

## Redefinition of Genus *Malenchus* Andrassy, 1968 (Tylenchomorpha: Tylenchidae) with Additional Data on Ecology

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**Abstract:** *Malenchus* is the second specious genus in Tylenchidae. In the presented study, we examined 22 populations including 12 type/paratype species. Detailed morphology was recovered using light microscopy, scanning- and transmission- electron microscopy. All population and type slides were recorded as picture and video vouchers, which are available online. We have compared inter- or intraspecific variations and extracted taxonomically informative traits. Amended definitions of the *Malenchus* as well as the closely related *Ottolenchus* were given based on a combination of morphology and recent molecular data, and their phylogeny were analyzed in a context of Tylenchidae. Furthermore, we test different fungi and moss as a food resource of *Malenchus* and their feeding behavior is also discussed.

**Key words:** *Duosulcius*, *Filenchus*, morphology, *Ottolenchus*, taxonomy, Tylenchomorpha, *Zanenchus*.

Tylenchidae is one of the most important soil inhabiting nematode groups (Andrassy, 1981), and species belonging to Tylenchidae may constitute up to 30% of the nematode individuals in a soil sample (Yeates and Bird, 1994; Ferris and Bongers, 2006). As early-branching Tylenchomorpha, they do not comprise economically important plant parasites and are characterized by ancestral characters, such as weak feeding apparatus, undifferentiated nonmuscular corpus, filiform tails, and four cell rows in uterus (Luc et al., 1987; Siddiqi, 2000; Bert et al., 2008). Knowledge of their food resources remains limited, albeit, given their numeric importance, this subject is important for tropic guild analysis or soil quality evaluation. Furthermore, their small body size and a lack of clearly homologous characters prevented us from deriving a consistent systematic framework. As a result, the delimitation of taxa in this group remains poorly documented and highly uncertain (Bongers and Bongers, 1998; Yeates, 2003; Ferris and Bongers, 2006).

In this study, we focus on the cosmopolitan genus *Malenchus*, which is the second most specious (after *Filenchus*) in Tylenchidae. Although several species have been proposed, morphology details have been often only poorly described. The only genus review was made more than 30 years ago based on a limited number of morphological details (Andrassy, 1981). Recently, molecular methods have revealed a phylogenetic position for the genus *Malenchus* (Yaghoubi et al., 2015; Qing et al., 2016, 2017), but the need for a review is growing. In this present study, we examined type or paratype of 12 species together with 10 populations worldwide. We do not intend to establish new nor to synonymize current taxon but rather to

summarize morphological variations and analyze the results in a phylogenical context, as most of the taxonomically important characters are generally absent or incomplete in the original description (Qing et al., 2017).

### MATERIALS AND METHODS

All specimen examined in this study are listed in Table 1. Classification of *Malenchus* and Tylenchidae follows Geraert (2008). Geographic distributions were plotted using QGIS 2.8.2 based on original descriptions and other reports (Andrassy, 1981; Geraert and Raski, 1986; Gómez-Barcina et al., 1992; Geraert, 2008; Holovachov, 2014; Mundo-Ocampo et al., 2015; Panahandeh et al., 2015a; Panahandeh et al., 2015b; Yaghoubi et al., 2015; Qing et al., 2016). Measurements and drawings from slides were prepared manually with a drawing tube mounted on an Olympus BX51 DIC Microscope (Olympus Optical, Tokyo, Japan), equipped with an Nikon DS-FI2 camera (Nikon Corporation, Tokyo, Japan) for photography. All examined populations as well as type slides were recorded as a video clips mimicking a multifocal observation through a light microscope (LM) following the video capture and editing procedures (De Ley and Bert, 2002). The resulting virtual specimens are available at <http://nematodes.myspecies.info>. Extraction and examination of female reproductive system was based on the method of Geraert (1973) and Bert et al. (2008). Illustrations were prepared based on LM drawings and modified by Adobe Illustrator CS3 and Adobe Photoshop CS6.

For scanning electron microscopy (SEM), specimens from DESS were gradually transferred to water, then dehydrated in a battery of ethanol solutions and dried by critical point dried with CO<sub>2</sub>. After mounting on stubs samples were coated with gold and observed with a JSM-840 EM (JEOL, Tokyo, Japan) at 12 kV. For transmission electron microscope (TEM), specimens were fixed, ultra-thin sections were cut and sections were stained as detailed by Qing et al. (2017). Sections were observed with a JEOL JEM 1010.

Received for publication March 23, 2017.

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We thank Marjolein Couvreur for SEM and Myriam Claeys for TEM analyses. The first author thanks the China Scholarship Council (CSC) for providing a grant. This work was also supported by a special research fund UGent 01N02312.

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This paper was edited by Zafar A. Handoo.

TABLE 1. Number of cuticle annules and width in different species or populations of the genus *Malenchus*.<sup>a</sup>

Species and voucher no.	Specimen no.	Pharynx	Vulva/cloacal	Total	Annules width	Material	Slide no.
<i>M. acarayensis</i>	2♀♀	66, 68	188, 207	320, 356	1.26 ± 0.08	Qinling, China	XQ048
<i>M. acarayensis</i> C173	5♀♀1♂	59–64/83	163–166/231	280–291/303	1.23 ± 0.10/1.03	Qing et al., 2017	XQ148
<i>M. bryophilus</i> C171	3♀♀	55–70	203–219	351–382	1.38 ± 0.13	Qing et al., 2017	XQ149
<i>M. exiguus</i>	3♀♀1♂	72–74/81	194–198/310	330–333/426	1.14 ± 0.09/1.01	Qing et al., 2016	XQ090, XQ091
<i>M. leioderis</i>	1♀	79	208	340	1.05	Paratypes, Geraert and Raski, 1986	UGMD103431
<i>M. macrodorus</i>	3♀♀1♂	72–75/79	180–185/229	304–320/311	1.43 ± 0.24/1.68	Paratypes, Geraert and Raski, 1986	UGMD103434, UGMD103435
<i>M. malawiensis</i> <sup>b</sup>	1♂1♂	55/63	185/284	295/360	1.43/1.03	Paratypes, Siddiqi, 1979	UGMD100230, UGMD100231
<i>M. nanellus</i>	1♂1♂	63/92	202/234	320/325	0.93/0.84	Paratypes, Siddiqi, 1979	UGMD100223, UGMD100224
<i>M. novus</i>	1♀	60	236	378	1.71	Qing et al., 2016	XQ088
<i>M. ovalis</i> C140	3♀♀1♂	62–64/68	162–164/314	263–277/380	1.19 ± 0.11/1.06	Qing et al., 2017	XQ155
<i>M. ovalis</i> <sup>c</sup>	1♀	71	219	343	1.2	Paratype, Siddiqi, 1979	UGMD 100229
<i>M. pachycephalus</i> C116	3♀♀1♂	46/50	115–118/177	194–196/260	2.34–2.38/1.68, 1.96	Qing et al., 2017	XQ156
<i>M. pachycephalus</i> C161	3♀♀1♂	48–51	129–132/152	209–220/210	2.38 ± 0.15/2.03	Qing et al., 2017	XQ157
<i>M. pachycephalus</i> <sup>d</sup>	3♀♀	58–60	185(?), 138–142	320(?), 229–225	2.15 ± 0.07	Gomez-Barcina et al., 1992	UGMD103002 UGMD103003 UGMD103004
<i>M. parthenogeneticus</i>	1♀	66	199	293	1.02	Paratypes, Geraert and Raski, 1986	UGMD103432
<i>M. parvus</i>	2♀	51, 54	143–147	281–305	1.35 ± 0.14	Paratypes, Brzeski, 1988	UGMD 100851
<i>M. sextineatus</i>	4♀	70–75	182–185	289–296	0.76 ± 0.03	Holotype, Qing et al., 2016	UGMD104304
<i>M. tantulus</i> <sup>e</sup>	1♂1♂	64/70	173/245	308/334	1.4 ± 0.31	Paratypes, Siddiqi, 1979	UGMD100225
<i>M. solerjacae</i>	4♀♀	49–50, 59?	133–135, 196?	230–241, 320?	2.3 ± 0.3	Paratypes, Brzeski, 1988	UGMD 100852
<i>M. undulatus</i>	2♀♀	50, 51	139, 141	251, 273	1.81 ± 0.05	Qing et al., 2017	XQ158
<i>M. williamsi</i>	1♂1♂	67	194/294	320/395	1.6/1.2	Paratypes, Geraert and Raski, 1986	UGMD103428
<i>Malenchus</i> sp. C163	3♀♀	80–85	271–280	436–460	0.98 ± 0.1	Qing et al., 2017	XQ159
<i>Duosulcius acutus</i>	1♀	131	364	>550	0.97	Paratypes, Siddiqi, 1979	UGMD 100227

<sup>a</sup> Annuli number is arranged as ♀/♂ when both genders are present. Annuli width is given in average ± SD, measured as: total length of 30 annuli anterior to vulva/cloacal divided 30. All number counts start from anterior end. Slides number: UGMD: Deposited in Zoology Collections, Ghent University Museum, Belgium. XQ: first author's own collections. LM photo and video vouchers are available in <http://nematodes.myspecies.info>.

<sup>b</sup> Paratype of *Neomalenchus malawiensis* Siddiqi, 1979, synonym of *M. malawiensis* (Siddiqi, 1979) Andrassy, 1981.

<sup>c</sup> Paratype of *Neomalenchus ovalis* Siddiqi, 1979, synonym of *M. ovalis* (Siddiqi, 1979) Andrassy, 1981.

<sup>d</sup> Slide probably contain a mixed population, the counts marked with (?) are probably not belongs to *M. pachycephalus*.

<sup>e</sup> Paratype of *M. tantulus* Siddiqi, 1979, synonym of *M. acarayensis* by Geraert and Raski (1986).

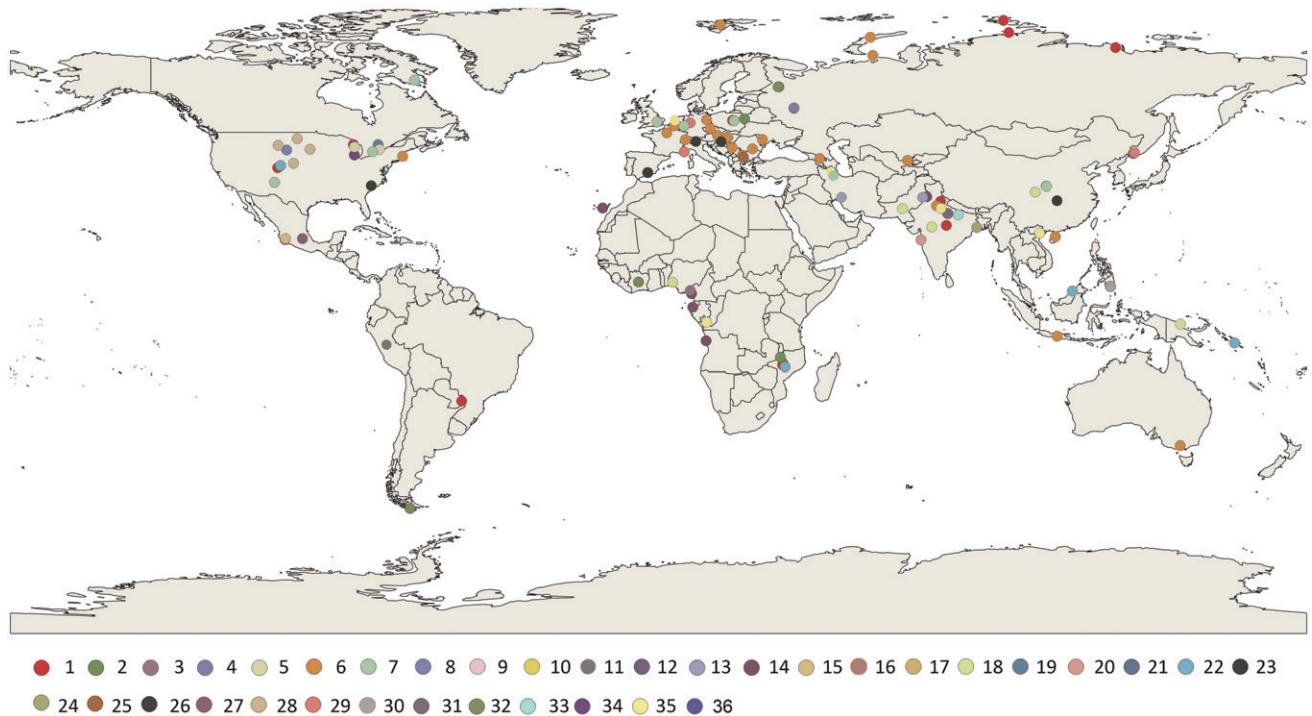


FIG. 1. World distribution of *Malenchus* species. Species are labeled with different colors. 1. *M. acarayensis*. 2. *M. Andrassyi* Merny, 1970. 3. *M. angustus*. 4. *M. anthrisculus*. 5. *M. bryanti* Knobloch, 1976. 6. *M. bryophilus* (Steiner, 1914) Andrassy, 1981. 7. *M. exiguus* (Massey, 1969) Andrassy, 1981. 8. *M. fusiformis*. 9. *M. graciosus*. 10. *M. holochmatus*. 11. *M. herrerae*. 12. *M. kausari*. 13. *M. labiatus* Maqbool and Shahina, 1985. 14. *M. laccocephalus* Andrassy, 1981. 15. *M. leiodermis* Geraert and Raski, 1986. 16. *M. machadoi* (Andrassy, 1963) Andrassy, 1968. 17. *M. macrodorus*. 18. *M. nanellus* Siddiqi, 1979. 19. *M. neosulcus* Geraert and Raski, 1986. 20. *M. nobilis*. 21. *M. novus* Mukhina and Kazachenko, 1981. 22. *M. ovalis* (Siddiqi, 1979) Andrassy, 1981. 23. *M. pachycephalus* Andrassy, 1981. 24. *M. pampinatus*. 25. *M. paramonovi*. 26. *M. parthenogeneticus*. 27. *M. parvus*. 28. *M. platycephalus* (Thorne and Malek, 1968) Andrassy, 1981. 29. *M. pressulus* (Kazachenko, 1975) Andrassy, 1981. 30. *M. sexlineatus*. 31. *M. shaheenae*. 32. *M. solovjovae*. 33. *M. subtilis*. 34. *M. truncatus*. 35. *M. undulates* Andrassy, 1981 and 36. *M. williamsi*.

To test the feeding type, four fungal species (*Flammulina velutipes*, *Lepista nuda*, *Botrytis cinerea*, and *Pleurotus* sp.) were inoculated on potato dextrose agar medium with three repeats for each species and incubated at

25°C for 10 d until the mycelium covered the culture plates. Forty individuals of *M. pachycephalus* and *M. acarayensis* were transferred to each plate and nematodes were extracted by Baermann tray after 2 months.

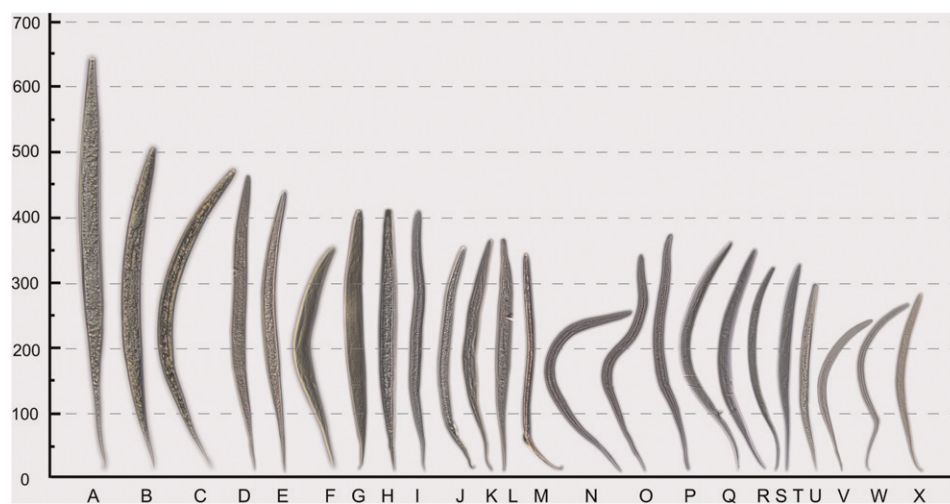


FIG. 2. Body habitus and size in genus *Malenchus*. Size measured in  $\mu\text{m}$  and shown in longitudinal axis. A. *M. novus* from China. B, C. *M. pachycephalus* C116 from China. D. *M. williamsi* from Chile. E. *M. solovjovae*, from Poland. F, G. *M. pachycephalus* C161 from Belgium. H–J. *M. pachycephalus* C161 from Belgium. K, L. *M. exiguus* from China. M. *M. undulates*, from Philippines. N–P. *M. acarayensis* from Spain. Q, R. *M. tantulus*, from Malawi. S, T. *M. nanellus*, from Nigeria. U. *M. parvus*, from Mexico. V–X. *M. sexlineatus*, from Philippines. Female ventral views: A, B, D, G, H, L, T. Female lateral views: C, E, F, I, K, N, O, P, R, S, U, V, X. Male later view: J, M, Q, W.



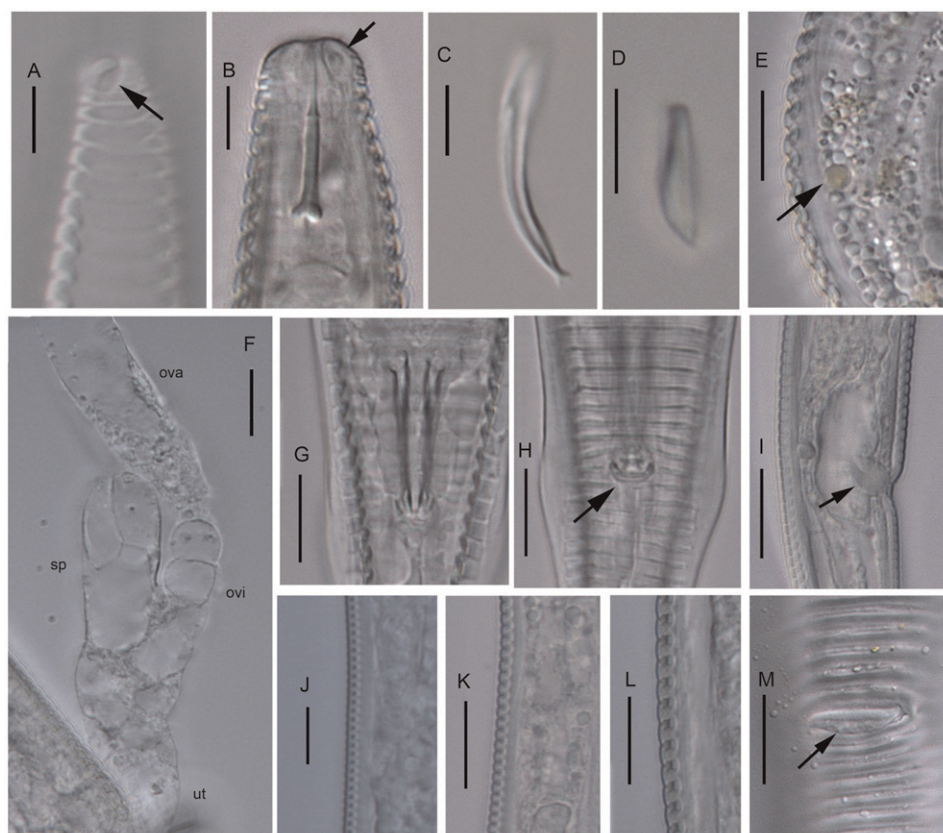


FIG. 3. Selected anatomic structures in *Malenchus pachycephalus* (A–I, L, M), *M. sexlineatus* (J) and *M. exiguus* (K). A. Lateral view of amphideal fovea. B. Head region, arrow indicates ventral view of amphideal fovea. C. Spicule, after dissection. D. Gubernaculum, after dissection. E. Anterior part of intestine, arrow indicates brown granule. F. Female gonad after dissection. G. Ventral view of spicule. H. Ventral view of cloacal, arrow indicates distal end of spicule and gubernaculum. I. Lateral view of vulva region, arrow indicates swollen vagina. J. Folded cuticle of type 1. K. Folded cuticle of type 2. L. Folded cuticle of type 3. M. Ventral view of vulva, arrow indicates epiptygmata. ova = ovarium, ovi = oviduct, sp = spermatheca. Scale bar: A–D, J = 5  $\mu$ m; E–I, K–M = 10  $\mu$ m.

To test moss as a potential host, *Eurhynchium* sp. was isolated from soil habited by *M. pachycephalus*, rinsed five times with distill water to remove attached detritus and then carefully transplant to culture plates with 1% agar in tap water. Controls plates were made using 1% agar in tap water to compare with the two treatments. Forty individuals were transferred to each plate and directly checked in binocular every 3 d for 2 months.

## RESULT AND DISCUSSION

### Taxonomic overview

The genus *Malenchus* was established by Andr  ssy (1968) with *M. machadoi* (formerly *Aglenchus machadoi* Andr  ssy, 1963) as the type species. Later, several new genera have been erected and later synonymized with this genus, for details see Geraert (2008). Within the

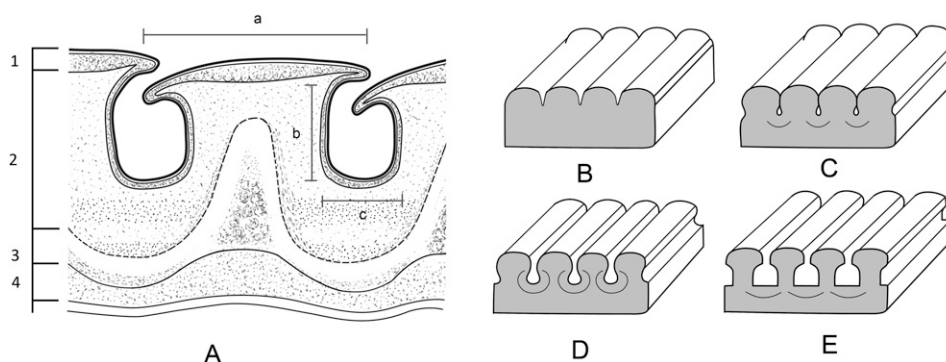


FIG. 4. Diagrammatic example of cuticle layers in *Malenchus* (A) and the variation of the cuticle as observed based on LM observation (B–E). A. Illustration of ultrastructure in *M. pachycephalus* based on TEM, adapted from Qing et al. (2016). (1) epicuticle. (2) Cortical zone. (3) Median zone. (4) Basal zone. (a) Annuli width. (b) Groove depth. (c) Groove width. B–E. Schematically representation of the most common cuticle appearances in Tylenchidae. B. *Filenchus* type with indistinct annuli. C–E. cuticle types in *Malenchus*.

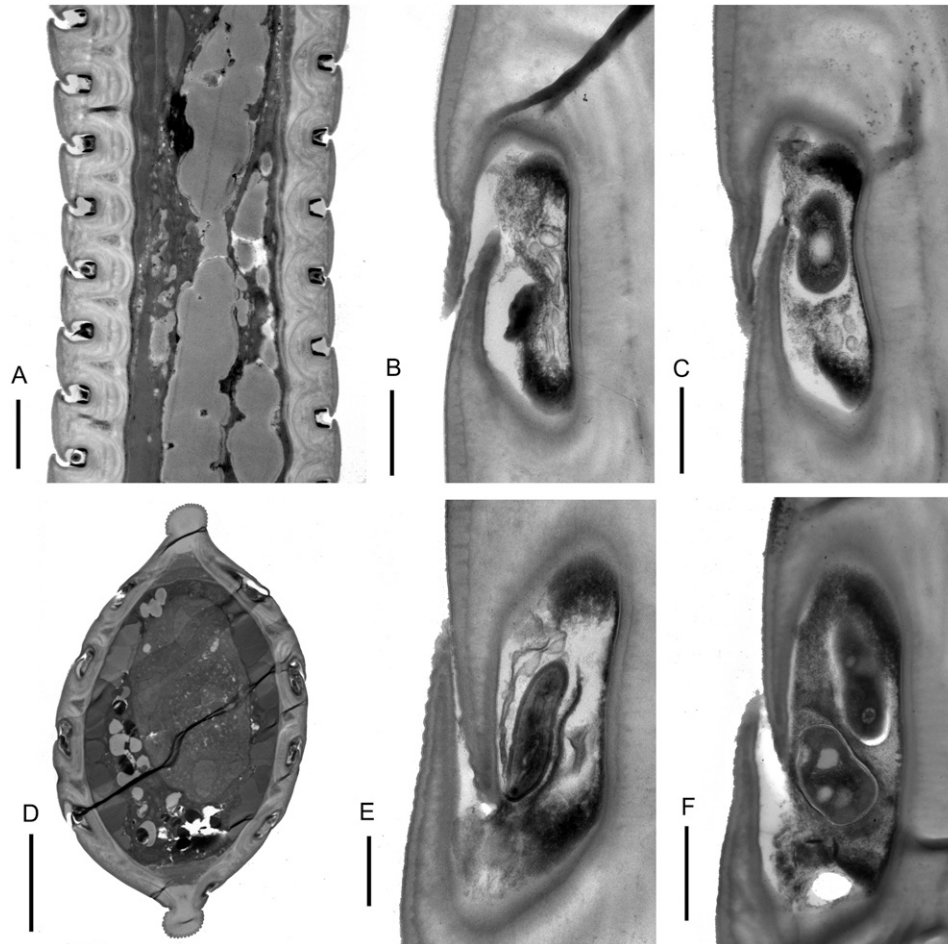


FIG. 5. Cuticle ultrastructure of *M. pachycephalus* C161. A. Longitudinal section in female middle body. B, C, E, F. Unknown organisms present in annulation grooves. D. Cross section in female middle body. Scale bar: A = 2  $\mu\text{m}$ ; B, C, E, F = 0.5  $\mu\text{m}$ ; D = 5  $\mu\text{m}$ .

genus, three subgenera are valid: *Malenchus*, *Neomalenchus*, and *Telomalenchus*. *Neomalenchus* was initially established as a genus for species with indistinct median bulb in *Malenchus* (Siddiqi, 1979), but this genus was synonymized (Andrássy, 1981) in his comprehensive review of *Malenchus* and later considered as a subgenus (Siddiqi, 2000). Subgenus *Telomalenchus* was introduced to accommodate three species (*M. williamsi* Geraert and Raski, 1986; *M. parthenogeneticus* Geraert and Raski, 1986 and *M. leiodermi* Geraert and Raski, 1986) with straight amphideal aperture and less lateral incisures (four or six) (Siddiqi, 2000). Although Andr  ssy (2007) synonymized *Telomalenchus* with *Fraglenchus*, such an action was rejected by Geraert (2008). Currently, *Malenchus* contains 36 valid species and 3 *nomen nuda* (Geraert, 2008; Mundo-Ocampo et al., 2015; Qing et al., 2016).

#### Geographic distribution

*Malenchus* is a cosmopolitan genus and is reported from all continents except for Antarctica (Fig. 1). Among them, *Malenchus bryophilus* (Steiner, 1914) Andr  ssy 1981, and *M. acarayensis* Andr  ssy, 1968 are

the most frequently encountered species, whereas 18 species are only reported once from their type location (*M. angustus* Talavera and Siddiqi, 1996; *M. anthrisulcus* (Sumenkova, 1988) Ebsary, 1991; *M. fusiformis* (Thorne and Malek, 1968) Siddiqi, 1979; *M. graciosus* Andr  ssy 1981; *M. holochmatus* (Singh, 1971) Siddiqi, 1986; *M. herrerae* Mundo-Ocampo, Holovachov and Pereira, 2015; *M. kausari* Khan and Ahmad, 1991; *M. macrodorus* Geraert and Raski, 1986; *M. nobilis* Andr  ssy, 1981; *M. pampinatus* Andr  ssy, 1981; *M. paramonovi* Katalan-Gateva and Alexiev, 1985; *M. parvus* Brzeski, 1988; *M. sexlineatus* Qing, S  nchez-monge, Janssen, Couvreur and Bert, 2016; *M. shaheenae* Khan and Ahmad, 1991; *M. solovjovae* Brzeski, 1988; *M. subtilis* Lai and Khan, 1988; *M. truncatus* Knobloch, 1976; *M. parthenogeneticus* Geraert and Raski, 1986; *M. williamsi* Geraert and Raski, 1986).

#### General morphology

The body size of *Malenchus* ranges from 250  $\mu\text{m}$  to 900  $\mu\text{m}$ , the largest species is *M. novus*, whereas *M. sexlineatus*, *M. bryanti*, and *M. parvus* are the three smallest species (Fig. 2). A ventrally arcuate habitus is the most common appearance, but a straight or “S”



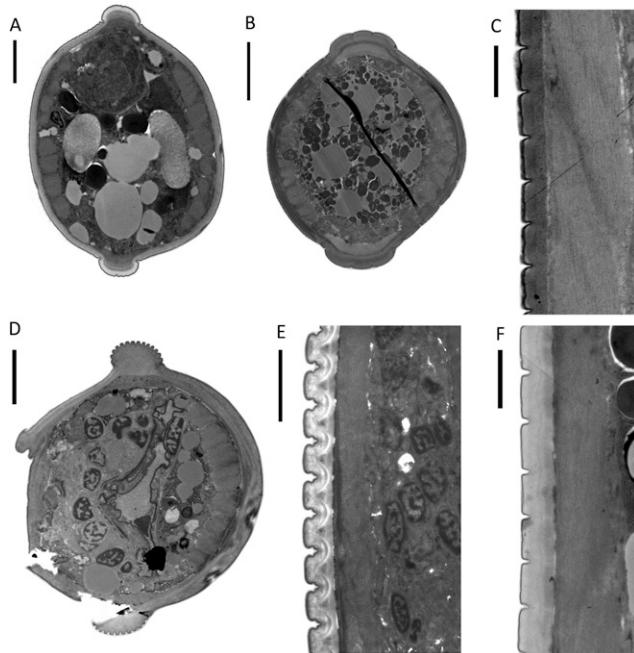


FIG. 6. Ultrastructure of cuticle and lateral region in *Malenchus* and *Filenchus*. A, C, *F. discrepans*. B, F, *F. vulgaris*. D, E, *M. acarayensis*. A, B, D. Cross section of female middle body. C, E, F. Longitudinal section in female middle body. Scale bar: A, D, E = 2  $\mu\text{m}$ ; B = 4  $\mu\text{m}$ ; C, F = 1  $\mu\text{m}$ .

shape can also occasionally be found. Body behind vulva usually tapers markedly so that width at anus is about half of that at vulva, but an elongated-cylindrical shape similar to that of other genera in Tylenchidae is also possible (e.g., *Malenchus* sp. C163 nested within *Malenchus* clade [Qing et al., 2017] but with elongated-cylindrical shape behind vulva).

#### Cuticle annulation

The cuticle in genus *Malenchus* is generally thick and folded between annuli (Figs. 3J–L; 4C–E; 5A) (Andrássy, 1981), in contrast to the typical finely striated *Filenchus* (Figs. 4B; 6C,F). The cuticle surface is smooth in most species but longitudinal striae can be observed occasionally under SEM (Fig. 7I,J). Annulations are prominent with a width of 0.76 to 2.38  $\mu\text{m}$ , conspicuous even under low magnification. Although with some variations, the annulation number (especially from anterior to vulva/cloacal) and width shows different ranges interspecifically and is a taxonomically useful reference (see details in Table 1).

The cuticle has been considered as an important generic character ever since this genus was proposed (Siddiqi, 1979; Andrássy, 1981; Geraert, 2008). However, a recent study shows that annulations can vary from prominent and folded to rather faint (Qing et al., 2017). These variations can be explained by different combinations of annuli width ( $a$  in Fig. 4A), groove height ( $b$  in Fig. 4A) and groove width ( $c$  in Fig. 4A) and therefore can be roughly clustered into three groups: (i) with indistinct folded part (Figs. 3J; 4C),

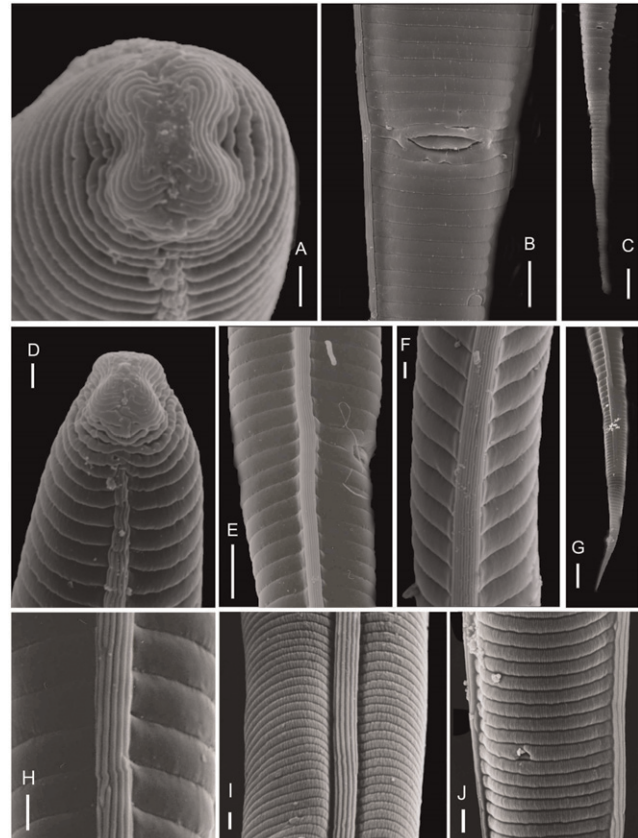


FIG. 7. SEM of female *M. pachycephalus* C116 (A–H) and *M. nanellus* (I, J). A, en face view. B, Vulva. C, Ventral view of tail. D, Lateral view of female head. E, Lateral view of vulva region. F, Lateral view of middle body showing smooth cuticle surface. G, Lateral view of tail. H, Lateral region of tail showing small ridges are stopped or interrupted. I, Lateral region appears slightly crenated due to the extension of the cuticle annulations until ridge beneath. J, Ventral view of anus showing cuticle surface with longitudinal striae. Scale bar: A, D, F, H, I, J = 1  $\mu\text{m}$ ; B, C, E, G = 5  $\mu\text{m}$ .

annuli narrow and groove hardly visible in LM ( $a > 4c$ , usually annuli width less than 1.2  $\mu\text{m}$ ), represented by species *M. sexlineatus*, *M. parthenogeneticus*, *M. leiodermis*, and *Malenchus* sp. C163; (ii) with moderated folded cuticle annuli width (Figs. 3K; 4D), groove narrow but visible ( $4c < a < 2c$ , usually annuli width 1.2–1.6  $\mu\text{m}$ ), with species *M. parvus*, *M. acarayensis*, *M. exiguus*, *M. nanellus*, and *M. ovalis*; (iii) cuticle prominently folded (Figs. 3L; 4E), with spacious grooves and wide annuli ( $a < 2c$ , usually annuli wider than 1.6  $\mu\text{m}$ ); typical species include *M. pachycephalus*, *M. solovjovae*, *M. pressulus*, and *M. novus*.

Within each type, the groove appears with a narrow opening, forming a nearly enclosed space. In TEM, this groove lumen was embedded by unknown organisms which resemble conidia, zoospore, or hypha of fungus (Fig. 5B,C,E,F). Remarkably, we recovered 18S rDNA of the fungus *Malassezia* sp. from *M. pachycephalus*, the sequence similar to a fungus associated with *Malenchus* sp. in forest soil (Renker et al., 2003). Such fungal sequences have been obtained five times during our

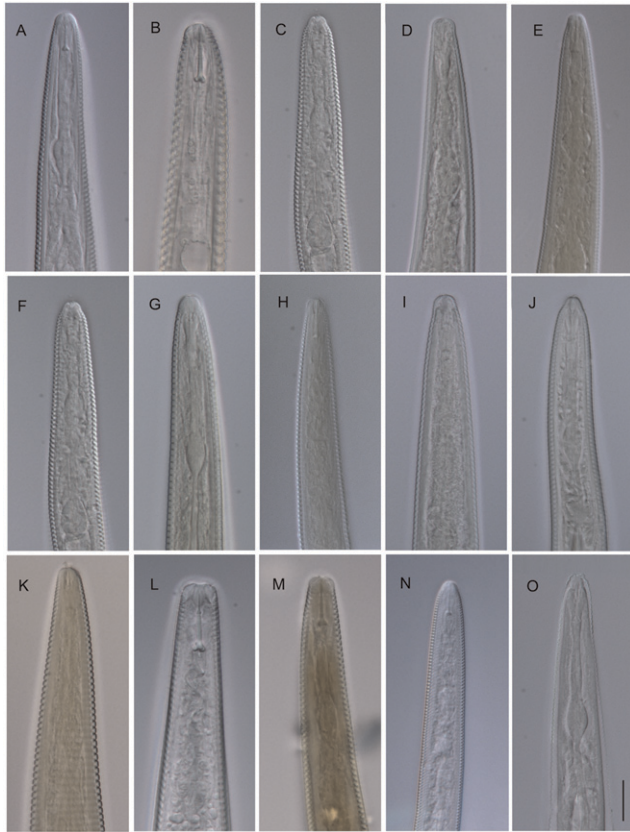


FIG. 8. Anterior part of different species in genus *Malenchus*. All specimens are from examined type/paratypes, except for *M. exiguus* from Chinese population. More picture and video vouchers see <http://nematodes.myspecies.info>. A. *M. exiguus*. B. *M. pachycephalus*. C. *M. parvus*. D. *M. leioderms*. E. *M. nanellus*. F. *M. ovalis*. G. *M. solovjovae*. H. *M. tantulus*. I. *M. williamsi*. J. *M. acarayensis*. K. *M. macrorodrus*. L. *M. novus*. M. *M. malawiensis*. N. *M. sexlineatus*. O. *M. parthenogeneticus*. Scale bar = 10  $\mu$ m.

studies on *Malenchus* using “nematode specific” primers (Qing et al., 2017). Fungi from the genus *Malassezia* are opportunists, causing infection in humans and animals; they are commonly isolated from the skin and scalp of humans (Cunningham et al., 1990;

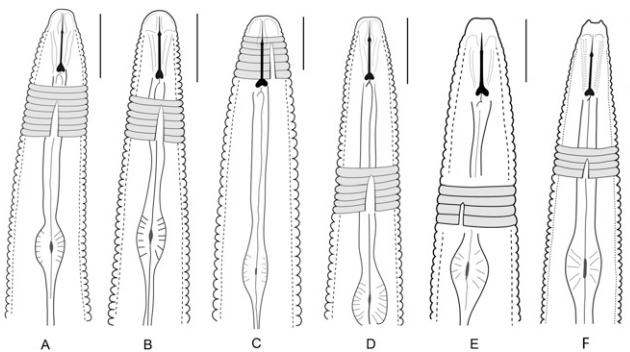


FIG. 9. Illustration of anterior part of five *Malenchus* species showing general head shape, stylet and start position of lateral lines. A. *M. acarayensis*. B. *M. exiguus*. C. *M. pachycephalus*. D. *M. nanellus*. E. *M. leioderms*. F. *M. labiatus*. Adapt from Andr  ssy (1981), Geraert and Raski (1986), and Maqbool and Shahina (1985). Scale bar = 10  $\mu$ m.

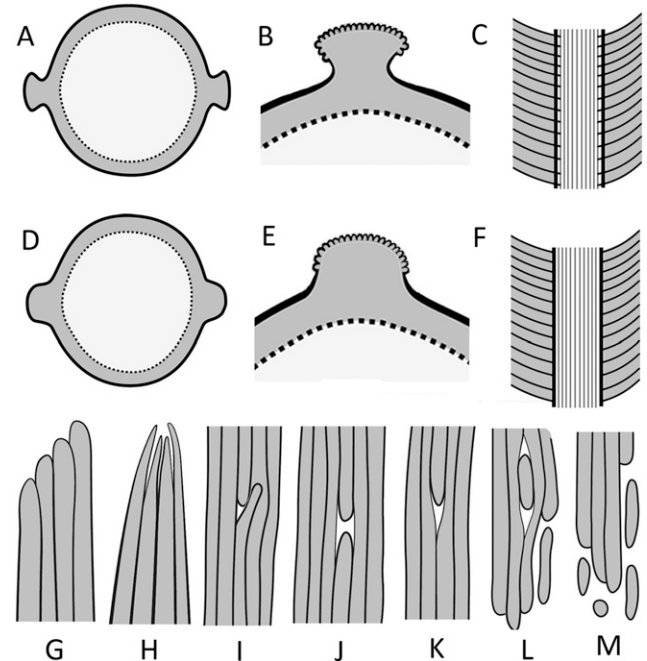


FIG. 10. Illustration of lateral region in genus *Malenchus*. A–C. Longitudinal lateral ridge narrow at the base, forming overlap with transversal crenation at two image plane and appears as crenated margin. A, B. Cross section of lateral ridge. C. Lateral view of lateral ridge. D–F. Transversal crenation cannot reach bottom of lateral ridge, no overlap from lateral view and appears as smooth margin. D, E. Cross section of lateral ridge. F. Lateral view of lateral ridge. G, H. Anterior start of lateral ridge. I–K. Lateral ridge with small ridges stopped or interrupted. L, M. Posterior end of lateral ridge.

Marcon and Powell, 1992; Hay and Midgley, 2010) and also from insects (Zhang et al., 2003). Although it has been reported from several species (*Malenchus* spp., *Meloidogyne* sp., *Acroboloides* sp., and *Cephalobus* sp.) and assumed to be selectively associated with nematodes (Renker et al., 2003) as a vector (Karab  rkl   et al., 2015) or in random adherence (Adam et al., 2014), the relationship of *Malassezia* and nematodes remain unknown. In this study, the recovered unknown cuticula-associated organisms confirm the association of nematodes with another organism, and such an organism is likely to be *Malassezia* sp.

#### Cuticle ultrastructure

The ultrastructure in the cuticle was conventionally divided into four layers (Decraemer et al., 2003): (i) epicuticle, (ii) cortical zone, (iii) median zone, (iv) basal zone (including basal lamina) and all of these layers are present in *Malenchus* (Fig. 4A). The epicuticle and cortical and median zones generally resemble those of other Tylenchomorpha, whereas the radial striae in the basal zone are reduced in *M. pachycephalus* and *M. acarayensis* (Qing et al., 2017). Although the cuticle ultrastructure shows intergeneric variation within Tylenchomorpha (Johnson et al., 1970; Mounport et al., 1991, 1993b, 1997; Valette et al., 1997), a radially striated layer in the basal zone was considered



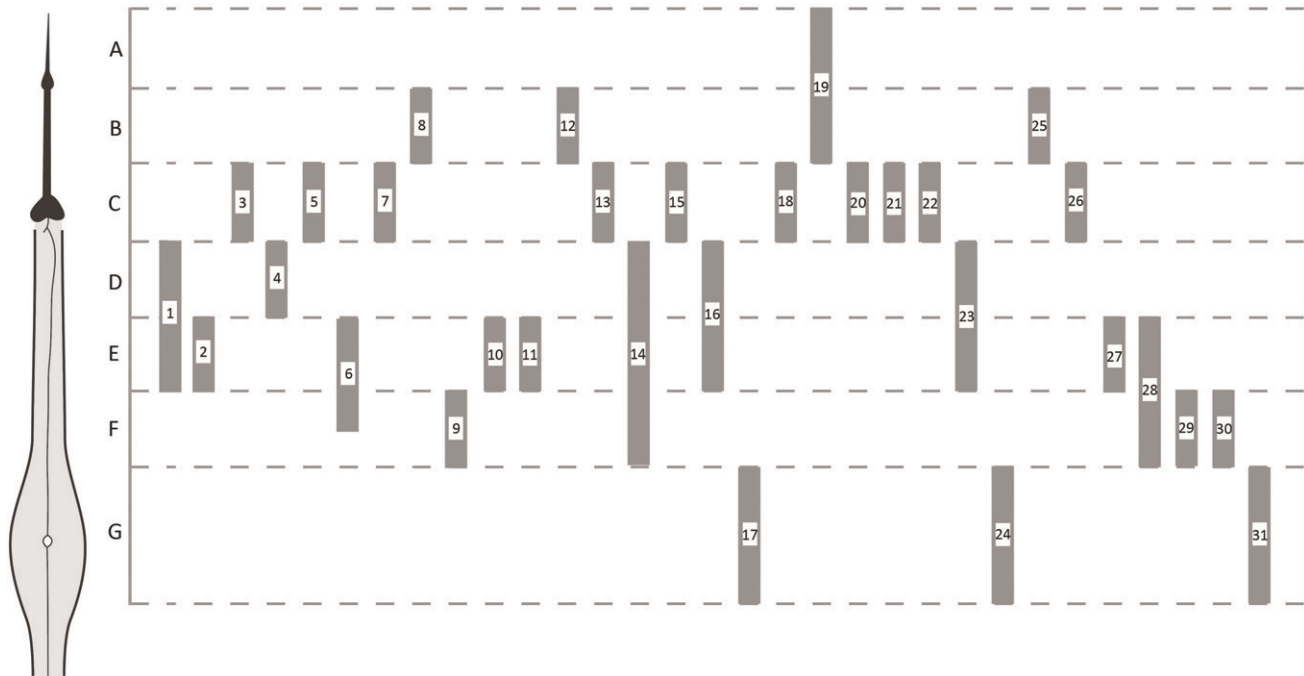


FIG. 11. The relative origin positions of lateral lines in genus *Malenchus*. A. Anterior of stylet. B. Mid-region of stylet. C. Level of knob. D. Anterior of procorpus. E. Mid-region of procorpus. F. Base of procorpus. G. Median bulb region. 1. *M. acarayensis*. 2. *M. andrassyi*. 3. *M. angustus*. 4. *M. bryanti*. 5. *M. bryophilus*. 6. *M. exiguus*. 7. *M. graciosus*. 8. *M. herrerae*. 9. *M. kausari*. 10. *M. labiatus*. 11. *M. laccocephalus*. 12. *M. machadoi*. 13. *M. macrodorus*. 14. *M. nanellus*. 15. *M. neosulcus*. 16. *M. nobilis*. 17. *M. novus*. 18. *M. ovalis*. 19. *M. pachycephalus*. 20. *M. pampinatus*. 21. *M. parvus*. 22. *M. pressulus*. 23. *M. sexlineatus*. 24. *M. shaheenae*. 25. *M. solovjovae*. 26. *M. subtilis*. 27. *M. truncatus*. 28. *M. undulatus*. 29. *M. leiodermis*. 30. *M. parthenogeneticus*. 31. *M. williamsi*.

to be always present (Decraemer et al., 2003; Geraert, 2006). Although several structural cuticular elements are homoplasious within Nematoda, at less inclusive taxonomic levels (e.g., on a family or genus level) the cuticle appears to be a more reliable phylogenetic marker (Decraemer et al., 2003). Thus, the divergent cuticle structure supports *Malenchus* as an evolutionarily divergent lineage within Tylenchomorpha and this character therefore important to define the genus.

#### Head region

The head of genus *Malenchus* is generally elevated, dorso-ventrally compressed (Andrássy, 1981) but more continuous in some species such as *M. exiguus*, *M. parthenogeneticus*, and *M. williamsi* (Figs. 8,9). Stylet usually delicate, comparable to *Filenchus*, but can be robust in some species (e.g., *M. macrodorus*, *M. novus*, *M. pachycephalus*, and *M. solovjovae*). Cone part of stylet always heavier sclerotized but distinctly shorter ( $1/3$ – $1/2$  vs shaft) and thinner than shaft (Fig. 3B). Basal knobs flattened, directed backwards, forming a triangle-like base in stylet.

Amphideal fovea is usually invisible in Tylenchidae but is conspicuous spindle shaped (=large inner sacks) in *Malenchus* (Fig. 3A,B), a trait that corresponds to molecularly defined lineages and thus potentially useful in *Malenchus* delimitation (Qing et al., 2017). The amphideal fovea is wrapped in cuticular outgrowths, which form the finer clefts (Gómez-Barcina et al., 1992)

resulting in either an S shaped (Andrássy, 1981) or straight (Geraert and Raski, 1986) amphideal aperture. Although the aperture shape can change during development by the modification of the two outgrowths, it never switches from S shaped to straight (Qing et al., 2016). The most common S-shaped aperture varies among species and can be roughly divided into two groups: (i) aperture starts with large round to oval-shaped hole, sharply narrowing to a slit and ending at head base, represented by *M. macrodorus*, *M. nanellus*, *M. pachycephalus*, *M. solovjovae*, and *M. sexlineatus*; (ii) the aperture slit is equally wide throughout its length, represented by *M. acarayensis*. Interestingly, the S-shape aperture is also present in some *Filenchus* species, which have only two lateral field incisures such as *F. normanjonesi*, *F. facultativus*, and *F. helenae* (Raski and Geraert, 1986b), but not in *F. fungivorus* (Bert et al., 2010), and never in *Filenchus* with four incisures. This is in line with the molecularly-based observation that *Filenchus* species with two incisures are more closely related to *Malenchus* than *Filenchus* species with four incisures (Atighi et al., 2013; Qing et al., 2017).

#### Lateral region

The lateral region is prominent, two incisures delimit a single narrow but elevated ridge (=protruding band, by Geraert and Raski [1986]; Geraert [2008]). In LM, it appears as a plain ridge but in SEM or TEM several small ridges can be discerned (Figs. 5D; 6D; 7F,H,I).



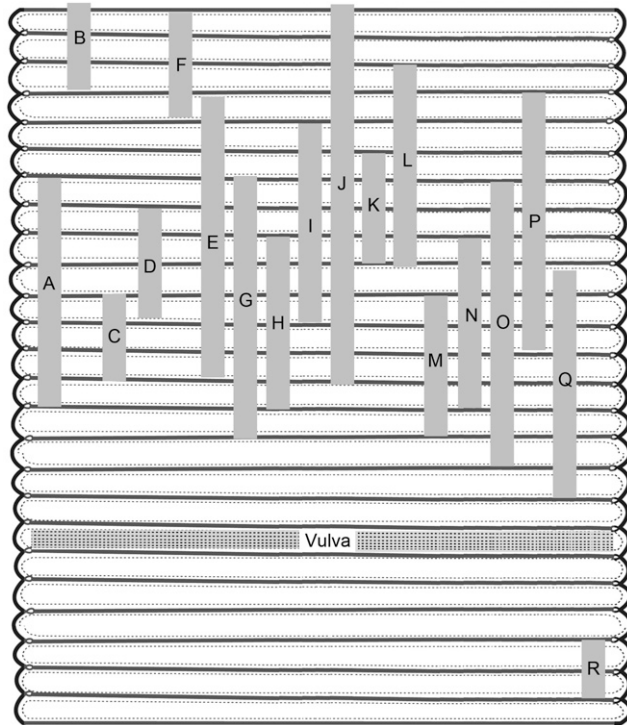


FIG. 12. The relative position of prophasms in *Malenchus*. All prophasms located dorsal side near lateral lines, bars here only shows range of phasms locations measured by number of annulations anterior/posterior to vulva. A. *M. acarayensis*. B. *M. andrassyi*. C. *M. bryanti*. D. *M. bryophilus*. E. *M. exiguus*. F. *M. sexlineatus*. G. *M. macrodorus*. H. *M. malawiensis*. I. *M. nanellus*. J. *M. nobilis*. K. *M. ovalis*. L. *M. pachycephalus* (Andrassy's population). M. *M. pachycephalus* C161. N. *M. parthenogeneticus*. O. *M. parvus*. P. *M. pressulus*. Q. *M. solovjovae*. R. *M. williamsi*. Based on data from Andrassy (1981), Geraert and Raski (1986), and Qing et al. (2016) and this study.

This feature is different from genus *Filenchus* (Fig. 6A, B) as well as other known species in Tylenchomorpha (Baldwin and Hirschmann, 1975; Mounport et al., 1991, 1993a, 1993b, 1997; Valette et al., 1997). The number of these small ridges is an interspecific variable, ranging from 3 to 14 based on SEM (Geraert and Raski, 1986; Brzeski, 1988; Gómez-Barcina et al., 1992; Mundo-Ocampo et al., 2015; Qing et al., 2016). However, even based on SEM the actual number can be underestimated, as small ridges can be present below the elevated ridge of the lateral region and these are hard to observe based on a single SEM image plane (Figs. 7I, 10B). Therefore, a cross section is crucial to determine the correct number of small ridges, which can be up to 22 based on TEM (Figs. 5D, 6D) (Qing et al., 2017).

The boundary of lateral lines sometimes appears to be a crenated margin, based on LM (Knobloch, 1976; Siddiqi, 1979; Andrassy, 1981; Geraert and Raski, 1986; Siddiqi, 2000). However, unlike other species, this crenated lateral field appears to correspond with the width of the ridge base, and if narrow then the crenation can extend below the lateral ridge that appears as a crenated margin from a lateral view (the longitudinal

TABLE 2. Detail counts of female gonad cellular architecture.<sup>a</sup>

Species	Oviduct		Spermatheca	Uterus	
	Rows	Cells per row	Cells	Cell rows	Cells per row
<i>M. pachycephalus</i>	2	3	16 (+2)	4	5
<i>M. acarayensis</i>	2	4	17 (+2)	4	5
<i>M. ovalis</i>	2	3	14 (+2)	4	5
<i>Malenchus</i> sp. C163	2	4	10 (+2)	4	5

<sup>a</sup> Numbers in brackets indicate connecting cells between spermatheca and uterus.

lateral ridge overlap with transversal crenation in two focus planes, see Fig. 10A–C), while if the base is wide there is no overlap and the margin of the lateral field is smooth (transversal crenation cannot reach to bottom of longitudinal ridge, see Fig. 10D–F).

The beginning of the lateral field range from few annuli after the head to the median bulb level (Figs. 9, 11) and ends at 1/4 to 1/3 of the tail. Interestingly, at least two start patterns have been observed (Fig. 10G, H), and the number of small ridges can be reduced at the anterior- or posterior-most part (Fig. 10I–M); they are clearly dissimilar to *Cephalenchus* (Mizukubo and Minagawa, 1985; Raski and Geraert, 1986a), which start from single ridge then hierarchically split three times to form five small ridges (six incisures). The start position of the lateral field has been used in species diagnosis and is indeed, consistent intraspecifically and varies interspecifically, based on our observations of 22 examined populations over 18 species. However, interpopulation differences have also been observed, for example the lateral field of *M. nanellus* starts at knob level (Troccoli and Geraert, 1995), the mid-region of procorpus (Siddiqi, 1979; Andrassy, 1981; Siddiqi and Khan, 1983; Geraert, 2008) or even at the base of the procorpus (Geraert and Raski, 1986). If this is a matter of real variation or the presence of cryptic species (the examined paratype start at mid-region of the procorpus, other different reports may be cryptic species) remains to be investigated, but based on our data the starting position of the lateral ridge is a consistent character and taxonomically informative. This also concurs with the key to species provided by Andrassy (1981) and Geraert (2008).

#### Prophasms

The phasms usually occurs in the lateral region of the tail, but in Tylenchidae, it is situated postmedian, just outside the lateral fields and termed prophasms (Siddiqi, 1978). In females, the position ranges from 2 to 8 annuli anterior to 4 to 5 posterior vulva. Andrassy (1981) considered the prophasms position as taxonomically informative at species level, ignoring the considerable variation presented in the same paper. Similar variations are also observed in this present study (Fig. 12); the intraspecific variation is often as large as the interspecific variation. Even in the same specimen

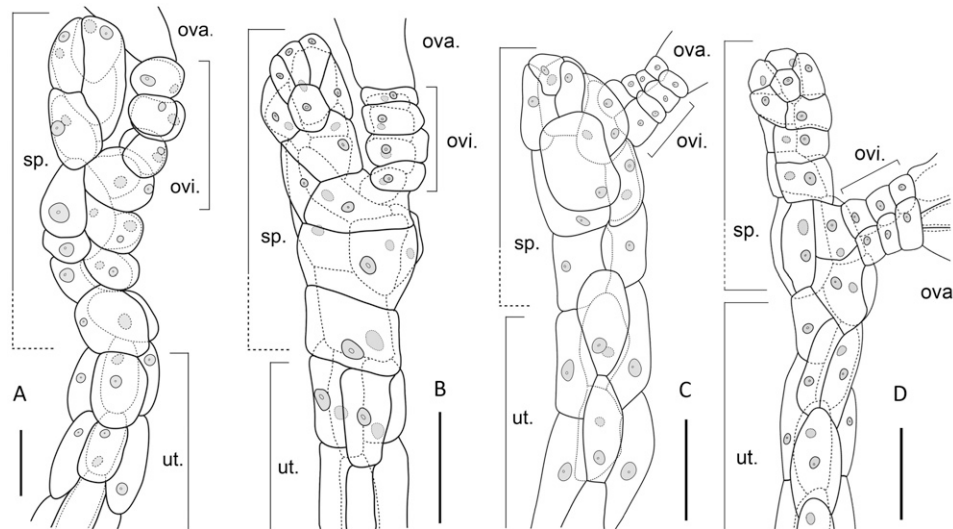


FIG. 13. Line drawings of the cellular composition of oviduct, spermatheca and distal part of uterus of representative of the genus *Malenchus*. A. *M. pachycephalus*. B. *M. acarayensis*. C. *Malenchus* sp. C163. D. *M. ovalis*. Scale bar: A–C = 10  $\mu$ m; D = 5  $\mu$ m.

both prophasms can differ in up to 5 annuli from one another. Hence, this character is not reliable to distinguish species, except for *M. williamsi* with an unusual but conserved prophasmid position (post-vulval *vs* anterior to vulva in other species, see Fig. 12).

#### Reproductive system

Female reproductive system monodelphic, ovary outstretched with oocytes arranged in a single row. Uterine sac spacious with thickened wall, eggs only present exceptionally (nongravid) (Brzeski, 1988), post-vulval uterine sac (PUS) about half of body width. Vagina has well-developed muscles, perpendicular to body or slightly anteriorly direct. Vulva sunken, cavity shape with epiptygmata and lateral flaps (=dikes in Andr  ssy [1981]).

On the basis of dissected gonoducts, the oviduct comprises two rows of three (*M. pachycephalus*, *M. acarayensis*, and *M. ovalis*) or four cells (*Malenchus* sp. C163), the spermatheca is offset, comprises 10 to 17 cells (Table 2) and is connected to the uterus by two cells (uterus except for *M. ovalis*), and the uterus cells are arranged in four regular rows (=quadricolumella) of five cells (Figs. 3F,13; Table 2). Our observations concur with other gonoduct studies of Tylenchidae (Bert et al., 2006); the oviduct and uterus rows have been considered as an evolutionary stable structure: two oviduct cell rows were considered as an apomorphy of the order Rhabditida and four rows in uterus were typical for Tylenchidae and Anguinidae (Geraert, 1983; Bert et al., 2006; Geraert, 2006; Bert et al., 2008). The cell number of the spermatheca is intraspecifically consistent in all examined specimens, supporting spermatheca number as a species-specific indicator (Bert et al., 2006, 2008). However, additional observa-

tions based on more species are necessary to validate this character for *Malenchus* species identification. According to *in vivo* observations, the spermatheca of *Malenchus* appear as rounded to elongated and offset or bilobed-offset. However, examination of the expelled *M. ovalis* gonoduct shows that the bilobed appearance is the result of the non-offset part of spermatheca being filled with sperm. This confirms the observations of Qing et al. (2016) who presumed that the observed bilobed structure is the effect of sperm cells in the proximal part of the uterus and further limits the use of this trait in species diagnosis (Geraert and Raski, 1986).

The vulva is delimited by a depression of the cuticle, usually a gradual sinking that extends over two or three adjacent annuli, this in contrast to a sharp sink of one annulus in *Filenchus*. Lateral flaps (i.e. lateral dikes by Andr  ssy [1981] or vulval membranes by Carta et al. [2009]) are the cuticular outgrowths lateral and perpendicular to vulval slit. Two-annuli-long lateral flaps (Fig. 14B) is most common but they can be also indistinct (e.g., *M. pachycephalus*, *M. solovjovae*, and *M. macrodorus*) or extend to 7 to 8 annuli (*M. williamsi*) (Fig. 14C). Interestingly, lateral flaps usually reduced in

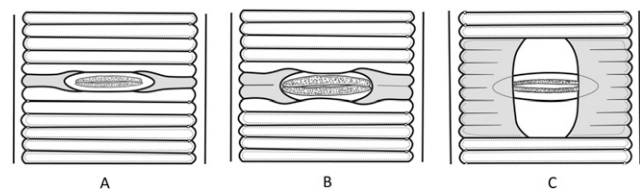


FIG. 14. Ventral view of typical vulval flap and epiptygmata in the genus *Malenchus*. A. Flap occupies one annulus without overlapping on vulva. B. Flap occupies about two annuli slightly overlapping vulva. C. Flap occupies more than four annuli covering half of vulva.

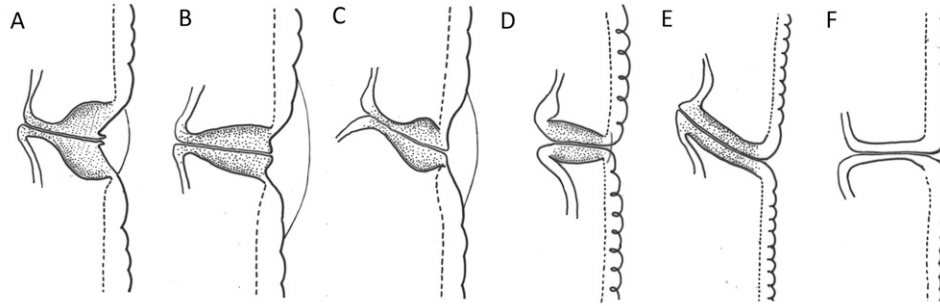


FIG. 15. Vagina with different types of swollen walls. A–C. Vagina with swollen wall in distal part, present in *Coslenchus* and *Aglenchus*. D, E. Vagina with swollen wall in more proximal or middle part, present in *Malenchus*. F. Thin and straight wall, most common type in Tylenchidae.

species with wider annuli. Epitygmata (Fig. 14A–C) are found in all studied specimens and are considered as cuticular protrusions of the vaginal wall (Siddiqi, 2000). Although indistinct in LM, they can be clearly distinguished in SEM (Fig. 7B). A vagina with swollen walls (Figs. 3I; 15D,E) is most promising character we recovered. Although it has been noticed by several authors (Siddiqi, 1979; Andr  ssy, 1981; Geraert, 2008), none of them used it as generic delimitation character. A swelling of the proximal or middle part of the vagina is presents in all examined *Malenchus* and we consider this character as an important generic character. In *Aglenchus* and *Coslenchus*, the vulva is also swollen but more in the distal part (Fig. 15) and this trait may have evolved independently.

The male is generally less frequent than the female. Testis single, spermatogonia normally arranged in one row, spermatids few, indistinct. Spermatozoa always round but size can differ among species, filling proximal part of vesicula seminalis. Cloacal opening bears prominent cone with protruding lips. Spicules are variable in size and shape and thus taxonomically important in some species (Nickle, 1970; Hechler, 1971; Geraert and De Grisse, 1982; Adams and Nguyen, 2002). Within Tylenchomorpha spicule is less informative in species diagnosis; however four characters are potentially useful on genus level: (i) curvature; (ii) the length/diameter ratio; (iii) the presence/absence of a velum; and (iv) the shape of spicule tip (Geraert and De Grisse, 1982; Geraert, 2006). The typical “tylenchid-like” shape of capitulum, shaft, and blade varies among the four examined species (Fig. 16). Remarkably, the spicule tip is twisted in *M. pachycephalus* and *M. acarayensis*, the edges curve in at level of blade but abruptly twist 180   and curve outward at the end of the blade, which appears as a C shape in the cross view of distal end (Figs. 3C,G; 16). Such a structure is unique to Tylenchidae. The gubernaculum is similar to other Tylenchomorpha (Clark et al., 1973; Wen and Chen, 1976; Wang and Chen, 1985), being centrally concave with ridge and two curved sides expanding laterally (Figs. 3D,H; 16E,F).

#### Revised generic definitions

Genus *Malenchus* Andr  ssy, 1968

Syn. *Neomalenchus* Siddiqi, 1979

*Mukazia* Siddiqi, 1986

*Paramalenchus* Sumenkova, 1988

Body straight or ventrally arcuate, dorso-ventrally flattened in cross view. Cuticle thick, most species have prominent folded annuli, occasionally with faint annuli, 0.76 to 2.38   m, conspicuous even under low magnification. Head can be dorso-ventrally compressed or more rounded, with **pouch-like amphideal fovea**. Amphideal aperture usually S shaped, but can also straight. Basal plate of cephalic framework is not flat (appears as M shaped). Stylet weakly sclerotized, cone part of stylet always heavier sclerotized but distinctly shorter (1/3–1/2 *vs* shaft) and thinner than shaft. Basal knobs flattened, directed backwards, forming a triangle-like base in stylet. **Lateral field with offset ridge, comprising 6–22 small sub-ridges**, starting from stylet to level of median bulb and ending at middle of tail. Pharynx slender, median bulb from very weak to moderately developed, valvular apparatus present. Basal bulb short, pyriform. Female reproductive system monodelphic, prodelphic, straight, PUS about half of body width. Prophasmid dorso-lateral, usually anterior but rarely posterior to vulva. Vulva sunken, usually in a definite vulval cavity. Lateral flaps often present in species with narrow annuli

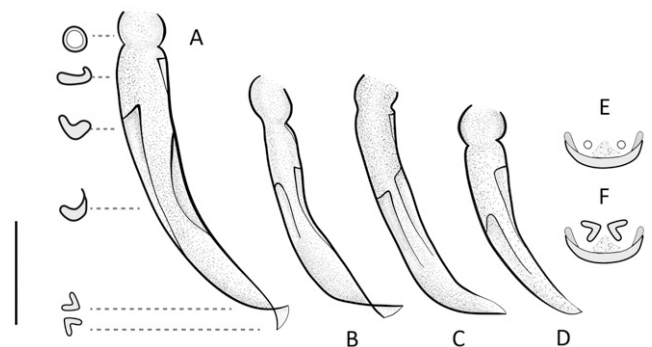


FIG. 16. Spicules and gubernaculum in four *Malenchus* species. A, E, F. *M. pachycephalus*. B, C. *M. acarayensis*. D. *Malenchus* sp. C163. A, B, D. Lateral view of spicule. C. Lateral-ventral view of spicule. E, F. Distal end of spicule and gubernaculum. Scale bar = 5   m.



TABLE 3. Comparison of generic definitions of *Malenchus*.<sup>a</sup>

This study	Andrássy, 1968	Siddiqi, 1979
1: Most species have prominent folded annuli, occasionally with faint annuli	1: Prominent annulations of cuticle	1: Thicker and folded annuli
2: Head can be dorso-ventrally compressed or more rounded, with <b>pouch-like amphideal fovea</b>	2: Elevated head, dorso-ventrally compressed	2: Cephalic region is elevated (about four or more adjacent annuli high, is striated and prominently compressed dorso-ventrally)
3: Basal plate of cephalic framework is not flat (appears as M shaped)	3: No description about basal plate of cephalic framework	3: Basal plate of cephalic framework is not flat (appears as M shaped)
4: <b>Lateral field with offset ridge, comprising many small sub-ridges</b>	4: Plain and conspicuous lateral fields	4: Lateral fields with two closely spaced incisures, in cross-section each field appearing as a narrow, rounded ridge
5: <b>Vagina with swollen wall</b> in proximal or middle part	5: No description about vagina wall	5: No description about vagina wall
6: Body behind vulva markedly tapering in most species, but can also elongated	6: Markedly narrowing body behind vulva	6: Body behind vulva markedly tapering so that width at anus is about half of that at vulva, overall shape is elongate-fusiform

<sup>a</sup> Most important generic characters proposed in this study are marked in bold.

(less than 1.8  $\mu\text{m}$ ), but reduced or absent in species with wider annuli (more than 1.8  $\mu\text{m}$ ). Epitygmata present but may obscure in LM. **Vagina with swollen wall** in proximal or middle part. Body behind vulva markedly tapering so that width at anus is about half of that at vulva in most species, but can also be elongated behind vulva. Tail similar in both sexes. Male less frequent than females. Cloacal lips protruding. Bursa adanal, short, heavily curved. Spicule ventrally curved, tip is twisted. Gubernaculum small.

*Comments on amended generic definitions*

Based on characters recovered in the present study as well as available molecular evidence (Qing et al., 2016, 2017), we propose an amended definition of the genus *Malenchus* emphasizing on amphideal fovea, lateral region and vaginal structure. The most important traits of *Malenchus*, in comparison with earlier definitions, are presented in Table 3.

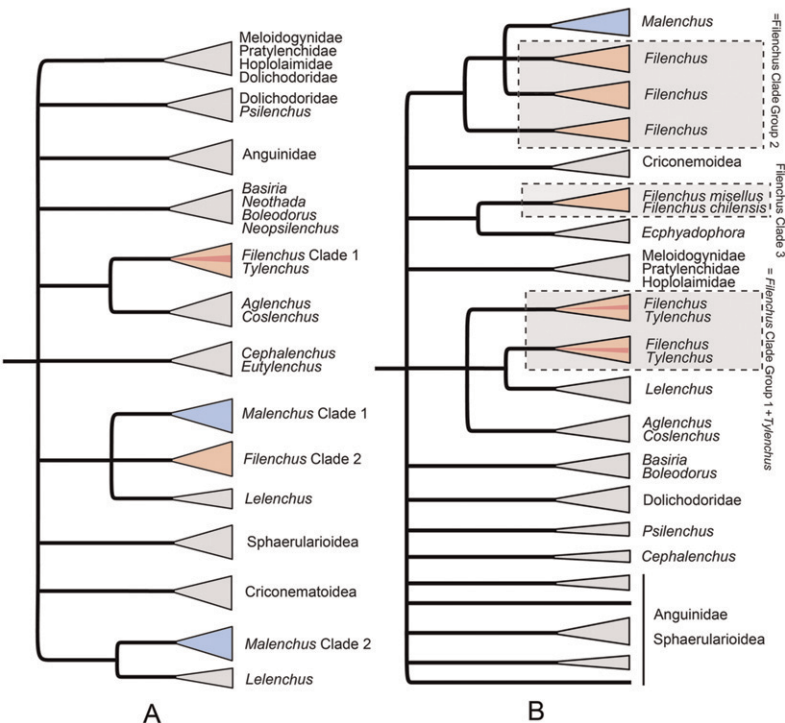


FIG. 17. Phylogenetic position of the genus *Malenchus* in Tylenchomorpha. A. Inferred from 28S rDNA. The genus *Malenchus* is separated in two separated clades without morphological support. *Filenchus* clade1 has four lateral incisures. *Filenchus* clade 2 has one ridge forming two incisures. B. Inferred from 18S rDNA, genus *Malenchus* is monophyletic, *Filenchus* has three clade/clade groups. Trees modified from Qing et al. (2017). *Malenchus* clades indicate in blue, *Filenchus* in red.

TABLE 4. Comparison of generic definitions of *Ottolenchus*.<sup>a</sup>

This study	Husain and Khan, 1967 (as subgenus)	Wu, 1970	Siddiqi, 1979
1: Annulations usually less prominent, but can be relatively smooth	1: Body cuticle strongly annulated	1: Body annulation generally coarse	1: Cuticle less thick and annulation less prominent
2: Lateral region with <b>one offset ridge</b> which forms two incisures	2: Lateral field with only two crenate incisures	2: Lateral field with two incisures	2: Lateral field with two incisures
3: Head with low cephalic region, smooth and not prominently compressed	3: Head rounded with a slight depression at the base of lip region, without clear annulations	3: No description on cephalic framework, <i>en face</i> rectangular with four lips, two subdorsal and two subventral, lateral lip regions in the form of two depressed areas	3: Head with low cephalic region, smooth and not prominently compressed 3: Basal plat is somewhat flat and demarcates the cephalic region
4: <b>Amphideal fovea indistinct in LM</b>	4: No description on amphideal fovea	4: No description on amphideal fovea	4: No description on amphideal fovea
5: <b>Vagina wall not well swollen</b> , vulva not sunken	5: No description on vulva	5: No description on vagina wall, rudimentary membrane of vulva present or not distinct	5: No description on vagina wall, vulva closed
6: Body behind vulva not markedly tapers, elongate-cylindrical overall body shape	6: No description on body behind vulva	6: No description on body behind vulva	6: Body behind vulva not markedly tapers, elongate-cylindrical overall body shape

<sup>a</sup> Most important generic characters proposed in this study are marked in bold.

*Ottolenchus* are intimately related to *Malenchus* and *Filenchus* clades group 2 by sharing two incisures. Indeed, such a similarity has been noticed and repeatedly discussed (Siddiqi, 1979; Brzeski and Sauer, 1982; Raski and Geraert, 1986b; Brzeski, 1998; Siddiqi, 2000; Geraert, 2008). The two prevailing opinions are either *Ottolenchus* as a valid genus distinguished from *Filenchus* spp. by two incisures and ventral curved amphideal aperture (Siddiqi, 2000) or a synonym of *Filenchus* due to the high variability of lateral incisures (some species show faint interrupted inner lines in SEM) and an amphideal aperture similar with other known *Tylenchus* spp. and *Filenchus* spp. (Raski and Geraert, 1987; Andr ssy 2007; Geraert, 2008). Molecular analysis indicates the two-incisures *Filenchus* (Fig. 6A) is separated from four-incisures *Filenchus* (Figs. 6B,17) and suggests the lateral region is an important character to define taxa (Qing et al., 2017). In such a scenario, we consider *Ottolenchus* as a valid genus and revised definitions are listed in Table 4. Given that SEM and other informative character are largely unknown in *Filenchus* or *Ottolenchus*, any action allocating species to one of the genera is difficult. Here we forward three taxonomic proposals for current *Filenchus*/*Ottolenchus* species: (i) species that fit definitions listed in Table 3 should move to *Malenchus*, (ii) species with two clear incisures, no pouch-like amphideal fovea, and nonswollen vaginas should move to *Ottolenchus* (further splits into more genera are still possible, as several molecular lineage present, but so far without morphological support), (iii) type species of *Filenchus* (*F. vulgaris*) bear four incisures, thus all four-incisures species should stay in *Filenchus*. Probably some *Tylenchus* species also need to be included in the latter group.

*Duosulcius* and *Zanenchus* also have two incisures. The examined paratype *D. acutus* Siddiqi, 1979 (slide No. UGMD 100227) morphologically fits our proposed *Malenchus* definition, except for an unknown lateral region (no SEM available) and the absence of a PUS. The paratype of *Z. nemorosus* (slide No. UGMD100645) was also examined, but no nematode found in the slide.

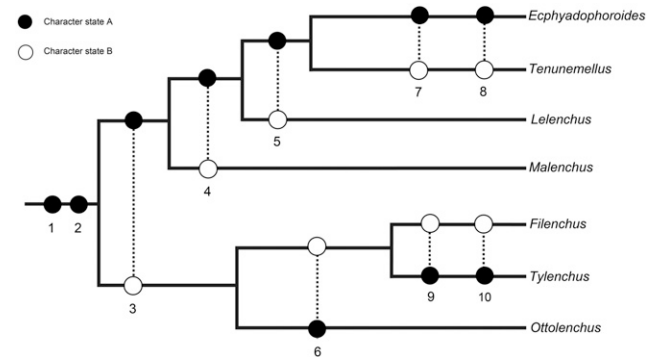


FIG. 18. Cladogram based on informative morphology trait and molecular phylogeny of Qing et al. (2017). The tree shows possible phylogeny relationship of genus *Malenchus* and other related genera based on morphology characters. *Ottolenchus* is treated as valid genus (*vs* synonym of *Filenchus* [Raski and Geraert, 1986b; Brzeski, 1998; Geraert, 2008]) as two and four incisures *Filenchus* nested in divergent lineages (see Table 5 and Fig. 17). Character states are arranged as A/B. Character 1. Filiform tail. 2. Monodelphic female. 3. Conspicuous pouch shape amphideal fovea/indistinct amphideal fovea. 4. Vagina wall thin/vagina wall well developed. 5. Bursa rectangular/bursa simple with convex margins. 6. Lateral region one ridge forming two incisures/lateral region with four incisures. 7. Cuticle coarsely annulated/cuticle relatively smooth. 8. Cuticle with longitudinal lines/cuticle without longitudinal lines. 9. Heavily sclerotized stylet with cone half of total length/weakly sclerotized stylet with cone less than half. 10. Large, round amphideal aperture confined to labial plate/slit-like aperture extending 3–4 annuli beyond labial plate.

TABLE 5. Recovered habitats of different species in genus *Malenchus*

Species	Habitats	Reference/Comments
<i>M. acarayensis</i>	Tropical rain forest litter Sand dune forest Soil around white birch ( <i>Betula papyrifera</i> ) near bog and lake area Soil around roots of tomato Grass root, park near lake Forest soil around root of <i>Albizia prosera</i> , <i>Quercus incana</i> , and <i>Terminallia belerica</i> Soil around root of <i>Quercus rotundifolia</i>	Andrássy, 1968 Wasilewska, 1970 Knobloch, 1976 Siddiqi, 1979, Syn. <i>M. tantulus</i> Andrássy, 1981, Syn. <i>M. cognatus</i> Lal and Khan, 1988 Gomez-Barcina et al., 1992
<i>M. andrassyi</i>	Flooded rice field Soil around <i>pennisetum purpureum</i> Soil around root of pear ( <i>Pyrus communis</i> ); mango ( <i>Mangifera indica</i> ); wheat ( <i>Triticum aestivum</i> ) Forest soil	Merny, 1970 Siddiqi, 1979 Maqbool and Shahina, 1985 Coosemans, 2002
<i>M. angustus</i>	Soil around moss	Talavera and Siddiqi, 1996
<i>M. anthrisculus</i>	Rhizosphere of <i>Anthriscus sylvestris</i> in flood land meadow	Sumenkova, 1988 Syn. <i>Para-malenchus anthrisculus</i> .
<i>M. bryanti</i>	Soil around white birch ( <i>Betula papyrifera</i> ) near bog and lake area Moss soil	Knobloch, 1976 Andrássy, 1981
<i>M. bryophilus</i>	Arctic island Moss from rock; near root of reed grass; root of willow; sandy soil in the vicinity lake; moss from soil; forest litter; forest soil Meadow, moss Grassland	Loof, 1971 Andrássy, 1981 Coomans, 1989 Bert et al., 2003
<i>M. exiguus</i>	Root of grass in <i>Picea engelmanni</i> infected by Engelmann spruce beetle Soil around maple tree ( <i>Acer saccharum</i> ); birch tree ( <i>Betula</i> sp.); <i>Dryas</i> sp. near lake area; red cedar ( <i>Thuja plicata</i> ); spruce ( <i>Picea glauca</i> ); Douglas fir ( <i>Pseudotsuga menziesii</i> ); spruce ( <i>Picea engelmanni</i> ); trembling aspen ( <i>Populus tremuloides</i> ); alpine fir ( <i>Abies lasiocarpa</i> ); pine ( <i>Pinus contorta</i> ); wet moss; grass Soil around root of horse chestnut ( <i>Aesculus hippocastanum</i> ). Root of strawberries near lake Soil from deciduous forest near the root of birch tree ( <i>Betula</i> sp.) Rhizosphere of <i>Bromus</i> sp.	Wu, 1970, Syn. <i>Ottolenchus sulcus</i> Siddiqi, 1979 Szczygiel, 1974 Qing et al., 2016 Panahandeh et al., 2014 Thorne and Malek, 1968 Syn. <i>Tylenchus fusiformis</i>
<i>M. fusiformis</i>	Prairie soil	
<i>M. graciosus</i>	Moss <i>Sphagnum</i> sp. from virgin forest	Andrássy, 1981
<i>M. herrerae</i>	Epiphyte moss associated with coffee plants	Mundo-Ocampo et al., 2015
<i>M. hexalineatus</i>	Tropical rainforest, litter under of <i>Lithocarpus illanosii</i>	Qing et al., 2016
<i>M. holochmatus</i>	Rhizoids of moss	Singh, 1971, Syn. <i>Tylenchus holochmatus</i>
<i>M. kausari</i>	Soil around roots of grass <i>Cyanodon dactylon</i>	Khan and Ahmad, 1989
<i>M. labiatus</i>	Soil near root of sugarcane ( <i>Saccharum officinarum</i> ) Rhizosphere of sugarcane	Maqbool and Shahina, 1985 Yaghoubi et al., 2015
<i>M. laccocephalus</i>	Moss from Muhapa tree; moss from trunks in rain-forest Soil around root of pear ( <i>Pyrus malus</i> )	Andrássy, 1981 Maqbool and Shahina, 1985 Syn. <i>M. pyri</i>
<i>M. leioderms</i>	Freshwater soil beneath thick tundra Volcanic soil of a pine-oak forest	Geraert and Raski, 1986 Brzeski, 1988
<i>M. machadoi</i>	Moss from Moua tree	Andrássy, 1963
<i>M. malawiensis</i>	Soil around roots of <i>Eucalyptus saligna</i> ; around root of <i>Pennisetum purpureum</i>	Siddiqi, 1979, Syn. <i>Neomalenchus malawiensis</i>
<i>M. nanellus</i>	Soil around root of maize ( <i>Zea mays</i> ) in experimental plot Moss from trunk of a willow; Sand soil in the vicinity of a small lake Soil near root of sugarcane ( <i>Saccharum officinarum</i> ) Benthos from stagnant brooklet, mud; border of mangroves under pandanus tree; sagu tree; coconut plantation, among grass; secondary rainforest, clay under leaves; cowpat puddle with duck-weed; Bank of swamp Soil around root of fern and moss in forest Rhizosphere of grasses	Siddiqi, 1979 Andrássy, 1981 Maqbool and Shahina, 1985 Trocchi and Geraert, 1995 Qing et al., 2016 Panahandeh et al., 2015b
<i>M. neosulcus</i>	<i>Sphagnum</i> sp. moss from virgin forest	Geraert and Raski, 1986
<i>M. nobilis</i>	Soil around grass root from a garden	Andrássy, 1981
<i>M. novus</i>	Soil near the root of <i>Echinopanax elatum</i> , <i>Abies nephrolepis</i> , and <i>Pinus koraiensis</i> Deciduous forest soil around root of <i>Quercus</i> sp.	Mukhina and Kazachenko, 1981 Qing et al., 2016
<i>M. ovalis</i>	Soil around roots of Chili ( <i>Capsicum annuum</i> ) Soil around root of <i>Quercus rotundifolia</i> Wet humus from the base of a palm	Siddiqi, 1979, Syn. <i>Neomalenchus ovalis</i> Gomez-Barcina et al., 1992 Andrássy, 1981

(Continued)



TABLE 5. Continued.

Species	Habitats	Reference/Comments
<i>M. pachycephalus</i>	Fern grass; soil around root of <i>Alnus glutinosa</i> ; soil around grass root, dry moss Soil around root of <i>Quercus rotundifolia</i> Soil from deciduous forest Moss mixed with soil from base of birch tree ( <i>Betula</i> sp.) in forest	Andrássy, 1981 Gomez-Barcina et al., 1992 Qing et al., 2016 Qing et al., 2017
<i>M. pampinatus</i>	Soil around grass root	Andrássy, 1981
<i>M. paramonovi</i>	Rhizosphere soil from mixed forest of scots pine ( <i>Pinus sylvestris</i> ) and spruce ( <i>Picea</i> sp.)	Katalan-Gateva and Alexiev, 1989
<i>M. parthenogeneticus</i>	Freshwater soil beneath thick tundra	Geraert and Raski, 1986
<i>M. parvus</i>	Sandy soil near <i>Vaccinium</i> sp. root	Brzeski, 1988
<i>M. platycephalus</i>	Soil near root of grass and aquatic plants near river; brush thicket	Thorne and Malek, 1968, Syn. <i>Tylenchus platycephalus</i>
<i>M. pressulus</i>	Soil of coniferous forest  Soil of grass root Beech forest soil Rhizosphere of <i>Vaccinium</i> sp. in forest	Kazachenko, 1975 Syn. <i>Aglenchus pressulus</i> Andrássy, 1981 Zell, 1988 Wisniewska and Kowalewska, 2015
<i>M. shaheenae</i>	Soil around root of unidentified wild trees in forest	Khan and Ahmad, 1991
<i>M. solovjovae</i>	Sandy soil near root of various shrubs, close to a lake; sandy soil near birch tree ( <i>Betula</i> sp.)	Brzeski, 1988
<i>M. subtilis</i>	Forest soil around root of Bakan ( <i>Melia azadirach</i> )	Lal and Khan, 1988
<i>M. truncatus</i>	Soil under moss and leaf litter in low, bog-like area near woods	Knobloch, 1976
<i>M. undulatus</i>	Rainforest litter; tropical, soil under leaf Rhizosphere of grasses Soil from moss	Andrássy, 1981 Panahandeh et al., 2015b Qing et al., 2017
<i>M. williamsi</i>	Freshwater soil beneath thick tundra	Geraert and Raski, 1986

Since no further data were available, the validations for these two genera remain unknown.

#### Phylogenetic implications

The main limitation of the current molecular phylogenies is the discordance of 18S rDNA and 28S rDNA genes. Specifically, *Malenchus* appears as a monophyletic genus according to 18S rDNA but splits into two well-supported clades in 28S rDNA (Qing et al., 2017). Although 18S rDNA is expected to be more informative and reliable since for 28S rDNA substations are saturated, the low coverage of the 18S alignment limits its conclusive power (Qing et al., 2017). Furthermore, *Lelenchus* is closely related to *Malenchus* and *Filenchus* clades group 2 according to 28S rDNA phylogenies but sister to *Filenchus* clades group 1 in the 18S rDNA phylogeny (Yaghoubi et al., 2015; Qing et al., 2016, 2017). However, the latter position is not fully supported (PP = 99, BS = 62) and also possibly influenced by long-branch attraction (Qing et al., 2017). Here we assume *Lelenchus* together with *Ecphyadophoroides* and *Tenunemellus* are sister groups to *Malenchus* (Fig. 18), based on a combination of morphology and molecular evidence: (i) *Lelenchus* and *Tenunemellus* are closely related to *Malenchus* based on 28S rDNA (Qing et al., 2017, unpubl. data); (ii) both share prominent pouch-like amphideal fovea; (iii) both present a similar S-shaped amphideal aperture and labial sensilla arrangement (see SEM from Raski and Geraert [1985], Brzeski and Sauer [1982]). Interestingly, *Ecphyadophora tenuissima* de Man, 1921 is, albeit with low support, closely related to *Malenchus* (Holterman et al., 2008; van Megen et al., 2009), and although this genus

has a pore-like amphideal aperture, its inner fovea is very likely to be pronounced (see drawings from Tarjan [1957], Raski et al. [1982]), thus further emphasising the importance of amphideal fovea.

In conclusion, the phylogeny of *Malenchus* cannot be resolved based on current approaches. Given that LM-derived characters are relatively less informative for *Malenchus* and related taxa, any further taxonomic action devoid of SEM and informative molecular data should not be made. Hence, other available or new technologies are needed to extract more informative genes and/or morphological data.

#### Observations on ecology

The species in genus *Malenchus* generally appear in an undisturbed environment, preferably forest soil, often associated with moss or litter or aquatic sediments (all known species have at least once reported from these habitats). Occasionally, *Malenchus* is also found in agricultural fields (few populations from *M. acarayensis*, *M. andrassyi*, *M. labiatus*, *M. laccocephalus*, and *M. ovalis*, for details see Table 5). Allocation of the feeding behavior in Tylenchidae is a recurrent discussion point among nematologists (Bongers and Bongers, 1998). Normally, *Malenchus* species are considered as epidermal and root hair feeders (Bongers and Bongers, 1998) or algal, lichen and moss feeders and parasites of lower and higher plants (Siddiqi, 1986, 2000; Andrássy, 2007). The feeding studies in Tylenchidae (Okada et al., 2002; Okada and Kadota, 2003; Okada et al., 2005) suggested a fungal-feeding habit for three *Filenchus* species. Our feeding test on four different fungal species and one

moss species failed to culture either *M. pachycephalus* or *M. acarayensis*. However, we observed numerous brown to green granules consistently presented in the anterior intestine of two analyzed *Malenchus* species, but not for other fungal feeding nematodes from the same sample (Fig. 3E). Interestingly, such pigments resemble to moss and/or soil algae and this is consistent with the most reported habitats of *Malenchus*, indicating that moss and/or algae are likely to be a natural food resource. However, the direct feeding on moss or algae was not observed, thus further study is necessary to understand the exact feeding behavior of *Malenchus* as well as other Tylenchidae.

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