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A new isolate of *Mesorhabditis monhystera* (Bütschli, 1873) Dougherty, 1955 (Rhabditida: Rhabditidae): re-evaluated with molecular data and scanning electron microscopic observations

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Research Paper

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Abstract

A new isolate of *Mesorhabditis monhystera* (Bütschli, 1873) Dougherty, 1955 is described and illustrated with morphological and molecular data. The phylogenetic analysis based on the D2/D3 segment of 28S rDNA using the Bayesian inference method, revealed monophyly of the genus *Mesorhabditis* as the subordinate taxa clustered in one clade. The clade further divided into two subclades representing the *Monhystera*-group and *Spiculigera*-group with 100% posterior probability values. However, GenBank sequences of several species constituting the *Monhystera*-group, showed high similarity and very little genetic divergence (98–99%) of up to 4–5 bases. In order to ascertain the status of those isolates, detailed morphological comparison is provided along with a pictorial key. A sequence-based phylogeography of haplogroups of *Mesorhabditis* using the median-joining network method, was also inferred. The results suggested the need for morphological validation of a species before its sequences are deposited in GenBank.

Introduction

Most of the species of *Mesorhabditis* Osche, 1952 belonging to the *Monhystera*-group, are poorly described and illustrated. Males have not been reported in *Mesorhabditis acuminata* (Kreis, 1929) Dougherty, 1955; *Mesorhabditis capitata* Loof, 1964; *Mesorhabditis cranganorensis* (Khera, 1968) Andrássy, 1983; *M. dunensis* Khera, 1971; *Mesorhabditis sambharensis* Khera, 1971; and *Mesorhabditis signifera* (Baranovskaya, 1959) Baranovskaya, 1962. Only a few species such as *M. acuticauda* (Shokoohi *et al*., 2014), *Mesorhabditis minuta* (Boström, 1991; Abolafia & Peña-Santiago, 2009) belonging to the *Spiculigera*-group, and *Mesorhabditis microbursaris* (Mahboob and Jahan, 2021) belonging to the *Monhystera*-group have been described based on scanning electron microscopic observations. Likewise, *Mesorhabditis acidophila* (Borgonie *et al.,* 2010) and *Mesorhabditis monhystera* have been molecularly characterized. Lately, Launay *et al.* (2020) studied the relationship between the isolates of the *Monhystera*-group based on the D2/D3 domain of large subunit 28 rDNA.

The present study provides a detailed description of *M. monhystera* (Bütschli, 1873) Dougherty, 1955 based on morphometrics and morphological observations, molecular characterization and scanning electron microscopy (SEM). A phylogenetic analysis based on the D2/D3 segment of 28S rDNA, presents the precise status of the genus *Mesorhabditis* among closely related taxa. A pictorial key comparing the species of the *Monhystera*-group, is also provided. The phylogeography of haplogroups of *Mesorhabditis* using the median-joining network method (Bandelt *et al*., 1999) is used to infer the degree of mutation/divergence among the different isolates.

Materials and methods

Collection, extraction and culturing of nematodes

The sample containing *M. monhystera* was collected from soil contaminated with slaughter wastes in Vessu, Anantnag, Jammu and Kashmir, India. The samples were stored in plastic bags and brought to the laboratory. To extract nematodes, the samples were processed through Cobb's (1918) sieving and decanting methods and the modified Baermann (1917) funnel technique. Stock cultures of nematodes were maintained in 1.2% nematode growth medium.

Light microscopic observations

For light microscopy (LM), nematodes were fixed in 4% formaldehyde, dehydrated to pure glycerine (Seinhorst, 1959) and later mounted on slides using the wax ring technique (De Maeseneer & D'Herde, 1963). The nematodes were measured with an ocular micrometer and drawn using a drawing tube. LM photographs were taken with a Jenoptik digital camera, 'ProgRes' (Jena, Germany), mounted on an Olympus BX-51 DIC microscope.

Scanning electron microscopic observations

For SEM, live nematodes were picked from one-week-old culture. The nematodes (15 males and 15 females) were fixed in SEM fixative (1.6% paraformaldehyde and 2.5% glutaraldehyde) for 24 h at 4°C. The fixed nematodes were washed three times in phosphate buffer, dehydrated in ethanol series (30%–100%) and dried using hexamethyldisilazane. The dried nematodes were later mounted on stub and coated with 10 nm gold before being observed under 10–15 kV under a Hitachi TM4000 Plus scanning electron microscope (Hitachi, Singapore).

Molecular profiling

For DNA extraction, ten live individuals were transferred to an Eppendorf tube containing 20 µl lysis buffer (Williams *et al.*, 1992). The sample was kept at −20°C in a refrigerator for 24 h, and then incubated in a thermal cycler at 65°C for 45 min, followed by 15 min at 95°C. The samples were cooled at 4°C and stored at −20°C. For DNA amplification, 5 µl lysate was used in a 20 µl polymerase chain reaction (PCR) reaction mix following the manufacturer's protocol (GeNei, Bengaluru, India). The sequence of the D2/D3 expansion region of large subunit 28 s rDNA was amplified using the forward primer D2A 5′–ACAAG TACCGTGAGGGAAAGTTG–3′ and the reverse primer D3B 5′–TCCTC GGAAGGAACCAGCTACTA–3′. For amplification, 5 ml DNA lysate was used in a 20 ml PCR mix. The PCR parameters included: initial denaturation at 95°C for 5 min; followed by 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 1 min; and final extension for 10 min at 72°C. Aliquots of 5 ml of the PCR products were sized with a low DNA mass ladder and separated by electrophoresis in 1% agarose gel stained with ethidium bromide and observed under the Ultraviolet Transilluminator Dolphin View Gel Documentation system. Sequencing was done in both directions.

Evaluation of the phylogenetic framework

The obtained sequences were edited in Chromas version 2.6.6. (Technelysium Pty Ltd, www.technelysium.com.au), aligned and a consensus sequence generated in BioEdit (Hall, 1999). The consensus sequence of 579 base pairs was submitted to GenBank with accession number ON693986. The sequence of *M. monhystera* (Bütschli, 1873) Dougherty, 1955 was aligned with GenBank sequences of 37 closely related taxa, in MEGA X (Kumar *et al.*, 2018) using the CLUSTAL_x alignment tool (Thompson *et al.*, 1997). The ambiguously aligned sequences were removed using the online version of Gblocks 0.91b (Castresana, 2000). The phylogenetic tree with 481 characters in the final dataset was inferred by the Bayesian inference method, MrBayes version 3.1.2 (Huelsenbeck & Ronquist, 2001). For the analyses, the best model under the Akaike information criterion was determined to be (GTR + G + I) using jModelTest version 2.1.3 (Darriba *et al.*, 2012). The Akaike-supported model, log-likelihood, state frequency of nucleotides, substitution rate across the sites, proportions of invariable sites, the shape parameter of the gamma distribution and rate of variation were examined during analysis. The obtained values of the above parameters were as follows: −InL = 2567.7772; freqA = 0.2457, freqC = 0.2052, freqG = 0.3231, freqT = 0.2260; R (AC) = 0.7687, R(AG) = 2.2213, R(AT) = 0.9403, R(CG) = 1.4047, R(CT) = 7.1119, R(GT) = 1.0000; p-inv = 0.1870; and gamma shape = 0.6110. The analysis was run with the Markov chain Monte Carlo for 4×10^6 generations. 'Burn-in' samples were discarded every 2000 generations, and a consensus tree with a minimum 50% majority rule was used for analysis. The tree was visualized, edited and saved with FigTree 1.4.0 (Rambaut, 2014).

Percentage similarity and genetic divergence

The percentage similarity and genetic divergence (base differences) among the sequences of selected species of *Mesorhabditis* were estimated as per Mahboob *et al.* (2022). The number of base differences per sequence was computed in MEGAX with 262 positions including parts of both loci in the final dataset.

Systematics

Class: Chromadorea Inglis, 1983
Order: Rhabditida Chitwood, 1933
Suborder: Rhabditina Chitwood, 1933
Infraorder: Rhabditomorpha De Ley & Blaxter, 2002
Superfamily: Rhabditoidea Örley, 1880
Family: Rhabditidae Örley, 1880
Genus: Mesorhabditis Osche, 1952

Diagnosis. Rhabditidae. Gonochoristic or hermaphroditic individuals with small to medium-sized, 400–944 µm long body. Cuticle finely transversely annulated. Lip region usually offset from adjoining body, rarely continuous. Lips well separated, rounded to globular, each with raised setose outer labial sensilla. Amphidial aperture small, on lateral lips. Stoma tubular, long. Cheilostomal walls usually not cuticularized. Gymnostom cuticularized constituting long tubular part of stoma. Stegostom with distinct metastegostomal swellings, each armed with two small denticles. Pharynx rhabditoid type with cylindrical corpus, swollen metacorpus, usually with zipper-like lumen and basal bulb with double-chambered haustrulum. Female reproductive system monodelphic, prodelphic. Ovary reflexed, oviduct continuing into a distinct spermatheca. Vagina obliquely oriented with post-equatorial vulval opening. Male reproductive system monorchic; vas deferens with paired ejaculatory glands. Spicules separated or distally fused, short to long and slender. Bursa well-developed or rudimentary, peloderan or leptoderan, anteriorly open. Genital papillae 5–9 pairs. Tail short conical to elongate conoid, moderately long. Phasmids at the level of or posterior to the anus.

Type species: *Mesorhabditis spiculigera* (Steiner, 1936) Dougherty, 1955

Other species

Mesorhabditis acidophila Borgonie, Dierick, Houthoofd, Willems, Jacobs and Bert, 2010

Mesorhabditis acuminata (Kreis, 1929) Dougherty, 1955

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Mesorhabditis acuticauda Ahmad, Shah and Mahamood, 2010
Mesorhabditis africana Andrássy, 1982
Mesorhabditis anisomorpha (Sudhaus, 1978) Andrássy, 1983
Mesorhabditis belari (Nigon, 1949) Dougherty, 1953
Mesorhabditis capitata Loof, 1964
Mesorhabditis carmenae Abolafia and Peña-Santiago, 2009
Mesorhabditis cranganorensis (Khera, 1968) Andrássy, 1983
Mesorhabditis denticulatus Mahboob and Jahan, 2021
Mesorhabditis dunensis Khera, 1971
Mesorhabditis franseni Fuchs, 1933
Mesorhabditis inarimensis (Meyl, 1953) Dougherty, 1955
Mesorhabditis irregularis (Körner in Osche, 1952) Dougherty, 1955
Mesorhabditis kherai (Sudhaus, 1976) Sudhaus, 2011
Mesorhabditis kinchegensis Nicholas, 1998
Mesorhabditis labiata (Völk, 1950) Dougherty, 1955
Mesorhabditis littoralis Yeates, 1969
Mesorhabditis longespiculosa (Schuurmans Stekhoven, 1951) Dougherty, 1955
Mesorhabditis longistomis Massey, 1974
Mesorhabditis megachilis (Sudhaus, 1978) Andrássy, 1983
Mesorhabditis microbursaris (Steiner, 1926)
Mesorhabditis minuta Boström, 1991
Mesorhabditis miotki (Sudhaus, 1978) Andrássy, 1983
Mesorhabditis monhystera (Bütschli, 1873) Dougherty, 1955
Mesorhabditis oschei (Körner in Osche, 1952) Dougherty, 1955
Mesorhabditis paucipapillata (Paetzold, 1955) Paetzold, 1958
Mesorhabditis riparia (Brzeski, 1985) Sudhaus, 2011
Mesorhabditis sambharensis Khera, 1971
Mesorhabditis scanica (Allgén, 1949) Sudhaus, 2011
Mesorhabditis signifera (Baranovskaya, 1959) Baranovskaya, 1962
Mesorhabditis simplex (Cobb, 1893) Sudhaus, 2011
Mesorhabditis spiculigera (Steiner, 1936) Dougherty, 1953
Mesorhabditis striatica Dassonville and Heyns, 1984
Mesorhabditis sudhausi Andrássy, 1982
Mesorhabditis szunyoghyi Andrássy, 1961
Mesorhabditis vernalis (Andrássy, 1982)

Material examined

The voucher material representing nine females and nine males in good condition, was examined (figs 1–4).

Measurements

For measurements, see table 1.

Description

Adult. Medium-sized nematodes, almost straight after fixation, tapering at both extremities, more in the posterior region. Cuticle 1–2 µm thick, annulated with transverse striations and punctations all over the body except tail region. Punctations conspicuous up to two stoma length in the anterior region. Lateral fields with four prominent bands/ridges. Lip region offset, about twice of its length. Lips six, globular, well separated, each with raised setose labial sensilla. Amphidial apertures slit-like, labial, minute, and indistinguishable under LM. Stoma long, 4–5 times longer than wide, constitute 11–12% of total pharyngeal length. Cheilostom a short tube with cuticularized walls. Gymnostom a long tube with parallel walls covering larger part of the stoma. Stegostom having two setose denticles at each metastegostomal swelling. Pharynx well-developed, highly muscular, covering about 20–26% of total body length; procorpus long, highly muscular with convoluted lumens (in some specimens), corpus lumen without conspicuous striation or zipper-like pattern, expanded posteriorly into a swollen metacorpus of about 14–18 × 10–15 µm in dimension; isthmus a narrow tube of 15–28 µm long, expanding posteriorly to form a well-developed pyriform basal bulb of about 17–20 µm × 12–15 µm dimension containing highly cuticularized grinder and double-chambered haustrulum. Nerve ring encircling the mid of isthmus at about 57–58% of total pharyngeal length from anterior end. Secretory–excretory duct opening at posterior level of nerve ring at 68–71% of pharyngeal length from anterior end. Cardia conoid, 3–5 µm long. Intestinal cells large with prominent nuclei. Rectum 1.3–1.6 times longer than anal body diameter. Tail conoid, shorter than vulva–anus distance or about 10–11% of the total body length. Phasmids open at the level of anus.

Female. Reproductive system monodelphic, prodelphic; ovary dorsally reflexed often reaching up to spermatheca. Oocytes arranged in three rows at the distal end of the ovary followed by two tiers and a single tier proximally. Oviduct indistinguishable. Spermatheca ovoid to oblong, axial with many spermatozoa followed by the uterus. Vagina thick-walled, 4–6 µm long or 1/4 of the corresponding body diameter obliquely oriented. Vulva posterior at about 72%–76% of body length from the anterior end with lips not protruded.

Male: Similar to female in general morphology except more arcuate ventrally in the posterior region. Testis monorchic, dorsally reflexed (lateral in few specimens). Seminal vesicle well differentiated, swollen containing numerous rounded minute sperms. Vas deferens a muscular narrow tube extending proximally into ejaculatory glands. Spicules small with prominent knob-like capitulum, indistinguishable neck, and slender calamus with a fused distal end. Gubernaculum trough-shaped, covering about 50% of spicule length. Bursa leptoderan, rudimentary, anteriorly open. Genital sensilla papilliform, nine pairs; two pairs precloacal and seven postcloacal pairs out of which three postcloacal pairs inside and four pairs outside bursal flaps, oriented dorsally; Tail conoid, constituting 13–15% of the total body length, usually shorter than vulva–anus distance.

Habitat and locality

The present population of *M. monhystera* (Bütschli, 1873) Dougherty, 1955 was collected from the soil sample contaminated with slaughter wastes in Vessu, Anantnag, Jammu and Kashmir, India at coordinates 33°40′17″ N 75°07′45″ E.

Voucher materials

Nine females and nine males on slides of *M. monhystera* (kmr/dist/Meso/1–10) were deposited in the Nematode Collection, Department of Zoology, Aligarh Muslim University, Aligarh, Uttar Pradesh, India.

Remarks

Mesorhabditis monhystera has been originally reported from soil around the roots of Plantago, Germany (Bütschli, 1873) and subsequently from multiple terrestrial and also aquatic habitats of

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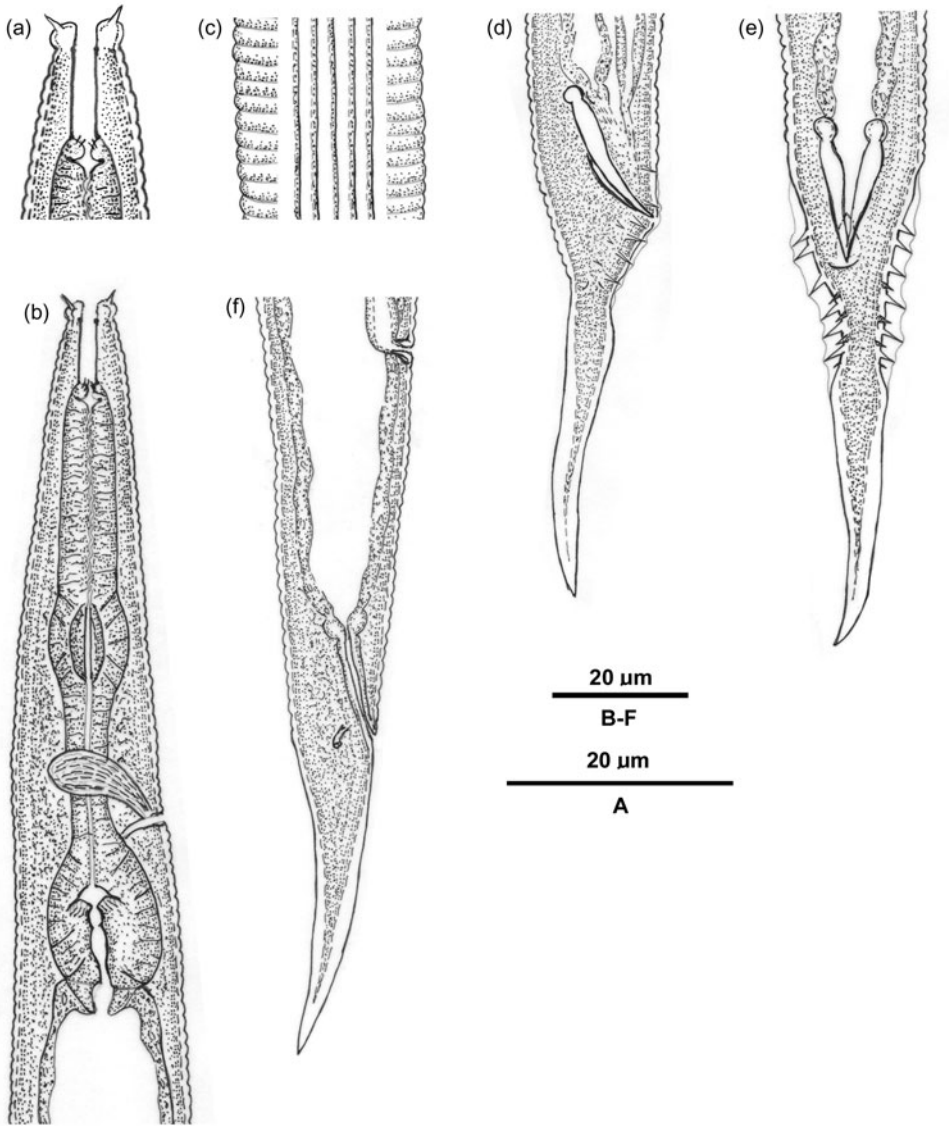

Fig. 1. Line drawing of *Mesorhabditis monhystera* (Bütschli, 1873) Dougherty, 1955. (a–c, f) female and (d, e) male: (a) anterior end; (b) pharyngeal region; (c) lateral field with four prominent bands; (d) tail region (lateral view); (e) tail region (ventral view); and (f) tail region (lateral view). Scale bar = 20 µm.

France (Launay *et al.*, 2020) and the United States (Chitwood & Chitwood, 1934, 1937; Sudhaus, 2018). The present population of *M. monhystera* (Bütschli, 1873) Dougherty, 1955 resembles those described by earlier workers (Bütschli, 1873; Sudhaus & Fitch, 2001; Andrássy, 2005) including the original population, in most of the characteristics. However, the only population showing dissimilarity with the present population in the morphological characteristics is *M. monhystera apud* Abolafia & Peña-Santiago (2009) that shows differences in the type of lip region (weakly vs. distinctly offset); length of labial sensilla (smaller vs. larger); and number (three vs. four) of ridges in the lateral fields, male tail and spicule shapes (with distinguishable vs. indistinguishable) calamus and the configuration of genital papillae (three vs. two) precloacal pairs. Coincidentally, *M. monhystera apud* Abolafia & Peña-Santiago (2009) resembles *M. vernalis* Andrássy, 1982 in all the above characteristics although the number of genital papillae is greater (nine vs. six) pairs.

Emended diagnosis

Mesorhabditis monhystera is characterized by small to medium-sized individuals with cuticle annulated with transverse striations and punctations; lateral fields with four prominent bands; lip region offset with six well-separated globular lips, each with a tentaculate labial sensilla; amphidial apertures labial, small, elliptical slit-like; corpus lumen occasionally striated, metacorpus swollen, basal bulb having a grinder with double-chambered haustrulum; reproductive system mono-prodelphic, vagina obliquely oriented, vulva posteriorly located at about 65%–76% from the anterior end without protruded lips; rectum usually 1.3–1.6 times longer than the anal body diameter; phasmids opening at the level of the anus; tail conoid, usually shorter than vulva–anus distance; male with spicules fused distally, each comprising of distinct knobbed manubrium, indistinguishable calamus, slender lamina; gubernaculum covering about 50% of the spicule length; bursa leptoderan

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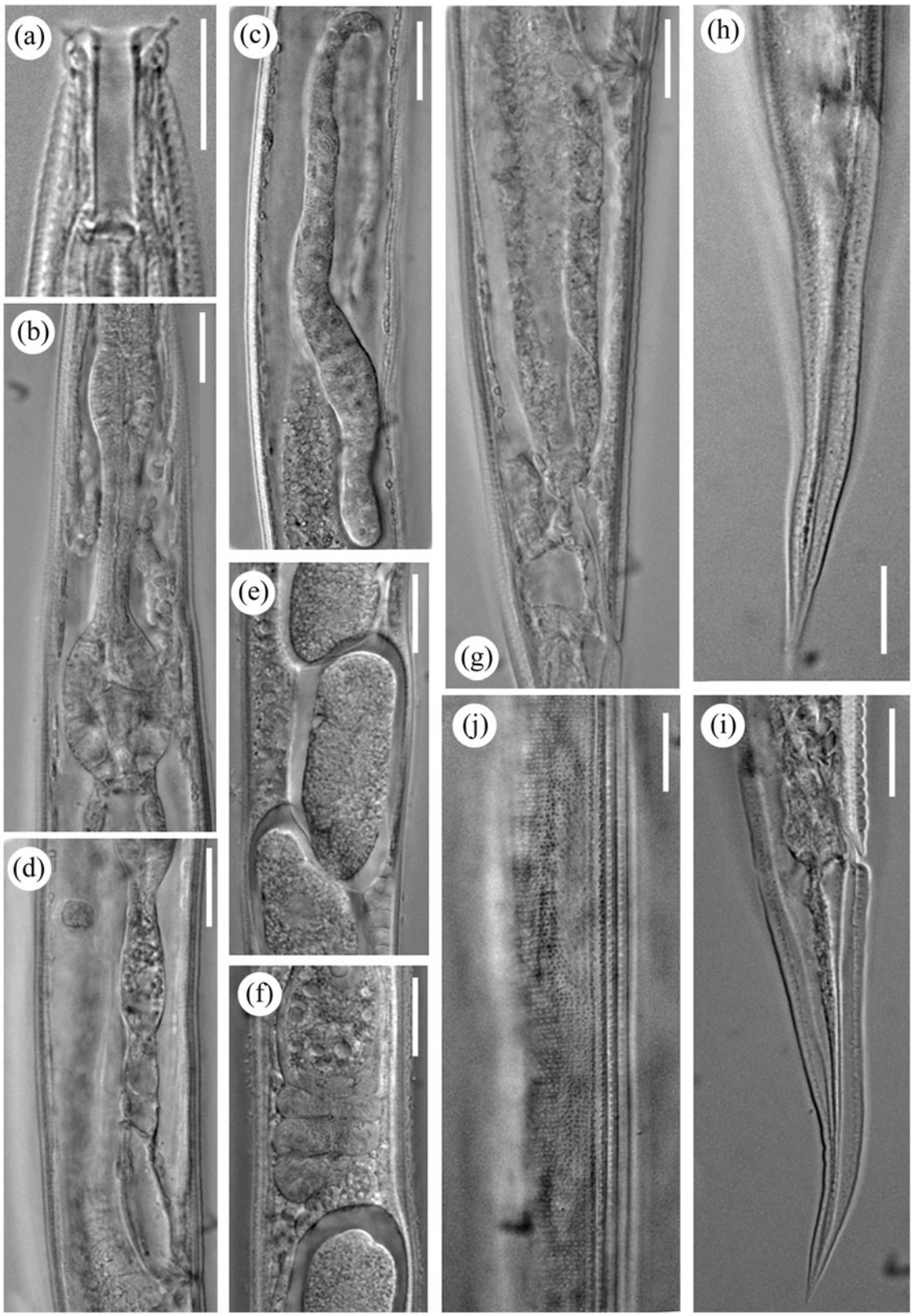

Fig. 2. Light micrograph of *Mesorhabditis monhystera* (Bütschli, 1873) Dougherty, 1955 (female): (a) anterior end; (b) pharyngeal region; (c) distal part of the reproductive system showing ovarian flexure; (d) proximal part of the reproductive system showing seminal vesicle filled with sperms, and uterus; (e, f) part of reproductive system showing seminal vesicle, columella and uterus containing egg; (g) vulva–anus region; (h, i) tail region; and (j) lateral field with four bands. Scale bars = 10 μm.

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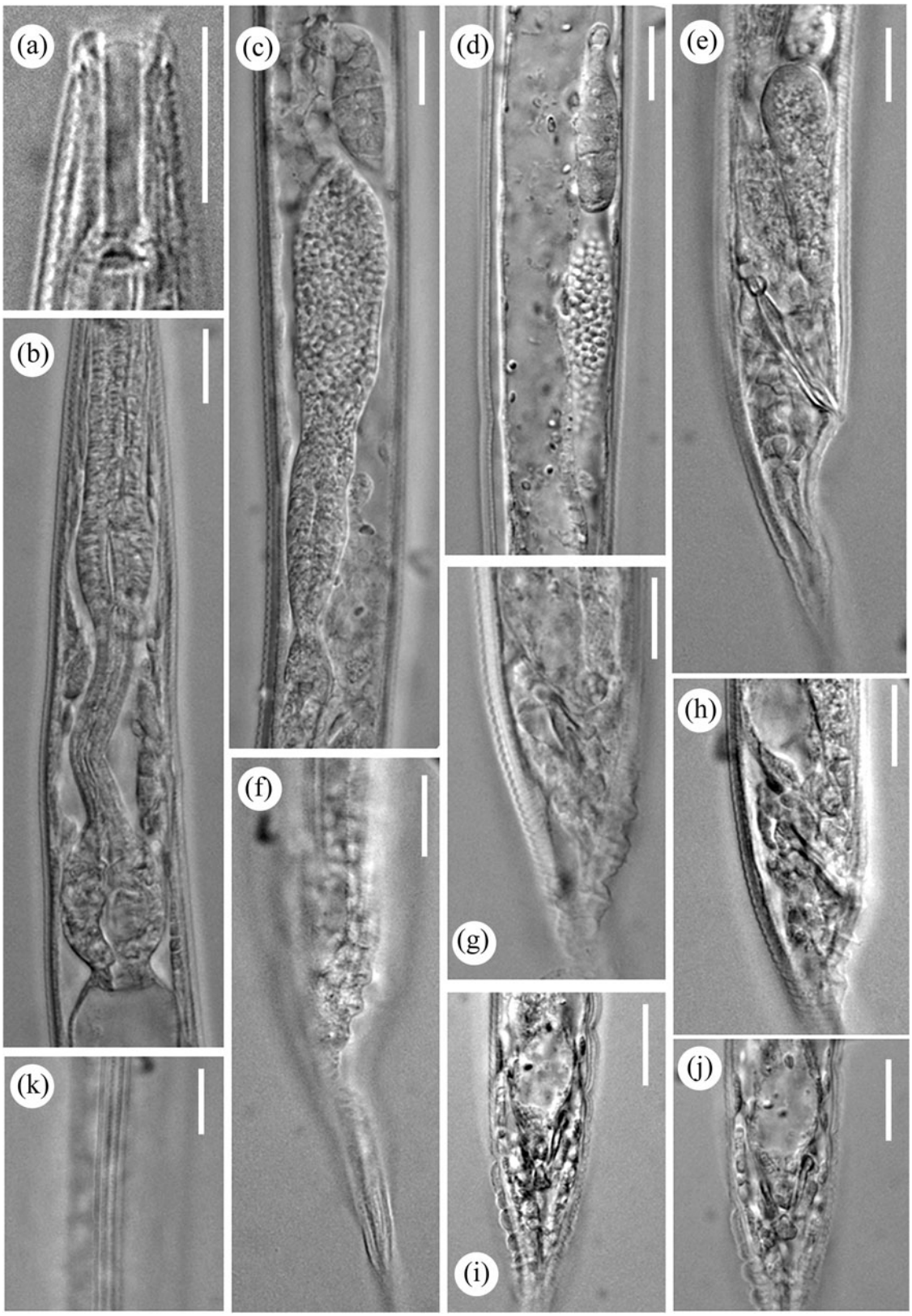

Fig. 3. Light micrograph of *Mesorhabditis monhystera* (Bütschli, 1873) Dougherty, 1955 (male): (a) anterior end; (b) pharyngeal region; (c, d) genital branch with dorsally and laterally reflexed testis, respectively; (e) tail region showing spicule and gubernaculum; (f–h) tail region showing arrangement of genital papillae (lateral view); (i, j) tail region (ventral view); and (k) lateral field with four bands. Scale bars = 10 μm.

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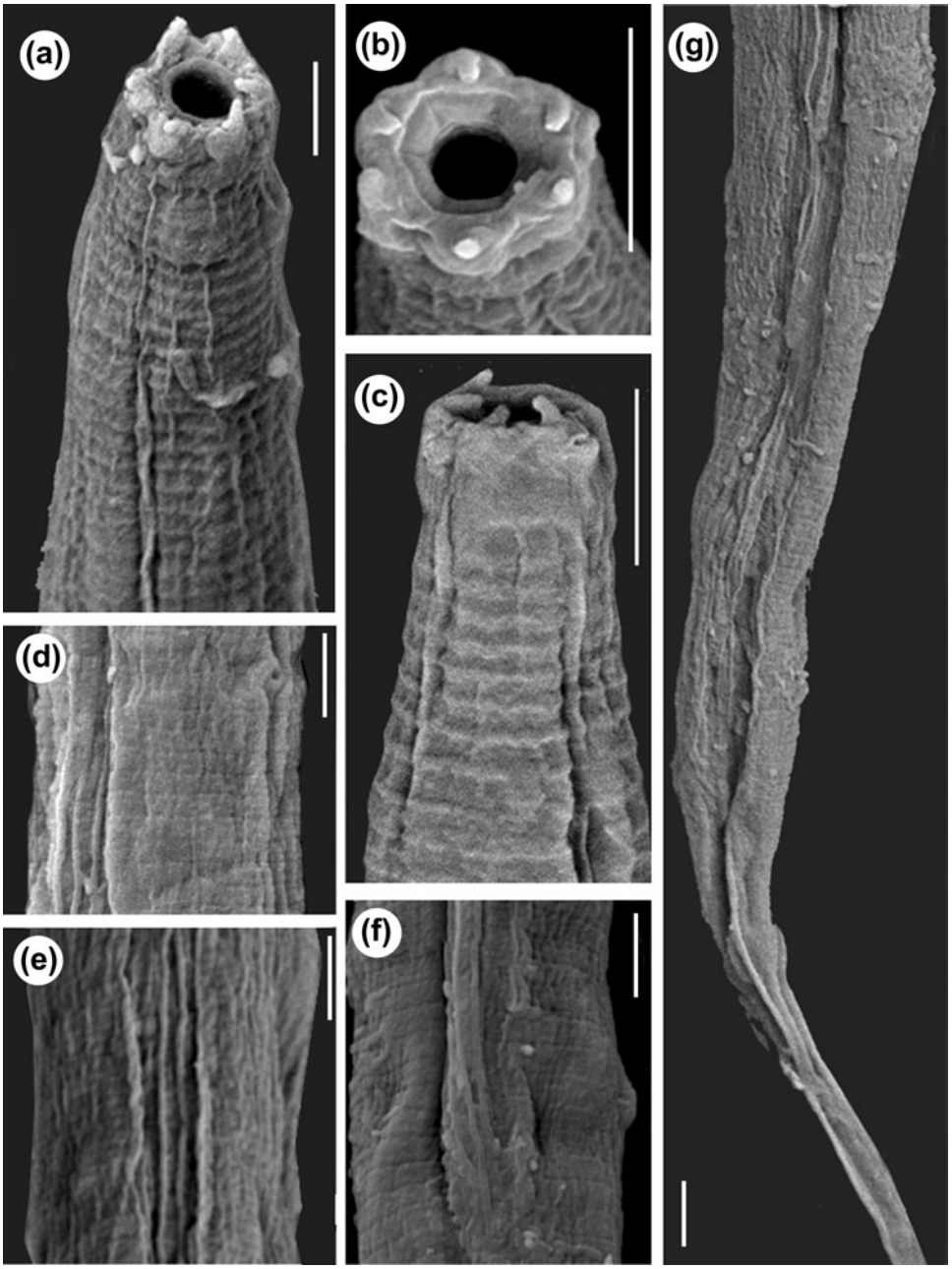

Fig. 4. Scanning electron micrograph of *Mesorhabditis monhystera* (Bütschli, 1873) Dougherty, 1955: (a, c) anterior region; (b) *en face* view; (d) body region showing excretory pore; (e) body region showing lateral fields; (f) mid-body showing vulval lips; and (g) posterior region from the vulva to tail. Scale bars: 5 μm.

largely rudimentary; genital papillae nine pairs; and two pairs precloacal and seven postcloacal pairs out of which three postcloacal pairs inside and four pairs outside bursal flap.

Discussion

Status of the genus Mesorhabditis *among closely related genera*

The Basic Local Alignment Search Tool (BLAST) results revealed similarities of the present population of *M. monhystera* with the monodelphic taxa *viz*., *Crustorhabditis* Sudhaus, 1974*; Distolabrellus* Anderson, 1983; *Mesorhabditis* Osche, 1952 and *Parasitorhabditis* Fuchs, 1937. These groups resembled in homologous traits such as offset lip region (except *Parasitorhabditis obtusa* possessing continuous lip region), vulva situated far posterior (except *Rhabpanus ossiculum* with a slightly posterior vulva) and tail hemispheroid to short conoid. However, the DNA sequence of the present population did not show similarity with monodelphic species of *Cruznema* Artigas, 1927 in the BLAST results. Nevertheless, the members of the genus *Cruznema* were included in the phylogenetic analysis due to being representatives of the family Rhabditidae and sharing some degree of homology. *Panagrolaimus* sp. (LT908055) was selected as an outgroup with few common traits such as monodelphic female gonad with vulva situated far posterior and conoid tail. Besides these traits, *Panagrolaimus* spp. also showed similarity in having continuous lip region, fused lips and metastegostom without

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Table 1. Morphometric data of *Mesorhabditis monhystera* (Bütschli, 1873) Dougherty, 1955.

Character	Female	Male
n	(9 ♀♀)	(9 ♂♂)
body length	612.8 ± 71.0 (548–766)	357 ± 24.8 (326–405)
body diameter	30.6 ± 3.4 (25–36)	20.6 ± 2.7 (17–25)
a	20.2 ± 2.3 (16.2–23.9)	17.4 ± 1.5 (14.8–19.2)
b	4.2 ± 0.5 (3.8–5.4)	3.0 ± 0.1 (2.7–3.3)
c	8.7 ± 0.9 (7.8–10.9)	7.2 ± 0.5 (6.3–7.9)
c′	4.7 ± 0.4 (4.0–5.4)	3.6 ± 0.5 (3.1–4.7)
V/T	74.6 ± 1.1 (72–76)	52.9 ± 4.0 (50–53)
G1	45.9 ± 6.0 (38–53)	–
lip region (height)	4.0 ± 0.0 (4–4)	3.1 ± 0.3 (3–4)
lip region (diameter)	7.4 ± 0.5 (7–8)	6.0 ± 0.0 (6–6)
stoma length	17 ± 0.0 (16–18)	14.1 ± 0.3 (14–15)
stoma diameter	4.0 ± 0.0 (4–4)	3.0 ± 0.0 (3–3)
pharynx length	146.1 ± 5.2 (140–154)	117.3 ± 4.2 (111–123)
nerve ring from anterior end	85.9 ± 3.2 (82–90)	70.3 ± 7.3 (63–85)
secretory–excretory pore from anterior end	102.0 ± 5.2 (96–110)	77.3 ± 6.0 (71–90)
rectum length	23.4 ± 2.7 (18–26)	14.1 ± 1.6 (10–15)
anal body diameter	15.1 ± 1.5 (13–16)	13.8 ± 2.1 (10–17)
vulva–anus distance	78.9 ± 9.0 (68–95)	–
spicule length	–	20.0 ± 1.5 (18–23)
gubernaculum length	–	10.2 ± 1.2 (8–12)
tail length	70.8 ± 4.0 (64–80)	50.1 ± 4.9 (43–60)

Measurements are in µm and in the form: mean ± standard deviation (range).
Note: *a*, total body length/body diameter; *b*, total body length/pharynx length; *c*, total body length/tail length; *c′*, tail length/anal body diameter; *V/T*, vulva percentage with respect to total body length/male gonad percentage with respect to total body length; and *G1*, female genital branch percentage with respect to total body length.

conspicuous denticles as observed in members of *Matthesonema* Osche, 1955 and *Parasitorhabditis*, although warts were observed in some species of *Parasitorhabditis*.

The tree topology of the selected genera revealed two major clades: one clade comprised the taxa of *Mesorhabditis* belonging to both *Monhystera*-groups and *Spiculigera*-groups suggesting monophyly; and the other clade represented the taxa of the genera *Cruznema*, *Distolabrellus* and *Teratorhabditis*, although *Cruznema* diverged earlier than the latter two genera. Moreover, the genus *Parasitorhabditis* formed a separate clade and diverged earlier than the rest of the groups.

Our analysis agreed well with the results of Launay *et al.* (2020) based on 28S rDNA and 1nternal transcribed spacer 2 region in the placement of the different isolates of the genus *Mesorhabditis* (*Monhystera*-group). However, the present analysis did not totally conform to that of Shokoohi *et al.* (2014) based on small subunit (SSU) 28S rDNA, where *Cruznema* formed a clade with *Buetschlinema* Sudhaus, 2011 and diverged earlier than the genera *Teratorhabditis* and *Distolabrellus*. Also, the placement of the genus *Parasitorhabditis* and the species *M. anisomorpha* showed conflict where *Parasitorhabditis* diverged first and both *M. anisomorpha* and *M. longispiculosa* clustered together. The present analysis also differs from the phylogenetic inference made by Valizadeh et al. (2017) in the placement of *Parasitorhabditis* close to *Mesorhabditis*, although it showed agreement in the placement of *M. longispiculosa* and *M. anisomorpha*. However, the placement of *Cruznema* also differed as it clustered with *Pellioditis* Dougherty, 1953 and *Rhabditella* Cobb, 1929 (fig. 5).

Molecular status of the congeners of Mesorhabditis *(*Monhystera*-group)*

The phylogenetic tree demonstrated monophyletic status of the genus *Mesorhabditis* that formed a major clade of the subordinate taxa. The members were further divided into two subclades representing *Monhystera*-group and *Spiculigera*-group with 100% posterior probability values. The members of *Monhystera*-group further diverged into two groups with good branch support values: the isolates of *M. monhystera* with accession numbers (MT710269; ON693986; MT710271), clustered together showing similarity but differed with nearest *M. denticulatus* (MW763072) with 100% posterior probability values, whereas, most species – *viz.*, *M. belari* (EF417149; MT710238), *M. paucipapillata* (MT710240), *M. cranganorensis* (MT710263), *M. microbursaris* (MT710259); *M. vernalis* (MT710258), *M. littoralis* (MT710253), *M. simplex* (MT710249), and *M. franseni* (MT710247) of the *Monhystera*-group as reported by Launay *et al.* (2020), clustered together in another group (fig. 5).

The sequence of the present population of *M. monhystera* (ON693986) showed 95% similarity and three (3) base divergence with another isolate of *M. monhystera* (MT710271); however, it showed 91% similarity and thirty-one (31) base divergence with *M. denticulatus*. In this context, several species of *Monhystera*-group with sequence deposited in GenBank, were observed to demonstrate high similarity (98–99%) with very little base divergence (0–5 bases) from each other. The sequences of *M. belari* (EF417149; MT710238) and *M. paucipapillata* (MT710240) with 99% similarity, 0–1 base divergence along with 0.0–1.0 standard error appeared to be conspecific. Likewise, the sequences of *M. cranganorensis* (MT710263) and *M. microbursaris* (MT710259) with 99% similarity and 0 base divergence with 0.0 standard error, and *M. simplex* (MT710249) and *M. franseni* (MT710247) with 98% similarity and 1 base divergence with 1.0 standard error also indicated overlap. Although *M. vernalis* (MT710258) and *M. littoralis* (MT710253) clustered in one clade with 81% similarity, there was only 1 base difference with standard error (1.0) (tables 2 and 3). The Haplotype network of the isolates of *Mesorhabdittis* sampled from different geographical locations and inferred using the median-joining network method, revealed three distinct clusters originating from the ancestral stock. Considerable differences based on allele frequency could be noted between the haplotypes of *M. denticulatus* and *M. monhystera* and between *M. longispiculosa* and *M. anisomorpha* but the cluster representing *M. vernalis*, *M. littoralis*, *M. belari*, *M. paucipapillata*, *M. cranganorensis*, *M. microbursaris M. simplex* and *M. franseni* showed insignificant genetic deviation and little change in allele frequency (figs 6 and 7). Launay *et al.* (2020),

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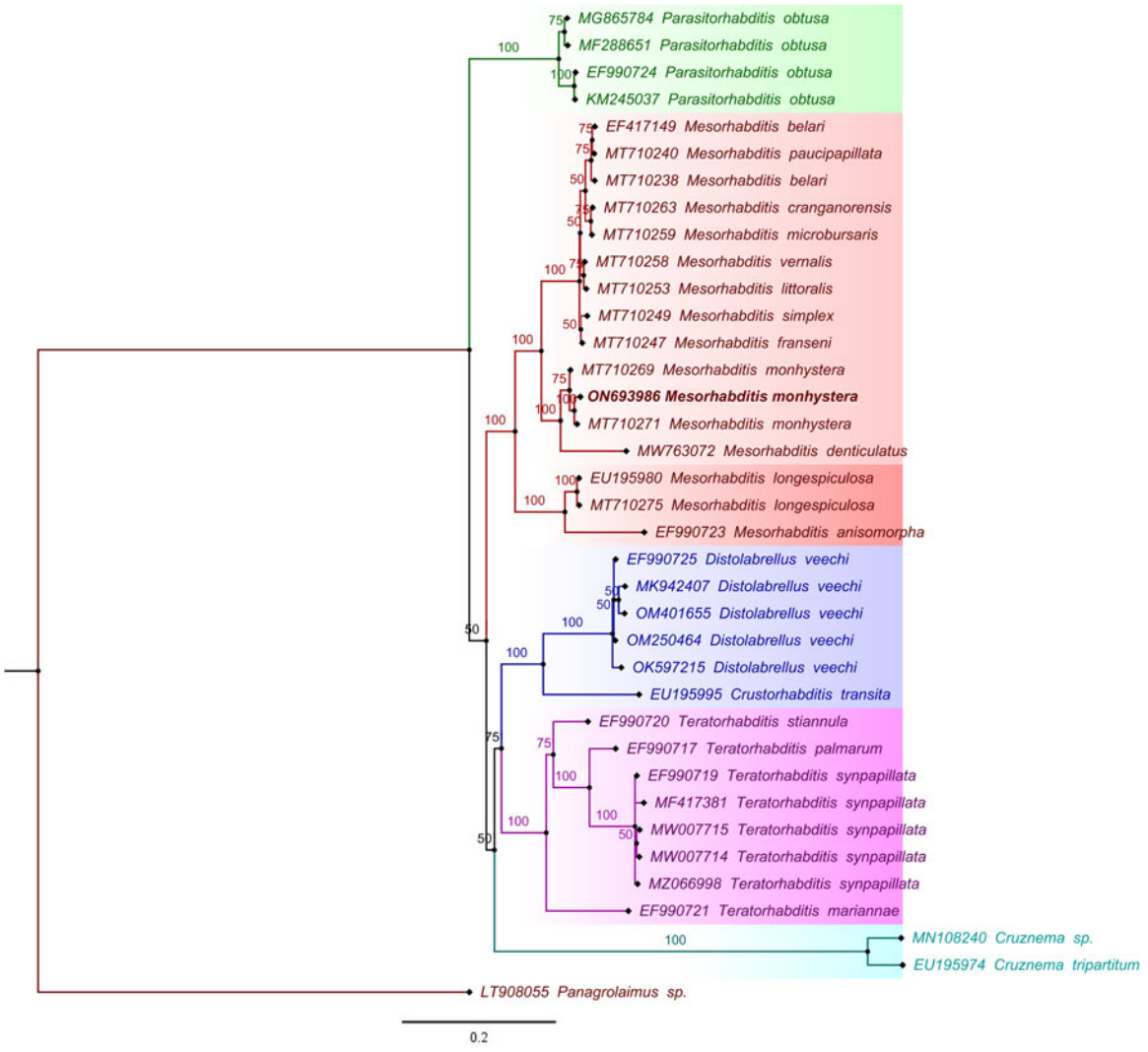

Fig. 5. Bayesian phylogenetic tree based on the D2/D3 domain of large subunit 28S rDNA inferred in MrBayes version 3.1.2 (Huelsenbeck & Ronquist, 2001). The evolutionary history was evaluated using the GTR + I + G model. The tree topology indicated the status of the present population of *Mesorhabditis monhystera* (Bütschli, 1873) Dougherty, 1955 among the congeners. The consensus tree with a minimum 50% majority rule was used for analysis. The posterior probability values are reflected at appropriate clades. The scale bar shows the number of substitutions per site.

based on cross-breeding experiments, reported the above-mentioned, largely pseudogamous isolates to be true species and emphasized that little genetic variation was sufficient for transition to a new species in the asexual regime, while intraspecific genetic diversity accumulated with sexuality and recombination. However, in view of the bifurcated findings, a revision based on the complete SSU (18S rDNA) and LSU (28S rDNA) is required to resolve the status of the above species in addition to the validation of its identity in the light of original types.

Morphological status of the congeners of Mesorhabditis *(*Monhystera*-group)*

The species of *Mesorhabditis* represent widely distributed, r-selective bacterivores reported from enriched habitats including rotten wood, plant residues and sediment at the shore of freshwater bodies. The salient characters include: rounded, separated lips, each with one thorn-like sensillum; long stoma having glottoid apparatus with two setose denticles at each sector; pharyngeal sleeve absent or very small; zipper-like corpus lumen, swollen metacorpus; mono-prodelphic gonad; female tail mostly elongate conoid, males occasionally rare with well-developed to the reduced bursa, and spicules distally fused and two pairs of precloacal genital sensilla. Owing to the heterogeneity, the members are divided into two species groups *viz*., *Monhystera*-groups and *Spiculigera*-groups (Sudhaus, 2011). The differentiation of the groups is mainly based on the reproductive mode and the male features. The *Monhystera*-group mainly represents small–medium-sized females that are largely hermaphroditic/parthenogenetic or pseudogamous (amictic) with males rare or few while the *Spiculigera*-group usually contains large-sized, gonochoristic individuals with fair representation of males. The males of the *Monhystera*-group usually show small spicules with rudimentary bursa not enclosing all genital sensilla which often are inconspicuous or reduced in number. The females usually possess a tail shorter than vulva–anus distance. On the other hand, males of the *Spiculigera*-group usually possess large-sized spicules and well-developed bursa enclosing 9–10 pairs of genital sensilla including phasmids.

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Table 2. Percentage similarity within the species of genus *Mesorhabditis* based on the D2/D3 domain of the large subunit 28S rDNA sequences along with information on the microhabitat and country-wise location (similarity statistics are as follows: minimum = 67.3; maximum = 100; mean = 90.3; and standard deviation = 7.5).

Serial number	Accession number	Species	Habitat	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	ON693986	*Mesorhabditis monhystera*	soil	100														
2	MT710271	*M. monhystera*	soil	95	100													
3	MW763072	*Mesorhabditis denticulatus*	*Phyllophaga* sp.	91	91	100												
4	EF417149	*Mesorhabditis belari*	–	89	92	88	100											
5	EU195980	*Mesorhabditis longespiculosa*	–	86	89	84	90	100										
6	MT710258	*Mesorhabditis vernalis*	rotting leaves along river	89	92	89	**99**	90	100									
7	MT710263	*Mesorhabditis cranganorensis*	rotting banana	89	92	88	**99**	89	**99**	100								
8	MT710238	*M. belari*	soil and vegetal matter	89	93	89	**99**	89	**99**	**99**	100							
9	MT710240	*Mesorhabditis paucipapillata*	soil	89	92	89	**99**	90	**99**	**99**	**99**	100						
10	MT710249	*Mesorhabditis simplex*	rotting *Ficus auriculata* fruit	89	93	88	**98**	90	**99**	**99**	**98**	**98**	100					
11	MT710247	*Mesorhabditis franseni*	heap of leaves	88	92	88	**98**	89	**98**	**98**	**98**	**98**	**98**	100				
12	MT710259	*Mesorhabditis microbursaris*	soil	89	92	88	**98**	89	**99**	**99**	**98**	**99**	**98**	**98**	100			
13	EF990723	*Mesorhabditis anisomorpha*	–	82	85	80	85	92	84	85	84	85	85	84	84	100		
14	MT710275	*Mesorhabditis longespiculosa*	tunnel of beetle larva	85	89	84	89	100	89	89	89	89	89	89	89	92	100	
15	MT710253	*Mesorhabditis littoralis*	rotting fruit	71	74	70	80	72	81	80	80	81	80	81	80	67	72	100

Bold values indicate close relationship based on high degree of similarity.

Table 3. Base differences per nucleotide among the sequences of the species of genus *Mesorhabditis* based on of D2/D3 domain of the large subunit 28S rDNA.

Serial number	Accession number	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	ON693986	*Mesorhabditis monhystera*		1.8	5.4	5.5	6.4	5.6	5.6	5.4	5.5	5.6	5.5	5.6	7.3	6.4	5.7
2	MT710271	*M. monhystera*	3		5.3	5.4	6.2	5.5	5.5	5.3	5.4	5.5	5.4	5.5	7.2	6.2	5.5
3	MW763072	*Mesorhabditis denticulatus*	31	30		5.8	7.3	6.0	5.9	5.8	5.8	6.0	5.9	5.9	7.9	7.3	6.0
4	EF417149	*Mesorhabditis belari*	33	32	42		6.2	1.4	1.7	1.0	0.0	2.0	1.7	1.7	7.4	6.2	1.6
5	EU195980	*Mesorhabditis longespiculosa*	46	45	60	43		6.1	6.2	6.2	6.2	6.2	6.1	6.2	5.4	0.0	6.2
6	MT710258	*Mesorhabditis vernalis*	33	32	42	**2**	42		1.7	1.6	1.4	1.4	1.0	1.7	7.5	6.1	1.0
7	MT710263	*Mesorhabditis cranganorensis*	34	33	43	**3**	43	**3**		1.9	1.7	1.7	1.4	0.0	7.4	6.2	1.9
8	MT710238	*M. belari*	32	31	41	**1**	44	**3**	**4**		1.0	2.2	1.9	1.9	7.4	6.2	1.9
9	MT710240	*Mesorhabditis paucipapillata*	33	32	42	**0**	43	**2**	**3**	**1**		2.0	1.7	1.7	7.4	6.2	1.6
10	MT710249	*Mesorhabditis simplex*	33	32	42	**4**	43	**2**	**3**	**5**	**4**		1.0	1.7	7.6	6.2	1.7
11	MT710247	*Mesorhabditis franseni*	32	31	41	**3**	42	**1**	**2**	**4**	**3**	**1**		1.4	7.5	6.1	1.4
12	MT710259	*Mesorhabditis microbursaris*	34	33	43	**3**	43	**3**	**0**	**4**	**3**	**3**	**2**		7.4	6.2	1.9
13	EF990723	*Mesorhabditis anisomorpha*	62	61	77	61	33	62	61	62	61	63	62	61		5.4	7.5
14	MT710275	*M. longespiculosa*	46	45	60	43	0	42	43	44	43	43	42	43	33		6.2
15	MT710253	*Mesorhabditis littoralis*	34	33	43	3	43	1	4	4	3	3	2	4	63	43	

The values below the diagonal indicate the base differences, while those above the diagonal (in blue) indicate standard errors.
Bold values indicate close relationship indicating very less base divergence.

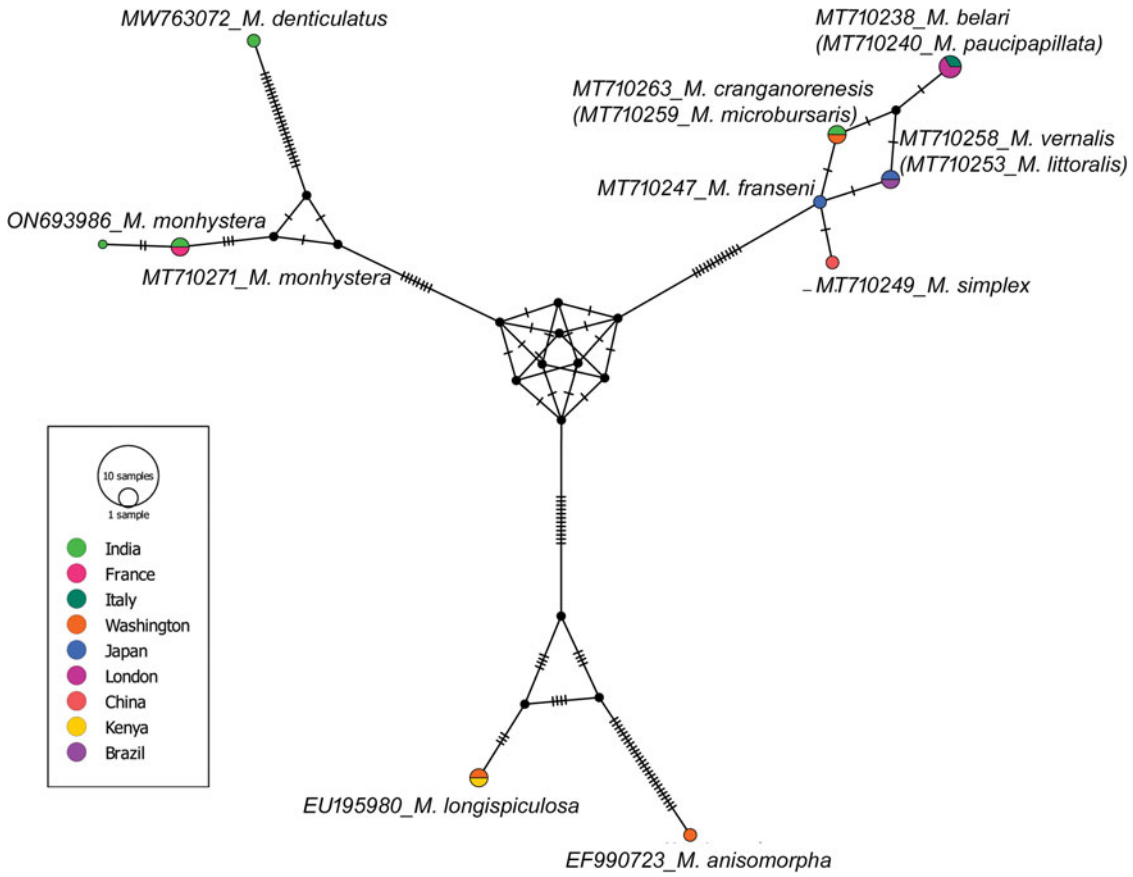

Fig. 6. Haplotype network of the isolates of *Mesorhabditis* inferred using the median-joining network method (Bandelt *et al.*, 1999) based on the D2/D3 domain of large subunit 28 s rDNA. The network was evaluated using the POPART (Leigh & Bryant, 2015). The circle represents the haplotype and its size indicates allelic frequency of the haplotype. Hatch marks between nodes indicate degree of divergence and the colour of the circle shows the geographical location of the isolate of *Mesorhabditis*. Note: nucleotide diversity (pi) = 0.131852; segregating sites = 89; parsimony-informative sites = 47; Tajima's D statistic (D) = 3.48433; and p (D ≥ 3.48433) = 0.

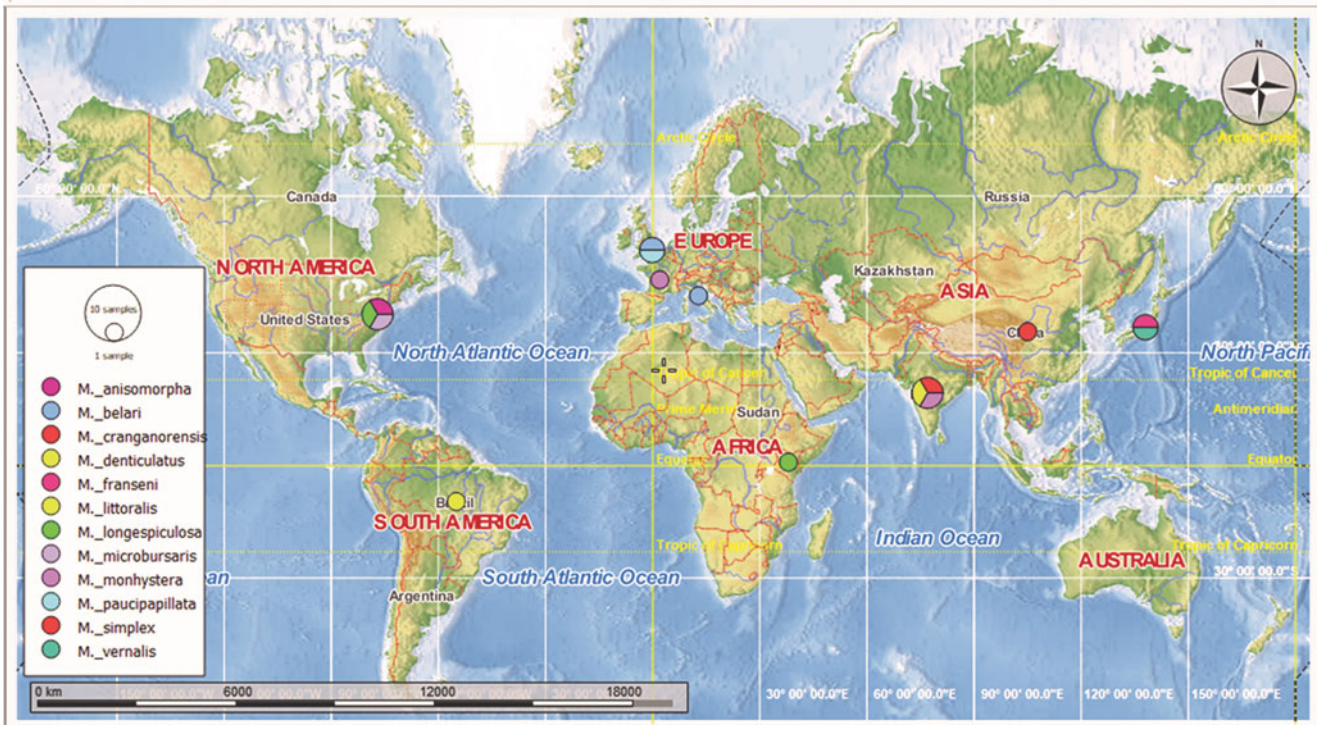

Fig. 7. The geographical location of D2/D3 based sequences of the isolates of *Mesorhabditis*. The sampling location of such isolates was used to place the sequences on the map. The geographical location of the taxa was evaluated using the POPART (Leigh & Bryant, 2015). The colour represents different taxa of *Mesorhabditis*.

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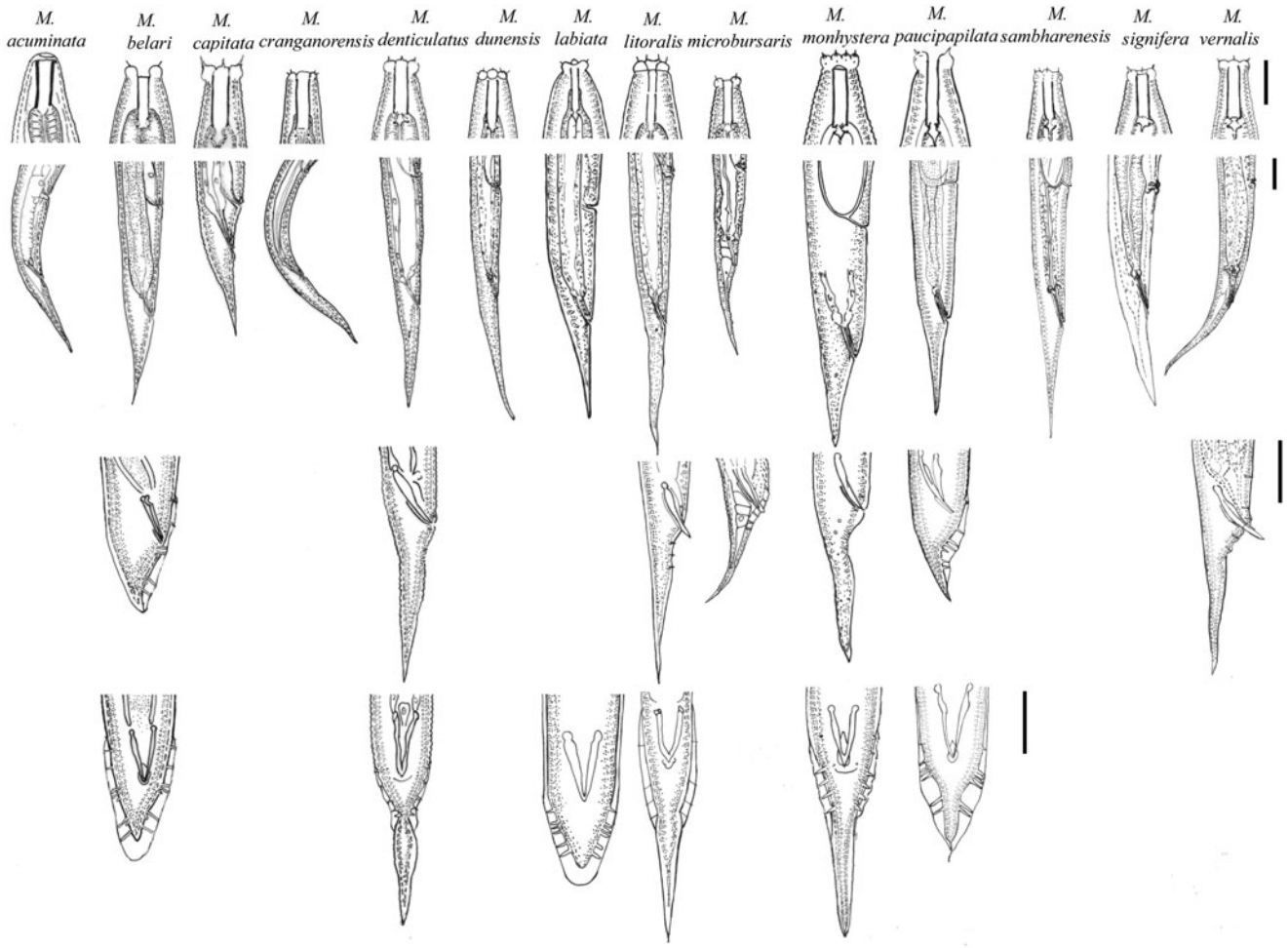

Fig. 8. Pictorial key for the comparison of the species of genus *Mesorhabditis* (*Monhystera*- group) based on female anterior region (scale bar = 10 µm); female posterior region (lateral view) and male tail region (lateral and ventral view) (scale bar = 20 µm. [The type species except *Mesorhabditis monhystera* (after Sudhaus & Fitch, 2001) were redrawn from the original descriptions].

The molecular data of the present population of *M. monhystera* and the other previously reported isolates (MT710269; ON693986; MT710271) revealed close relationships with *M. denticulatus* Mahboob and Jahan, 2021; however, the present population of *M. monhystera* showed closeness to *M. denticulatus* and *M. litoralis* Yeates, 1969 in appearance of lip region and labial sensilla, shape and size of the male tail, and similar shape of spicules. However, *M. monhystera* could be differentiated from the latter two species in having females with conoid (vs. slender) tails and arrangement of genital papillae in five (vs. nine) pairs. *Mesorhabditis denticulatus* significantly differed from *M. litoralis* in having relatively smaller females (411–538 µm vs. 600–720 µm); smaller *b* (3.3–4.5 vs. 5.2–6.0) and V (66–70% vs. 72–82%) values; vulval lips protruded (vs. not protruded); stoma wide (vs. narrow), 4 times (vs. 5–6 times) longer than wide; phasmidial opening posterior (vs. at level of anus); gubernaculum relatively small (vs. large) covering about (50% vs. 70%) of the spicule length; and precloacal genital papillae one pair (vs. two pairs) in *M. litoralis apud* Yeates (1969) (fig. 8).

Besides the close morphological relationships of *M. littoralis* with *M. monhystera* and *M. denticulatus*, the molecular analysis showed that *M. littoralis* positioned with *M. vernalis* Andrássy, 1982, but significantly differed from the latter in a combination of characters *viz*., larger females (600–720 µm vs. 410–560 µm); greater *b* (5.2–6.0 vs. 3.6–4.4) value; stoma wide (vs. narrow); spicules with indistinguishable (vs. distinguishable) neck; gubernaculum relatively smaller (vs. larger) covering about (50% vs. 70%) of the total spicule length; and genital papillae five (vs. six) pairs with one pair (vs. three pairs) of precloacals in *M. vernalis apud* Andrássy (1982) (fig. 8).

The molecular phylogenetic tree constructed from the existing GenBank sequences for *M. belari* Nigon, 1949 and *M. paucipapillata* Paetzold, 1955 showed close relationships of the two which markedly differed in original description in the type of bursa (peloderan vs. leptoderan), tail shape (with vs. without spike) and the number and arrangement of genital papillae (eight pairs vs seven pairs) with two precloacal pairs (vs. one precloacal pair) in *M. paucipapillata apud* Paetzold (1955) (fig. 8).

Likewise, no congruence could be observed between the molecular data obtained from GenBank for *M. cranganorensis* Khera, 1968 and *M. microbursaris* (Steiner, 1926) Andrássy, 1983 and the morphological features of both species in original and subsequent descriptions. Despite being similar on account of body size (405–615 µm) and overlapping morphometric values, *M. cranganorensis* showed significant differences in having greater *a* (28–31 vs. 18–24) value; cuticle with fine or smooth (vs. coarse and prominent) annulations; continuous (vs. offset) lip region with reduced (vs. well-developed globular) lips; female tail slender

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with blunt tip (vs. conoid with fine terminus); and recorded without (vs. with) males in *M. microbursaris apud* Zeidan & Geraert (1989) (fig. 8).

Although molecular characterization is regarded as the most reliable tool for identification, morphological characters cannot be undermined. At a time when classical taxonomists are diminishing, the molecular data submitted to GenBank on the name of an old species becomes very critical. It is instead better to give a new name to a molecularly-characterized population if the holotype and paratype of the proposed species could not be compared.

Conflict of interest. None.

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Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guidelines onthe care and use of laboratory animals.

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