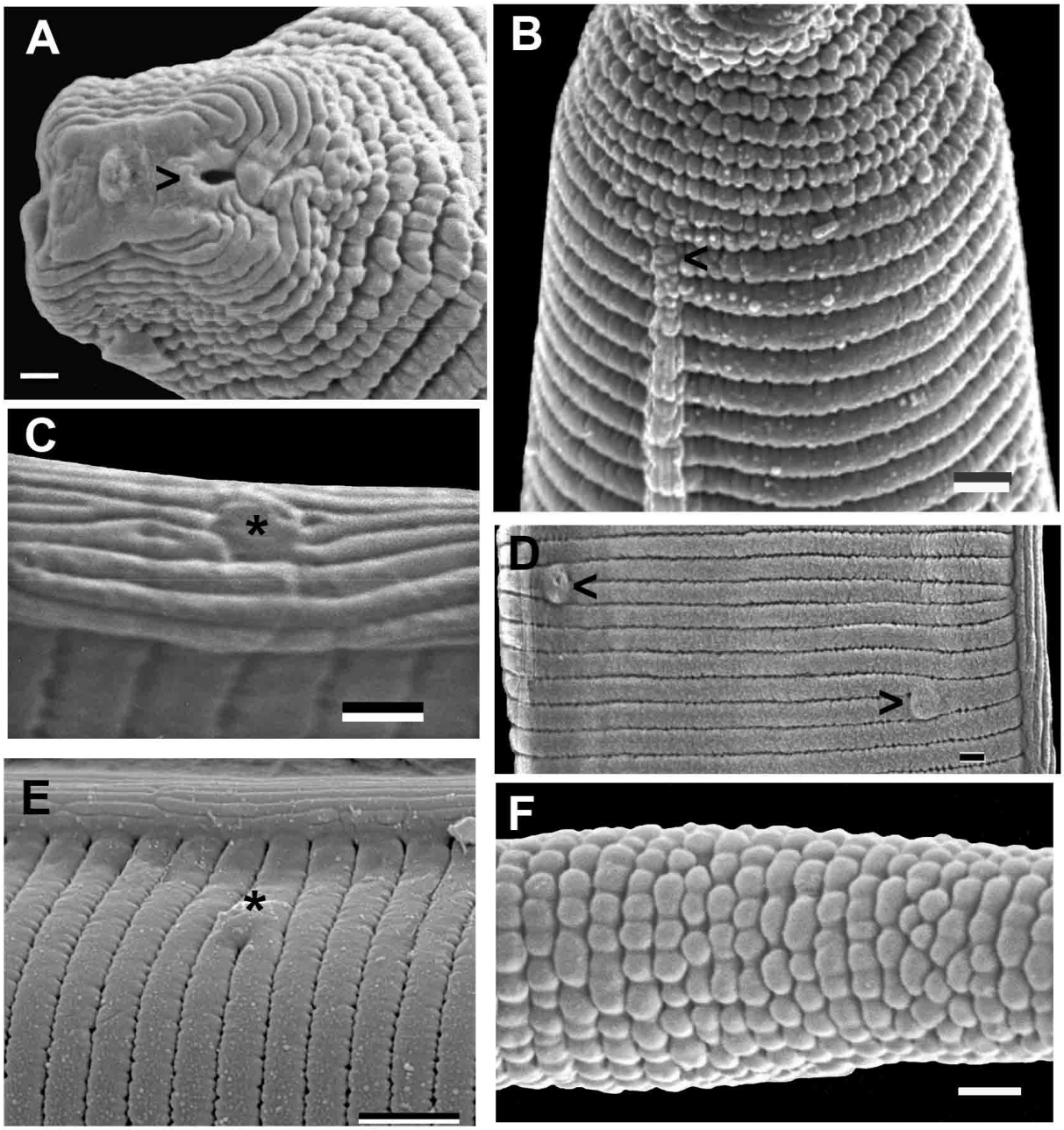

Fig 4. SEM photographs of a male of *Malenchus herrerai* n. sp. A: Face/lateral view of cephalic region showing cephalic annulations and the amphidial opening (black arrow); B: Lateral view, showing ornamentation of cephalic annulations. Black arrow indicates point where lateral field starts; C: Lateral field showing deirid position (*); D: Dorsal view showing the position of prophasmids (black arrows); E: Close up of a prophasmid (*); F: Asymmetric ornamentation of annules on the tail. (Scale bars: A, B, C, D = 1 µm; E, F = 2 µm).

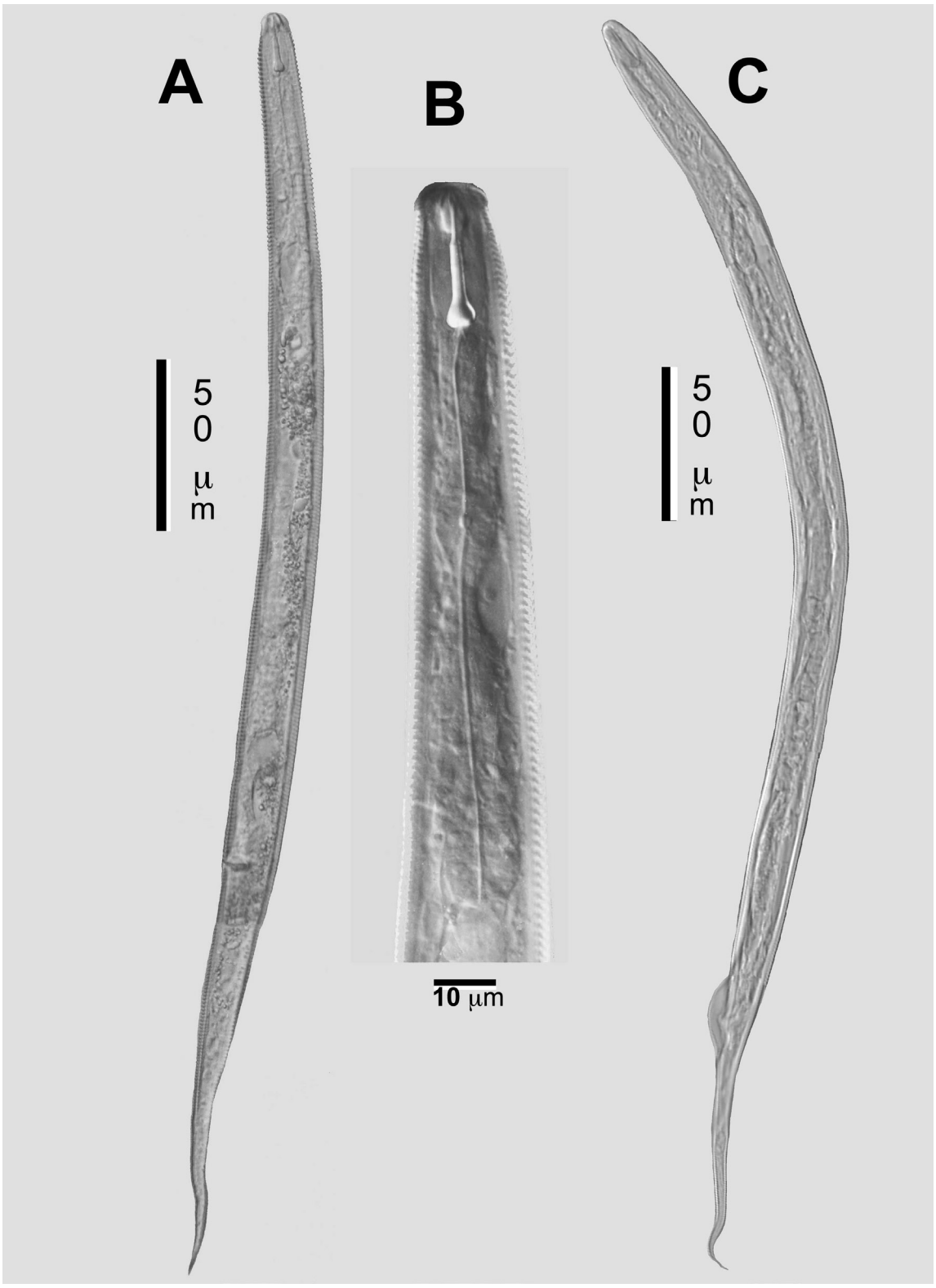

Fig. 5. Light micrographs of an entire female and male of *Malenchus herrerai* n. sp. A: Entire body of female; B. Anterior region of female showing a close up of the stylet and esophagus region; C: Entire body of male.

Table 1. Morphometrics of *Malenchus herrerai* n. sp. from an epiphytic moss plant from the Santa Fe farm, La Merced region, Peru. All measurements are in microns (µm) and in the format: mean ± standard deviation (range). Abbreviations for morphological characters follow Bert and Geraert (2000), unless it is stated otherwise.

Character[z]	Holotype	Female Paratypes	Male Paratypes
n	-	12	7
L	419.7	415.2 ± 14.0 (382.5-432.5)	396.82 ± 17.3 (396.8-450.5)
L’	353.4	342.5 ± 11.5 (315.2-353.4)	301.37 ± 15.9 (301.4-347.7)
a	19.6	22.8 ± 1.5 (18.8-24.5)	21.6 ± 21.6 (21.9-21.9)
b	4.50	5.1 ± 0.4 (4.5-5.9)	4.1 ± 4.0 (4.1-4.1)
c	15.0	17.3 ± 1.2 (14.1-18.4)	22.8 ± 22.6 (22.7-23.0)
c'	6.50	6.5 ± 0.9 (3.9-7.5)	8.9 ± 8.6 (8.6-8.7)
Head to vulva	283.0	275.4 ± 9.0 (254.0-286.6)	-
Stylet	17.8	17.0 ± 0.7 (15.3-17.9)	17.73 ± 0.4 (17.3-18.2)
Pharynx	97.4	94.4 ± 6.6 (74.4-100.0)	85.46 ± 5.3 (85.5-97.7)
Procorpus	20.4	21.1 ± 2.3 (18.4-27.5)	18.18 ± 2.4 (15.9-22.7)
MB L	12.2	11.3 ± 1.2 (9.2-13.3)	9.09 ± 1.6 (9.1-13.6)
MB W	8.6	7.8 ± 0.9 (7.1-10.2)	13.64 ± 0.3 (13.2-14.1)
Isthmus	25.5	25.9 ± 1.1 (23.4-27.5)	25 ± 2.2 (22.7-29.5)
Basal bulb	18.3	18.6 ± 1.2 (16.3-20.4)	12.73 ± 3.4 (12.7-21.8)
E. pore	87.2	86.1 ± 1.1 (84.2-87.7)	72.73 ± 4.3 (72.7-84.10)
Deirid	87.7	87.1 ± 2.0 (83.6-89.8)	70.45 ± 5.4 (70.5-84.1)
n. r.	71.4	66.9 ± 3.5 (60.1-71.4)	54.55 ± 3.5 (54.6-64.6)
V-a	70.3	67.7 ± 5.2 (61.2-78.0)	-
Max. body width	21.4	20.9 ± 1.5 (19.3-24.4)	18.18 ± 1.6 (15.9-20.5)
Anal body width	10.2	11.3 ± 2.2 (9.2-18.3)	9.09 ± 1.1 (9.1-12.7)
Ovary	160.1	154.3 ± 20.1 (122.4-185.6)	-
PUS	10.2	9.6 ± 0.9 (8.2-11.2)	-
Spermatheca	22.4	25.4 ± 7.4 (13.3-37.7)	-
Tail	66.3	71.9 ± 5.6 (57.1-76.5)	95.45 ± 8.4 (89.6-112.5)
Annul.	1.8	1.5 ± 0.2 (1.3-1.8)	-
Testis	-	-	218.19 ± 24.6 (159.1-222.7)
Spicule	-	-	15.91 ± 2.2 (15.9-22.7)
Gubernaculum	-	-	6.82 ± 0.8 (5.5-8.2)
Bursa	-	-	35.71 ± 4.1 (27.3-40.9)
T %	-	-	54.98 ± 6.6 (36.3-55.0)

[z] MB L = length of medium bulb, MB W = width of medium bulb, n. r. = distance of the nerve ring from the anterior end, PUS = post uterine sac, T% = length of testis as a percentage of body length

DISCUSSION

In the present study, we are consistently adopting the term "cephalic region" to describe the anterior end of the nematode. In previous species descriptions of *Malenchus*, authors referred to "labial region", "lip region", or "head" (Knobloch, 1976; Geraert, 2008;). The amphidial apertures in *Malenchus* are difficult to resolve with LM. Observations based on SEM show these structures as being very elaborated, relatively large, and irregular in shape; starting interiorly within the lateral side of the labial plate and expanding to the lateral side of the cephalic region. The most anterior and visible part of the amphids could be oval or slit shaped and about 1 µm in length. Posterior to this portion, a fissure (external) showing cuticular flat outgrowths with an irregular zigzag appearance, covers an internally wide portion of the amphidial aperture, ending on a lobule formed by the terminal lateral side of the head median annuli.

Similar amphid morphology has been observed and described in other Tylenchidae such as *Filenchus* Andrássy, 1954 (Bert *et al*., 2010). Gómez-Barcina *et al.* (1992) interpreted the amphidial apertures in the subgenus *Malenchus* as very wide, (internally) straight clefts covered by (external) cuticular outgrowths so that finer zigzag clefts remain. In *M. herrerai* n. sp., these cuticular outgrowths include a set of flaps located close to the amphidial opening, projecting from the dorsal side followed by an additional set of flaps on the lateral side of the head. These cuticular outgrowths seem to be characteristic in all observed specimens and they are only found in the subgenus *Malenchus*, but not in the subgenus *Telomalenchus*, where the amphidial aperture is a straight, slightly oblique slit.

The feeding habits of *Malenchus* species are still uncertain, although some authors considered *Malenchus* a fungal feeder (Siddiqi, 2000; Renker *et al*., 2003). Accordingly, *M. herrerai* n. sp. was recovered from an above ground environment embedded in a moss plant. The size and morphology of the stylet may reflect a phytophagous relationship between both the moss plant and the nematode. However, studies on the biology, including host-parasite relationships, are still needed to elucidate its feeding group.

Andrássy (1981) revised the genus *Malenchus* and its worldwide distribution; from the 23 revised species, only two species are reported from South America: *M. acarayensis* Andrássy, 1968 and *M. piauhyensis* Monteiro, 1974 from Paraguay and Brazil, respectively. Therefore, *M. herrerai* n. sp. is the third species reported from that geographic area. Unfortunately, molecular data for this species are not available. In spite of that, *M. herrerai* n. sp. has been described in accordance with traditional diagnostic characters and is thus ascribed to the subgenus *Malenchus*. Nevertheless, we propose that a combination of molecular and morphological data is fundamental for revising generic characters and for a needed phylogenetic analysis of the group.

Based on classical taxonomy, the genus *Malenchus* has been placed in the family Tylenchidae along with other small plant-parasitic nematodes, although in different subfamilies according to different authors, Duosulciinae (Siddiqi, 1979; Siddiqi, 2000) or Tylenchinae (Andrássy, 1981; Geraert, 2008). Small tylenchs such as *Malenchus* species and other Tylenchidae genera have not been related to agricultural crop damage, and therefore, they have been almost entirely ignored in molecular phylogenetic studies. Few molecular studies focusing on tylenchs (Holterman *et al*., 2006; Holterman *et al*., 2009; van Megen *et al*., 2009; Bert *et al*., 2010) have included the genus *Malenchus* (i.e., only *M. andrassyi* Merny, 1970). In these previous molecular phylogenies, *M. andrassyi* is a sister taxon of *Ottolenchus discrepans* Andrássy, 1954 with strong node support favoring Siddiqi's hypothesis (i.e., both genera under Duosulciinae). On the other hand, both taxa were not closely related to other Tylenchidae genera such as *Tylenchus*, *Filenchus*, and *Aglenchus*. Nevertheless, the hypothesis of relationships between *Malenchus* and other Tylenchidae genera needs to be further improved with broader taxon sampling. A better representation of *Malenchus* species is also crucial for testing hypothesis of monophyly and its valid status as a genus as well as its subgenera.

Identification key

Morphological terminology to illustrate some of the characters for *Malenchus* is not consistently applied. Andrássy (1981) recommends considering the following characters for species diagnosis: shape and length of the body; width of cuticular annuli; anterior end and bordering of lateral fields; shape and width of labial region; length of spear; number of annuli along the esophagus and the same with body between vulva and anus; absolute and relative length of tail; relation between tail length and vulva/anus distance; shape of tail tip and length of spicules. However, in consequence of the morphological uniformity in *Malenchus*, there are few distinctive characters to separate species. Expanding our knowledge on the morphology in combination with other characters, including molecular and feeding behavior, may provide sound information on the phylogeny of this group (Table 2).

Table 2. Key to Malenchus species (After Geraert, 2008).

Among the characters used for differentiating the species are structure and origin of lateral field, stylet length, tail length and shape, annulus width, annuli with longitudinal cuticular ornamentation or smooth, body length and head shape. Not used are the crenation or non-crenation of the lateral field and the simple or bilobed spermatheca.

1 Lateral field with six lines ..... *M (T.) williamsi*
- Lateral field with four lines (two closely related species) 2
- Lateral field with two lines (under light microscope) 3
2 Length = 305-340 µm; tail = 44-51 µm; cuticle crenated ..... *M (T.) parthenogeneticus*
- Length = 355-480 am; tail = 52-77µm; cuticle smooth (except anterior) ..... *M (T.) leiodermis*
3 Length = 700-865 µm ..... *M (M.) novus*
- Length below 600 µm 4
4 Tail very long, 130 µm or more; c' = 15-17 5
- Tail shorter, 50-120 µm; c' = 6-16 6
5 Stylet 12 µm long, tail tip twisted ventrally, clasper-like; annuli 1.1-1.3 µm wide at mid-body ... *M (M) pampinatus*
- Stylet 10 µm; tail tip straight; annuli 1.7-2 µm wide ..... *M (M.) platycephalus*
- Stylet 8-9 µm; tail tip filiform, annuli 2.5 µm wide ..... *M (M.) anthrisulcus*
6 Annuli 3 µm wide at mid-body ..... *M (M) fusiformis*
- Annuli less than 2.5 µm wide 7
7 Lateral field originating at level of stylet 8
- Lateral field originating between stylet base and end of median bulb 10
8 Body longer than 500 µm; head much narrower than adjacent body ..... *M (M.) machadoi*
- Body shorter than 500 µm; head not or only slightly narrower than adjacent body 9
9 Annuli 1-1.3 µm at mid-body, stylet 9-11 µm ..... *M (M) ovalis*
- Annuli 1.3-1.8 µm at mid-body, stylet 15-18 µm ..... *M (M.) herrerai* sp. n.
- Annuli 1.7-1.9 µm at mid-body, stylet 9.5-10 µm ..... *M (M.) undulatus*
- Annuli 1.9-2.3 µm at mid-body, stylet 12 µm ..... *M. (M.) pachycephalus*
- Annuli 2-2.5 µm at mid-body, stylet 8-9.5 µm ..... *M (M) solovjovae*
10 Tail short (c' = 6-7), nearly equal in length to vulva-anus distance 11
- Tail longer (c' =8- 16), mostly longer than vulva-anus distance 13
11 Stylet 10-12 µm; head truncate, anteriorly flattened; annuli 1.5 µm wide at mid-body ..... *M (M.) truncatus*
- Stylet 7-10 µm; head narrowing, anteriorly rounded 12
12 Stylet 8-1 0 µm; annuli 1.2-1.5 µm wide at mid-body ..... *M. (M.) parvus*
- Stylet 7-8.5 µm; annuli 1.8-1.9 µm wide at mid-body ..... *M (M.) bryanti*
13 Lateral field originates at level of stylet knobs or 1-3 annuli behind 14
- Lateral field originates 1/4-3/4 of procorpus 17
14 Stylet 8.5-9 µm, delicate ..... *M. (M.) subtilis*
- Stylet 9-9.5 µm, delicate ..... *M (M.) bryophilus*
- Stylet 10-12 µm, delicate 15
- Stylet 13-14 µm, robust ..... *M (M.) macrodorus*
15 Tail tip curved (*M. holochmatus* is probably the same species) ..... *M (M.) gratiosus*
- Tail tip straight 16
16 Head truncate, broad, 6.5-7 µm at base, with one or two basal annuli only ..... *M (M.) pressulus*
- Head rounded, 5-6 a µm wide at base, entirely annulated (with 6 small annuli) ..... *M (M.) neosulcus*
17 Lip region with distinct disc-like structure at apex ..... *M (M) labiatus*
- Lip region without disc-like structure 18
18 Stylet 7-9 µm 19
- Stylet 9- 12 µm 20
- Stylet 12-14 µm basal knobs asymmetrical; body length about 500 µm ..... *M (M.) laccocephalus*
(*M. pyri* is considered a synonym).
19 Tail short (c' = 8-12), 1-1.7 vulva-anus distance, lateral field starts at 1/3-112 of procopus ..... *M (M.) acarayensis*
- Tail longer (c' = 11-16), 1.5-2.1 vulva-anus distance, lateral field starts at 1/2-end of procorpus ..... *M (M.) nanellus*
20 Tail 1.5-2 vulva-anus distance, annuli 1.1-1.6 µm ..... *M (M.) exiguus*
- Tail 1.1-1.5 vulva-anus distance, annuli 1-1.3 µm 21
21 Stylet 11-12 µm, L = 490-590 am, tail terminus dorsally curved ..... *M. (M.) nobilis*
-Stylet 9-11 µm, L = 360-490 am, tail terminus straight or curved ..... *M (M.) andrassyi*
(*M (M.) paramonovi* could be the same species).

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