BRILL

Nematology 0 (2021) 1-13

Nematology
brill.com/nemy

Description of *Trichodorus marylandi* n. sp. (Nematoda: Trichodoridae) from Maryland, USA

Wilfrida DECRAEMER [1,*], Mihail R. KANTOR [2,**], Zafar A. HANDOO [2] and Sergei A. SUBBOTIN [3,4]

[1] *Nematology Research Unit, Department of Biology, Ghent University, K.L. Ledeganckstraat 35, B9000 Ghent, Belgium*
[2] *Mycology and Nematology Genetic Diversity and Biology Laboratory, USDA, ARS, Northeast Area, Beltsville, MD 20705, USA*
[3] *California Department of Food and Agriculture, Plant Pest Diagnostic Center, 3294 Meadowview Road, Sacramento, CA 95832, USA*
[4] *Center of Parasitology of A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences, Leninskii Prospect 33, Moscow 117071, Russia*

Received: 4 July 2021; revised: 6 August 2021
Accepted for publication: 6 August 2021; published online: 7 October 2021

Summary – During nematode surveys in natural vegetation, a new species, described herein as *Trichodorus marylandi* n. sp., was identified in addition to *Paratrichodorus* species and *Nanidorus minor* from Maryland, USA, and three putative new *Trichodorus* species from California. *Trichodorus marylandi* n. sp. is about 1 mm long with a medium-sized onchiostyle 54 (47-59) μm long. Males are characterised by a single ventromedian cervical papilla located anterior to the secretory-excretory pore, all three ventromedian precloacal supplements located anterior to the retracted spicules, and spicules 42 (38-44) μm long showing a minor indentation at the level of the posterior border of the capsule of the protractor muscles. Females possess a short barrel-shaped vagina when the body is relaxed, and two sublateral body pores on each side, one up to four body diam. anterior to the vulva and one advulvar in position. A rare observation of a 'hood' ventrally attached to a moulting male juvenile was observed and illustrated. *Trichodorus marylandi* n. sp. is morphologically closest to *T. obtusus*. Based on recent information gained from molecular data for *Trichodorus* species that has revealed the occurrence of several cryptic species, *T. proximus* is temporarily accepted as a valid species until molecular data become available from topotype specimens. Phylogenetic relationships within trichodorids, as inferred from the analyses of the D2-D3 expansion segments of 28S rRNA and ITS2 rRNA gene analysis and a statistical parsimony network showing the phylogenetic relationships between *COI* haplotypes of *T. obtusus* and *T. marylandi* n. sp., are also provided.

Keywords – *COI* haplotypes, D2-D3, ITS2-rRNA, molecular, morphology, morphometrics, *Nanidorus minor*, *Paratrichodorus* sp., phylogeny, taxonomy, *Trichodorus obtusus*, *Trichodorus* sp.

Currently, 39% of the 117 valid species of the virus vector family Trichodoridae Thorne, 1935 have been recorded from the USA, one-third of these species belonging to *Trichodorus* Cobb, 1913 (Subbotin *et al*., 2020; Decraemer & Subbotin, 2021; Decraemer *et al*., 2021). During nematode surveys in natural vegetation in Maryland and California, USA, several known and unidentified species of *Trichodorus* were collected as well as *Nanidorus minor* (Allen, 1957) Siddiqi, 1980 and some unidentified *Paratrichodorus* Siddiqi, 1974 species. So far, six trichodorid species have been recorded from Maryland, USA, among them two species of *Trichodorus*: *T. obscurus* Allen, 1957 and *T. primitivus* (de Man, 1880) Micoletzky, 1922 (Subbotin *et al*., 2020). A third *Trichodorus* species for Maryland has now been collected and appears new to science.

Ten valid trichodorid species have been recorded from California (Subbotin *et al*., 2020). Recently, Subbotin *et al*. (2020) recorded and molecularly characterised three known species of *Trichodorus* from California: *T. californicus* Allen, 1957, *T. intermedius* Rodriguez-M & Bell, 1978, and *T. obscurus*, in addition to eight unidentified species of the genus. The unidentified species *Trichodorus* sp. 1 in the former paper was later described as *T. pseudoaequalis* Decraemer & Subbotin, 2021 (Decraemer &

* Corresponding author, e-mail: wilfrida.decraemer@UGent.be
** ORCID: https://orcid.org/0000-0001-7609-104X

DOI 10.1163/15685411-bja10132

Subbotin, 2021). In the current contribution, molecular analyses identified three additional unidentified species of the genus (*Trichodorus* sp. 13, *Trichodorus* sp. 14 and *Trichodorus* sp. 15).

The main objective of this study was to describe the new species from Maryland as *T. marylandi* n. sp. using light microscopy (LM) observations, and to assess the diagnostic value of morphological and molecular characters.

Materials and methods

Nematode samples

The nematode samples used in this study were collected from the rhizosphere of grasses in one location in Maryland (N59.907879; E10.800483) and two locations in California, USA (Table 1). The samples consisted of soil and root fragments collected at a depth of up to 10 cm. Nematode specimens were extracted from soil using the centrifugal flotation method (Jenkins, 1964) and used for the morphological and molecular studies.

Light microscopic study

At Ghent University, Belgium, specimens were examined and measured. Drawings and microphotographs were made using a Nikon Eclipse microscope with Nomarski differentiated interference contrast equipped with a Nikon camera and DS-L4 tablet.

Molecular study

DNA was extracted from single nematode specimens using the proteinase K protocol. DNA extraction and PCR were used as described by Subbotin (2021). The following primer sets were used in this study: the forward ITSA (5′-ATC GAT GAA GAA CGC AGC-3′) (Boutsika *et al*., 2004) and the reverse PXb481 (5′-TTT CAC TCG CCG TTA CTA AGG-3′) (Vrain *et al*., 1992) primers for amplification of the ITS2 rRNA gene, the forward D2A (5′-ACA AGT ACC GTG AGG GAA AGT TG-3′) and the reverse D3B (5′-TCG GAA GGA ACC AGC TAC TA-3′) primers (Subbotin *et al*., 2020) for amplification of the D2-D3 expansion segments of 28S rRNA gene and the forward COIF5 (5′-AAT WTW GGT GTT GGA ACT TCT TGA AC-3′) and the reverse COIR9 (5′-CTT AAA ACA TAA TGR AAA TGW GCW ACW ACA TAA TAA GTA TC-3′) primers (Powers *et al*., 2014) for amplification of the partial *COI* gene. Sequencing was done at Genewiz. New sequences were deposited in the GenBank database and accession numbers are given in Table 1 and the phylogenetic trees.

Phylogenetic and sequence analyses of the D2-D3 of 28S rRNA and ITS2 rRNA gene sequences were done

Table 1. The species and populations of stubby root nematodes from California, Oregon and Washington states, USA, used in the study.

Species	Location	Host	Sample code	GenBank accession number			Source
				Partial 28S rRNA gene sequence	ITS rRNA gene sequence	Partial *COI* gene sequence	
Trichodorus marylandi n. sp.	USA, Maryland	Grasses	CD3327, CD3433, CD3454a, b, c, c2, d, e, f, k, l, o, p, q	MZ409462-MZ409475	MZ409448	MZ408106, MZ408107	M. Kantor, Z. Handoo
Trichodorus sp. 13	USA, California, Madera County	Forest	CD3474	MZ409459	–	–	S.A. Subbotin
Trichodorus sp. 14	USA, California, Marin County, Bon Tempe Lake	Forest	CD3448	MZ409458	–	–	S.A. Subbotin
Trichodorus sp. 15	USA, California, Madera County	Forest	CD3471	MZ409476	–	–	S.A. Subbotin
Nanidorus minor	USA, Maryland	Grasses	CD3449g, k	MZ409460, MZ409461	–	–	M. Kantor, Z. Handoo
Paratrichodorus sp.	USA, Maryland	Grasses	CD3449l	MZ409477	–	–	M. Kantor, Z. Handoo

as described by Subbotin *et al*. (2020). New *COI* gene sequences were aligned with the sequences of *T. obtusus* Cobb, 1913 (Shaver *et al*., 2016). The alignment of *COI* gene sequences was used to construct the phylogenetic network estimation using statistical parsimony (SP) as implemented in POPART software (http://popart.otago.ac.nz) (Bandelt *et al*., 1999).

Results and discussion

Trichodorus marylandi[*] n. sp.
(Figs 1-4)

MEASUREMENTS

See Table 2.

DESCRIPTION

Male

Body *ca* 1 mm long, largely cylindrical, J-shaped. Body cuticle thin (1.0-1.5 μm), non-swollen when properly fixed. Lip region rounded, marked by four slightly raised sets of double papillae composed of a cephalic papilla and a subdorsal or subventral papilla of the outer crown of labial papillae. Amphidial fovea cup-shaped with amphidial aperture a large transverse slit located just posterior to lip region. Stoma small, strengthening rods 4-5 μm long (average). Pharyngostome with medium-sized (average 63 μm or *ca* one-third of pharynx length) ventrally curved onchiostyle, with onchium about half as long. Slender mid-part of pharynx usually slightly curved upon fixation, gradually widening posteriorly to a more or less marked glandular bulb. Pharyngeal bulb with five pharyngeal gland nuclei with anterior ventrosublateral pair small and often difficult to observe, dorsal nucleus located more anteriorly (about mid-bulb) than posterior ventrosublateral pair of gland nuclei, which are located close to base of bulb. Pharyngeal bulb offset from intestine or flanked by anterior dorsal intestinal overlap (5-17 μm); overlap may be the result of fixation. Cardia weakly developed. Nerve ring around isthmus. A single ventromedian cervical papilla (CP1) located anterior to secretory-excretory pore (S-E pore). Lateral cervical pore (LP) on both sides located posterior to nerve ring and anterior to CP1. Single testis occupying on average one-fifth of reproductive system. Sperm cells small, round, 5.0-6.5 μm in diam., with small round to oval nucleus (2.0-3.5 μm). Spicules curved ventrally, manubrium slightly widened but hardly marked, spicule blade non-striated, slightly tapering to a minor indentation near level of posterior border of capsule of protractor muscles, or on average at 19 μm from anterior spicule end, at level of blade indentation in a young male (old cuticle still present in posterior body region) spicule with a set of short spines. Gubernaculum with typical keel-like thickening posteriorly. Three protruding ventromedian precloacal supplements (SP1-SP3), posteriormost supplement (SP1) just anterior to retracted spicule. In young males supplements not protuberant. One pair of small postcloacal papillae close to cloacal opening and just posteriorly flanked by a pair of caudal pores. Tail short, less than anal body diam. long, terminal cuticle not thickened.

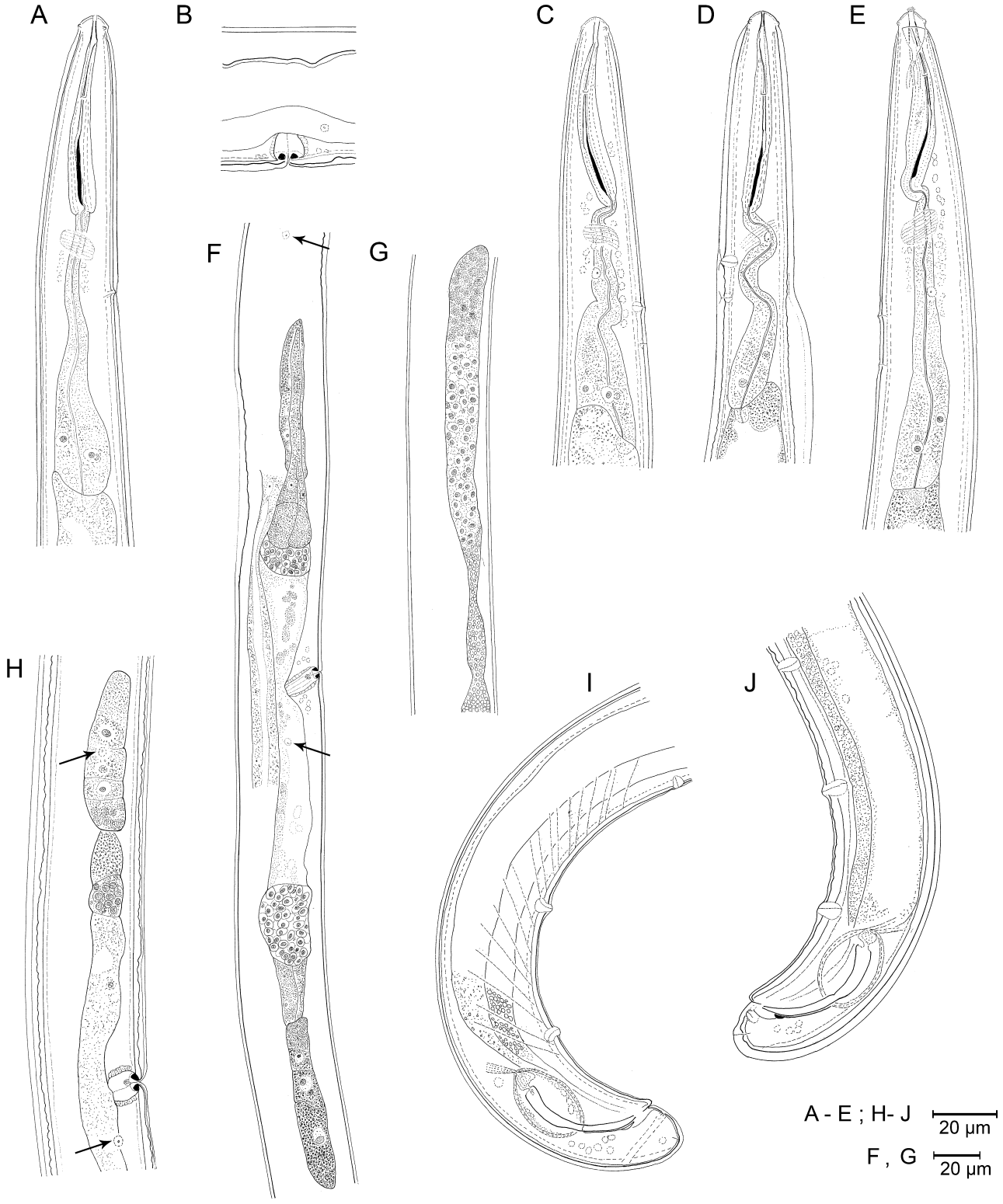
Female

Body largely straight or slightly ventrally curved. Digestive system as in male. S-E pore located at level of pharyngeal isthmus. Reproductive system didelphic-amphidelphic with both genital branches about equally developed. At tip of germinal zone of reflexed ovaries, two large, finely granular oviduct cells can be observed showing an extension (often difficult to differentiate) towards growing zone of the ovary (Fig. 3), spermatheca 12-45 μm long and filled with sperm, sperm small with round to oval nucleus 2.0-3.5 μm in diam. Vagina barrel-shaped when relaxed and *ca* one third of corresponding body diam. (cbd) long or elongate cylindrical and up to half cbd long upon contraction of vaginal constrictor muscles. Vaginal sclerotised pieces small rounded triangular, 2 μm in size on average, obliquely orientated, on average 1.5 μm apart at tip. Vulva a pore in ventral view. One postadvulvar body pore present on each side of body at 21 μm (average) from vulva and one body pore on each side of body on average 4.5 cbd anterior to vulva. Tail minute with anus subterminal and a pair of (sub)terminal caudal pores.

Juveniles

J2 and J3 were observed as well as moulting male juveniles. Juveniles similar in appearance to females but possessing slightly shorter onchiostyle, 42 μm in J2, 47-51 μm in J3; a reserve onchium, 21 μm in J2, 13.0-16.5 μm long in J3 and developing reproductive system or genital primordium of 14 μm (J2), and 32-62 μm long (J3). J4 moulting into a male showed shedding and presence of a 'hood' attached ventrally to old body cuticle. However, similar to previous observations, no shed onchium of the onchiostyle was seen in loosened head region of hood (Morton, 1967; Morton & Perry, 1968; Tanha Maafi & Decraemer, 2002). A male with partially

[*] The species name refers to its type location in the state of Maryland and is genitive.


A
B
C
D
E
F
G
H
I
J
A - E ; H- J
20 µm
F , G
20 µm

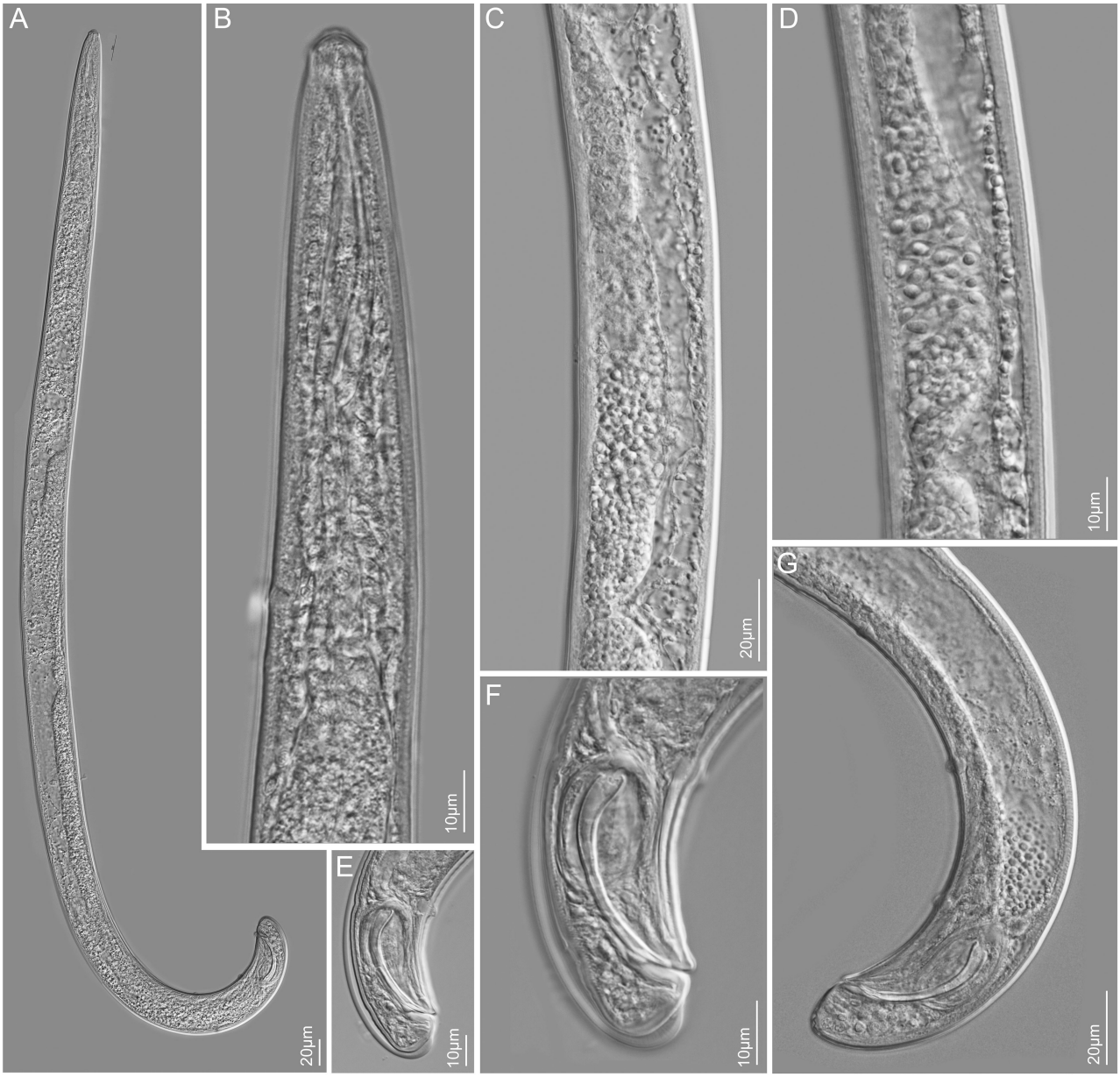


Fig. 2. Light microphotographs of males of *Trichodorus marylandi* n. sp. A: Total view; B: Anterior body region; C: Testis; D: Detail of testis with sperm; E: Tail region with focus on gubernaculum; F: Tail region and copulatory apparatus; G: Posterior body region. (A: holotype; other figures representing paratype males.)

Fig. 1. Line drawings of *Trichodorus marylandi* n. sp. A, C-E: Pharyngeal region; B: Vaginal region; F: Female reproductive system; anterior branch showing connection of oviduct with tip ovary, arrows indicating sublateral body pores; G: Testis; H: Anterior genital branch of female, arrows indicating sublateral body pores; I, J: Posterior body region in male. (A, B, F, H: Paratype females; C, G, I: Holotype male; D, E, J: Paratype males.)

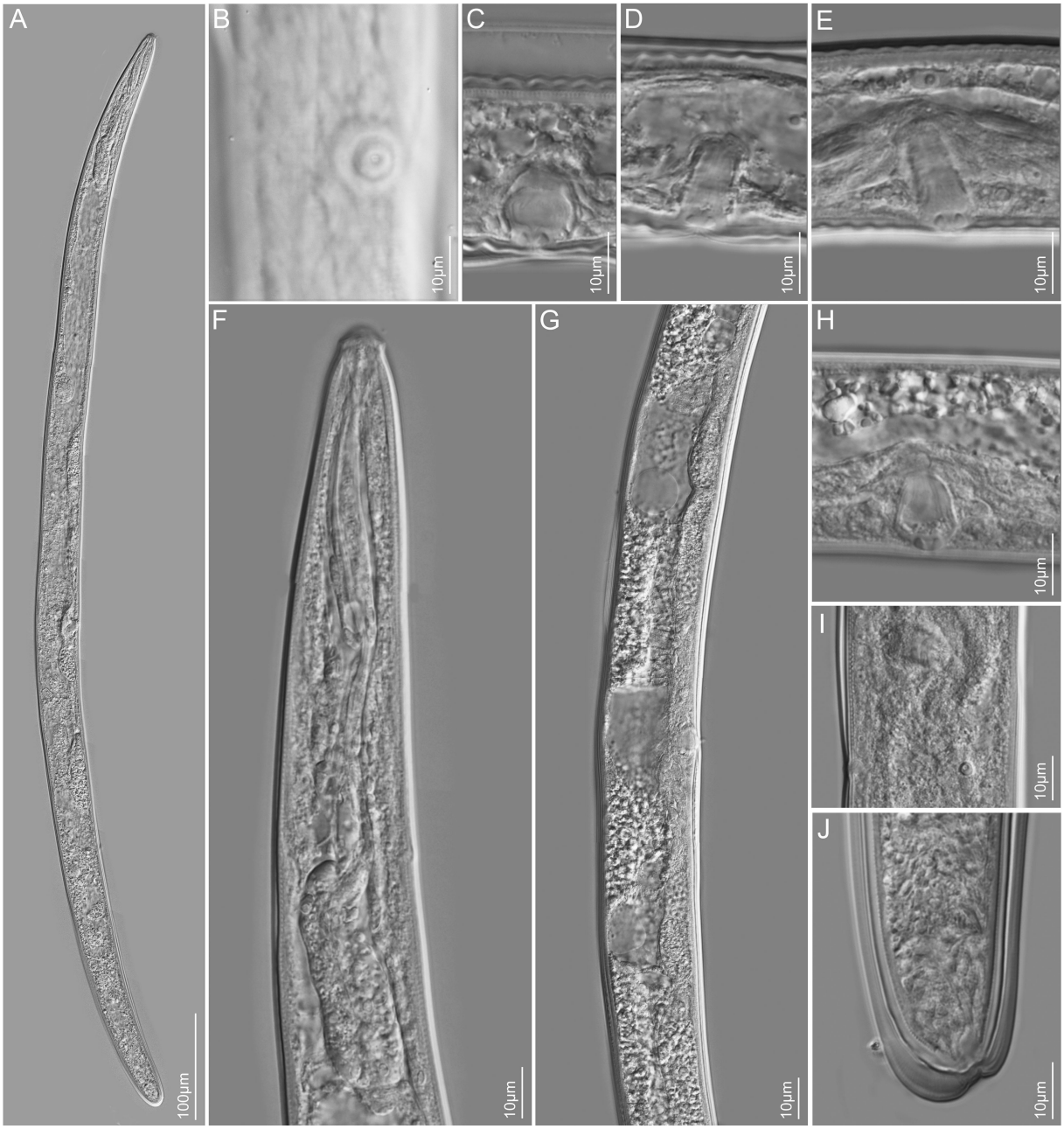


Fig. 3. Light microphotographs of female paratype of *Trichodorus marylandi* n. sp. A: Total view; B: Vulva in ventral view; C-E, H: Vaginal region; F: Pharyngeal region; G: Reproductive system; I: Vaginal region in oblique dorsal view showing body pore; J: Tail region.

attached old cuticle and hood was fully developed with sperm present in testis plus three precloacal supplements, although these not papilla-like or protruding. Observation and recording of moulting Trichodoridae in collected soil samples is exceptional (Tanha Maafi & Decraemer, 2002) but illustrations of ecdysis are even rarer. To our knowledge, Morton (1967) was the only author illustrating a break in the old body cuticle encircling the body in the region of the onchiostyle base and the formation of a 'hood' attached to the old body cuticle at a single

Fig. 4. Light microphotographs of male juveniles of *Trichodorus marylandi* n. sp. A: Entire moulting juvenile; B: Anterior body region in moulting juvenile; C: Testis in moulting juvenile; D: Detail of 'hood' of moulting juvenile male; E: Tail region of moulting juvenile; F: Posterior region of juvenile male showing two incipient posterior supplements; G: Spicule in moulting juvenile with arrow pointing to bristles; H: Gubernaculum in moulting juvenile male.

point at the dorsum. However, the author did not specify the moulting developmental stage illustrated. After several decades of studying Trichodoridae, we observed for the first time the presence of a 'hood' attached not dorsally but ventrally on the body in a J4 moulting into a male.

Remark

The formation of a hood has only been described for Trichodoridae but according to Morton (1967), a similar way of shedding occurs in some animal parasites such

Table 2. Morphometric data of fixed type specimens of *Trichodorus marylandi* n. sp. All measurements are in μm and in the form: mean ± s.d. (range).

Character	Male		Female
	Holotype	Paratypes	Paratypes
n	–	12	14
L	940	913 ± 96 (775-1075)	927 ± 98 (696-1054)
a	26.1	24.6 ± 2.5 (19.8-27.9)	22.9 ± 32.6 (18.8-27.9)
b	6.9	6.2 ± 0.6 (5.0-7.3)	6.6 ± 0.5 (4.8-6.7)
c	67.1	69.6 ± 13.0 (48.1-93.4)	–
c′	0.7	0.6 ± 0.1 (0.5-0.8)	–
V	–	–	53.8 ± 1.7 (51.1-56.4)
G_1	–	–	22.9 ± 5.7 (16.7-27.4)
G_2	–	–	23.8 ± 3.4 (21.2-30.6)
T	63.1	61.6 ± 11.4 (55.0-98.6)	–
Onchium	27	26.5 ± 1.7 (23.0-30.0)	28.0 ± 1.4 (26.0-31.0)
Onchiostyle	51	52 ± 2.5 (47-54)	54 ± 2.9 (48-59)
Pharynx length (along lumen)	135	149 ± 10.3 (119-166)	150 ± 9.0 (134-164)
Pharyngostome	61	63 ± 3.2 (57-67)	64 ± 2.1 (61-68)
Anterior to nerve ring	72	71 ± 4.4 (65-75)	69 ± 2.8 (66-75)
Anterior to S-E pore	103	100 ± 8.5 (86-114)	94 ± 6.5 (84-107)
Anterior to CP1	91	88 ± 7.4 (74-100)	–
Anterior to LP	–	80 ± 10.6 (65-101)	–
Anterior to guiding ring	24.0	24.2 ± 1.9 (20.0-26.0)	26.4 ± 1.1 (24.0-28.0)
Pharyngeal bulb (length)	35	46 ± 6.5 (34-54)	44 ± 8.0 (32-61)
Pharyngeal bulb (diam.)	16.0	12.5 ± 1.7 (10.0-16.0)	15.5 ± 1.9 (12.0-19.0)
cbd (cardia)	28	31.5 ± 2.9 (27-37)	34.4 ± 2.9 (30-40)
cbd (mid-body)	36	37 ± 3.7 (31-44)	40 ± 4.4 (34-47)
Anal body diam.	21.5	22.0 ± 2.5 (19.0-28.5)	–
Cloacal opening to SP1	51	48 ± 4.6 (42-56)	–
SP1 to SP2	54	46 ± 2.9 (41-54)	–
SP2 to SP3	74	53 ± 5.7 (45-74)	–
Anterior to testis	333	368 ± 47.8 (292-463)	–
Spicule length	41	42 ± 1.7 (38-44)	–
Gubernaculum	13	17.0 ± 2.9 (14.0-21.0)	–
Anterior ovary length	–	–	53 ± 19.4 (32-95)
Anterior branch length	–	–	197 ± 42.2 (149-289)
Posterior ovary length	–	–	56 ± 14.9 (28-84)
Posterior branch length	–	–	217 ± 36.5 (153-296)
Anterior end to vulva	–	–	499 ± 57.0 (356-574)
Vagina length	–	–	13.0 ± 3.0 (9.5-16.5)
Vaginal sclerotised pieces length	–	–	2.0 ± (1.0-2.3)
Distance between sclerotised pieces	–	–	1.5 ± 0.2 (2.0-2.5)
Postadvulvar body pore from vulva	–	–	20.7 ± 8.3 (0.0-37)
Anterior body pore from vulva	–	–	178 ± 57.7 (121-302)
Tail length	14.0	13.5 ± 1.6 (11.0-16.5)	–
Cuticle at tail tip	1.5	6.0 ± 1.1 (4.5-8.5)	–
L/spicule length	20.4	22.0 ± 2.4 (19.0-26.2)	–
L/onchiostyle	15.2	15.0 ± 1.6 (11.0-18.0)	18.0 ± 1.6 (13.1-18.7)
% S-E pore from ant.	11.0	11.0 ± 0.5 (10.5-11.9)	10.0 ± 0.5 (8.9-10.9)

Abbreviations: cbd = corresponding body diam.; CP1 = cervical papilla 1; LP = lateral cervical pore; SP1-SP3 = precloacal supplement 1-3.

as *Trichonema* sp. (Lapage, 1933 *in* Poynter, 1954), with the formation of a bulge of the old cuticle that remains attached at the anterior end in the region of the pharynx.

Type habitat and location

Trichodorus marylandi n. sp. was recorded from the rhizosphere of grasses in Maryland, USA, GPS: N59.907879 E10.800483.

Type material

Type material composed of a holotype male, 12 paratype males, 14 paratype females and five paratype juveniles. Holotype (male): slide T-757t deposited in the United States Department of Agriculture Nematode Collection, Beltsville, MD, USA. The holotype slide also includes one paratype male and one young paratype male. Paratypes (females, males and juveniles): same data and repository as holotype, slideT-7511p (two females, one male), T-7512p (two females, one juvenile), 7513p (one female, one juvenile), T-7514p (one male, one juvenile), and T-7515p (one male), Additional paratype males, females and juveniles on slide numbers T-7516p (two females), T-7617p (one male. one female, one juvenile, one young male), and T-7518p (one male) at Collection of Nematology Research Unit, Department of Biology, Ghent University, Ghent Belgium; slides T-7519 (one male, one female, one young male), T-7520p (one male), and T7521p (one male, one female) at University of California, Riverside, CA, USA; and slides T-7522p (1 one female) and T7523p (two females, one male) at FERA, Plant Pest Disease Cultures and Collections, York, UK.

Zoobank ID: urn: lsid: zoobank.org: act:7EF73E76-A8CB-4DOC-A34A-A4C4DF8906A92

Diagnosis and relationships

Trichodorus marylandi n. sp. is medium-sized to long (913 (696-1075) μm) with a medium-sized onchiostyle (54 (47-59) μm long (average)). It is characterised in the male by the possession of a single ventromedian CP1 located anterior to the S-E pore. Males possess three ventromedian precloacal supplements, the posteriormost one (SP1) located shortly anterior to the retracted spicules and the spicules are 42 (38-44) μm long, with a slightly widened manubrium gradually tapering to a minor blade indentation at the level of the posterior border of the capsule of the suspensor muscles (= protractor muscles). Gubernaculum with a typical keel-like posterior end. Females are characterised by a short barrel-shaped vagina when relaxed that is less than one-third of cbd long, or vagina elongated when vaginal constrictor muscles are contracted and up to half as long as cbd, triangular vaginal sclerotised pieces small, 2 (1.0-2.3) μm on average, rounded triangular in longitudinal optical section and vulva pore-like in ventral view. On each body side one sublateral body pore at about 4.5 cbd anterior to vulva and one postadvulvar sublateral body pore. The multi-entry (described as polytomous key) identification key codes based on Decraemer & Baujard (1998) for the male of *T. marylandi* n. sp. are: A223, B22, C22, D1, E0, F3, G0, H23, I13, J52, K32, L42, M17, N6, O3, P2 and for the female: A231, B22, C1, D1, E23, F13, G1, H3, I1, J1, K3, L12, M1?, N1, O1, P26, Q1, R1, S3.

To select the most similar *Trichodorus* species to *T. marylandi* n. sp. the multi-entry identification key of Decraemer & Baujard (1998) supplemented by *Trichodorus* species described thereafter (update unpubl.) was used. For the male, sorting of the species of the genus based on the prime character D (number of ventromedian cervical papillae) resulted in four species groups characterised by 0, 1, 2 and 3 CP respectively. The new species falls within a group of 15 species characterised by a single CP. For this group, a further selection based on character G (number of precloacal supplements (SP) within the region of the retracted spicules) grouped the new species with *T. eburneus* De Waele & Carbonell, 1983, *T. proximus* Allen, 1957 and *T. obtusus*. *Trichodorus marylandi* n. sp. differs from *T. eburneus* by the small sperm cells with a small round to circular nucleus *vs* large sperm cells with a large sausage-shaped nucleus. Small sperm cells with a small nucleus were also observed in *T. proximus*, but not described for *T. obtusus*.

For *Trichodorus* females, the following diagnostic characters were used to group similar species based on the supplemented multi-entry identification key of Decraemer & Baujard (1998): character S (geographic distribution) and character G (vulva shape). The new species groups with *T. aequalis* Allen, 1957, *T. carlingi* Bernard, 1992a, *T. elefjohnsoni* Bernard, 1992b, *T. paucisetosus* Bernard, 1992a, *T. obtusus*, and *T. proximus*. *Trichodorus marylandi* n. sp. differs from all except *T. proximus* in the shape of the sperm cells (feature unknown for *T. obtusus* before its synonymisation with *T. proximus*).

Notes

The taxonomic status of *T. obtusus*, the type species of *Trichodorus*, remains under discussion until molecular data can be provided based on topotypes. The original

description of *T. obtusus*, based on specimens from about the roots of grasses at Arlington, Virginia, USA, was brief with illustrations restricted to the lip and onchiostyle region and the spicule region, including the posteriormost precloacal supplement and tail (Cobb, 1913). Thorne (1974) provided additional information and illustration of a female specimen from material collected from turf near Milbank, SD, USA. He considered *T. obtusus* to most closely resemble *T. proximus* Allen, 1957 (collected from roots of St Augustine grass, at Tampa, FL, USA) but differing from it mainly by a shorter onchiostyle 40-45 μm (Thorne, 1974) *vs* 48-65 μm (male), 49-70 μm (female) in *T. proximus* (Allen, 1957). Loof (1975) recalculated the onchiostyle length in the *T. obtusus* type as 75 μm and spicule as 56 μm from the Cobb formula, meaning that these measurements fit within the range known for *T. proximus* (spicules = 48-65 μm). The measurements of both species overlap. Additional information on measurements of *T. proximus* collected in Iowa prairies by Norton *et al*. (1982) agrees with the original description. The only other difference between both species lies in the presence of an anterior pair of lateral body pores that is present in *T. proximus* and not described in *T. obtusus*. This feature can be easily overlooked and was not observed by Norton *et al*. (1982). Based on the overlapping morphological data of both species, Decraemer (1995) considered *T. proximus* a junior synonym of *T. obtusus*. Hunt (1993) considered *T. obtusus* as *species inquirenda*.

Based on the more recent information gained from molecular data for *Trichodorus* species, which indicates the occurrence of several cryptic species, we suggest accepting *T. proximus* as a valid species until molecular data become available from topotype specimens of both it and the type species.

Molecular characterisation of *T. marylandi* n. sp. and its relationships within other trichodorids

The D2-D3 of 28S rRNA gene sequences

The alignment included 87 sequences of stubby root nematodes and four sequences used as outgroups. Fourteen new sequences of *T. marylandi* n. sp., two new sequences of *N. minor*, and one new sequence for *Trichodorus* sp. 13, *Trichodorus* sp. 14, *Trichodorus* sp. 15, and *Paratrichodorus* sp. were also included in the analysis. Phylogenetic relationships within Trichodoridae inferred from the analysis of this gene fragment are presented in Figure 5. Intraspecific variation for *T. marylandi* n. sp. reached 0.8% (6 bp) and this species differed from *T. obtusus* (KM276666, North Carolina, USA) by 9.0-9.4% (59-67 bp). *Trichodorus marylandi* n. sp. had a sister relationship with *T. obtusus*. Sequence of *Paratrichodorus* sp. from Maryland differed from two sequences of *P. pachydermus* (Seinhorst, 1954) Siddiqi, 1974 by 8.0, 8.6% (58 bp, 62 bp). Sequences of *N. minor* from Maryland differed from those of Californian *N. minor* by 1.0% (7 bp). Sequences of two putative species (*Trichodorus* sp. 13, *Trichodorus* sp. 14) from California, clustered within Clade III, whereas *Trichodorus* sp. 15 belonged to Clade I.

The ITS2 rRNA gene sequences

The alignment contained ten sequences of *Trichodorus*, including a new sequence of *T. marylandi* n. sp. and one sequence of *Paratrichodorus* sp. as an outgroup and was 1176 bp in length. Phylogenetic relationships of *T. marylandi* n. sp. with *Trichodorus* spp. belonging to Clade I and other related species is given in Figure 6. Relationships of *T. marylandi* n. sp. with other trichodorids were unresolved.

Fig. 5. Phylogenetic relationships within trichodorid nematodes with *Trichodorus marylandi* n. sp. Bayesian 50% majority rule consensus tree from two runs as inferred from the D2-D3 expansion segments of the 28S rRNA gene sequence alignment under the GTR + I + G model. Posterior probabilities more than 70% are given for appropriate clades. New sequences are indicated in bold letters.

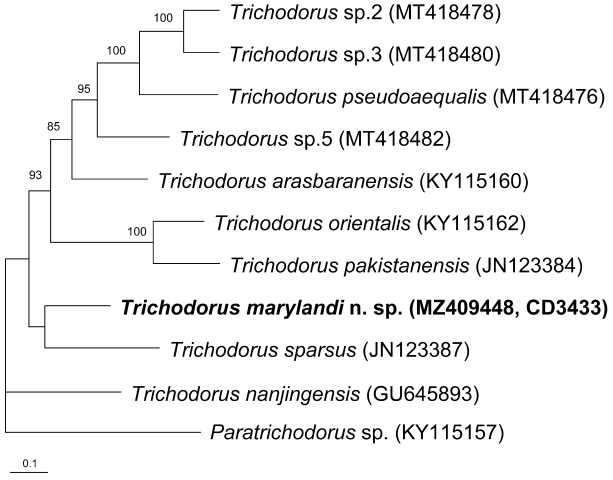
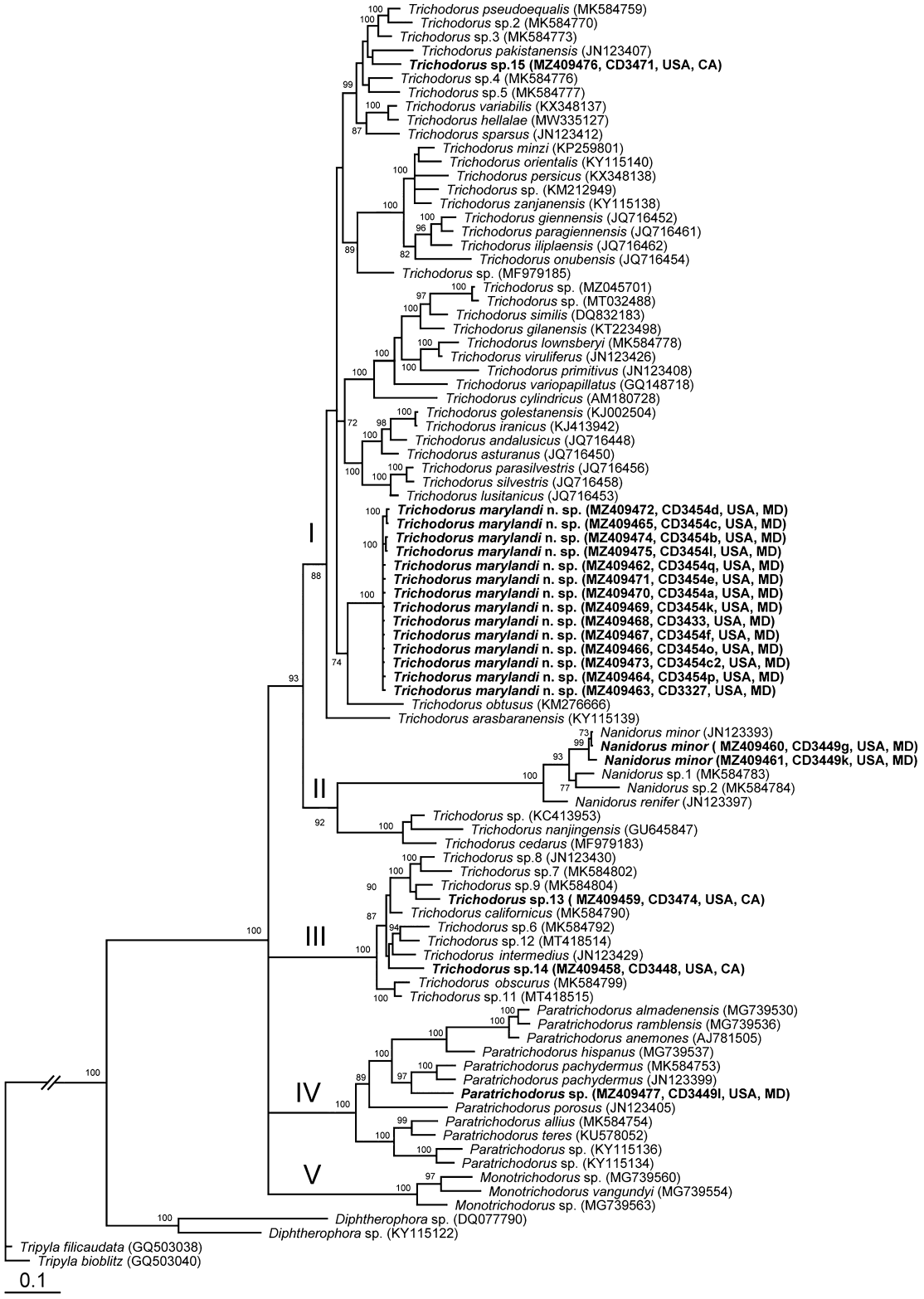


Fig. 6. Phylogenetic relationships of *Trichodorus marylandi* n. sp. with *Trichodorus* spp. belonging to the Clade I and other related species (Subbotin *et al*., 2020) as inferred from Bayesian analysis using ITS2 rRNA gene sequences under the GTR + I + G model. Posterior probability more than 70% is given for appropriate clades. New sequences are indicated in bold letters.


Trichodorus pseudoequalis (MK584759)
Trichodorus sp.2 (MK584770)
Trichodorus sp.3 (MK584773)
Trichodorus pakistanensis (JN123407)
Trichodorus sp.15 (MZ409476, CD3471, USA, CA)
Trichodorus sp.4 (MK584776)
Trichodorus sp.5 (MK584777)
Trichodorus variabilis (KX348137)
Trichodorus hellalae (MW335127)
Trichodorus sparsus (JN123412)
Trichodorus minzi (KP259801)
Trichodorus orientalis (KY115140)
Trichodorus persicus (KX348138)
Trichodorus sp. (KM212949)
Trichodorus zanjanensis (KY115138)
Trichodorus giennensis (JQ716452)
Trichodorus paragiennensis (JQ716461)
Trichodorus iliplaensis (JQ716462)
Trichodorus onubensis (JQ716454)
Trichodorus sp. (MF979185)
Trichodorus sp. (MZ045701)
Trichodorus sp. (MT032488)
Trichodorus similis (DQ832183)
Trichodorus gilanensis (KT223498)
Trichodorus lownsberyi (MK584778)
Trichodorus viruliferus (JN123426)
Trichodorus primitivus (JN123408)
Trichodorus variopapillatus (GQ148718)
Trichodorus cylindricus (AM180728)
Trichodorus golestanensis (KJ002504)
Trichodorus iranicus (KJ413942)
Trichodorus andalusicus (JQ716448)
Trichodorus asturanus (JQ716450)
Trichodorus parasilvestris (JQ716456)
Trichodorus silvestris (JQ716458)
Trichodorus lusitanicus (JQ716453)
Trichodorus marylandi n. sp. (MZ409472, CD3454d, USA, MD)
Trichodorus marylandi n. sp. (MZ409465, CD3454c, USA, MD)
Trichodorus marylandi n. sp. (MZ409474, CD3454b, USA, MD)
Trichodorus marylandi n. sp. (MZ409475, CD3454l, USA, MD)
Trichodorus marylandi n. sp. (MZ409462, CD3454q, USA, MD)
Trichodorus marylandi n. sp. (MZ409471, CD3454e, USA, MD)
Trichodorus marylandi n. sp. (MZ409470, CD3454a, USA, MD)
Trichodorus marylandi n. sp. (MZ409469, CD3454k, USA, MD)
Trichodorus marylandi n. sp. (MZ409468, CD3433, USA, MD)
Trichodorus marylandi n. sp. (MZ409467, CD3454f, USA, MD)
Trichodorus marylandi n. sp. (MZ409466, CD3454o, USA, MD)
Trichodorus marylandi n. sp. (MZ409473, CD3454c2, USA, MD)
Trichodorus marylandi n. sp. (MZ409464, CD3454p, USA, MD)
Trichodorus marylandi n. sp. (MZ409463, CD3327, USA, MD)
Trichodorus obtusus (KM276666)
Trichodorus arasbaranensis (KY115139)
Nanidorus minor (JN123393)
Nanidorus minor (MZ409460, CD3449g, USA, MD)
Nanidorus minor (MZ409461, CD3449k, USA, MD)
Nanidorus sp.1 (MK584783)
Nanidorus sp.2 (MK584784)
Nanidorus renifer (JN123397)
Trichodorus sp. (KC413953)
Trichodorus nanjingensis (GU645847)
Trichodorus cedarus (MF979183)
Trichodorus sp.8 (JN123430)
Trichodorus sp.7 (MK584802)
Trichodorus sp.9 (MK584804)
Trichodorus sp.13 (MZ409459, CD3474, USA, CA)
Trichodorus californicus (MK584790)
Trichodorus sp.6 (MK584792)
Trichodorus sp.12 (MT418514)
Trichodorus intermedius (JN123429)
Trichodorus sp.14 (MZ409458, CD3448, USA, CA)
Trichodorus obscurus (MK584799)
Trichodorus sp.11 (MT418515)
Paratrichodorus almadenensis (MG739530)
Paratrichodorus ramblensis (MG739536)
Paratrichodorus anemones (AJ781505)
Paratrichodorus hispanus (MG739537)
Paratrichodorus pachydermus (MK584753)
Paratrichodorus pachydermus (JN123399)
Paratrichodorus sp. (MZ409477, CD3449l, USA, MD)
Paratrichodorus porosus (JN123405)
Paratrichodorus allius (MK584754)
Paratrichodorus teres (KU578052)
Paratrichodorus sp. (KY115136)
Paratrichodorus sp. (KY115134)
Monotrichodorus sp. (MG739560)
Monotrichodorus vangundyi (MG739554)
Monotrichodorus sp. (MG739563)
Diphtherophora sp. (DQ077790)
Diphtherophora sp. (KY115122)
Tripyla filicaudata (GQ503038)
Tripyla bioblitz (GQ503040)
I
II
III
IV
V
0.1

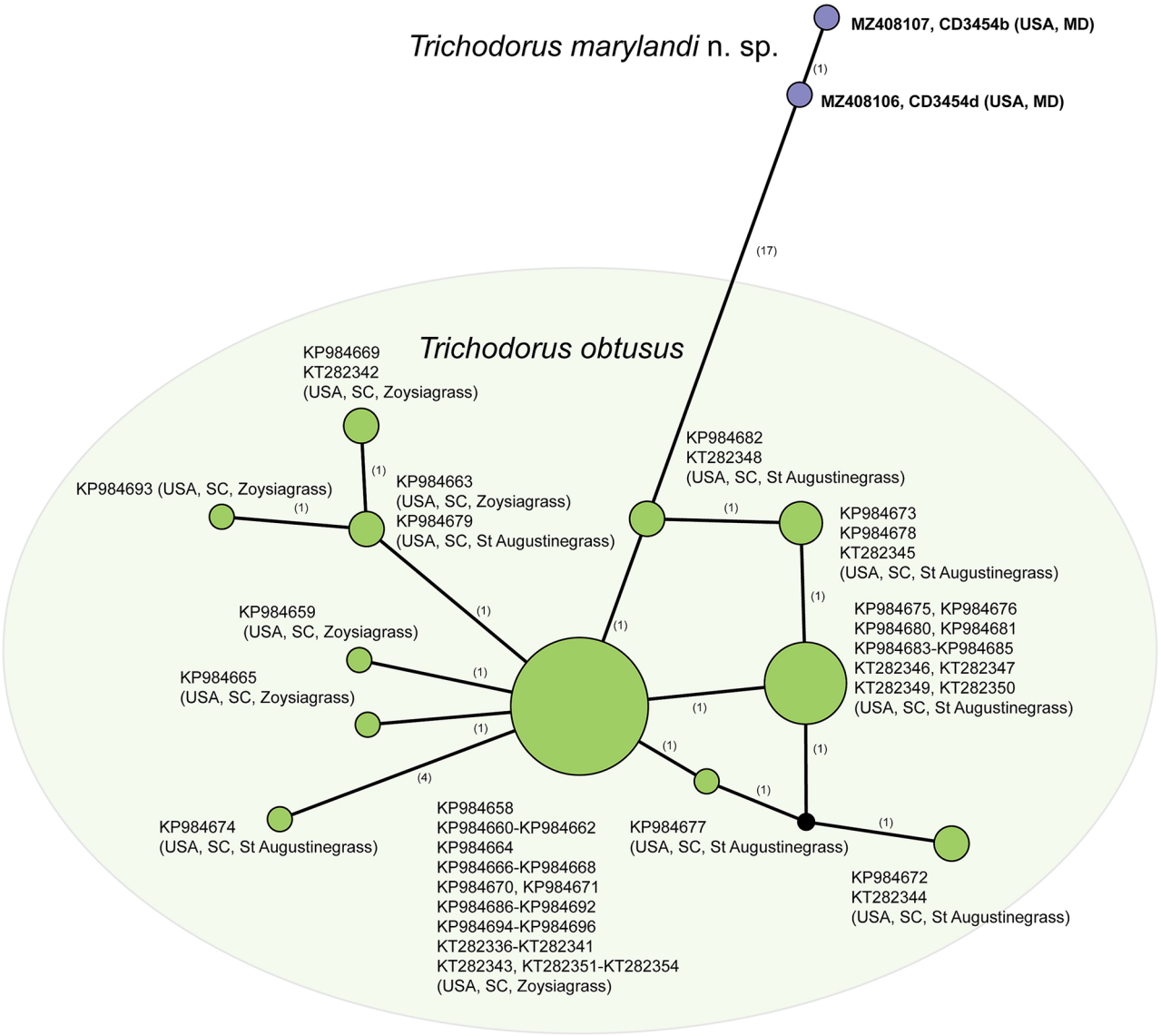


Fig. 7. Statistical parsimony network showing the phylogenetic relationships between *COI* haplotypes of *Trichodorus obtusus* and *T. marylandi* n. sp. (dot represents missing haplotypes). Pie chart sizes are proportional to the number of samples with a particular haplotype. New sequences are indicated in bold letters.

The COI *gene sequences*

The alignment included two new sequences of *T. marylandi* n. sp. and 58 sequences of *T. obtusus* (South Carolina, USA). *Trichodorus marylandi* n. sp. differed from *T. obtusus* by 18-22 bp (2.7-3.7%). Statistical parsimony network showing the phylogenetic relationships between *COI* haplotypes of *T. obtusus* and *T. marylandi* n. sp. is given in Figure 7.

Acknowledgements

The authors thank Marjolein Couvreur (Ghent University, Belgium) and Julie Burbridge (CDFA, Sacramento, CA, USA), and Stephen Rogers of USDA-ARS, MNGDBL for technical assistance. Mihail Kantor was supported in part by an appointment to the Research Participation Program at the Mycology and Nematology Genetic Diversity and Biology Laboratory USDA, ARS,

Northeast Area, Beltsville, MD, USA, administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the US Department of Energy and USDA-ARS.

References

Allen, M.W. (1957). A review of the nematode genus *Trichodorus* with description of ten new species. *Nematologica* 2, 32-62. DOI: 10.1163/187529257X00635

Bandelt, H., Forster, P. & Röhl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16, 37-48. DOI: 10.1093/oxfordjournals.molbev.a026036

Bernard, E.C. (1992a). Terrestrial nematodes of Alaska I. Trichodoridae (Nemata). *Journal of Nematology* 24, 67-77.

Bernard, E.C. (1992b). *Trichodorus elefjohnsoni* n. sp. (Nemata: Trichodoridae) from undisturbed Appalachian forest. *Journal of Nematology* 24, 78-83.

Boutsika, K., Brown, D.J.F., Philips, M.S. & Blok, V.C. (2004). Molecular characterisation of the ribosomal DNA of *Paratrichodorus macrostylus*, *P. pachydermus*, *Trichodorus primitivus* and *T. similis* (Nematoda: Trichodoridae). *Nematology* 6, 641-654. DOI: 10.1163/1568541042843432

Cobb, N.A. (1913). New nematode genera found inhabiting fresh waters and non-brackish soils. *Journal of the Washington Academy of Sciences* 9, 432-444.

De Waele, D. & Carbonell, E. (1983). Two new species of *Trichodorus* (Nematoda: Diphtherophorina) from Africa. *Nematologica* 28, 387-397. DOI: 10.1163/187529282X00196

Decraemer, W. (1995). *The family Trichodoridae: stubby root and virus vector nematodes*. Dordrecht, The Netherlands, Kluwer Academic Publishers.

Decraemer, W. & Baujard, P. (1998). A polytomous key for the identification of species of the family Trichodoridae Thorne, 1935 (Nematoda: Triplonchida). *Fundamental and Applied Nematology* 21, 37-62.

Decraemer, W. & Subbotin, S.A. (2021). Molecular and morphological characterisation of *Trichodorus* species (Nematoda: Trichodoridae), with description of *T. pseudoaequalis* n. sp. from California and other states of the USA. *Nematology* 23, 317-344. DOI: 10.1163/15685411-bja10044

Decraemer, W., Magnusson, C. & Bert, W. (2021). Morphological and molecular characterisation of *Trichodorus hellalae* n. sp. (Nematoda: Trichodoridae) from Norway. *European Journal of Plant Pathology* 161 91-105. DOI: 10.1007/s10658-021-02306-8

Hunt, D.J. (1993). *Aphelenchida, Longidoridae and Trichodoridae: their systematics and bionomics*. Wallingford, UK, CAB International.

Jenkins, W.R. (1964). A rapid centrifugal-flotation method for separating nematodes from soil. *Plant Disease Reporter* 48, 692.

Loof, P.A.A. (1975). Taxonomy of Trichodoridae. In: Lamberti, F., Taylor, C.E. & Seinhorst, J.W. (Eds). *Nematode vectors of plant viruses*. London & New York, Plenum Press, pp. 103-127.

Morton, H.V. (1967). *The bionomics and life cycle of* Trichodorus christiei. Ph.D. Dissertation, University of Florida, Gainesville, FL, USA.

Morton, H.V. & Perry, V.G. (1968). Life cycle and reproduction potential of *Trichodorus christiei*. *Nematologica* 14, 11 [Abstr.]

Norton, D.C., Dunlap, D. & Williams, D.D. (1982). Plant-parasitic nematodes in Iowa: Longidoridae and Trichodoridae. *Proceedings of the Iowa Academy of Sciences* 89, 15-19.

Powers, T.O., Bernard, E.C., Harris, T., Higgins, R., Olson, M., Lodema, M., Mullin, P., Sutton, L. & Powers, K.S. (2014). COI haplotype groups in *Mesocriconema* (Nematoda: Criconematidae) and their morphospecies associations. *Zootaxa* 3827, 101-146. DOI: 10.11646/zootaxa.3827.2.1

Poynter, D. (1954). Second ecdysis of infective nematode larvae parasitic in the horse. *Nature* 173, 781. DOI: 10.1038/173781a0

Seinhorst, J.W. (1954). On *Trichodorus pachydermus* n. sp. (Nematoda: Enoplida). *Journal of Helminthology* 28, 111-114.

Shaver, B.R., Marchant, S., Martin, S.B. & Agudelo, P. (2016). 18S rRNA and COI haplotype diversity of *Trichodorus obtusus* from turfgrass in South Carolina. *Nematology* 18, 53-65. DOI: 10.1163/15685411-00002944

Siddiqi, M.R. (1974). Systematics of the genus *Trichodorus* Cobb, 1913 (Nematoda: Dorylaimida), with descriptions of three new species. *Nematologica* 19(1973), 259-278.

Subbotin, S.A. (2021). Molecular identification of nematodes using Polymerase Chain Reaction (PCR). In: Perry, R.N., Hunt, D.J. & Subbotin, S.A. (Eds). *Techniques for work with plant and soil nematodes*. Wallingford, UK, CAB International, pp. 218-239.

Subbotin, S.A., Cid del Prado Vera, I., Inserra, R.N., Chizhov, V.N. & Decraemer, W. (2020). Molecular characterisation of some stubby root nematodes (Nematoda: Trichodoridae) from the USA and other countries. *Nematology* 22, 39-57. DOI: 10.1163/15685411-00003279

Tanha Maafi, Z. & Decraemer, W. (2002). Description of *Trichodorus gilanensis* n. sp. from a forest park in Iran and observations on *Paratrichodorus tunisiensis* (Siddiqi, 1963) Siddiqi, 1974 (Nematoda: Diphtherophorina). *Nematology* 4, 43-54. DOI: 10.1163/156854102760082195

Thorne, G. (1974). *Nematodes of the Northern Great Plains*. Part II. *Technical Bulletin* 41. Brookings, South Dakota, USA, Agricultural Experiment Station, South Dakota State University. https://openprairie.sdstate.edu/agexperimentsta_tb/5

Vrain, T.C., Wakarchuk, D.A., Levesque, A.C. & Hamilton, R.I. (1992). Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. *Fundamental and Applied Nematology* 15, 563-573.