***Sigmolenchus sinuosus* n. gen., n. sp. a new member for the family
Tylenchidae Örley, 1880 (Nematoda, Rhabditida)**

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Summary - *Sigmolenchus* n. gen., is proposed as a new member to the family Tylenchidae. The new genus is established based upon light and scanning electron microscopic (LM and SEM) observations; and phylogenetic analyses based upon small, partial large and internal transcribed spacer sequences of ribosomal DNA (SSU, LSU D2-D3, ITS rDNA) and mitochondrial cytochrome c oxidase I gene (*COI* mtDNA) sequences. The new genus is at first characterized by its slender body, but is mainly characterized by dorsoventrally flattened smooth and elevated lip region and an elongate sinuous amphidial opening, appearing as a wavy slit with three peaks, originating from the vicinity of the labial plate, extending backward, reaching and passing the first body annulus. It has an anteriorly rounded outline under LM, knobbed stylet, its conus about half the total length, lateral field with a single band, metacarpus with a distinct valve, vulva with small lateral flaps and common males with simple vulva and tylenchoid spicules. The new genus was morphologically compared with six tylenchid genera viz. *Chilenchus*, *Ecphyadophoroides*, *Labrys*, *Lelenchus*, *Sakia* and *Tenunemellus*, mainly by having small slender body and dorsoventrally flat lip region; and was placed under the subfamily Ecphyadophorinae by its slender body, elevated smooth cephalic region and long amphidial openings. It was recovered from the muddy soils of mangrove forests in southern Iran. Currently *Sigmolenchus sinuosus* n. gen., n. sp., is monotypic. In SSU phylogeny, the new genus formed a not highly supported sister relation with *Filenchus discrepans*, and in LSU phylogeny, it occupied a placement inside a major clade including several Tylenchinae genera.

Keywords - LSU rDNA, new genus, phylogeny, SSU rDNA, wavy amphidial opening.

Members of the family Tylenchidae Örley, 1880 constitute main soil fauna, comprising up to 30% of the nematodes in a given soil sample (Ferris & Bongers, 2006). According to Geraert (2008), the family includes five subfamilies Atylenchinae Skarbilovich, 1959, Boleodorinae Khan, 1964, Ecphyadophorinae Skarbilovich, 1959, Tylenchinae Örley, 1880 and Tylodorinae Paramonov, 1967. Based on the recent study (Qing & Bert, 2017b), the number of genera and species under the family reach 44 and 412 respectively, but the number of the species are still increasing (Panahandeh *et al.*, 2019a,b; Jalalinasab *et al.*, 2019). The two genera *Discopersicus* Yaghoubi, Pourjam, Álvarez-Ortega, Liébanas, Atighi & Pedram, 2016 and *Labrys* Qing & Bert, 2017a are the last genera added to the family.

During our recent samplings in southern Iran, a population of a Tylenchidae nematode was recovered from saline sediments in mangrove forests of Hormozgan province. The SEM data revealed it has a unique form of amphidial openings, and detailed comparisons using morphological and molecular data, corroborated its status as a new genus. Thus, the present study aims to describe this new genus using the combination of traditional and molecular criteria and decipher its phylogenetic affinities with other genera in the family.

Materials and methods

SAMPLING, NEMATODE EXTRACTION AND MORPHOLOGICAL STUDY

Several sediment samples were collected from Persian Gulf coastline near Khamir port in Hormozgan province, south of Iran during April 2018. The new genus was recovered from one sample using the improved centrifugal sugarflotation technique of Jenkins (1964) (Heip, 1974), killed and transferred to anhydrous glycerine according to Seinhorst (1959, 1962) and De Grisse (1969) and mounted on permanent slides. The specimens were measured using a Nikon Eclipse E600 light microscope, photographed using an Olympus DP72 digital camera attached to an Olympus BX51 light microscope equipped by differential interference contrast (DIC) optics. The drawings of the new genus were done by means of a drawing tube attached to the Nikon microscope. The cross section of a female mid-body was done after examining the selected individual according to Atighi *et al.* (2013).

DNA EXTRACTION, PCR AND SEQUENCING

Genomic DNA was extracted from DESS-preserved specimens according to Yoder *et al.* (2006). Each DNA sample was extracted from one female individual after examination on temporary slide. In total, 10 DNA samples from 10 females were taken. DNA samples were stored at -20°C until used as PCR templates. To amplify the SSU rDNA, the forward primer 1096F (5'-GGTAATTCTGGAGCTA ATAC-3') and reverse primer 1912R (5'-TTTACGGTCAGAACTAGGG-3'); and forward primer 1813F (5'-CTGCGTGA GAGGTGAAAT-3') and reverse primer 2646R (5'-GCTACCTTGTTACGACTTTT-3') (Holterman *et al.*, 2006) were used. The D2-D3 domains of LSU rDNA were amplified using the forward primer D2A (5'- ACAAGTACCGTGAGGGAAAGT-3') and reverse primer D3B (5'-TGCGAAGGAACCAGCTACTA-3') (Nunn, 1992). The ITS region was amplified using the forward primer TW81 (5'-GTTTCCGTAGGTGAACCTGC-3') and reverse primer AB28 (5'-ATATGCTTAAGTTCAGCGGGT-3') as described in Vovlas *et al.* (2008). Also the mitochondrial cytochrome oxidase c subunit I (*COI*) gene was amplified with primers LCO1490 (5'-GTCAACAAATCATAAAGATATTGG-3') and HC02198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.*, 1994). The newly obtained sequences of the new genus were deposited into the GenBank database under accession numbers MK611963, MK611964, MK611965 for near full-length SSU rDNA, MK611958, MK611959, MK611960 for D2-D3 expansion segments of LSU rDNA, MK611961, MK611962 for partial ITS rDNA and MK611957 for *COI* mtDNA.

SCANNING ELECTERON MICROSCOPY

Three female and three male specimens preserved in glycerin were selected for observation under SEM following the protocol of Abolafia (2015). The nematodes were hydrated in distilled water, dehydrated in a graded ethanol and acetone series, critical point-dried, coated with gold and observed with a Zeiss Merlin microscope (5kV) (Zeiss, Oberkochen, Germany).

PHYLOGENETIC ANALYSES

The newly obtained sequences were manually checked, edited and assembled. Two separate SSU and LSU datasets were prepared for SSU and LSU phylogenies. Two aphelenchoidid species were used as outgroups in both analyses. The alignment of selected sequences for each dataset was performed using the Q-INS-i algorithm of MAFFT v.7.205 (Kato & Standley, 2013). The poorly aligned and divergent regions of each alignment were eliminated using the online version of Gblocks 0.91b using all of the three less stringent parameters (http://molevol.cmima.csic.es/castresana/Gblocks_server.html). The best-fitting substitution model of each dataset was selected using PAUP*/MrModeltest.2 (Nylander, 2004). The Bayesian analysis was performed using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) under the GTR + I + G model for both datasets with a random starting tree and five independent runs and 1×10^7 generations. The Markov chains were sampled every 100 generations. The burn-in phase was set at 25% of the converged runs. A 50% majority rule consensus tree was generated and posterior probabilities (PP) were calculated for each clade using the Markov chain Monte Carlo (MCMC) method within a Bayesian framework. Tracer v1.5 software (Rambaut & Drummond, 2009) was used to visualise the results of each run in order to check the effective sample size of each parameter. The ML analyses were performed using RaxmlGUI 1.1 software (Silvestro & Michalak, 2012) with the same nucleotide substitution model as in the BI in 1×10^3 bootstraps (BS) for both datasets. The output files of the used phylogenetic programs were visualised using Dendroscope v.3.2.8 (Huson & Scornavacca, 2012). Bayesian posterior probability (BPP) and ML BS values exceeding 50% were plotted on Bayesian 50% majority rule consensus trees after redrawing in CorelDRAW® software version 18. By lacking sufficient ITS and *COI* sequences of Tylenchidae genera in GenBank database, no further analyses were performed using these sequences.

Results

*Sigmolenchus** n. gen.

*The generic name refers to the wavy amphidial opening of the new genus, made from sigmoid = having a serpentine shape, resembling the Greek sigma (S) letter [ancient Greek σιγμοειδής (sigmoeidēs), from σίγμα (sigma)].

DESCRIPTION

Tylenchidae, Ecphyadophorinae. Slender nematodes with short body, narrowing toward both extremities more so towards distal end by having an elongate conical tail. Cuticle smooth under LM, finely annulated under SEM, lacking longitudinal lines. Lateral line a simple band with smooth margins. Anterior end rounded in LM. SEM images revealed cephalic region is elevated, smooth, and dorso-ventrally flattened. Cephalic papillae four, distinct, the labial plate small, rounded, slightly elevated, including six small pore-like labial sensilla, and the oral opening as a small rounded pore in the center. The amphidial openings long wavy sinuous slits with three peaks, originating at close to labial plate, extending laterally backward, reaching and passing the first body annulus. Stylet knobbed, the knobs three, slightly directed backward, well visible in fresh material in water, its cone 47-56% of the total stylet. Pharynx tylenchoid, the metacarpus ovate with small valve, the pharyngeal bulb pyriform to saccate. Excretory pore at metacarpus level. Intestine simple, rectum and anus functional. Reproductive system monodelphic-prodelphic, its parts visible (hardly), the ovary outstretched, oviduct hardly seen, spermatheca rectangular with rounded corners to ovate, axial, crustiform quadricolumellate, uterus simple, vulva with small lateral flaps under SEM and post-vulval uterine sac (PUS) short. Tail elongate conical, its tip finely rounded. Males abundant, similar to females in general morphology except for their characters related to sexuality, bursa simple, small and spicules slender, tylenchoid.

TYPE SPECIES

Sigmolenchus sinuosus n. gen., n. sp.

NO OTHER SPECIES

DIAGNOSIS AND RELATIONSHIPS

The new genus has a slender small body and is mainly characterized by smooth, dorsoventrally flattened elevated lip region and elongate wavy sinuous amphidial openings having three peaks, originated close to labial plate, extending backward, reaching and passing the first body annulus. By having a slender and small body as well as a dorsoventrally flattened smooth cephalic region, the new genus resembles six Tylenchidae genera, the four genera *Chilenchus* Siddiqi, 2000,

Ecphyadophoroides Corbett, 1964, *Lelenchus* Andrssy, 1954 and *Tenunemellus* Siddiqi, 1986 from Ecphyadophorinae and two genera *Labrys* and *Sakia* Khan, 1964 from Tylenchinae. Compared to all four aforementioned genera, the new genus differs by its amphidial opening status (elongate sinuous amphidial opening, appearing as a wavy slit, originating from the vicinity of the labial plate, extending backward, reaching and passing the first body annulus). The detailed comparisons with abovementioned genera follow:

Compared to *Chilenchus* by knobbed (vs not) stylet with a conus about 52% of its total length (vs third) and lateral line number (two vs four).

Compared to *Ecphyadophoroides*, by lacking longitudinal ridges all over the body (vs present) and simple bursa (vs rather rectangular, projecting outward and backward).

Compared to *Labrys* by higher cephalic region lacking an elongate offset labial plate (vs present).

Compared to *Lelenchus*, a closely similar genus by the status of the amphidial opening, as already discussed.

Compared to *Sakia* by elevated cephalic region lacking a *Sakia*-type labial pattern (Panahandeh *et al.*, 2019b).

Compared to *Tenunemellus* by simple bursa (vs rectangular, lobed and projected outwardly and backwardly).

***Sigmolenchus sinuosus* * n. gen., n. sp.**

*The specific epithet refers to sinus-like slit of the amphidial opening.
(Figs 1-4)

MEASUREMENTS

See Table 1.

DESCRIPTION

Female

Body slender, slightly ventrally arcuate after fixation. Cuticle finely striated with annuli about 0.9-1.2 μm apart at mid-body under SEM. Lateral fields visible in mid-body under LM in lateral view and cross sections, the lines smooth under SEM. Cephalic region elevated, smooth, dorso-ventrally flattened, anteriorly rounded and continuous with body contour, as long as wide, 4-6 μm , with

weak framework. the amphidial openings long wavy sinuous slits with three peaks, originating at close to labial plate, extending laterally backward, reaching and passing the first body annulus. Stylet with three slightly posteriorly directed rounded knobs, its conus comprising 47-56% of the total length. Dorsal pharyngeal gland orifice 1.0-1.3 μm posterior to stylet knobs. Pharynx tylenchoid with corpus tubular, median bulb (metacarpus) fusiform, with small distinct valve, isthmus slender, and basal bulb elongate, saccate to pyriform and set off from intestine. Cardia small, 2-4 μm long and dome-shaped. Intestine simple, rectum, as long as the anal body diameter, and anus distinct, reduced. Nerve ring encircling isthmus. Excretory pore hardly observed, anterior to posterior to median bulb (observed in four females). Reproductive system monodelphic-prodelphic, occupying 23-39% of total body length, composed of an outstretched ovary with oocytes at first in single row at proximal region, increasing distally, oviduct indistinct, spermatheca ellipsoid, sometimes rectangular, apparently axial, functional, filled with spheroid sperm, crustaformeria quadricolumellate, uterus simple, vagina perpendicular to body axis to slightly anteriorly directed, vulva a transverse slit with small lateral flaps under SEM, post-vulval uterine sac 9-15 μm long or 0.8-1.3 times body diam. at vulval region. Tail elongate, filiform, remarkably narrowing distally at 1/4 to 1/5 of its length, filiform at posterior end with faintly pointed tip.

Male

Common and functional. Similar to female in its general morphology except for character states associated with sexual differences. Testis single, genital branch occupying 40-48% of total body length. Spermatocytes arranged in multiple rows followed by spheroid spermatozoa. Spicules tylenchoid, arcuate, sickle-like. Gubernaculum simple, small and linear in lateral view. Tail similar to that of female. Bursa adcloacal, simple, with smooth margins.

Type habitat and locality

Recovered from sediments collected from mangrove forests in Khamir port, Hormozgan provine, south of Iran in April 2018. GPS coordinates: 26°58.548' N, 55°40.433' E.

Type material

Holotype female, 10 paratype females and seven paratype males were deposited in Nematode Collection of Zoology Museum of Ghent University, Ghent, Belgium with accession numbers UGMD_XXXXXX and UGMD_XXXXXX, and nine paratype females and seven males were deposited in the WaNaCo collection, Wageningen, The Netherlands (<http://www.waneco.eu/>) with accession numbers WT XXXX and WT XXXX.

MOLECULAR PHYLOGENETIC STATUS

Three near full-length SSU (1588-1677 nt long), three LSU D2-D3 (703-704 nt long), two ITS (560-569 nt long) and one *COI* sequences were generated for the new genus (accession numbers already given). The BLAST search using the longest SSU sequence, MK611965, revealed its identity with currently available SSU sequences of Tylenchidae is less than 86%. The BLAST search using the longest LSU sequence, MK611959, revealed its identity with currently available LSU sequences of Tylenchidae is less than 87%. For phylogenetic analyses, two independent SSU and LSU datasets were prepared (species names, accession numbers and outgroups in Figs 5 and 6). The SSU dataset included 76 sequences and the LSU dataset included 77 sequences.

Fig. 5 represents the Bayesian phylogenetic tree inferred using the SSU dataset. In this tree, the new genus was in poorly supported sister relation with *Filenchus discrepans* (Andrássy, 1954) Andrásy, 1972, both of which forming a sister clade to several *Malenchus* spp. The Ecphyadophorinae genera *i.e.* *Lelencus*, *Ecphyadophora* de Man, 1921 and *Sigmatylenchus* n. gen. are distantly related in this tree, showing the subfamily is not monophyletic. Fig. 6 represents the Bayesian phylogenetic tree inferred using the LSU dataset. In this tree, the new genus has fell into a maximally supported major clade including sequences from several *Labrys* spp., *Malenchus* spp., *Lelencus leptosoma* de Man, 1880 (KX156322, KX156324) and *Filenchus discrepans* (KX156315, KX156317). The Ecphyadophorinae genera *i.e.* *Lelencus*, *Tenunemellus sheri* (Raski, Koshy & Sosamma, 1982) Siddiqi, 1986 (MG994966, MG994967) and *Sigmatylenchus* n. gen. are distantly related in this tree, showing the subfamily is not monophyletic. As already discussed, no phylogenetic analyses were performed using ITS and *COI* data.

Acknowledgements

The authors thank to the research activity "PAIUJA 2019/2020: EI_RNM02_2019" of the University of Jaén. SEM pictures were obtained with the assistance of technical staff (Amparo Martínez-Morales) and equipment of "Centro de Instrumentación Científico-Técnica (CICT)" from University of Jaén.

Discussion

The herein newly established genus, *Sigmolenchus* n. gen., has placed under the subfamily Ecphyadophorinae by its slender small body and mainly by its elevated dorso-ventrally flat smooth cephalic region and remarkably long amphididal openings. It is further characterized by type IV pattern of lip region of Tylenchidae *sensu* Geraert and Raski (1987), as schematically illustrated by Qing and Bert (2017). The subfamily is known by a remarkable variation in head shape, amphidial aperture, vulva, and bursa shape of its genera (Qing et al., 2018) forming an artificial group (see below), and lacking of molecular data for most tylenchid genera, including several Tylenchinae and Ecphyadophorinae members, prohibits a deep revision of the currently well-established frameworks and establishment of natural groups. The presently resolved SSU phylogeny could be compared with the resolved topology by Qing et al. (2018) and the most recently resolved topology by Panahandeh *et al.* (2019b). Although the general topology of these three trees could be regarded similar, but the aligning and post-editing techniques influence them. The presently resolved LSU phylogeny is also comparable to the resolved phylogeny in two aforementioned studies, and could be an update to them by adding the new genus. In conclusion, the new genus shows phylogenetic affinities with Tylenchinae members in both SSU and LSU phylogenies, but was placed under Ecphyadophorinae by its morphological homogeneity. This status is also seen for the other ecphyadophorid genera like placement of *Tenunemellus sheri* in Tylenchinae clade in LSU, representatives of *Lelenchus* in both SSU and LSU; and *Ecphyadophora* in SSU phylogeny (Figs 5 and 6).

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Table 1. Morphometric data for *Sigmolenchus sinuosus* gen. nov., sp. nov. All measurements are in μm and in the form: mean $\pm$ SD (range).

Characters	Female		CV	Male	
	Holotype	Paratypes		Paratypes	CV
n	1	19		14	
L	467	472 $\pm$ 25.3 (440-512)	5.4	469.2 $\pm$ 12.3 (450-490)	2.6
a	42.5	42.3 $\pm$ 3.6 (37.0-48.7)	8.6	45.0 $\pm$ 3.1 (41.5-53.0)	6.8
b	5.8	5.3 $\pm$ 0.5 (4.2-6.1)	10	5.3 $\pm$ 0.6 (4.3-6.2)	10.7
c	4.4	4.4 $\pm$ 0.3 (4.0-4.9)	6.2	4.3 $\pm$ 0.2 (4.0-4.7)	5.1
c'	13.4	12.9 $\pm$ 1.4 (10.7-15.6)	10.7	14.9 $\pm$ 1.9 (11.0-17.4)	12.8
V/T	61	63 $\pm$ 1.7 (61-67)	2.7	43.3 $\pm$ 2.6 (40-48)	6
V'	79.2	80.5 $\pm$ 4.7 (65-88)	5.9	-	-
Cephalic region height	4.5	4.6 $\pm$ 0.5 (4-6)	10.7	5.3 $\pm$ 0.4 (4.5-6)	7.2
Cephalic region width	5	5.1 $\pm$ 0.4 (4-6)	8.8	4.8 $\pm$ 0.3 (4-5)	6.8
Stylet length	10	9.4 $\pm$ 0.6 (9-11)	6.4	10.4 $\pm$ 0.7 (9-12)	6.6
Conus	4.5	4.8 $\pm$ 0.4 (4-6)	8.5	5.0 $\pm$ 0.7 (4-6)	13
M	45	52.4 $\pm$ 3.2 (47-56)	6.1	48.0 $\pm$ 4.5 (42-57)	9.4
Tail length/vulva-anus	1.4	1.5 $\pm$ 0.1 (1.3-1.6)	7.1	-	-
O	10	11.1 $\pm$ 1.2 (10.0-14.4)	10.9	9.7 $\pm$ 0.7 (8.7-11.1)	6.9
Median bulb	36	39.7 $\pm$ 3.7 (33-48)	9.3	39.2 $\pm$ 3.2 (33-45)	8.1
MB	45	44.7 $\pm$ 2.9 (40-51)	6.6	44.2 $\pm$ 2.2 (41-48)	5.1
Pharynx	80	90.5 $\pm$ 9.5 (75-118)	10.5	89 $\pm$ 9.9 (78-109)	11.1
PUS	10	11.8 $\pm$ 1.7 (9-15)	14.3	-	-
Body width	11	11.2 $\pm$ 0.8 (10-12)	7.2	10.5 $\pm$ 0.7 (9-12)	7.1
GL1	97	176.4 $\pm$ 25.0 (114-200)	14.2	204 $\pm$ 14.5 (186-228)	7.1
G1	40.3	27.3 $\pm$ 4.9 (23.2-39.1)	18.1	-	
Anal body width	8	8.4 $\pm$ 0.9 (7-11)	10.8	7.4 $\pm$ 1 (7-11)	13.9
Tail	107	107.1 $\pm$ 4.7 (100-118)	4.4	108.8 $\pm$ 6.2 (99-117)	5.7
Spicules	-	-		12.4 $\pm$ 0.8 (11-14)	6.3
Gubernaculum	-	-		3.1 $\pm$ 0.5 (2-4)	15.7

Legend of figures

Fig. 1. *Sigmolenchus sinuosus* n. gen., n. sp. A, F: Entire male and female, respectively; B, E: Male and female anterior body region; C, D: Male and female cephalic region; G: Midbody cross section of female; H: Reproductive system of male; I: Female posterior end; J: Lateral field; K: Male posterir end; L: Proximal part of reproductive system of female.

Fig. 2. *Sigmolenchus sinuosus* n. gen., n. sp. (light microscopy). A: Anterior body of female; B, C: Anterior body of male (upper arrow indicate amphidial slit, lower arrow indicate excretory pore in median bulb region); D, E: Female cephalic region (arrow indicate amphidial slit); F: Reproductive system of female; G: Female pharyngeal region; H: Cross section of female mid-body; I: Cloaca and bursa of male; J: Female tail; K: Vulva and filled elipsoid spermathecae; L: Lateral field; M: Female tail distal region; N: Spicule and gubernaculum. (scale bars = 10 μ m.)

Fig. 3. *Sigmolenchus sinuosus* n. gen., n. sp. (female, scanning electron microscopy). A: Entire body; B: Anterior body showing beginning of lateral fields; C: Cephalic region showing amphidial opening; D: Ventro-frontal view of cephalic region; E: Latero-frontal view of cephalic region showing labial plate and six sensilla; F: Ventral view of excretory pore (arrow); G: Ventral view of anterior body (arrow indicates excretory pore); H: Female posterior end (upper arrow, vulva, lower arrow, anus); I, J: Vulva in lateral and ventral view, showing small lateral flaps; K: Anus.

Fig. 4. *Sigmolenchus sinuosus* n. gen., n. sp. (male, scanning electron microscopy). A: Entire body; B, C: Cephalic region in ventral and lateral view, respectively; D: Body annuli; E: Anterior body region; F: Posterior body region; G: Bursa region.

Fig. 5. Bayesian 50% majority rule consensus tree inferred from SSU rDNA sequences of *Sigmolenchus sinuosus* n. gen., n. sp. under the GTR + I + G model using two aphelenchoidids as outgroup taxa. Bayesian posterior probabilities and maximum likelihood bootstrap values more than 50% are given for appropriate clades in the shape BPP/ML BS.

Fig. 6. Bayesian 50% majority rule consensus tree inferred from LSU rDNA sequences of *Sigmolenchus sinuosus* n. gen., n. sp. under the GTR + I + G model using two aphelenchoidids as outgroup taxa. Bayesian posterior probabilities and maximum likelihood bootstrap values more than 50% are given for appropriate clades in the shape BPP/ML BS.