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Morphological, morphometrical, and molecular characterization of *Metarhabditis amsactae* (Ali, Pervez, Andrabi, Sharma and Verma, 2011) Sudhaus, 2011 (Rhabditida, Rhabditidae) from India and proposal of *Metarhabditis longicaudata* as a junior synonym of *M. amsactae*

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Abstract

A new population of *Metarhabditis amsactae* from India is morphologically, morphometrically, and molecularly characterized. This material is characterized by having 0.65 to 1.14 mm length, lips rounded, and grouped in pairs, stoma with metastegostoma bearing setose denticles, pharynx with metacorpus slightly swollen and fusiform, nerve ring, and excretory pore located at isthmus level, female reproductive system didelphic-amphidelphic with vulva equatorial, female tail conical-elongate with acute tip, male tail conical with large and robust posterior filiform part, spicules free with hooked manubrium slightly bent ventrad, gubernaculum with narrow corpus, bursa open leptoderan with eight genital papillae and phasmids posterior to the GP8. Molecular studies based on 18S and 28S rDNA genes are provided for the first time for the species. In addition, integrated morphological, morphometrical, and molecular characters are compared with other previous records of the species. According to our analysis, *Metarhabditis longicaudata* and other material described as different species are proposed as new junior synonyms of *M. amsactae*.

Keywords

18S rDNA, 28S rDNA, ITS rDNA, *Metarhabditis amsactae*, *Metarhabditis longicaudata* n. syn. Molecular analysis, Morphology, New synonym, Taxonomy.

The genus *Metarhabditis* Tahseen, Hussain, Tomar, Shah and Jairajpuri, 2004 was proposed by Tahseen et al. (2004) under the family Rhabditidae Örley, 1880 with the type and only species *Metarhabditis andrassyana* Tahseen, Hussain, Tomar, Shah and Jairajpuri, 2004. This genus is characterized by having metastegostom with knobbed setose denticles and bursa bearing eight genital papillae. The genus was later revised by Sudhaus (2011) who transferred five species from the genera *Rhabditis* Dujardin, 1845 namely *Rhabditis adenobia* Poinar, 1971, *R. blumi* Sudhaus, 1974, *R. costai* Martins, 1985, *R. freitasi* Martins, 1985, and *R. rainai* Carta and Osbrink, 2005 and one species from *Oscheius* Andrássy, 1976 namely *Oscheius amsactae* Ali, Pervez, Andrabi, Sharma and Verma, 2011 into *Metarhabditis*. Recently, Abolafia and Peña-Santiago (2019a) described a new species, *M. giennensis* from Spain and provided a key for species identification. More recently, Tabassum et al. (2019) described other new species, *M. longicaudata* from Pakistan and its identity is discussed later in this paper.

One of the species recently transferred to the genus *Metarhabditis* is *M. amsactae* (Ali, Pervez, Andrabi, Sharma and Verma, 2011) Sudhaus, 2011. It is distinguished from all the other species of the genus by having large and robust posterior filiform part of the male tail (see keys to species identification provided by Abolafia and Peña-Santiago, 2019a). It was first described as *Oscheius amsactae* by Ali et al. (2011), who recovered some nematode specimens from a larva of the red-hairy caterpillar, *Amsacta moori* Butler (Lepidoptera: Arctiidae), collected in Kanpur, Uttar Pradesh, India. Since then, *M. amsactae* nematodes have been isolated from soil samples in different regions of India and Pakistan, many of them were previously identified as other species, however, such as *Oscheius ciceri* Shaheen, Ali and Asif, 2011, *Oscheius hussainii* Shaheen, Ali and Asif, 2011, *Oscheius gingeri* Pervez, Eapen, Devasahayan, and Jacob, 2012, and *Oscheius amsactae* Ali, Pervez, Andrabi, Sharma and Verma, 2011 and *Metarhabditis longicaudata* Tabassum, Salma and Nasir, 2019. Most of these studies, however, have characterized the species morphologically and morphometrically. Regarding molecular analysis, several Internal Transcribed Spacer (ITS) rDNA sequences, obtained from *M. amsactae* isolated in India, Philippines, and Pakistan have been deposited in the GenBank, but none of the nematode specimens used to obtain the sequences were morphologically characterized and vice versa. Hence, scanning electron microscopy images and reference molecular data for this species are still required. In this study, we therefore conducted the scanning electron microscopy (SEM) studies, and sequenced the ITS, and small-subunit (SSU) and large-subunit (LSU) rDNA of two *M. amsactae* isolated from Uttar Pradesh, India.

Materials and methods

Nematode sampling

A survey to obtain the nematodes was conducted in soils of the district Shamli (29.6189° N, 77.4329° E; 280 m altitude), Uttar Pradesh, India. This location has a semiarid and moderate-to-tropical monsoon (humid subtropical) predominant climate. The type of soil is sandy loam and loamy and the pH of soil samples ranged from 6.5 to 8.4. A total of eighty-nine soil samples were taken from meadows, pastures, agricultural fields, open fields, and orchards.

Each soil sample consisted of 1 kg of soil that was a mixture of five soil subsamples collected at 15 to 20 cm depth in five locations within each field (one sample from each corner of the field, and one from the center of the field). The soil was first made fine to remove any debris (i.e., rocks, pieces of wood or bark, leaves, etc.) and then moistened with distilled water using a spray bottle to facilitate the movement of nematodes. To recover insect-associated nematodes from these soil samples, the '*Galleria mellonella* baiting' method and the White (1927) trap method modified (Bedding and Akhurst, 1975) were used. Seven 4th instar *Galleria mellonella larvae* were buried in 250 ml of autoclaved plastic containers filled with the collected soil up to the brim. The plastic containers were then covered with tissue paper and muslin cloth. The containers were inverted upside-down and stored in the dark in an incubator at 27 ± 2°C for 7 days. The plastic containers were checked daily to recover dead insect larvae. Insect cadavers were rinsed with double-distilled water (ddH_2O) to remove soil particles and disinfected with 0.1% sodium hypochlorite before being placed on the modified White traps to obtain emerging infective juveniles. The White traps were incubated at 27 ± 2°C in an incubator and checked daily for the emergence of third-stage juveniles from the cadavers. Emerged third-stage juveniles migrate after 5 to 7 days to water surrounding the Petri dish and nematodes were collected regularly until nematode emergence ceased after 10 to 20 days (Bhat et al., 2018, 2019).

Emerged IJs were sterilized with 0.1% sodium hypochlorite and washed with ddH_2O, and finally stored in tissue culture flasks at 15°C. Third-stage juveniles were used within seven days after emergence (Aasha et al., 2019; Bhat et al., 2020a).

Nematode morphology and morphometry

Nematode third-stage juveniles were surface-sterilized with 1% NaOCl. Then, greater wax moth (*Galleria mellonella*) larvae were injected with 100 juvenile nematodes in sterile Petri plates using a 1 ml of insulin syringe. The male, female, and juvenile (third-stage) nematode generation were recovered from White traps as described above. All nematode generations were heat-killed in Ringer's solution and fixed in triethanolamine formalin (Courtney et al., 1955). Nematodes were infiltrated in glycerol by the Seinhorst method (Seinhorst, 1959) and processed further as described by Bhat et al. (2017). Briefly, the nematodes were kept in pure glycerol. Three females, specimens of five males, and 10 infective juvenile nematodes were mounted separately in a drop of glycerol on a clean glass slide. Paraffin wax was used to seal and to prevent the flattening of nematode specimens (Bhat et al. 2020b; Kajol et al., 2020). The morphology and morphometric analysis

of the specimens was conducted using light compound microscope (Magnus MLX) and phase-contrast microscope (Nikon Eclipse 50i). Twenty specimens of adults (male and female) and 20 of juveniles were analyzed. Morphometric analyses were carried out with the aid of in-built software of the phase-contrast microscope (Nikon DS-L1). The best-preserved specimens were also photographed using a Nikon Eclipse 80i (Nikon, Tokyo, Japan) light microscope provided with differential interference contrast optics (DIC) and a Nikon Digital Sight DS-U1 camera. Micrographs were edited using Adobe® Photoshop® CS. Nematode species were identified based on morphological and morphometric characters using the key provided by Abolafia and Peña-Santiago (2019a). Demanian indices (de Man, 1881) and other ratios were calculated. The terminology used for the morphology of the stoma and spicules/gubernaculum follows the proposals by De Ley et al. (1995) and Abolafia and Peña-Santiago (2017), respectively.

Scanning electron microscopy (SEM)

For the SEM, male and female specimens preserved in glycerin were selected for observation and processed according to the Abolafia's (2015) protocol. Thus, they were hydrated in distilled water, dehydrated in a graded mixture of ethanol-acetone series, critical point-dried with liquid carbon dioxide, and coated with gold. The mounts were examined with a Zeiss Merlin microscope (5 kV).

Nematode molecular characterization

Genomic DNA was isolated from approximately five hundred infective juvenile nematodes by using the Qiagen Blood and Tissue Analysis Kit following the manufacturer's protocol. A fragment of the rDNA gene containing the ITS regions (ITS1, 5.8S, ITS2) was amplified using primers 18S: 5′-TTGATTACGTCCCTGCCCTTT-3′ (forward), and 26S: 5′-TTTCACTCGCCGTTACTAAGG-3′ (reverse) (Vrain et al., 1992). The fragment containing the D2/D3 regions of the 28S rDNA gene was amplified using primers D2F: 5′-CCTTAGTAACGGCGAGTGAAA-3′ (forward) and 536: 5′-CAGCTATCCTGAGGAAAC-3′ (reverse) (Nadler et al., 2006). The 18S rDNA was amplified using primers NEM18SF: 5′-CGCGAATR GCTCATTACAACAGC-3′ (forward) and NEM18SR: 5′-GGGCGGTATCTGATCGCC-3′ (reverse) (Floyd et al., 2005). The Polymerase Chain Reaction (PCR) protocol for ITS, 18S, and D2/D3 rDNA gene amplification followed was described by Bharti et al. (2020) and Suman et al. (2020). Briefly, PCR master mix consisted of ddH_2O 16.8 µl, 10x PCR buffer 2.5 µl, dNTP mix (10 mM each) 0.5 µl, 1 µl of each forward and reverse primers, dream taq green DNA polymerase 0.2 µl, and 3 µl of DNA extract. The PCR profiles used were 1 cycle of 94°C for 3 min followed by 40 cycles of 94°C for 30 sec, 52°C for 30 sec for LSU (28S) rDNA or 55°C for 30 sec for ITS rDNA or 54°C for 30 sec for SSU (18S) rDNA, 72°C for 60 sec, and a final extension at 72°C for 10 min. PCR was followed by electrophoresis (45 min, 100 volts) of 5 µl of PCR product in a 1% TAE (Tris–acetic acid–EDTA) buffered agarose gel stained with ethidium bromide (Bhat et al., 2020c; Rana et al., 2020a). The amplified products were purified and Sanger sequenced in both directions by Bioserve Technologies Ltd. (Hyderabad, India). The obtained sequences were manually curated, trimmed, and submitted to the Center for Biotechnology Information (NCBI) under accession numbers, MT873043, MT872508, and MT872503 for ITS, 28S (D2/D3) and 18S rDNA regions, respectively for the isolate CJ6, and MT873044, MT872509, and MT872504 for the same respective genes for the isolate CJ13.

Sequence alignment and phylogenetic analyses

The sequences were edited and compared with those already present in GenBank using the Basic Local Alignment Search Tool (BLASTN) of the National Center for Biotechnology Information (NCBI) (Altschul et al., 1990). The newly obtained ribosomal LSU (D2/D3 rDNA), SSU (18S rDNA), and ITS (ITS1, 5.8S, ITS2) rDNA sequences were manually edited using BioEdit 7.2.6 (Hall, 1999) and aligned with other relevant segments of same rDNA gene sequences available in GenBank using Clustal W alignment in the program MEGA7 (Kumar et al., 2016). Poorly aligned regions were removed from the alignments using MEGA7. The base substitution model was evaluated using jModeltest2.1.10 (Darriba et al., 2012). Phylogenetic trees were elaborated using the Bayesian inference method as implemented in the program MrBayes 3.2.7 (Ronquist et al., 2012). For analysis in jModeltest, the HKY + I + G model was selected for the ITS tree, the GTR + I + G model was selected for the 18S tree, and the GTR + G was selected for the 28S tree. The selected models were initiated with a random starting tree and ran with the Markov chain Monte Carlo (MCMC) for 1×10^6 generations. The Bayesian tree was ultimately visualized using the FigTree program 1.4.4 (Rambaut, 2018). *Heterorhabditis downesi* (KU573061) was used as the outgroup and to root the trees for ITS1-5.8S-ITS2 rDNA tree, *Myolaimus*

byersi (KU180676) for LSU rDNA tree, and *M. byersi* (KU180665) for SSU rDNA tree.

The details of all the nematode species used in the molecular and phylogenetic study, including their updated nomenclature, accession numbers of rDNA genes, isolation source, and origin of the sequences are given in Table 4.

Results

Insect-associated nematode isolation

Nematodes of four genera: *Metarhabditis*, *Steinernema, Heterorhabditis,* and *Oscheius* were recovered from the eighty-nine soil samples collected in this study. Two soil samples, taken around the rhizosphere of sugarcane (*Saccharum officinarum* L.) and groundnut (*Arachis hypogaea* L.), contained *Metarhabditis* nematodes. Five samples were found positive for the presence of *Steinernema abbasi*, two for the presence of *Heterorhabditis indica,* and two for the presence of *Oscheius* sp. The rest of the samples were found negative for the presence of insect-associated nematodes. In this study, we characterized *Metarhabditis* nematodes. *Steinernema, Heterorhabditis,* and *Oscheius* nematodes are characterized in other studies (Bhat et al., 2020c, Rana et al., 2020b).

Systematics

Metarhabditis amsactae

(Ali, Pervez, Andrabi, Sharma and Verma, 2011) Sudhaus, 2011.

(Figs. 1-3 and Table 1)

Description

Adult: Body 0.72 to 1.14 mm long in females and 0.65 to 1.00 mm long in males, mostly straight rarely arcuate upon gentle heat killing with tapering to the anterior and posterior ends, more tapering toward the posterior end. The cuticle striated with scarcely prominent annuli 1.0 to 1.5 µm wide varying with body regions. Lateral fields were indistinct under light microscopy; however, four longitudinal lines are visible under scanning electron microscopy. The Lip region was almost continuous from contiguous body. Lips rounded and swollen, organized in doublets forming three pairs (one dorsal and two subventral) around the triradial oral orifice. Amphids small, oval, positioned at the base of lateral lips. Stoma rhabditoid type, 1.5 to 3.4 times the lip region width in length, with stomatal tube (gymno-promesostegostom) well developed. Cheilostom short with poorly refringent rhabdia; gymnostom tubular with cuticularized rhabdia, shorter than promesostegostom, this later surrounded by a thin pharyngeal collar; metastegostom isomorphic and isotopic having glottoid apparatus with three valves bearing two setose denticles per valve; telostegostom with minute rounded rhabdia. Pharynx rhabditoid, differentiated into cylinder-shaped pharyngeal corpus, 0.9 to 2.0 times the isthmus length, metacorpus slightly swollen, fusiform, isthmus relatively thick, weakly narrowing until its junction with the basal bulb, this more or less rounded, occasionally pyriform, with a weak to moderately developed valvular apparatus and faintly double-chambered haustrulum. Nerve ring surrounding the pharynx at the level of isthmus, 83 to 89% of neck length. Secretory–excretory pore at 79 to 86% of neck length, variable in position ranging from middle of isthmus to closely anterior to basal bulb. Deirids and hemizonid poorly visible, posterior to excretory pore, at 84 to 96% of neck length, at level of isthmus. Cardia small, conoid, surrounded by intestinal tissue. Intestinal lumen wider and dilated posterior to the basal bulb.

Female: Reproductive system didelphic-amphidelphic, the anterior and the posterior branches in sinistral and dextral position to intestine, respectively. Ovaries moderately developed, dorsally reflexed but with distal end not reaching to vulval level, anterior ovary slightly larger. Usually one or two small rounded pseudocoelomocytes observed in close proximity to the proximal end of ovaries. Oviducts proximally enlarged, connected to ovoid spermatheca frequently filled with sperm. Uteri well developed, differentiated into long glandular and muscular regions, filled with sperm and one to ten intrauterine eggs, 40–49 × 22–23 µm, in different stages of embryonation. Vagina thick-walled, often cuticularized, at right angle to the longitudinal body axis, with length equal to about one-third of the vulval body diameter. Vulva a wide transverse slit, with protruding lips, unremarkable or weak epiptygma but distinct cuticular flap. Rectum short, 1.0 to 1.8 times anal body diameter, allied with rectal glands at its junction with prerectum. Prerectum distinguishable from intestine in lacking prominent cell nuclei. Tail elongate conoid, gradually tapering to a fine terminus. Phasmids short tubular, located posterior to anus, about 38 to 42% of tail length.

Male: Similar to female in general morphology except for smaller size, posterior body curvature prominent and cuticular striations relatively fine. Reproductive system monorchic, with single testis reflexed ventrad anteriorly on the right side of the

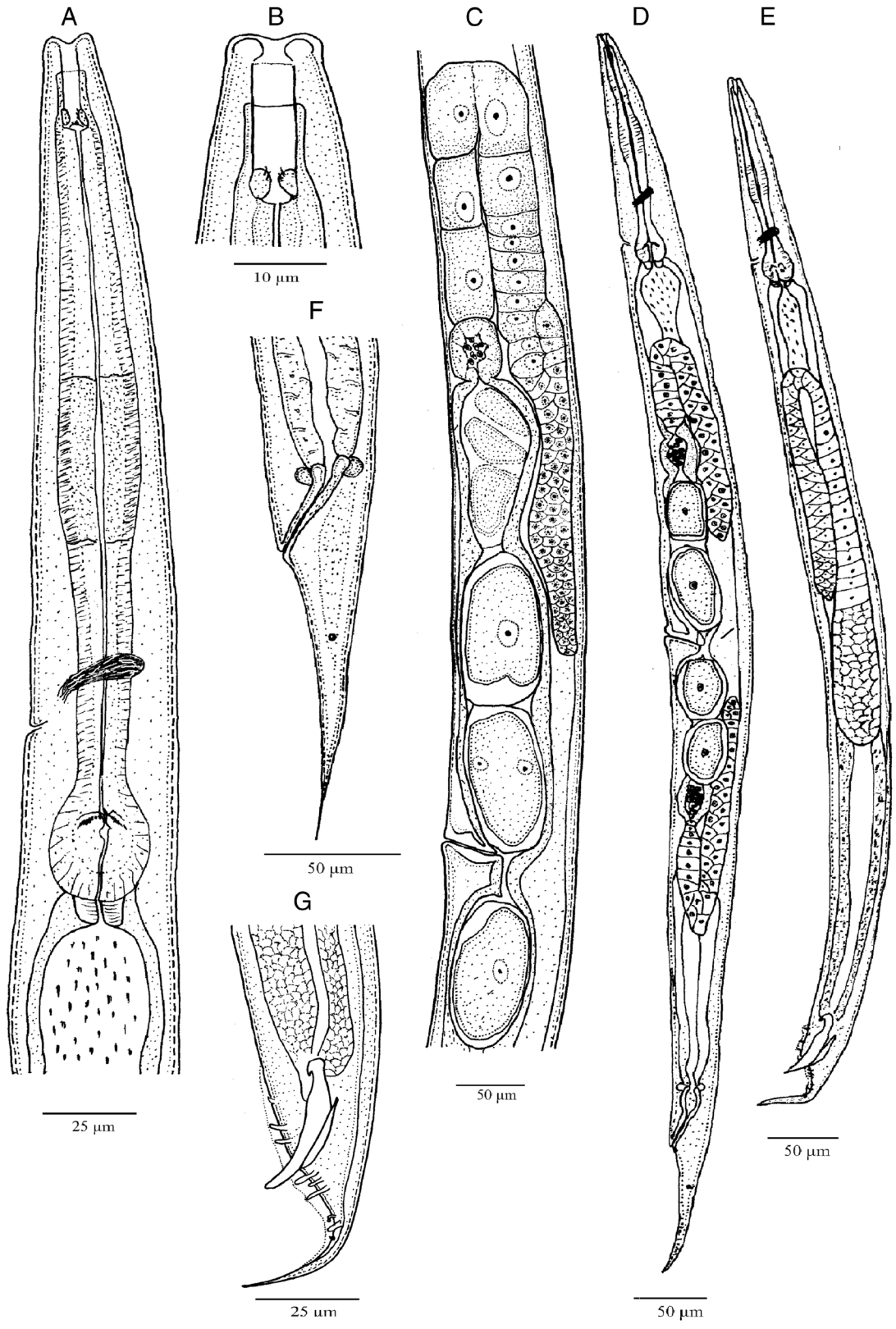

Figure 1: *Metarhabditis amsactae* (Ali, Pervez, Andrabi, Sharma and Verma, 2011) Sudhaus, 2011 (line drawing). A: Anterior region; B: Cephalic region; C: Anterior branch of the female reproductive system; D: Entire female; E: Entire male; F: Female posterior region; G: Male posterior region.

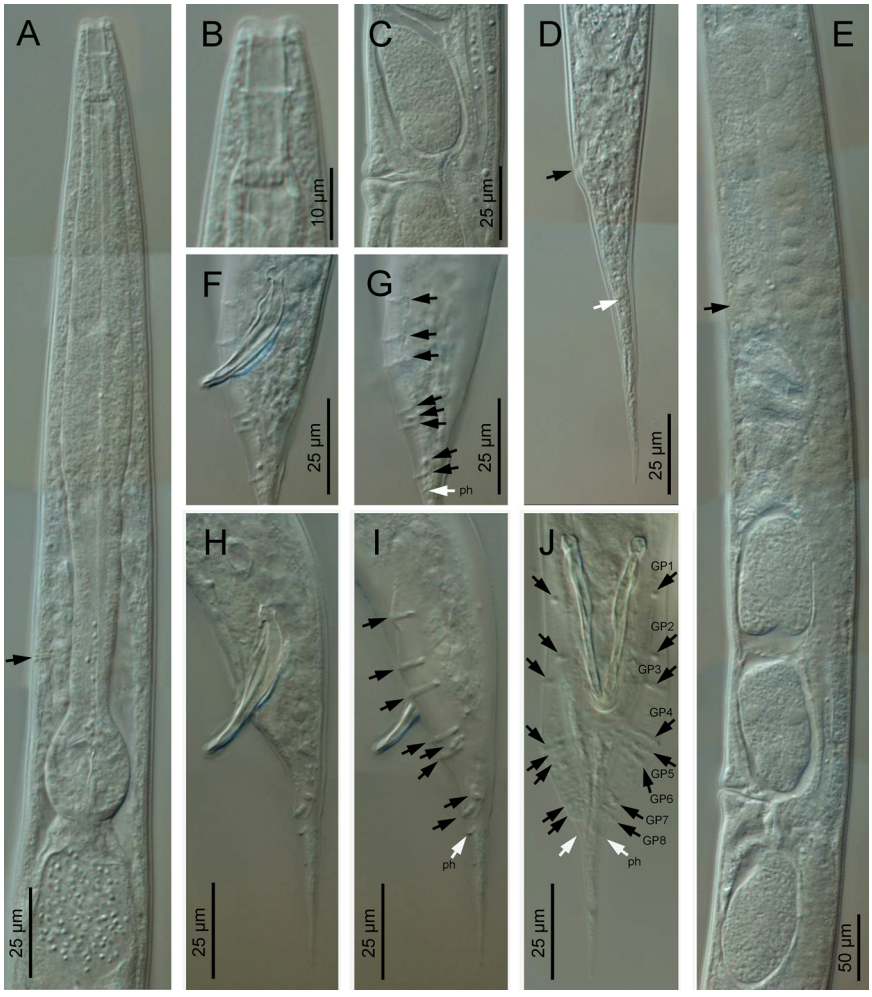

Figure 2: *Metarhabditis amsactae* (Ali, Pervez, Andrabi, Sharma and Verma, 2011) Sudhaus, 2011 (light microscopy). A: Anterior region (arrow pointing to the excretory pore); B: Cephalic region; C: Vagina region; D: Female posterior region (black arrow pointing to the anus, white arrow pointing to the phasmid); E: Anterior branch of the female reproductive system (black arrow pointing to the spermatheca); F-J: Male posterior region in lateral (F-I) and ventral (J) views, at spicules (F, H) and bursa (G, I) level (black arrows pointing to the genital papillae, white arrows pointing to the phasmids).

intestine. Vas deferens a broad tube, packed with sperm without delineation of seminal vesicles. Ejaculatory glands not observed. Spicules paired and symmetrical, ventrally arcuate, free with slightly bent ventrad manubrium, ventrally hooked, calamus short conoid and slightly ventrally curved lamina with ventrally bent finely rounded tip in lateral view. Gubernaculum well-developed, slightly ventrad curved with long manubrium and narrow corpus, 50 to 60% of spicule length. Three small gland-like cells are distinguishable around the anterior end of the cloaca. Tail conoid with posterior two-thirds abruptly tapering and reduced. Bursa anteriorly open, narrow, leptoderan, not enclosing large tail spike, having smooth margins and eight pairs (1+1+1/3+2+ph) of genital papillae, with GP1 and GP2 spaced, precloacal, GP3 slightly posterior to cloaca in most specimens, pairs GP4 to GP6 located at conoid part of tail and GP7 to GP8 located at posterior part of the bursa, dorsally directed. Phasmid small, tubular, located posterior to the GP8, at 45 to 50% of tail length.

Juveniles: Third-stage juveniles ensheathed in a cuticle of second stage juveniles. Sheath free anteriorly in third-stage juveniles, firmly bound to the posterior region of the body. Body lean, from anus to tail terminus. Cuticle with transverse striae. Lip region smooth; stoma opening closed. Stoma tubular. Pharynx with pharyngeal corpus and isthmus both

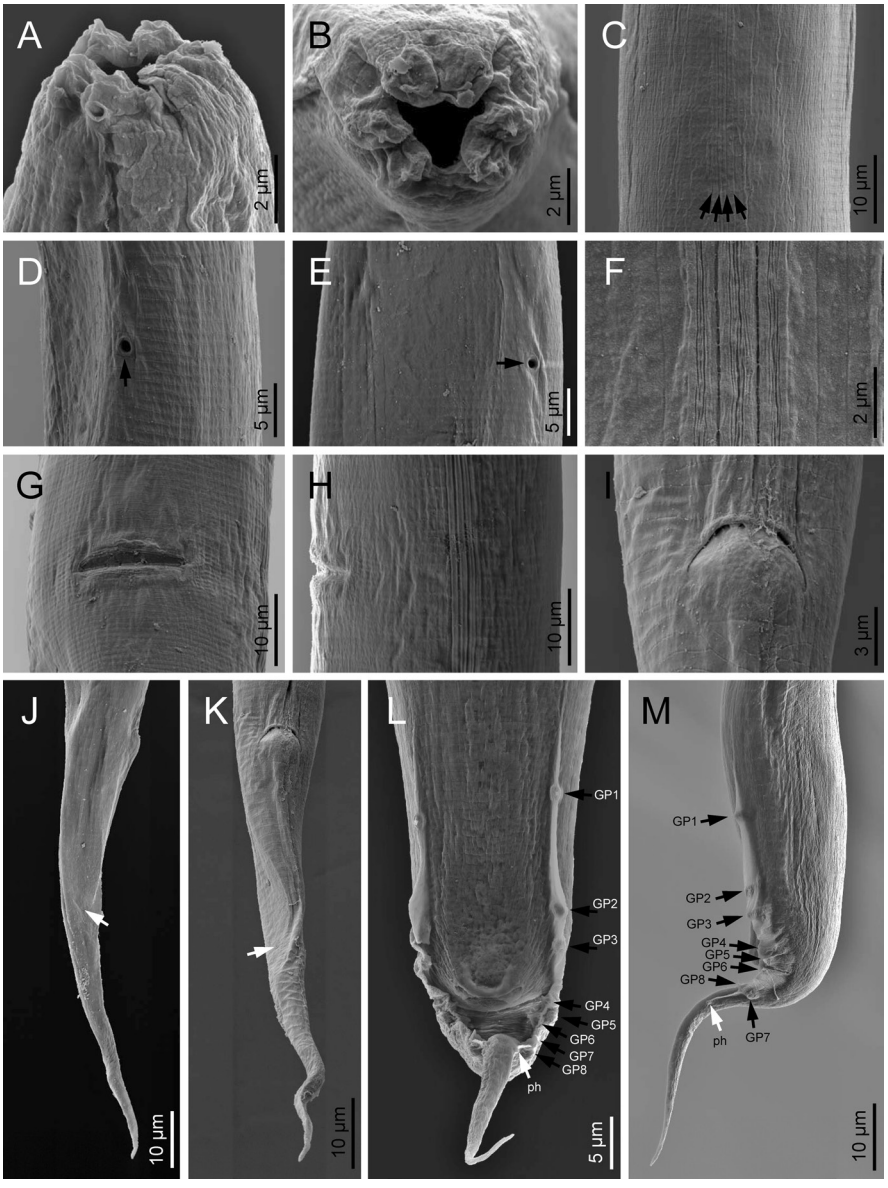

Figure 3: *Metarhabditis amsactae* (Ali, Pervez, Andrabi, Sharma and Verma, 2011) Sudhaus, 2011 (scanning electron microscopy). A, B: Cephalic and lip region in sublateral and frontal views, respectively; C, F: Lateral field (arrows pointing to the longitudinal incisures); D, E: Excretory pore (arrow) at ventral and lateral views, respectively; G, H: Vulva in ventral and lateral views, respectively; I: Female anus; J, K: Female posterior region in right lateral and ventral views, respectively (white arrows pointing to the phasmid); L, M; Male posterior region in ventral and lateral views (black arrows pointing to the genital papillae, white arrows pointing to the phasmids).

long and narrow, and basal bulb spheroid, valvate. Nerve ring and excretory pore located at isthmus level. Tail conoid with pointed terminus.

Molecular characterization

From the two populations of *Metarhabditis amsactae* molecularly analyzed in the present study from India, two sequences of 18S rDNA (865 and 869 bp), two of D2/D3 fragment of 28S rDNA (887 and 907 bp) and two of ITS1-5.8S-ITS2 rDNA (885 and 883 bp) have been obtained. Sequences of 18S and 28S rDNA are obtained for the first time for this species. A common aligned fragments resulted in 865 bp for the 18S rDNA, 879 bp for the 28S rDNA and 883 bp for the ITS rDNA, any of them show changes

Table 1. Morphometrics of *Metarhabditis amsactae* (Ali, Pervez, Andrabi, Sharma and Verma, 2011) Sudhaus, 2011 from India.

Characters	Female	Male	L3
n	20	20	20
Body length (*L*)	939 ± 119 (718-1135)	800 ± 91 (653-999)	383 ± 55 (305-475)
a (*L*/MBW)	16.4 ± 2.7 (10.9-21.1)	16.7 ± 2.0 (12.7-20.7)	18.9 ± 2.0 (15.7-22.9)
b (*L*/NL)	5.4 ± 0.6 (4.5-6.6)	4.9 ± 0.5 (3.8-5.9)	3.4 ± 0.5 (2.7-4.3)
c (*L*/T)	10.9 ± 1.7 (8.7-14.0)	13.7 ± 2.7 (9.8-20.9)	7.5 ± 1.2 (6.0-10.2)
c' (*T*/ABW)	4.1 ± 0.6 (2.9-5.2)	2.9 ± 0.5 (1.5-3.8)	4.8 ± 1.0 (3.0-6.0)
V (VA/*L* × 100)	51.2 ± 2.1 (46-56)	–	–
Lip region width	7.9 ± 1.4 (5-11)	7.1 ± 1.1 (6-10)	3.3 ± 0.5 (2-5)
Stoma length	18.4 ± 2.3 (14-22)	17.1 ± 2.3 (13-21)	12.8 ± 1.1 (11-15)
Stomatal tube width	5.0 ± 0.4 (2.5-3.5)	3.5 ± 0.7 (2.5-5.5)	?
Pharyngeal corpus length	74 ± 5.6 (68-98)	68 ± 4.5 (58-76)	35 ± 2.9 (30-42)
Metacorpus length	31 ± 3.9 (24-36)	30 ± 2.6 (24-33)	22 ± 2.4 (19-27)
Isthmus length	40 ± 5.5 (40-48)	39 ± 2.5 (35-41)	27 ± 2.6 (23-31)
Bulb length	29 ± 2.3 (26-34)	29 ± 2.6 (24-35)	16.8 ± 2.8 (12-24)
Pharynx length	175 ± 13.3 (156-195)	166 ± 7.3 (141-175)	101 ± 6.5 (88-112)
Nerve ring – anterior end	123 ± 15.0 (98-153)	112 ± 9.1 (92-125)	69 ± 10.9 (53-92)
Excretory pore–anterior end (EP)	137 ± 18.2 (110-166)	130 ± 9.1 (113-144)	78 ± 11.7 (56-103)
Deirid–anterior end	133 ± 16.0 (110-167)	125 ± 11.0 (107-147)	?
Neck length (stoma + pharynx, NL)	173 ± 15.0 (148-195)	163 ± 8.3 (144-176)	114 ± 6.1 (101-124)
Body width at neck base	43 ± 5.2 (32-50)	38 ± 4.2 (32-50)	18.7 ± 2.9 (14-25)
Mid-body width (MBW)	58 ± 10.4 (43-81)	48 ± 7.2 (40-66)	20.5 ± 3.3 (16-29)
Anterior genital branch or Testis	260 ± 38.2 (192-372)	198 ± 15 (188-222)	–
Posterior genital branch	278 ± 27.5 (229-321)	–	–
Vagina length	24.5 ± 4.1 (17-30)	–	–
Vulva–anterior end (VA)	480 ± 64 (380-579)	–	–
Rectum length	31 ± 6.4 (22-42)	–	15.0 ± 4.0 (9-23)
Anal body width (ABW)	22 ± 2.7 (16-28)	20.9 ± 2.5 (17-27)	11.2 ± 2.2 (9-17)
Tail length (T)	87 ± 10.5 (68-101)	63 ± 8.2 (49-62)	52 ± 5.9 (48-58)
Spicules length	–	41 ± 7.5 (34-49)	–
Gubernaculum length	–	19.6 ± 3.5 (16-28)	–

Notes: = Character absent. ? = Measurement unknown. Measurements in µm (except n, ratio, and percentage) and in the form: mean±standard deviation (range).

(substitutions, deletions or insertions) in their respective sequences.

Comparing with other sequences (unpublished) of the species available from GenBank, the 18S rDNA fragment, from a common aligned fragments with 794 bp, the present populations from India shows one (0.1%) change from the other sequence available from India (NM453373), 1 (0.1%) and 16 (2.0%) changes from the sequences submitted from Philippines (MT012150 and MT043860), respectively. For the 28S rDNA fragment, there are no other available sequences to compare. The ITS rDNA sequences,

from a common aligned fragments with 645 bp, show 3 (0.5%) or 6 (0.9%) changes from other sequences submitted from India (KP834432/KP834433/KY083045 and MH392568), respectively; 1 (0.2%) or 2 (0.3%) changes with respect to two sequences from Philippines (MT422254 and MT576957), while other two sequences (MT452472 and MT576957) deposited from Philippines show too much changes (51 and 64, respectively); the sequence submitted from Pakistan (MK973071) show 24 (3.7%) changes, the most of them consistent in two long contiguous deletions in the Pakistani sequence (10 and 12 gaps, respectively, after aligning sequences), which must be considered as *M. amsactae*.

Voucher material

Twenty females and twenty males of each isolate were deposited at the museum of the Department of Zoology, Chaudhary Charan Singh University Meerut, India. Ten females and ten males were deposited at the nematode collection of the Department of Animal Biology, Plant Biology and Ecology of the University of Jaén, Spain.

Diagnosis (based on the species and its synonyms)

Metarhabditis amsactae, including its synonyms, are characterized by having a body length of 0.72 to 2.07 mm in females and 0.65 to 1.50 mm in males, cuticle with very fine transverse striations; lips rounded and swollen grouped in pairs, stoma with metastegostoma bearing setose denticles, esophagus with metacorpus slightly swollen and fusiform, nerve ring and excretory pore located at isthmus level, female reproductive system didelphic-amphidelphic with vulva equatorial (V = 42-60), female tail conical-elongate with acute tip (65-148 µm long, c = 8.7-18.0, c' = 2.5-8.0), female phasmids located about the middle length of the tail, male tail conical (32-76 µm long, c = 9.8-37.0, c' = 1.0-3.8) with large and robust posterior filiform part, spicules free (24-60 µm long) with rounded manubrium slightly bent ventrad and hooked ventrally, gubernaculum 9-34 µm long, bursa open leptoderan with eight genital papillae (1 + 1/1/3 + 2 + ph) and phasmids posterior to the GP8.

Remarks

The material examined in this study agrees well with the type material described by Ali et al. (2011) and the redescription of Asif et al. (2013) as *M. amsactae*. Morphologically, the present material does not show important morphological differences with previous described populations. With respect to other populations described from different geographical regions of India (Shaheen et al., 2011; Pervez et al., 2012 as *Oscheius ciceri* and *O. hussaini*; Asif et al., 2013 as *Oscheius gingeri*), the material examined now shows close similitude to each other with only variations in body length, pharyngeal corpus, and isthmus length in adult generations (see Tables 2 and 3). The variation in morphometry in the present Indian population compared with the other populations can be attributed to differences in their geographical origin.

Recently, Tabassum et al. (2019) described a new species, *Metarhabditis longicaudata* Tabassum, Salma and Nasir, 2019 from Pakistan. According to its morphology, especially males with posterior filiform part well developed, robust, and bursa posteriorly appearing parallel along it at its proximal part (unfortunately, the LM Fig. 2C, D provided by these authors seems to be strongly stretched making the stoma too much long and narrow, and not agreeing with the line drawing Fig. 1C, D provided by these same authors), and morphometric characteristics (Tables 1 and 2), the specimens described are highly similar to *M. amsactae*. Given these considerations, we propose that *Metarhabditis longicaudata* is a junior synonym of *Metarhabditis amsactae*. Moreover, the specimens described as *M. amsactae* in the same study by these Pakistani authors do not present characteristics of this species, especially because the males lack posterior filiform part of the tail, spicules lack ventral bent or hooked manubrium, and females have a long rectum. In addition, the nematode population described as *M. rainai* (Carta and Osbrink, 2005) Sudhaus, 2011 in the same study by Tabassum et al. (2019) are morphologically very similar to *M. amsactae* nematodes (Tables 1 and 2) and, hence they were misidentified. ITS-phylogenetic trees support these conclusions as the sequences submitted to GenBank by these authors, *Metarhabditis* sp. (MK973071), cluster together with other *M. amsactae* (Fig. 4).

According to this, the updated list of synonyms of *Metarhabditis amsactae* is as follows:

Metarhabditis amsactae (Ali, Pervez, Andrabi, Sharma and Verma, 2011) Sudhaus, 2011

= *Oscheius amsactae* Ali, Pervez, Andrabi, Sharma and Verma, 2011

= *Oscheius ciceri* Shaheen, Ali and Asif, 2011

= *Oscheius hussainii* Shaheen, Ali and Asif, 2011

= *Oscheius gingeri* Pervez, Eapen, Devasahayan and Jacob, 2012

= *Metarhabditis longicaudata* Tabassum, Salma and Nasir, 2019

Table 2. Compendium of females of *Metarhabditis amsactae* (Ali, Pervez, Andrabi, Sharma and Verma, 2011) Sudhaus, 2011 populations and its synonyms.

Species	*M. amsactae*	*M. amsactae*	*M. amsactae* as *O. ciceri*	*M. amsactae* as *O. hussainii*	*M. amsactae* as *O. gingeri*	*M. amsactae*	*M. amsactae* as *M. longicaudata*	*M. amsactae* as *M. rainai*	*Metarhabditis* sp. as *M. amsactae*
Reference	Present study	Ali et al. (2011)	Shaheen et al. (2011)	Shaheen et al. (2011)	Pervez et al. (2012)	Asif et al. (2013)	Tabassum et al. (2019)	Tabassum et al. (2019)	Tabassum et al. (2019)
Country	India	India	India	India	India	India	Pakistan	Pakistan	Pakistan
Habitat	Rhizosphere of sugarcane and groundnut	Rhizosphere of mungbean	Rhizosphere of chickpea	Rhizosphere of pigeonpea	Rhizosphere of ginger	Decaying matter	Rhizosphere of mango tree	Decomposed guava fruit	Rhizosphere of chicko
L	718-1,135	658-786	964-1,018	902-989	1,418-1,813	786-902	1,366-1,684	1,769-2,078	1,546-1,694
a	10.9-21.1	19.7-22.9	20.1-22.5	24.4-25.5	18.5-21.2	19.2-23.5	14.0-18.0	11.7-20.0	15.0-18.0
b	4.5-6.6	4.1-4.8	5.7-5.9	3.8-4.3	5.1-5.3	4.1-5.0	5.5-7.8	7.0-8.0	6.0-8.0
c	8.7-14.0	8.9-12.1	10.3-12.9	10.2-12.7	12.1-13.2	9.6-11.2	12.0-16.0	12.0-18.0	13.0-19.0
c′	2.9-5.2	4.1-4.6	3.6-4.3	3.3-4.8	4.6	3.8-4.5	2.5.-4.4	4.0-8.0	3.0-4.0
V	46-56	50-58	43[a]	42[a]	51-60	51-55	48-56	49-58	50-54
Lip region width	5-11	7-8	8-11	6-8	8-12	9-10	11-15	12[a]	12[a]
Stoma length	14-22	16-18	18-19	22-23	19-21	20-25	22-28	26-30	22-26
Corpus length	68-98	96-115	92-100	133	95-170	50[a]	62-106	57[a]	42[a]
Isthmus length	40-48	32-44	30-40	44-56	28-57	35-45	30[a]	27[a]	33[a]
Bulb length	26-34	24-35	26-31	33-45	44[a]	25-35	19[a]	16[a]	17[a]
Nerve ring-ant. end	98-153	99-112	111-134	170-179	178-203	110-143	154-190	76[a]	95[a]
Excretory pore-ant. end	110-166	109-130	134-136	166-172	187-223	121-160	148-250	98[a]	112[a]
Pharynx length	156-195	159-178	167-176	220-225	189-284	177-218	206-265	250-279	218-247
Mid-body width	43-81	32-39	39-46	32-40	75-89	26-40	78-108	92-111	88-105
Anal body width	16-28	16-17	20-28	19-23	25-28	19-22	25-33	12-30	30-36
Tail length	68-101	65-80	75-96	77-87	115-129	81-100	94-112	100-148	84-132

Notes: [a]Measurement obtained from illustrations. All measurements are in µm (except ratio and percentage) and in the form of range.

Table 3. Compendium of males of *Metarhabditis amsactae* (Ali, Pervez, Andrabi, Sharma and Verma, 2011) Sudhaus, 2011 populations and its synonyms.

Species	*M. amsactae*	*M. amsactae*	*M. amsactae* as *O. cicero*	*M. amsactae* as *O. hussainii*	*M. amsactae* as *O. gingeri*	*M. amsactae*	*M. amsactae* as *M. longicaudata*	*M. amsactae* as *M. rainai*	*Metarhabditis* sp. as *M. amsactae*
Reference	Present study	Ali et al. (2011)	Shaheen et al. (2011)	Shaheen et al. (2011)	Pervez et al. (2012)	Asif et al. (2013)	Tabassum et al. (2019)	Tabassum et al. (2019)	Tabassum et al. (2019)
Country	India	India	Pakistan	Pakistan	India	India	Pakistan	Pakistan	Pakistan
Habitat	Rhizosphere of sugarcane and groundnut	Rhizosphere of mungbean	Rhizosphere of chickpea	Rhizosphere of pigeonpea	Rhizosphere of ginger	Decaying matter	Rhizosphere of mango tree	Descomposed guava fruit	Rhizosphere of chicko
L	653-999	594-804	754-973	855-889	673-821	683-868	1,154-1,325	1,100-1,392	1,234-1,498
a	12.7-20.7	16.6-19.3	19.4-20.8	25.0.-28.0	18.3-24.0	18.1-21.7	14.4-19.4	15.0-24.0	14.0-20.0
b	3.8-5.9	4.0-5.0	5.0-5.6	3.83-3.89	4.32-5.3	4.3-4.5	5.3-6.6	4.0-6.0	6.0-8.0
c	9.8-20.9	10.7-17.8	13.6-16.7	13.9-13.6	11.5-16.7	11.5-13.7	14.9-19.0	23.0-37.0	16.0-20.0
c'	1.5-3.8	2.6-2.8	2.1-2.7	3.2	2.8-3.1	2.5-3.0	2.1-3.7	1.0-2.0	2.0-3.0
Lip region width	6-10	7-8	8-11	6-8	7[a]	9-10	11-14	?	?
Stoma length	13-21	15-17	19	22-23	17-19	18-20	20-28	24-28	20-24
Corpus length	58-76	81-109	?	134	71-112	72[a]	?	?	?
Isthmus length	35-41	27-42	?	44-56	21-38	27[a]	?	?	?
Bulb length	24-35	20-36	?	33-45	?	23[a]	?	?	?
Nerve ring-ant. end	92-125	79-108	116-136	149-179	90-114	100-127	143-185	?	?
Excretory pore-ant. end	113-144	87-114	127-138	155-168	110-142	119-141	137-179	?	?
Pharynx length	141-175	134-169	149-172	223-228	142-187	155-190	184-256	211-256	204-236
Midbody width	40-66	31-45	39-46	30-35	32-39	26-40	64-69	54-88	70-98
Anal body width	17-27	16-20	26-30	19-21	16-19	20-24	21-40	26-34	27-32
Tail length	49-62	41-55	55-58	61-65	43-59	58-67	62-76	32-56	66-78
Spicules length (SL)	34-49	31-36	35-44	41-44	24-27	33-39	40-46	32-60	42-60
Gubernaculum length (GL)	16-28	13-17	19-20	14-18	9-10	14-20	20-34	13-23	16-22
GL/SL × 100	50-60	43-46	45-54	34-40	36	42-51	50-74	40	37-38

Notes: [a]Measurement obtained from illustrations; ? = Measurement unknown. All measurements are in µm (except ratio and percentage) and in the form of range.

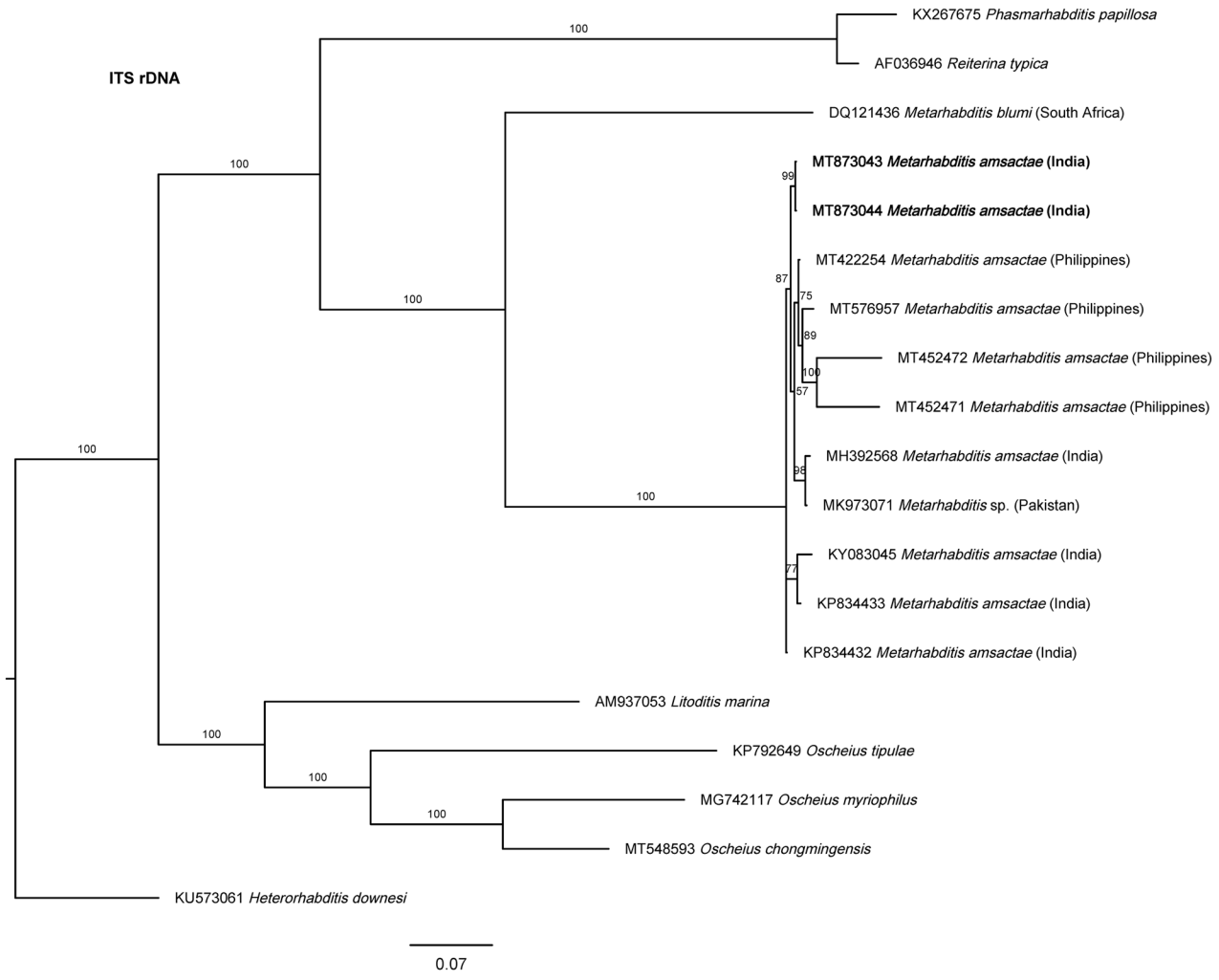

Figure 4: Bayesian Inference tree from previously and the newly sequenced *Metarhabditis amsactae* (bold) and other closely related species based on sequences of the Internal Transcribed Spacer (ITS1-5.8S-ITS2) rDNA region. Bayesian posterior probabilities (%) are given for each clade. The scale bar shows the number of substitutions per site.

Phylogenetic relationships

The phylogenetic relationships as inferred from the Bayesian Inference analysis between *Metarhabditis amsactae* and other closely related are provided based on ITS- (Fig. 4), 18S- (Fig. 5), and 28S- (Fig. 6) rDNA fragments. Based on the three phylogenetic trees, *M. blumi* (Sudhaus, 1974) Sudhaus, 2011 and *M. rainai* (Carta and Osbrink, 2005) Sudhaus, 2011 are sister species of *M. amsactae.*

The phylogenetic tree inferred using 18S rDNA gene sequences, shows three clusters that contain sequences of nematodes that have been suggested to belong to *Metarhabditis* (Fig. 5). One cluster is composed of *M. rainai* (AF083008, JQ237848, MT012133, MT012135 and MT012153) and one nematode isolate that might have been misidentified as *Rhabditis* sp (MN082353) but could correspond to *M. rainai*. A second cluster composed of *M. amsactae* (MT872504, MT872503) and three nematode isolates that might have been misidentified as *M. blumi* (MT043860, MT012150, MN453373). A third cluster composed of *M. blumi* (MF989442, U13935), and *Rhabditis* sp. (MN082355). As *M. amsactae* isolates that correspond to accession numbers MT872504 and MT872503, and the *M. blumi* isolate that correspond to accession numbers U13935 have been morphologically and molecularly characterized, we conclude that nematodes isolates with NCBI accessions MT043860, MT012150 and MN453373

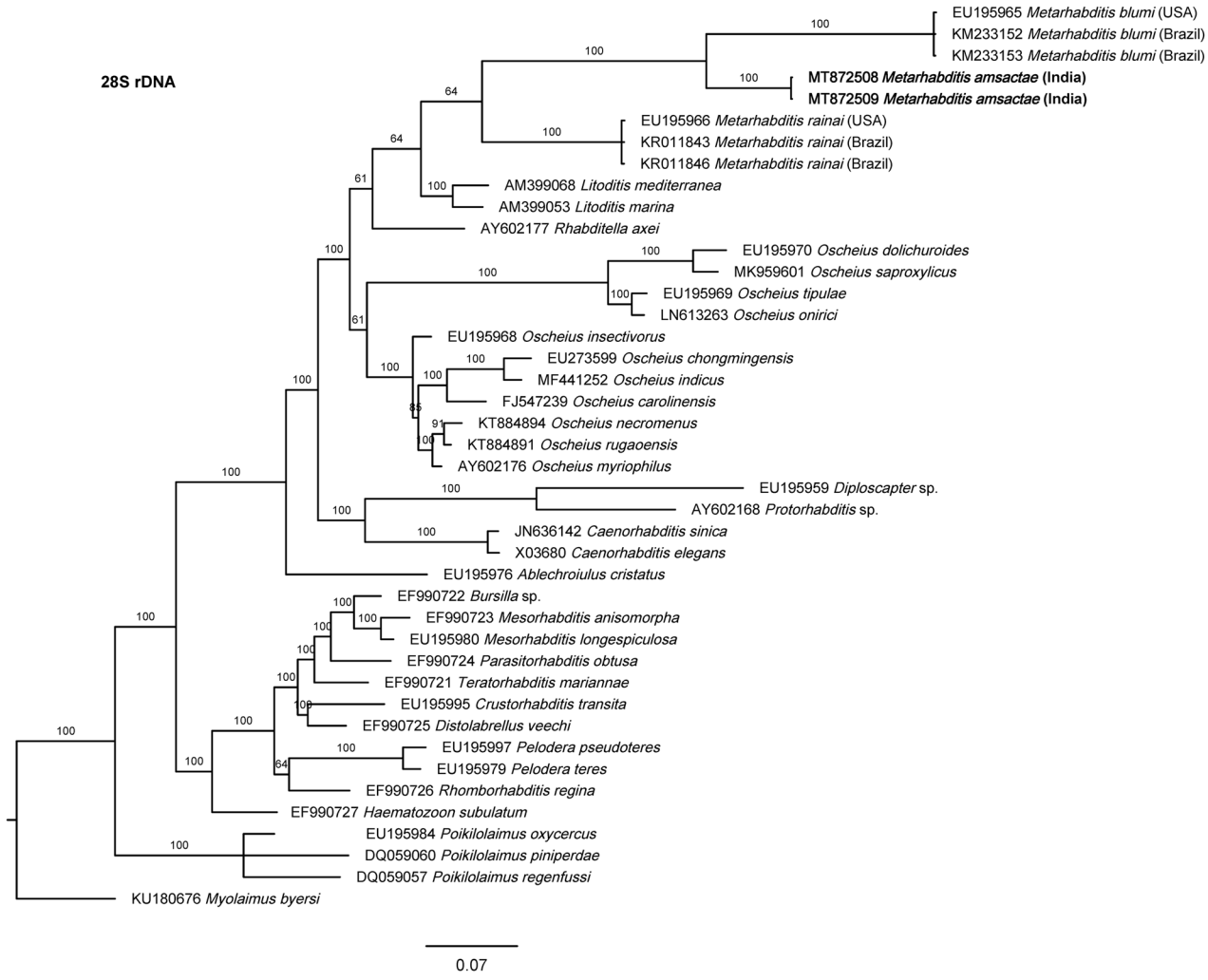

Figure 5: Bayesian Inference tree from the newly sequenced *Metarhabditis amsactae* (bold) and other closely related species based on sequences of the small subunit (18S) of rDNA region. Bayesian posterior probabilities (%) are given for each clade. The scale bar shows the number of substitutions per site.

are actually *M. amsactae* instead of *M. blumi*, and the nematodes isolate with NCBI accession MN082355 identified as *Rhabditis* sp. should correspond to *Metarhabditis* sp. This conclusion is also supported by sequence identity analysis. Comparing the nucleotide composition of a common 18S rDNA gene fragment of 723 bp in length of the *M. amsactae* specimens examined in this study (MT872503-4) and the nucleotide composition of *M. blumi* (U13935), *Rhabditis* sp. (MN082355), *M. rainai* (AF083008, JQ237848, MT012133, MT012135 and MT012153), *Rhabditis* sp. (MN082353), and *M. blumi* (MT043860, MT012150 and MN453373), we found 57 genetic changes (insertions, deletions or substitutions) between *M. amsactae* and *M. blumi*, 69 genetic changes between *M. amsactae* and *Rhabditis* sp. (MN082355), 82 changes between *M. amsactae* and *M. rainai*, 82 changes between *M. amsactae* and *Rhabditis* sp. (MN082353), 95 changes between *M. blumi* and *M. rainai*, and fewer genetic changes between *Rhabditis* sp. (MN082353) and *M. rainai*, and between the *M. amsactae* (MT872503-4) and *M. blumi* (MT043860, MT012150 and MN453373).

The phylogenetic tree inferred using 28S rDNA gene sequences show also three clusters containing *Metarhabditis* nematodes: one with *M. blumi*, one with *M. amsactae*, and one *M. rainai* (Fig. 6). Comparing the nucleotide composition of a common 28S rDNA

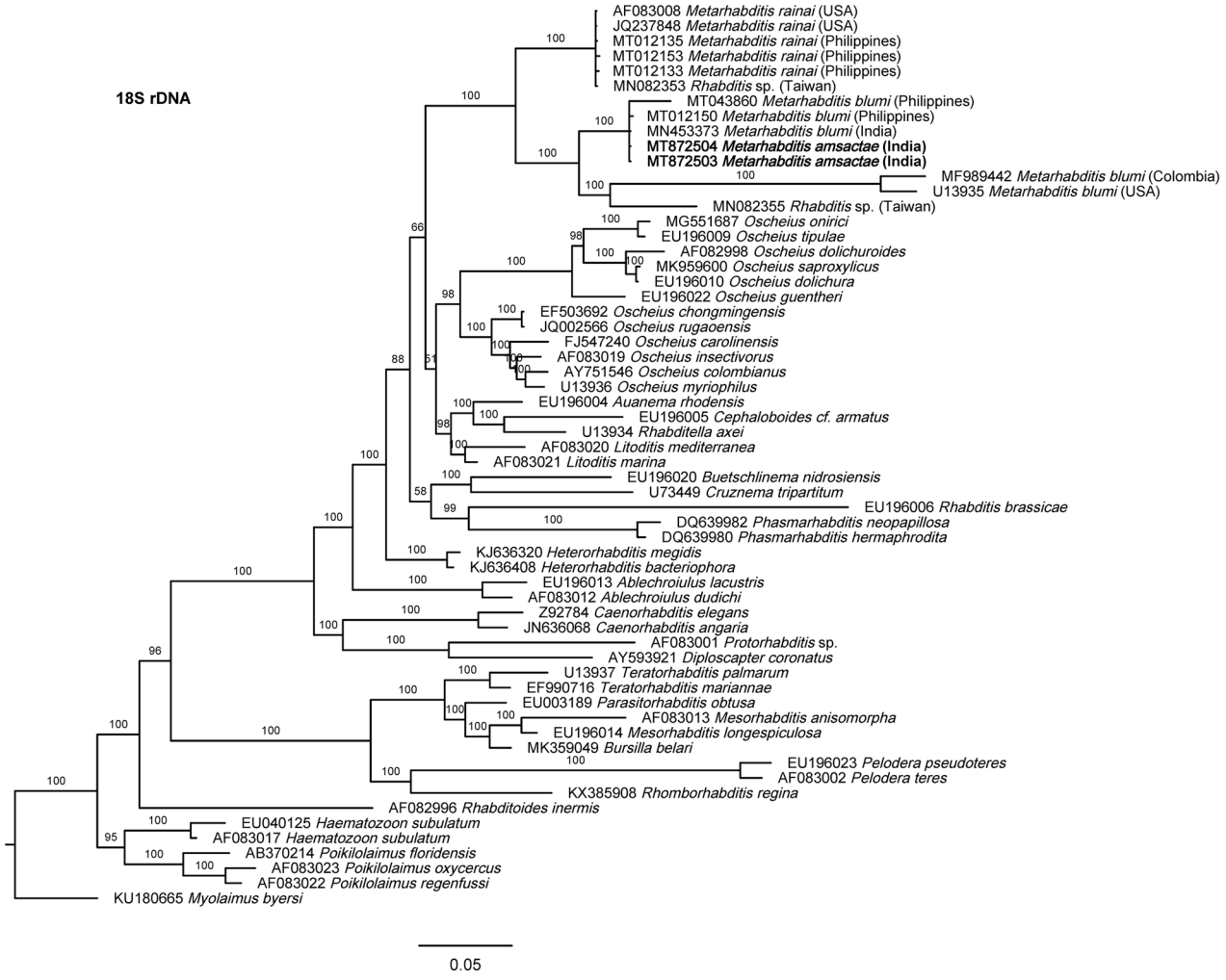

Figure 6. Bayesian Inference tree from the newly sequenced *Metarhabditis amsactae* (bold) and other closely related species based on sequences of the D2/D3 domain of large subunit (28S) of rDNA region. Bayesian posterior probabilities (%) are given for each clade. The scale bar shows the number of substitutions per site.

fragment of 343 bp in length of the *M. amsactae* specimens examined in this study (MT872508-9) and the nucleotide composition of *M. blumi* (EU195965, KM233152-3), and *M. rainai* (EU195966, KR011843-6), we found 75 genetic changes (insertions, deletions or substitutions) between *M. amsactae* and *M. blumi*, 80 genetic changes between *M. amsactae* and *M. rainai*, and 95 changes between *M. blumi* and *M. rainai*, suggesting that *M. amsactae, M. rainai* and *M. blumi* are sister species and that the nematode isolates characterized in this study belong to *M. amsactae*.

Finally, analyzing ITS rRNA gene sequences, we arrive to the same conclusions derived from the analysis of 28S- and 18S rDNA gene sequences. ITS-based phylogenetic tree show a clear cluster that separates *M. amsactae* and *M. blumi* (Fig. 4). Unfortunately, there are no available *M. rainai* sequences. Sequence comparisons show that a common ITS rDNA fragment of 802 bp in length of *M. amsactae* (MT873043-4) and of *M. blumi* (DQ121436) differ in 496 changes, which again support the status of the nematode isolates of this study as *M. amsactae* (Table 4).

Acknowledgments

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Table 4. Nematode species, GenBank accession number, and origin of the sequences used for phylogenetic study.

Species[a]	GenBank accession number			Country	Isolation source	Reference
	18S rDNA	28S rDNA	ITS rDNA			
Ablechroiulus cristatus	EU196013	EU195976		USA	Unknown	Kiontke et al. (2007)
Ablechroiulus dudichi	AF083012			USA	Unknown	Fitch (unpublished)
Auanema rhodensis	EU196004			USA	Unknown	Kiontke et al. (2007)
Buetschlinema nidrosiensis	EU196020			USA	Unknown	Kiontke et al. (2007)
Bursilla belari	MK359049			India	Soil	Palanisamy et al. (unpublished)
Bursilla sp.		EF990722		USA	Unknown	Kiontke et al. (2007)
Caenorhabditis angaria	JN636068			USA	Rotting fruits	Kiontke et al. (2011)
Caenorhabditis elegans	Z92784			UK	Unknown	Sulston and Waterston (1998)
Caenorhabditis elegans		X03680		USA	Unknown	Ellis et al. (1986)
Caenorhabditis sinica		JN636142		USA	Rotting fruits	Kiontke et al. (2011)
Cephaloboides cf. armatus	EU196005			USA	Unknown	Kiontke et al. (2007)
Crustorhabditis transita		EU195995		USA	Unknown	Kiontke et al. (2007)
Cruznema tripartitum	U73449				Unknown	Baldwin et al. (1997)
Diploscapter coronatus	AY593921			Netherlands	Soil	Helder et al. (2006)
Diploscapter sp.		EU195959		USA	Unknown	Kiontke et al. (2007)
Distolabrellus veechi		EF990725		USA	Unknown	Kiontke et al. (2007)
Haematozoon subulatum	EU040125			China	Soil	Li et al. (unpublished)
Haematozoon subulatum	AF083017			USA	Unknown	Fitch (unpublished)
Haematozoon subulatum		EF990727		USA	Unknown	Kiontke et al. (2007)

Heterorhabditis bacteriophora	KJ636408			Netherlands	Unknown	van Megen (unpublished)
Heterorhabditis downesi			KU573061	Ireland	Soil	Maher et al. (2016)
Heterorhabditis megidis	KJ636320			Netherlands	Unknown	van Megen (unpublished)
Litoditis marina	AF083021			USA	Unknown	Fitch (unpublished)
Litoditis marina		AM399053		Belgium	*Fucus* sp.	Derycke et al. (2008a)
Litoditis marina			AM937053	Greece	Decaying seaweeds	Derycke et al. (2008b)
Litoditis mediterranea	AF083020			USA	Unknown	Fitch (unpublished)
Litoditis mediterranea		AM399068		New Zealand	Unknown	Derycke et al. (2008a)
Mesorhabditis anisomorpha	AF083013			USA	Unknown	Fitch (unpublished)
Mesorhabditis anisomorpha		EF990723		USA	Unknown	Kiontke et al. (2007)
Mesorhabditis longespiculosa	EU196014	EU195980		USA	Unknown	Kiontke et al. (2007)
Metarhabditis amsactae	MT872503, MT872504	MT872508, MT872509	MT873043, MT873044	India	Soil	Present study
Metarhabditis amsactae			MT422254	Philippines	Soil	Dichusa et al. (unpublished)
Metarhabditis amsactae			MT452472, MT452471	Philippines	Soil	Sangcopan and Sumaya (unpublished)
Metarhabditis amsactae			MH392568, KY083045 KP834433, KP834432	India	Soil	Chavan et al. (unpublished)
Metarhabditis amsactae			MK973071	Pakistan	Soil	Tabassum et al. (unpublished)
Metarhabditis blumi	U13935			Germany	Unknown	Fitch et al. (1995)
Metarhabditis blumi	MT043860			Philippines	Soil	Andalan et al. (unpublished)
Metarhabditis blumi	MF989442			Colombia	*Bos indicus*	Chaves et al. (unpublished)
Metarhabditis blumi	MT012150			Philippines	Soil compost	Guadalquiver (unpublished)

Metarhabditis blumi	MN453373			India	Soil	Kandhasamy and Muthugounder (unpublished)
Metarhabditis blumi		EU195965		USA	Unknown	Kiontke et al. (2007)
Metarhabditis blumi		KM233152, KM233153		Brazil	Cow	Bossi et al. (2015)
Metarhabditis blumi			DQ121436	South Africa	Unknown	Jumba and Gray (unpublished)
Metarhabditis rainai	MT012135, MT012133			Philippines	Soil	Kabalu et al. (unpublished)
Metarhabditis rainai	MT012153			Philippines	Soil	Buldiman et al. (unpublished)
Metarhabditis rainai	JQ237848			USA	Citrus groves	Campos-Herrera et al. (2012)
Metarhabditis rainai	AF083008			USA	Unknown	Fitch (unpublished)
Metarhabditis rainai		EU195966		USA	Unknown	Kiontke et al. (2007)
Metarhabditis rainai		KR011843, KR011846		Brazil	Soil	de Brida et al. (2017)
Metarhabditis sp.			MK973071	Pakistan	Soil	Tabassum et al. (unplublished)
Myolaimus byersi	KU180665	KU180676		Sweden	Unknown	Holovachov et al. (2015)
Oscheius carolinensis	FJ547240	FJ547239		USA	Vermicompost	Ye et al. (2010)
Oscheius chongmingensis	EF503692			China	Soil	Zhang et al. (2008)
Oscheius chongmingensis		EU273599		China	Soil	Liu et al. (2012)
Oscheius chongmingensis			MT548593	China	*Spodoptera frugiperda*	Li et al. (unpublished)
Oscheius colombianus	AY751546			Colombia	*Cyrtomenus bergi*	Stock et al. (2005)
Oscheius dolichura	EU196010			USA	Unknown	Kiontke et al. (2007)
Oscheius dolichuroides	AF082998			USA	Unknown	Fitch (unpublished)
Oscheius dolichuroides		EU195970		USA	Unknown	Kiontke et al. (2007)
Oscheius guentheri	EU196022			USA	Unknown	Kiontke et al. (2007)
Oscheius indicus		MF441252		India	Soil	Kumar et al. (2019)
Oscheius insectivorus	AF083019			USA	Unknown	Fitch (unpublished)

Oscheius insectivorus		EU195968		USA	Unknown	Kiontke et al. (2007)
Oscheius myriophilus		AY602176		USA	Unknown	Kiontke et al. (2004)
Oscheius myriophilus			MG742117	Thailand	Soil	Nitjarunkul et al. (unpublished)
Oscheius myriophilus	U13936			USA	Soil	Fitch et al. (1995)
Oscheius necromenus		KT884894		Australia	*Oncocladosoma castaneum*	Carta et al. (2018)
Oscheius onirici	MG551687			Portugal	Unknown	Campos-Herrera (unpublished)
Oscheius onirici		LN613263		Italy	Soil	Torrini et al. (2015)
Oscheius rugaoensis	JQ002566			China	Soil	Zhang et al. (2012)
Oscheius rugaoensis		KT884891		Japan	*Chamberlinius hualienensis*	Carta et al. (2018)
Oscheius saproxylicus	MK959600	MK959601		Spain	Decaying wood	Abolafia and Pena-Santiago (2019b)
Oscheius tipulae		EU195969		USA	Unknown	Kiontke et al. (2007)
Oscheius tipulae			KP792649	Brazil	Soil	Campos-Herrera and Půža (unpublished)
Parasitorhabditis obtusa		EF990724		Germany	Bark beetles	Kiontke et al. (2007)
Parasitorhabditis obtuse	EU003189			USA	Unknown	Kiontke et al. (2007)
Pellioditis typica			AF036946	Kenya	Feces of an antelope	Adams et al. (1998)
Pelodera pseudoteres	EU196023	EU195997		USA	Unknown	Kiontke et al. (2007)
Pelodera teres	AF083002			USA	Unknown	Fitch (unpublished)
Pelodera teres		EU195979		USA	Unknown	Kiontke et al. (2007)
Phasmarhabditis hermaphrodita	DQ639980			Scotland	Nemaslug	MacMillan et al. (2006)
Phasmarhabditis neopapillosa	DQ639982			Scotland	*Arion ater*	MacMillan et al. (2006)
Phasmarhabditis papillosa			KX267675	South Africa	*Deroceras reticulatum*	Pieterse et al. (2017)
Poikilolaimus floridensis	AB370214			USA	Termites	Kanzaki et al. (2009)
Poikilolaimus oxycercus	AF083023			USA	Unknown	Fitch (unpublished)

Poikilolaimus oxycercus		EU195984	USA	Unknown	Kiontke et al. (2007)
Poikilolaimus piniperdae		DQ059060	Germany	Unknown	Hong et al. (2005)
Poikilolaimus regenfussi	AF083022		USA	Unknown	Fitch (unpublished)
Poikilolaimus regenfussi		DQ059057	Germany	Unknown	Hong et al. (2005)
Protorhabditis sp.	AF083001		USA	Unknown	Fitch (unpublished)
Protorhabditis sp.		AY602168	USA	Unknown	Kiontke et al. (2007)
Rhabditella axei	U13934		Germany	Unknown	Fitch et al. (1995)
Rhabditella axei		AY602177	USA	Unknown	Kiontke et al. (2004)
Rhabditis brassicae	EU196006		USA	Unknown	Kiontke et al. (2007)
Rhabditis sp.	MN082353, MN082355		Taiwan	Soil	Yang et al. (2020)
Rhabditoides inermis	AF082996			Unknown	Fitch (unpublished)
Rhabditoides regina		EF990726	USA	Unknown	Kiontke et al. (2007)
Rhomborhabditis regina	KX385908		Mexico	White grub	Jiménez-Cortés et al. (2016)
Teratorhabditis mariannae	EF990716	EF990721	USA	Soil compost	Kanzaki et al. (2008)
Teratorhabditis palmarum	U13937		USA	Unknown	Fitch et al. (1995)

Note: [a]Species names have been updated according their current nomenclature.

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