

Meloidoderita whittoni (Sledge & Christie, 1962) comb. n. (Tylenchida: Sphaeronematidae) and its parasitic habits on sweet gum (*Liquidambar styraciflua* L.)

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Summary – Morphological and molecular analyses of three populations of *Meloidoderita whittoni* (Sledge & Christie, 1962) comb. n. (syn. *Sphaeronema whittoni*; *Tumiota whittoni*) collected in Florida from sweet gum (*Liquidambar styraciflua* L.) indicated that this species is a representative of *Meloidoderita* rather than *Sphaeronema*, where it was included in the original description, or *Tumiota*, to where it was subsequently transferred. However, this species can be considered an atypical representative of the genus, because it differs from the other species of this genus in having females retaining the eggs inside a thin-walled uterus, which remains encased inside their body. After egg deposition inside the uterus, the female dies and its body is transformed into a persistent tanned sac with a thick cuticle, devoid of ornamentations, which protects the eggs like a heteroderid cyst. The female secretes from the vulva a gelatinous matrix, which becomes hardened in time and encases its body for protection against predation and parasitism by biological antagonists as has been suggested for other tylenchuloid nematodes. No egg deposition outside the female body was observed. Second-stage juveniles of this species have a semi-endoparasitic, rather than endoparasitic, habit as in other known *Meloidoderita* species. This is the first report of a sphaeronematid having a cyst stage fitting the definition of a heteroderid cyst. Phylogenetic relationships between some species of Tylenchuloidea and Criconematina are analysed using the partial 18S rRNA, the D2-D3 of 28S rRNA and the ITS rRNA gene sequences.

Keywords – 28S rDNA gene, cyst-forming tylenchuloid nematode, D2-D3, Florida, ITS rRNA, morphology, morphometrics, new combination, phylogeny, *Sphaeronema whittoni*, systematics, topotype, *Tumiota whittoni*.

In 1962, Sledge & Christie described a new tylenchuloid nematode that was infesting the roots of sweet gum (*Liquidambar styraciflua* L.), along the bank of Hatchet Creek, Alachua County, North Florida. Sweet gum is a native tree in hardwood forests throughout warm and temperate areas of North America. The authors identified this nematode as a new representative of the genus *Sphaeronema* Raski & Sher, 1952 on the basis of the spheroid shape of the swollen females, the vermiform males with atrophied pharynx and stylet, and the second-

stage juveniles (J2) having a robust stylet like those of tylenchuloids. They called this new *Sphaeronema* *S. whittoni* Sledge & Christie, 1962, and separated it from *S. californicus* Raski & Sher, 1952 and *S. minutissimum* Goodey, 1958 (the only *Sphaeronema* species known at that time) by its swollen females with a larger body size and J2 with a longer stylet than those in the other two species. Subsequently, Siddiqi (1986) considered the morphological characters of *S. minutissimum* and *S. whittoni* sufficient for the establishment of two new genera, *Good-*

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eyella Siddiqi, 1986, which was represented by *S. minutissimum* with the new combination of *Goodeyella minutissima* (Sledge & Christie, 1962) Siddiqi, 1986, and *Tumiota* Siddiqi, 1986, which was represented by *S. whittoni* with the new combination of *Tumiota whittoni* (Sledge & Christie, 1962) Siddiqi, 1986. These two genera were separated from *Sphaeronema*, which belongs with these two genera to the subfamily Sphaeronematinae, for having swollen females without protruding vulval lips, which are prominent in *Sphaeronema*. *Tumiota* was separated from *Goodeyella* for having pharyngeal glands extending over the intestine whereas in *Goodeyella* they remain enclosed in the basal bulb, and also by the absence of a circumoral elevation, which is present in females and J2 of *Goodeyella*. However, neither *Goodeyella* nor *Tumiota* were considered valid by other taxonomists, including Raski & Luc (1987) and Decraemer & Hunt (2013).

During a nematode survey conducted in North Florida, in 2015, a population of *Tumiota* (= *Sphaeronema*) *whittoni* was detected again on a sweet gum tree at the type locality of this species. The observation of the infested sweet gum feeder roots, using a stereomicroscope, revealed the presence of spheroid cysts that at low magnification resembled those of *Globodera* species. The cysts contained embryonated eggs and hatching J2. These J2 morphologically matched those of *T. whittoni*. However, the findings that we present in this study reveal that *T. whittoni* does not belong to *Tumiota* or *Sphaeronema*, but indeed to the genus *Meloidoderita* Poghossian, 1966, to which this species is transferred under the name of *Meloidoderita whittoni* (Sledge & Christie, 1962) comb. n. (syn. *Sphaeronema whittoni*; *Tumiota whittoni*).

In the original description of *M. whittoni* comb. n., Sledge & Christie (1962) illustrated and described the female, male and J2 of this species, but omitted the description of the cyst stage. The term “cyst” was, however, used in the description of the female without any differentiation of the morphology of the mature female from that of the cyst stage. The morphological description of the females also lacked details concerning the shape of the vulva and anus. The morphology of J2 and males was well elucidated, even if some morphological characters, such as the lines in lateral field and many morphometrics of J2, were omitted. The original description also did not report any information on the parasitic habits and development of the nematode life stages. A study was conducted to supplement the description of this nematode with additional information on its morphology, biology and taxonomic status. The main objectives of this work

were: *i*) to conduct the morphological characterisation of the life stages of this nematode that were not described in the original description; *ii*) to obtain information on the parasitic habits of this species; *iii*) to conduct the molecular characterisation of a *M. whittoni* comb. n. population from the type locality; and *iv*) to reconstruct the phylogenetic relationships among this species and other tylenchuloid nematodes using partial 18S rRNA, D2-D3 expansion segments of 28S rRNA and ITS rRNA gene sequences.

Materials and methods

NEMATODE POPULATIONS

A population of *M. whittoni* comb. n. was obtained from sweet gum at the type locality mentioned above, which is located eight miles east of Gainesville on highway 26, in Alachua County (latitude 29°41'15.4"N; longitude 82°13'15.3"W). Two additional populations were found on the same host in Hillsborough River State Park, Thonotosassa, in Hillsborough County and in the western section of Gainesville, in Alachua County. In all of the sampled sites sweet gum trees were associated with oak trees, mainly live oaks (*Quercus virginiana* Mill.). Soil and root samples from the three localities were collected with a shovel from the upper 10–40 cm soil surrounding the rhizosphere of sweet gum trees. Nematode populations were extracted from soil by rapid centrifugal-flotation methods (Jenkins, 1964). Sweet gum roots were separated from soil and observed for presence of cysts. Cysts and J2 from the type and other localities were placed in water in Syracuse dishes after their extraction from soil and roots. The J2 of the three populations were then used for molecular analyses and sequencing.

Other J2 and cysts from the Gainesville population were used to infest 3-month-old sweet gum seedlings grown in 20 cm diam. pots containing 1 l of a commercial artificial soil consisting of Canadian sphagnum 20%, composted pine bark 20%, peat moss 20%, Perlite 10% and vermiculite 30%. Two groups of sweet gum seedlings, each consisting of three seedlings of northern and southern provenance, respectively, were used. Each seedling was infested with five cysts and 20 J2 on 23 July 2015. Seedlings roots were observed for symptoms of nematode infestation on 23 October 2015 after 3 months of exposure to the nematode. At this sampling date, a small quantity (0.5 g) of feeder roots was removed from the seedlings and

observed with the aid of a stereomicroscope for the presence of nematode life stages. Then, seedlings were left for 1 year in the pots to allow the nematode to increase the populations. These populations not associated with roots from other trees, as occurs in samples collected directly from hard wood forests, facilitated the biological observations of the nematode. Feeder root segments removed from the seedlings were examined with light microscopy (LM).

The molecular study included the following populations of *M. whittoni* comb. n.: the topotype, Hatchet Creek, Alachua County (sample code: CD1802), a population from Gainesville (CD1803), a population from Thonotosassa (CD1759, CD1770, CD1799), and another two tylenchuloids, namely *M. polygoni* Golden & Handoo, 1984 from Maryland, USA (CA113) (Vovlas *et al.*, 2006), and juveniles of an unidentified sphaeronematid (CD942) collected in a coastal area of the Olympic National Park, WA, USA.

LIGHT MICROSCOPE STUDY

Live specimens of two populations of *M. whittoni* comb. n. from Hatchet Creek, the type locality, and Gainesville were immobilised by gently heating and then mounted in water agar on a slide for measurements. Specimens of the Gainesville population reared on sweet gum seedlings were used for photographs. Some of these specimens were also used for observations *in vivo* on water agar (Esser, 1986). Additional measurements and drawings were made using specimens killed and fixed in FP, hot aqueous 2% formaldehyde +1% propionic acid, dehydrated in ethanol vapour and mounted in dehydrated glycerin or in lactophenol (Hooper, 1970). Measurements of specimens were made with an ocular micrometer and drawings with a *camera lucida*. Photographs were taken using two Leica (Wild MPS 46/52 and Leica DFC 320) cameras mounted on Nikon (Optiphot) and Leica DM 2500 compound microscopes.

DNA EXTRACTION, PCR AND SEQUENCING

The DNA was extracted from several nematode individuals using proteinase K protocol as described by Subbotin *et al.* (2006). PCR protocols were as described by Tanha Maafi *et al.* (2003). The following primer sets were used for PCR: 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.*, 2006) for amplification of the D2-D3 expansion seg-

ments of 28S rRNA gene; the forward TW81 (5'-GTT TCC GTA GGT GAA CCT GC-3') and the reverse AB28 (5'-ATA TGC TTA AGT TCA GCG GGT-3') primers (Tanha Maafi *et al.*, 2003) for amplification of the ITS rRNA gene; and the forward G18SU (5'-GCT TGT CTC AAA GAT TAA GCC-3') and the reverse R18Ty11 (5'-GGT CCA AGA ATT TCA CCT CTC-3') and the forward F18Ty12 (5'-CAG CCG CGG TAA TTC CAG C-3') and the reverse R18Ty12 (5'-CGG TGT GTA CAA AGG GCA GG-3') primers (Chizhov *et al.*, 2006) for amplification of the partial 18S rRNA gene. The newly obtained sequences were submitted to the GenBank database under accession numbers KY704309-KY704317.

PHYLOGENETIC ANALYSIS

The newly obtained sequences of the 18S rRNA, D2-D3 of 28S rRNA and ITS rRNA genes were aligned with corresponding published gene sequences (Subbotin *et al.*, 2006; Vovlas *et al.*, 2006; Palomares-Rius *et al.*, 2010, Ashrafi *et al.*, 2012) using ClustalX 1.83 (Thompson *et al.*, 1997) with default parameters. The alignments were analysed with Bayesian inference (BI) using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001) under the GTR + I + G model. BI analysis for each gene was initiated with a random starting tree and was run with four chains for 1.0×10^6 generations. Two runs were performed for each analysis. The Markov chains were sampled at intervals of 100 generations. After discarding burn-in samples, other trees were used to generate a 50% majority rule consensus tree. Posterior probabilities (PP) are given on appropriate clades.

Results

NEMATODE POPULATIONS

The population densities of *M. whittoni* comb. n. recorded at the type locality were no less than 10 J2 and 2 cysts containing eggs ($500 \text{ cm}^3 \text{ soil}^{-1}$). Taking into consideration that one cyst contains an average of 110 eggs, the population of the nematode consisted of 2 J2 and 44 eggs ($100 \text{ cm}^3 \text{ soil}^{-1}$). Population levels were variable at the other two sites.

LIGHT MICROSCOPIC STUDY

The morphological and morphometric features of the topotype specimens of *M. whittoni* comb. n. matched those reported in the original description. However, some

morphometric values of J2 were greater than those in the original description because live rather than fixed specimens were used. Discrepancies, compared to the original description, were observed in the ratio c values of J2 and were due to the fact that the rectum is better discernible in live than in the fixed specimens that were measured in the original description. Despite these differences, the morphology of female, male and J2 of the populations studied fits that of *M. whittoni* comb. n., although the presence of cysts (Figs 1H, K; 2) and the omission of the description of this life stage in both the original and subsequent descriptions of this species cast doubt about the validity of the classification of this tylenchuloid nematode either as a representative of *Sphaeronema* as proposed by Sledge & Christie (1962) or *Tumiota* as proposed by Siddiqi (1986), because these two genera, in the subfamily Sphaeronematinae Raski & Sher, 1952, do not contain species with a cyst stage. Cystoid life stages are a characteristic of species of the genus *Meloidoderita* Poghosian, 1966, in the subfamily Meloidoderitinae (Kirjanova & Poghosian, 1973) Siddiqi, 1986. The presence of a cyst stage and the morphological features of *M. whittoni* comb. n. suggest that this species is a representative of *Meloidoderita* rather than *Sphaeronema* and *Tumiota*. These morphological considerations are supported by the results of the molecular analyses reported in the following sections, which indicate that the DNA sequences of the studied populations match those of representatives of *Meloidoderita*. This change in the classification of the nematode requires additional morphological information of the populations studied. Herein, we provide the combined original and subsequent morphological descriptions reported by Sledge & Christie (1962) and Siddiqi (1986), along with detail on new morphological and biological characteristics of this nematode.

***Meloidoderita whittoni* (Sledge & Christie, 1962)
comb. n.**

= *Sphaeronema whittoni* Sledge & Christie, 1962
= *Tumiota whittoni* (Sledge & Christie, 1962)
Siddiqi, 1986
(Figs 1-3)

MEASUREMENTS

See Table 1.

DESCRIPTION

Female

From Siddiqi (1986): "Body spherical to subspherical with protruding neck. Cephalic region elevated, sclerotization delicate. Stylet 19-21 μm long, with conus 54-57% of total stylet length, basal knobs rounded. Orifice of dorsal pharyngeal gland 3-4 μm behind stylet base. Precorpus elongate-cylindrical. Postcorpus spheroid, very muscular, with valve plates. Uterus thick walled, in mature females distended, packed with eggs. Vulva terminal, flush with body surface, lip not protruding." The females that we examined ($n = 10$) were whitish in colour and their dimensions fitted those of the original description ranging 224 to 370 vs 330 to 650 μm . The larger values reported by Sledge & Christie (1962) were due to the fact that cysts and females were lumped together when measured by these authors. Their stylet length (21.5-22 μm) was similar to that reported by Sledge & Christie (19 μm). The metacorpus valve was 10-12 μm long and 6.5-9.0 μm wide. Cardia, vulva and anus were difficult to localise in their globose body. Cuticle thickness ranged from 2-11 μm . Vulva and anus were visible only in dissected terminal portions of the cuticle removed from the female body at level of anus and vulva. In these cuticle sections, vulva and anus were localised in circular depressions (Fig. 3H, I). No vulval lips were present. Vulval slit length ranged from 2 to 5 μm . Eggs, produced after gonad maturation, were retained inside the uterus, which had a thin wall (less than 1 μm thick) rather than a thick wall as reported in the original descriptions. Whitish females were always encased in gelatinous matrix secreted from the vulva (Fig. 3M). The hardened gelatinous matrix is lost completely or partially when females become senescent or die. In these old females the cuticle becomes brownish. No egg deposition inside the gelatinous matrix outside the female body was observed.

Cyst

Body spherical to subspherical with protruding neck. Pharynx degenerate, not discernible. Cuticle brownish, unmarked, up to 14 μm thick. Vulva and anus not detectable. Cyst wall encasing and protecting uterus which is packed with eggs. After J2 hatch, cyst remaining filled with a spongy mass of egg chorions. Such cysts showing a broken neck, probably used by J2 to exit cysts.

Male

From Siddiqi (1986): "Body 390-470 μm , slender ($a = 32-39$). Cephalic region smooth, continuous. Lateral

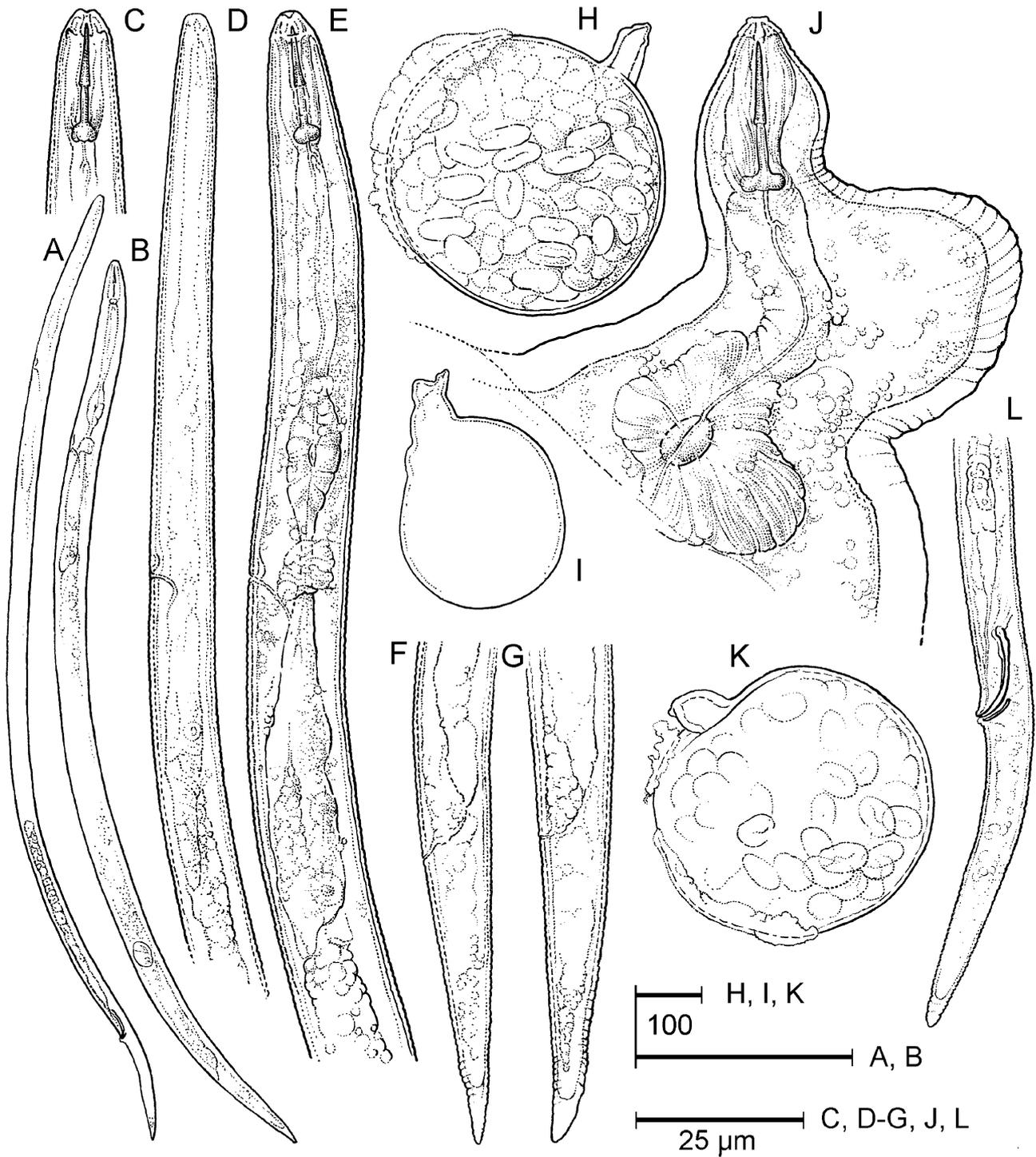


Fig. 1. Camera lucida line drawings of *Meloidoderita whittoni* comb. n. A, B: Male and female entire body; C: Anterior end of second-stage juvenile (J2); D, E: Pharyngeal region of male and J2; F, G: J2 tail; H, K: Cysts containing embryonated eggs and encrusted with residues of gelatinous matrix; I: Mature female entire body; J: Female anterior region; L: Male posterior end.

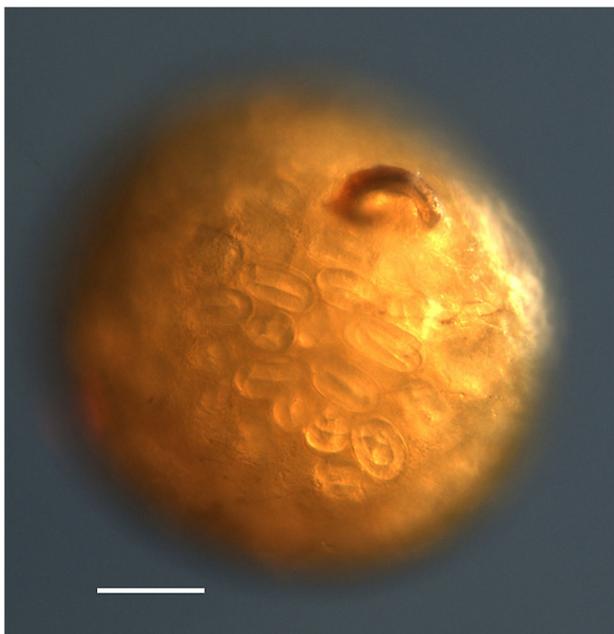


Fig. 2. Photomicrograph of *Meloidoderita whittoni* comb. n. cyst. (Scale bar: 100 μm .)

field obscure, oesophagus degenerate, stylet absent, testis single, gonoduct packed with hundreds of small round sperm. Spicule arcuate, pointed 12-14 μm long. Gubernaculum simple, tough-like, 5-6 μm long. Bursa absent. Tail conoid, with small round tip, slightly arcuate.”

The morphology of the males ($n = 2$) we examined fitted that of the description reported above. Our specimens showed the remnant of the stylet, *ca* 10.5 μm long.

Second-stage juvenile (J2)

From Siddiqi (1986): “Body 400-480 μm long. Stylet about 19 μm long, with conus 47-65% of stylet length and round basal knobs. Cuticle finely annulated. Cephalic region continuous, rounded, with weak sclerotization. Orifice of dorsal gland about 3 μm behind stylet base. Median oesophageal bulb elongate with refractive valve plates. Isthmus well defined. Oesophageal gland extending over intestine. Anus obscure. Tail tapering to a finely rounded terminus.”

The morphology of the live specimens ($n = 10$) of J2 females we examined fitted that of the original description reported above. Rectum and anus visible in live specimens, but obscure and not discernible in fixed specimens as in those measured in original description resulting in different values of ratio *c* which were smaller than in the original description: 8.7 ± 0.3 (8.3-9.2) *vs* 10.1 (8.7-13.0). Lateral field not discernible. However, in

specimens fixed in lactophenol we were able to observe a lateral field consisting of a small band marked by two lines. Genital primordium located posteriorly at 68.3-70.7% of body length. J2 females and males not separable. However, J2 males moulting in water into J3 and J4 which lose the stylet shaft and portion of the pharynx, retaining only the cone. They were distinguishable from J2 females which do not moult in water and develop only when feeding in the roots.

DIAGNOSIS

The morphological characters of the life stages of this new representative of the genus *Meloidoderita* are in agreement with those of the species of this genus. However, *M. whittoni* differs from the only four *Meloidoderita* species so far described, namely *M. kirjanovae* Poghosian, 1966, *M. polygona*, *M. safrica* Van den Berg & Spaul, 1982, and *M. salina* Ashrafi, Mugniéry, van Heese, van Aelst, Helder & Karssen, 2012, by the characters of the resistant stage containing the eggs (cystoid/cyst). *Meloidoderita* females of the species so far described deposit eggs in the uterine sac which has a thick, sclerotised, and variously ornamented wall. This sclerotised uterine sac is transformed into a cystoid body that is retained for some time inside the female body but, with the death of the female and decomposition of its body, becomes detached. *Meloidoderita whittoni* comb. n. females deposit eggs in the uterine sac as in the other *Meloidoderita* so far described, but this uterine sac has a thin wall and does not become a cystoid body. It remains permanently inside the female body, which does not disintegrate but has a thick, tanned and sclerotised cuticle, like that of *Globodera* cysts. The *M. whittoni* comb. n. female has a vulva flush with the body contour and a short vulval slit, 2-5 μm wide, unlike other *Meloidoderita* spp. females which have a protruding vulva with prominent lips and a longer vulval slit of 16-22 μm . No egg deposition has been observed outside the nematode body of *M. whittoni* comb. n., whereas in all other *Meloidoderita* spp., the females lay eggs in a gelatinous matrix outside the body. These morphological characters of the females separate *M. whittoni* comb. n. from other *Meloidoderita* species. With the exception of *M. salina*, which has J2 with a stylet of the same length as that of *M. whittoni* comb. n., the other species, *M. kirjanovae*, *M. polygona* and *M. safrica*, have a J2 with a shorter stylet of 13.2 (12.9-14.0), 15.0 (13.7-16.3) and 15.0 (14.0-15.9), respectively, *vs* 18.8 (18.6-19.3) μm . The J2 of *M. whittoni* comb. n. can be separated from that of *M. salina* by the longer tail of 53.5 (51.1-55.9) *vs* 38.7

Table 1. Morphometrics of live topotype of second-stage juvenile (J2) and a male of *Meloidoderita whittoni* comb. n. from the type locality compared with those reported for these life stages in the original description (Sledge & Christie, 1962). All measurements are in μm and in the form: mean \pm SD (range).

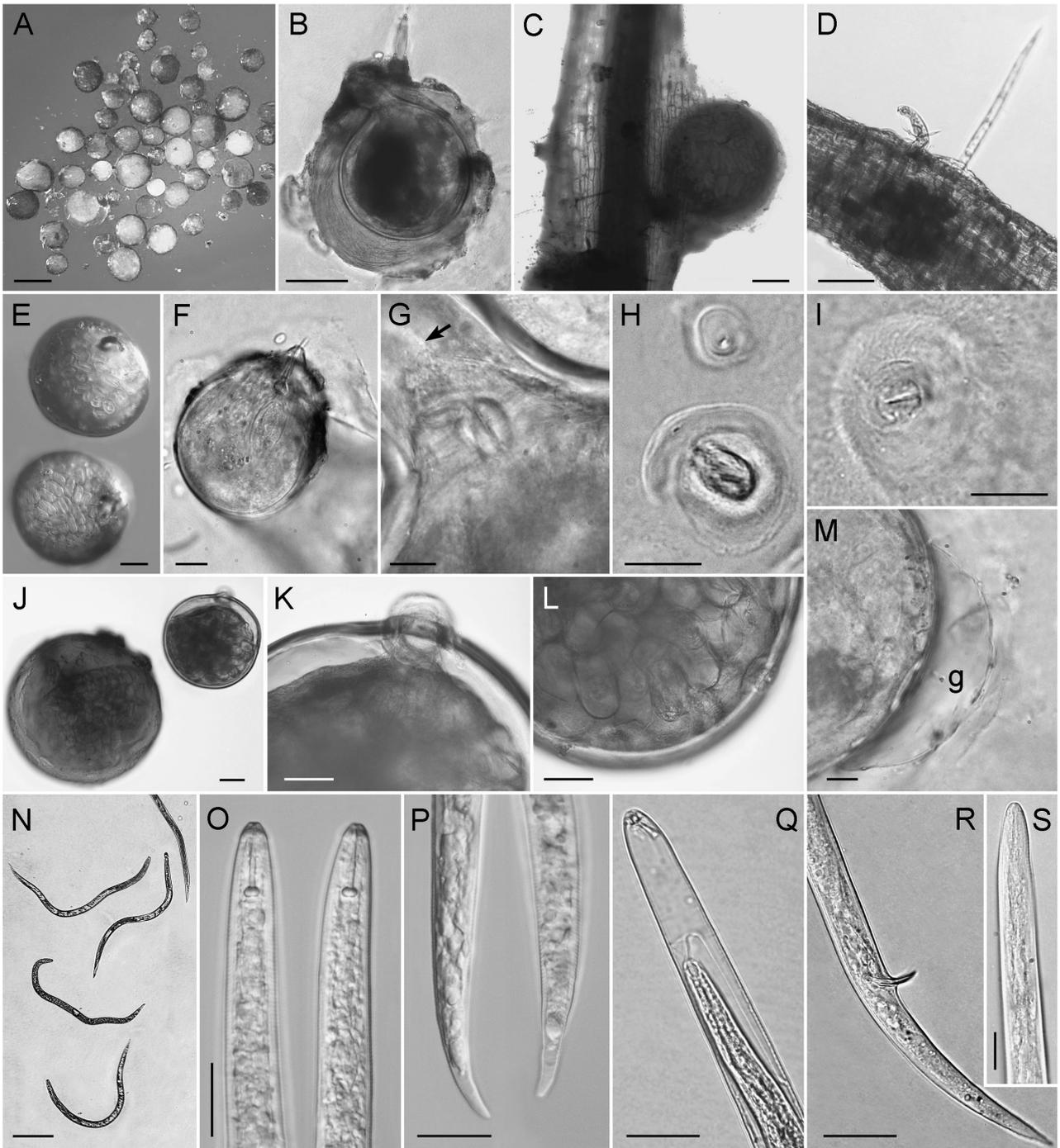
	J2 Topotype	J2 (Sledge & Christie, 1962)	Male topotype	Male (Sledge & Christie, 1962)
n	10	10	1	10
L	470 \pm 14.9 (453-503)	430 (400-480)	475.2	420 (390-470)
a	27.4 \pm 0.8 (26.1-28.6)	26.3 (24-29.8)	35.7	35.5 (32-39)
b	3.8 \pm 0.1 (3.7-4.0)	3.5 (2.8-4.2)	3.9	–
b'	3.3 \pm 0.1 (3.1-3.4)	–	–	–
c	8.7 \pm 0.3 (8.3-9.2)	10.1 (8.7-13)	8.1	8.3 (7.6-9.1)
c'	4.6 \pm 0.2 (4.3-5.0)	–	5.5	–
Max. body diam.	17.1 \pm 0.6 (16.8-18.8)	–	13.3	–
Body diam. at anus/cloaca	11.3 \pm 0.4 (10.8-11.8)	–	10.5	–
Stylet length	18.8 \pm 0.2 (18.6-19.3)	19	8.5	–
Stylet cone	10.2 \pm 0.3 (9.3-10.8)	–	–	–
Stylet shaft	8.6 \pm 0.3 (8.0-9.3)	–	–	–
Stylet knob height	2.8 \pm 0.2 (2.4-3.0)	–	–	–
Stylet knob width	4.7 \pm 0.2 (4.4-5.0)	–	–	–
DGO	4.2 \pm 0.3 (4.0-4.9)	3	–	–
Ant. end to metacarpus	70 \pm 1.1 (69-72)	–	–	–
Metacarpus valve length	4.9 \pm 0.2 (4.4-5.4)	–	–	–
Metacarpus valve width	2.7 \pm 0.2 (2.4-2.9)	–	–	–
Pharynx length	122 \pm 2.9 (116-127)	–	121	–
Ant. end to pharyngeal gland lobe	143 \pm 4 (135-149)	–	–	–
Pharyngeal overlap	21.6 \pm 1.9 (18.8-23.7)	–	–	–
Ant. end to excretory pore	94 \pm 2.3 (90-98)	–	87	–
Ant. end to genital primordium	325 \pm 8 (319-343)	–	–	–
Genital primordium to posterior end	134 \pm 8.7 (120-147)	–	–	–
Genital primordium length	11.9 \pm 1.3 (10.0-13.5)	–	–	–
Genital primordium width	7.1 \pm 0.9 (6.0-8.9)	–	–	–
Tail length	53.5 \pm 1.5 (51.0-55.9)	–	58.4	–
Spicule length	–	–	14.0	12.0-14.0
Gubernaculum length	–	–	5.0	5.6
Testis length	–	–	99.0	–
Hyaline tail terminus	7.8 \pm 1.3 (6.4-9.8)	–	–	–
Phasmid to posterior end	25.1 \pm 1.5 (23.0-27.7)	24-27	–	–
Excretory pore ant. end (% L)	20.1 \pm 0.9 (18.0-21.2)	–	–	–
Genital primordium (% L)	69.3 \pm 1 (67.9-70.7)	–	–	–
Hyaline region (% tail length)	14.4 \pm 2.2 (12.4-18.3)	–	–	–

(33.9-44.2) μm and a smaller ratio c value of 8.7 (8.3-9.2) vs 12.2 (9.9-13.9). Males of *M. whittoni* comb. n. can be separated from those of *M. kirjanovae*, *M. polygoni*, *M. safrica* and *M. salina* by the shorter spicules of 12-14 vs 15.1 (13.7-16.1), 17.9 (16.3-19.3), 18.8 (16.2-21.7) and 18.4 (15.4-21.1) μm , respectively.

PARASITIC HABITS

During the examination of sweet gum roots collected at the three sites, we did not find J2 inside the fibrous

roots. Infested roots showed J2 with their anterior portion of the body penetrating into the epidermis and other root tissues but with the posterior portion of the body protruding outside the root surface. These field observations were confirmed by the examination of roots of sweet gum seedlings exposed to the nematode for 1 year. Both groups of three seedlings, each of southern and northern provenance, were infested by the nematode. However, the nematode infestation persisted only in one seedling out of three in each group. We examined with a stereomicro-



scope 3.5 g of fibrous roots removed from the infested seedlings and were able to observe two cysts, ten females and six juveniles attached to the roots. Unlike the studied *Meloidoderita* species (Andrews *et al.*, 1981; Cohn & Mordechai, 1982), the J2 of *M. whittoni* comb. n. showed a semi-endoparasitic habit similar to species of *Tylenchulus* Cobb, 1913. After partial penetration into the root, the J2 moults into the J3 and J4, which are swollen and maintain the tapered, almost pointed, tail, until reaching the adult swollen female stage. The latter stage shows a bluntly rounded posterior body portion, which becomes subspherical with gonad maturation. The nematode very probably establishes a permanent feeding site in the root tissues as reported for *M. kirjanovae* (Cohn & Mordechai, 1982; Vovlas *et al.*, 2006). However, we do not have histological data to confirm this assumption. Young and whitish females produce a gelatinous matrix that exudes from the vulva and completely covers the body (Fig. 3B, M). Feeding activity by female is prolonged over time while gonad development and production of eggs enlarges the female body. At the end of the feeding activity, the thick cuticle of the female becomes brownish and tanned and the female is transformed into a cyst packed with eggs. Males are not parasitic. They were found in moulting J2 male populations (Fig. 3Q) maintained in water for 2 weeks at room temperature. Nematode J2 developed into males when maintained for longer than 2 weeks in water in the absence of a host.

PHYLOGENETIC RELATIONSHIPS

Phylogenetic relationships within Sphaeronematidae species and several other selected Criconematina species, representing different genera, are given from the analyses of the partial 18S rRNA gene sequences in Figure 4, the D2-D3 of 28S rRNA gene sequences in Figure 5 and the

ITS rRNA gene sequences in Figure 6. In all of these trees the Sphaeronematidae formed a highly or moderately supported clade where *Meloidoderita* and *Sphaeronema* shared a sister relationship. Molecular analysis revealed distinct differences in sequences of all genes of *M. whittoni* comb. n. from those of other studied *Meloidoderita* species: *M. kirjanovae*, *M. polygona*, and *M. salina*. The superfamily Tylenchuloidea Skarbilovich, 1947, containing the families Paratylenchidae, Sphaeronematidae, and Tylenchulidae, was paraphyletic in all of the obtained trees.

Discussion

Meloidoderita whittoni comb. n. is morphologically and biologically an atypical species in the genus *Meloidoderita*. Anatomical features of the females such as the thick cuticle, small vulva flush with the body contour, the presence of a uterus with thin walls and the transformation of the female body into a cyst similar to that of *Globodera*, would be sufficient elements to keep this species in a separate genus from *Meloidoderita*. Furthermore, in Siddiqi's (2000) diagnosis of the genus, mature females of the genus *Meloidoderita* have "...swollen body, without neck or tail", whereas *M. whittoni* has a well-defined neck region. The parasitic habits of this species are also dissimilar to those of *Meloidoderita* species, thereby supporting the separation of this species from this genus. However, *M. whittoni* comb. n. shares some morphological and physiological features with *Meloidoderita* species. These characters include the spherical shape of the female body, the formation of a resistant stage (cystoid body vs a cyst), and the secretion of a gelatinous matrix from the vulva, as reported in *M. kirjanovae* by Spiegel & Cohn (1985). *Meloidoderita whittoni* comb. n. DNA sequences

Fig. 3. Photomicrographs of *Meloidoderita whittoni* comb. n. life stages detached or attached to feeder root segments of sweet gum (*Liquidambar styraciflua*). A: Brown cysts and white females; B: Young female encased in hardened gelatinous matrix; C: Female body embedded in root cortex; D: Vermiform second-stage juveniles (J2) entering the root with the anterior body portion (right) and a shortened and swollen immature female encased in juvenile cuticles protruding with posterior portion of body from root surface (left); E: Cysts filled with embryonated eggs; F: Anterior end of twisted neck of young female, with stylet slightly protruded; G: Female neck region at level of pharyngeal median bulb, showing excretory duct (arrow); H: Female perineal area, showing vulval slit (bottom) and anus (top) located in circular depressions of cuticle; I: Vulval slit; J: Entire cysts; K, L: Particulars of anterior (K) and posterior (L) body portions of top right cyst in Figure 3J. Note, in K, the neck and the uterine sac detached from the thick body wall and, in L, the packed embryonated eggs enclosed in it and adhering to the thick cyst wall; M: Posterior portion of a young female secreting gelatinous matrix (g) from vulva; N: J2 in temporary water agar mount; O, P: Anterior and posterior end of glycerin infiltrated J2; Q: Male fourth-stage juvenile encased in J2 and J3 cuticles and with visible stylet conus in anterior body region; R: Male tail, lacking bursa and with protruded spicules; S: Male anterior end without stylet and with degenerate pharynx. (Scale bars: A = 500 μ m; B-E, J, N = 100 μ m; F-I, M, S = 10 μ m; K, L = 50 μ m; O-R = 20 μ m.)

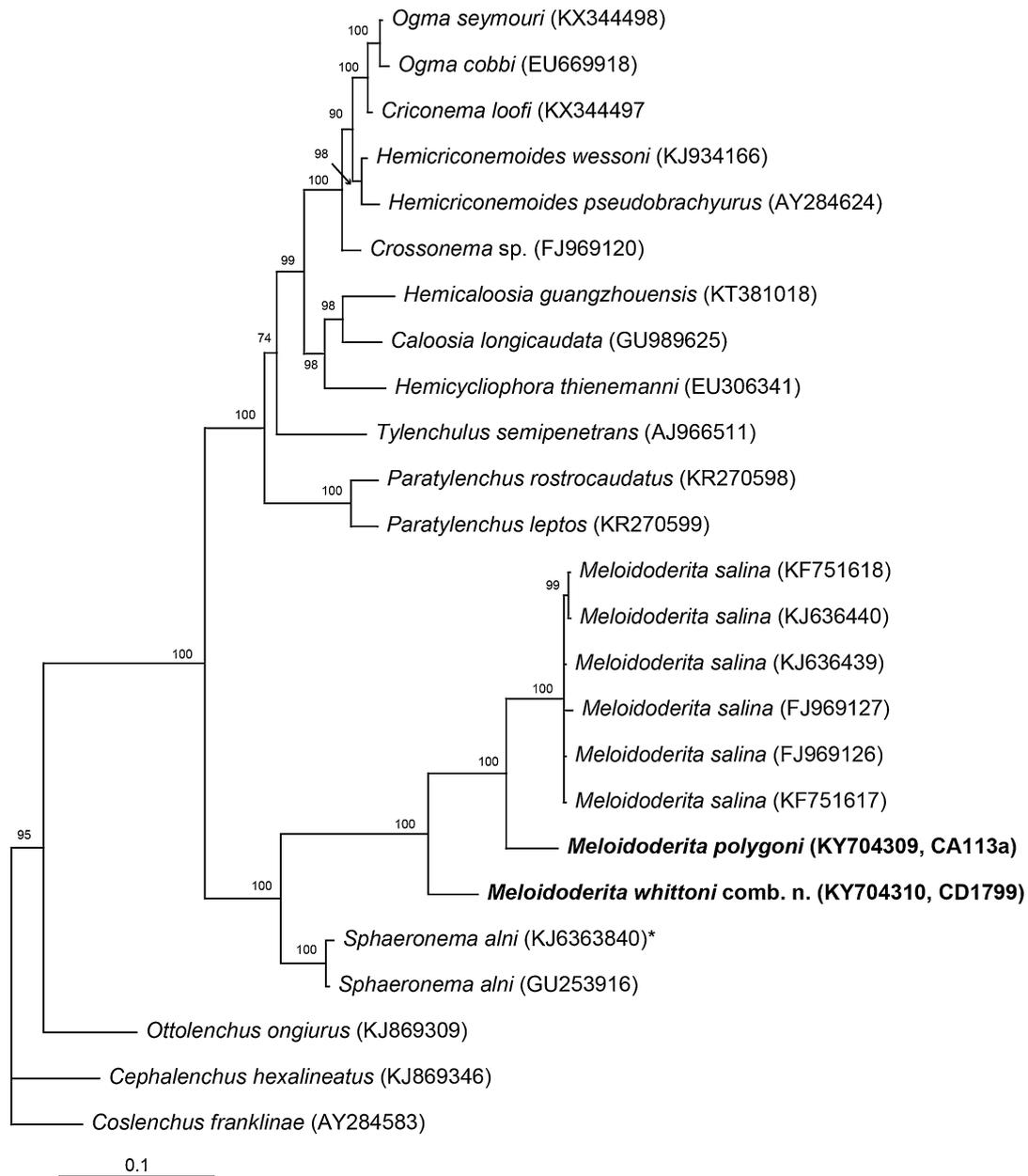


Fig. 4. Phylogenetic relationships within Sphaeronematidae and other Criconematina species: Bayesian 50% majority rule consensus tree as inferred from analysis of the partial 18S rRNA gene sequence alignment under the GTR + I + G model. Posterior probabilities equal or more than 70% are given for appropriate clades. Original sequences are indicated by bold font.

match those of other *Meloidoderita* species and cluster in the phylogenetic tree in the same clade. These characters support our decision to retain this species in the genus *Meloidoderita*. It is possible that the apparently aberrant morphological and biological characteristics of this

species were induced by the wet environment of the hardwood forests of North Florida where this species evolved. In North Florida, the hardwood forests remain flooded for several months during the summer. In this wet environment the number of biological antagonists, especially

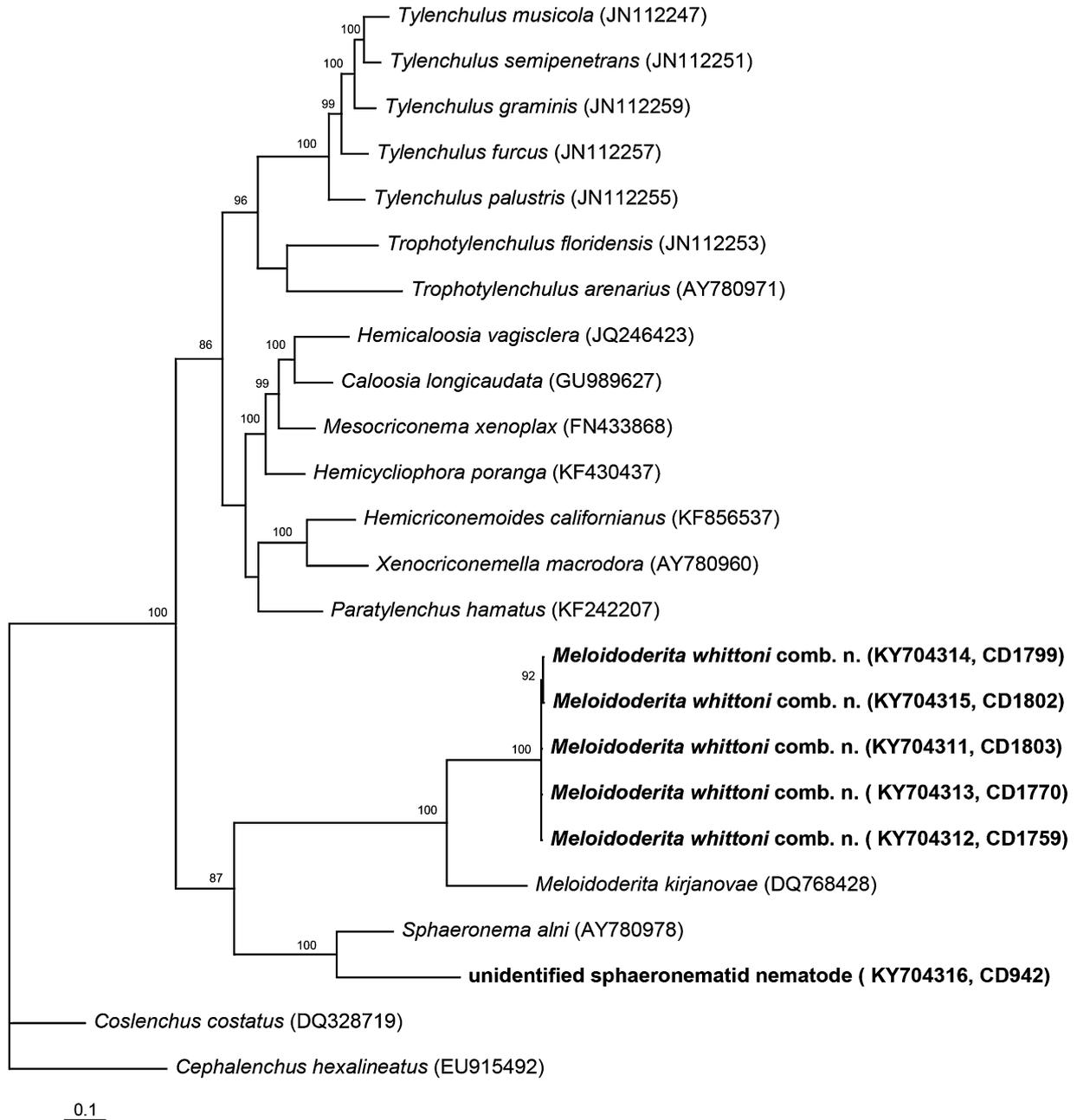


Fig. 5. Phylogenetic relationships within Sphaeronematidae and other Criconematina species: Bayesian 50% majority rule consensus tree as inferred from analysis of the D2-D3 of 28S rRNA gene sequence alignment under the GTR + I + G model. Posterior probabilities equal or more than 70% are given for appropriate clades. Original sequences are indicated by bold font.

fungi, is greater than that in a dry environment. The females of this species escape the parasitisation of these antagonists by remaining encased in a large mass of gelatinous matrix that probably has fungicidal properties, as re-

ported for the eggs and associated gelatinous matrix of *T. semipenetrans* (El-Borai *et al.*, 2002). On the other hand, the eggs may be better protected against antagonists in a hard cyst rather than in the gelatinous matrix. We would

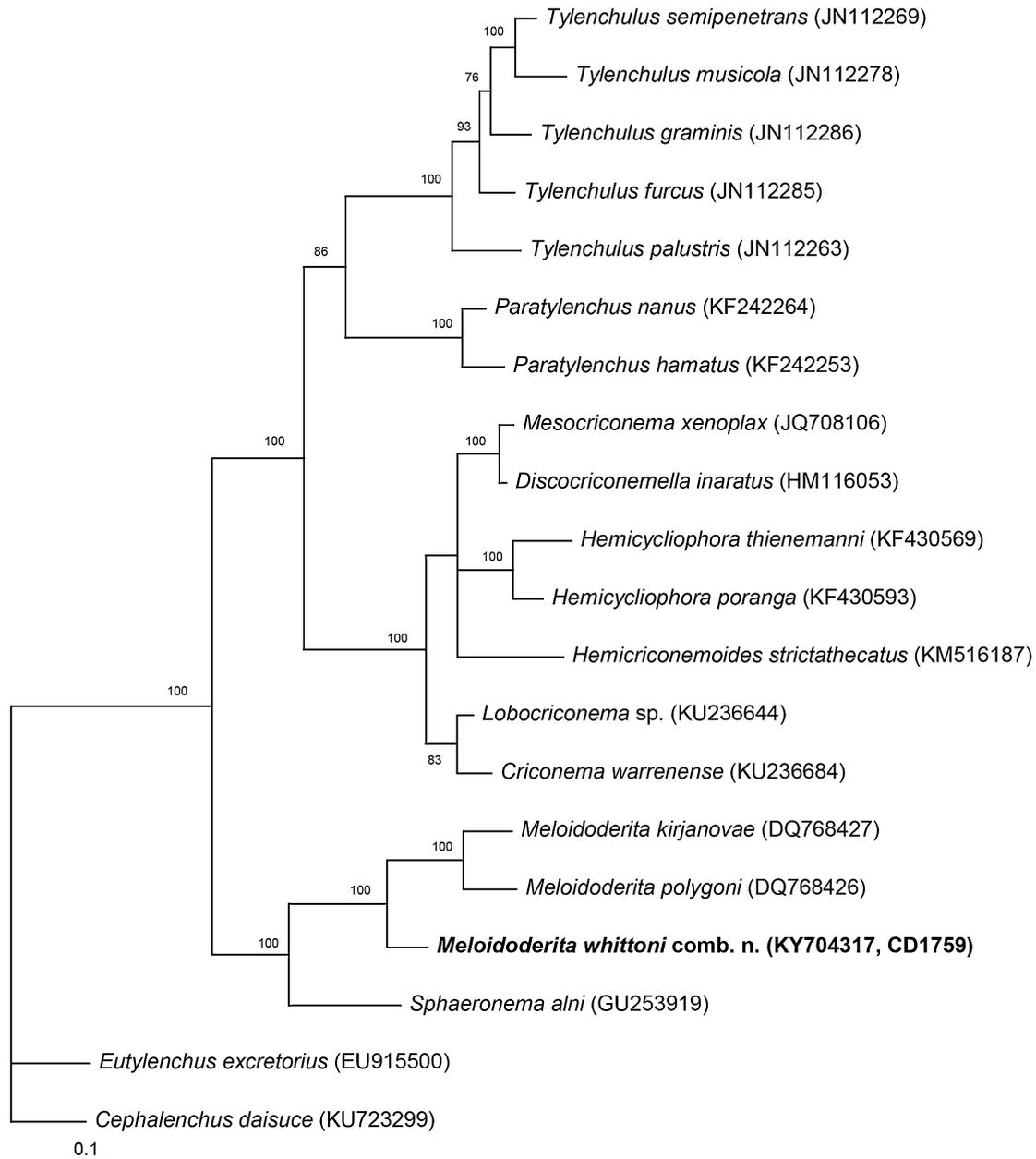


Fig. 6. Phylogenetic relationships within Sphaeronematidae and other Criconematina species: Bayesian 50% majority rule consensus tree as inferred from analysis of the ITS rRNA gene sequence alignment under the GTR + I + G model. Posterior probabilities equal or more than 70% are given for appropriate clades. Original sequences are indicated by bold font.

like to point out that the term ‘cyst’ is used only for heteroderids, because they have a life stage which, according to Luc *et al.* (1986), consists of “a persistent tannic sac which retains eggs and is derived from some or all components of female body”. These authors emphasise that

the *Meloidoderita* cystoid body cannot be called a cyst because “it is derived from the persistent wall of the uterus rather than the body wall as in Heteroderidae”. However, in the case of *M. whittoni* comb. n., the resistant non-living sac containing eggs is derived from a component of the

mature female body and thus fits the above definition of a cyst. To our knowledge, this is the first reported instance of the presence of a cyst in a tylenchuloid nematode.

On the basis of our observations, the diagnosis of females of species in the subfamily Meloidoderitinae, which only contains the genus *Meloidoderita*, should be emended after Siddiqi (2000) as follows: Meloidoderitinae. Small-sized (<0.5 mm). Marked sexual dimorphism. Mature female: Body fully swollen, without tail, pear-shaped or oval with neck present or absent, 0.21-0.45 mm long, 0.14-0.37 mm wide in type species. Vulva terminal, on a cone-like elevation of body or continuous with body contour. Longitudinal axis of body from head to vulva, anus shifted to dorsal side 44-180 μm from vulva in type species. Cuticle thick, may have spine-like outgrowths. Stylet ca 15-19 μm long, knobs prominent. Orifice of dorsal gland 3-7 μm from stylet base. Excretory pore opposite median bulb, latter very muscular with large refractive thickenings. Uterus spheroid, with thin to very thick walls, filling one-third to one-half of body cavity in young females, and most of body in old females. In old females, uterus becoming patchily sclerotised with a palmate branched surface pattern and transforming into a cystoid body, 0.18-0.36 \times 0.15-0.35 mm wide in type species. Maternal body wall withering and cystoid body serving as protective case for retained eggs, or maternal body-wall turning into a cyst, like that of *Globodera* spp., retaining a thin walled uterus enclosing the eggs. Several eggs laid in gelatinous material covering females and subsequently the cystoid bodies, or no eggs laid in gelatinous matrix covering and protecting females and in some cases cysts. Eggs 60-92 \times 35-50 μm in size.

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