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Morphological and molecular taxonomy of a new *Daptonema* (Nematoda, Xyalidae) with comments on the systematics of some related taxa

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A new species of *Daptonema* is described based upon morphological characters and 18S rRNA sequence. ***Daptonema matrona sp.* nov.** was collected in Pina Basin (north-eastern Brazil). It differs from all other species of the genus by the presence of reduced cephalic setae and straight spicules. These features require an adaptation of the generic diagnosis. Moreover, the females are characterized by intra-uterine development of the offspring, considered herein as their major autapomorphic feature. Molecular systematic analyses supported *Daptonema* matrona sp. nov. as a distinct genetic and evolutionary lineage. The data also indicate hypotheses of taxonomic synonymies amongst some related taxa from Xyalidae as well as the paraphyly of *Daptonema*.

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ADDITIONAL KEYWORDS: 18S rRNA – *Daptonema* taxonomy – integrative taxonomy – Xyalidae phylogeny.

INTRODUCTION

The phylum Nematoda is one of the most abundant taxa, showing one of the greatest species richness amongst the metazoans (Coomans, 2002; Lambshead, 2004). It has been estimated that there are 0.1 to 100 million species of nematodes (May, 1988; Hammond, 1992; Lambshead, 1993; Coomans, 2000). However, a large part of this diversity remains unknown, with just 26 646 species described in the literature (Hugot, Baujard & Morand, 2001). Until recently, there were no specialists on free-living marine nematodes in Brazil. The first records of marine nematode taxa were made by Cobb (1920). Later, Gerlach (1954, 1956a, b, 1957a, b) and Meyl (1956, 1957) conducted surveys that resulted in the description of 209 species, 106 of which were new to science. At present, 50 families, 285 genera, and 230 species have been recorded from marine and estuary environments along the Brazilian coast (Venekey, 2007).

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Nematode taxonomy often has a controversial history not only as a result of the greater development of systematics, but also because relatively few nematologists produce detailed classifications and provide identification keys (De Ley, Decraemer & Eyualem-Abebe, 2006). Moreover, the Nematoda are considered a taxon difficult to identify largely because of the small body size (< 1 mm) of the majority of the species. In order to accelerate the discovery and classification of nematode diversity, new tools and approaches are needed (Blaxter & Floyd, 2003; De Ley *et al.*, 2005). Although nematodes have been studied for over 350 years, the lack of objective criteria for assessing the homology amongst morphological characters has hindered the phylogenetic reconstruction of the phylum (Meldal *et al.*, 2007). Moreover, its ultrastructure and ontogenesis are not sufficiently understood and the lack of fossil records makes comparisons quite deficient (Meldal *et al.*, 2007). Lorenzen (1981) provided the first phylogenetic system based on cladistic principles. However, morphological characters alone appear insufficient in resolving more complex phylogenetic relationships (Decraemer & Smol, 2006).

Xyalidae is a well-defined family, most of the representatives of which are marine animals. However, the relationships proposed for some genera and subgenera remain open to question (Nicholas & Trueman, 2002). For instance, *Theristus* Bastian, 1865 and *Daptonem*a Cobb, 1920, both with many species described, present a particular difficulty in relation to taxonomy and systematics. For some time, *Daptonema* was a subgenus of *Theristus*, which included *Pseudosteineria*, *Trichotheristus*, *Cylindrotheristus*, *Mesotheristus*, *Pseudotheristus*, and *Spirotheristus*. Currently, the first four are considered junior synonyms of *Daptonema,* considered a distinct genus (Lorenzen, 1977).

Many of the taxonomic problems described above could be solved with the use of molecular methods. DNA barcoding for example, could lead to the identification of unknown species and provide a source for the phylogenetic ordination of the groups for which the taxonomy and relationships are currently controversial (Blaxter & Floyd, 2003; Tautz *et al.*, 2003; Bhadury *et al.*, 2006).

Molecular methods can also be used as tools for studies on population genetics and on issues of biogeography and species complexes (Blaxter, 2001; Monis, Andrews & Saint, 2002; Derycke *et al.*, 2008) as well as being considered a potential method for simplifying and accelerating Nematoda assessment and identification in ecological and biomonitoring studies (Hebert *et al.*, 2003; Rogers & Lambshead, 2004).

Nuclear ribosomal RNAs (rRNAs) have been studied extensively and are genomic regions that are often chosen for studies on evolutionary processes (Bhadury *et al.*, 2006). Sequences of a small subunit of rDNA (18S) have confirmed a number of controversies regarding previous taxonomic hypotheses and have revealed a number of unexpected relationships, altering substantially the dynamics of nematode systematics (De Ley, 2006). 18S is considered a good marker, as part of a highly conserved genomic region, serving as a reference point for studies on evolutionary divergence amongst taxa, especially in species differentiation (Powers, 2004; Bhadury *et al.*, 2006).

Recently, a large number of scientific papers have discussed the nature of the taxonomic problems and potential strategies that can be used to accelerate the pace of the discovery and classification of biodiversity (Blaxter & Floyd, 2003; Mallet & Willmott, 2003; Sites & Marshall, 2003). Many of the debates centre on the merits of morphology and DNA sequences in the differentiation of species and phylogenetic estimates (Hebert *et al.*, 2003; Lipscomb, Platnick & Wheeler, 2003; Scotland *et al.*, 2003). Dayrat (2005) argued that taxonomy needs to be integrative, employing various types of data (morphological, genetic, ecological, biological cycle) in the study of species. In one attempt, Martin *et al.* (2008) used the synergy of distinct datasets (integrative taxonomy) for a better systematic organization of the relationships of a group from Polychaeta, in which the taxonomy and systematics are controversial.

Following the principle of 'integrative taxonomy', the present study is aimed at the taxonomic assessment of a new lineage from the genus *Daptonema* and assesses its phylogenetic relationships with other congeneric species as well as species from related taxa within the family.

MATERIAL AND METHODS

Sampling procedure

Samples were carried out in the Pina Basin, an estuarine area in an urban zone (08 °04′03″S, 34 °52′16″W to 08 °05′06″S, 34 °53′58″W) located on the coast of the state of Pernambuco (Brazil). To obtain the biological material, five random replicates were sampled, using a corer 5.0 cm in length and 2.5 cm in inner diameter. Four of the samples were fixed in 4% formaldehyde and one was fixed in 70% alcohol. Specimens of *Daptonema matrona* sp. nov. were sorted. The individuals fixed in 70% alcohol were conserved in tubes with the same solution and those fixed in 4% formaldehyde were transferred to glycerine (Seinhorst, 1959) and later mounted on permanent glass slides, following the method described by De Grisse (1969).

Morphological observations

Twenty-two individuals (11 males and 11 females) were selected and drawn under an optical microscope with a drawing tube. The body length was measured under a ×10 objective. The pharynx and tail length were measured under a ×40 objective. Additional measures were obtained by ×100 objective. After drawing, body measurements were taken using an analogue curvimeter. Specimens were also recorded by digital camera photography coupled to the optical microscope and scanning electron microscopy. The identification of the genus was carried out by using the key provided by Warwick, Platt & Somerfield (1998). The identification of the new species was carried out by comparing the features observed herein with those provided by Deprez *et al.* (2005).

Abbreviations

a, body length divided by maximum body diameter; b, body length divided by pharynx length; c, body length divided by tail length; c′, tail length divided by anal body diameter; L, body length; mbd, maximum body diameter; ph, pharynx length; ph bd, pharynx base diameter; t, tail length ; abd, anal body diameter; b. cav, buccal cavity length ; hd, head diameter; exc. p, position of secretory-excretory pore from anterior body end; exc. pbd, body diameter at level secretory-excretory pore; n. ring, position of nerve ring from anterior body end; n. ringbd, body diameter in nerve ring region; Amph%, percentage of diameter amphidial fovea in relation to corresponding body diameter; amphd pos, distance of amphidial fovea from anterior end; els, external labial setae length; cs, cephalic setae length; ts, caudal setae length; spic, spicule length (along the spicule)'; gub, gubernaculum length ; V%, position of the vulva as percentage of body length from anterior end; v, position of vulva from anterior body end; vbd, body diameter in vulva region; The name of body regions were based on Coomans (1979).

All measurements are in micrometres.

DNA extraction, PCR, and sequencing

Based on the remarkable morphological distinctiveness of the sample, a single specimen was used to develop a molecular operational taxonomic unit (MOTU) as proposed by Blaxter & Floyd (2003), Blaxter (2004), and Blaxter *et al.* (2005).

DNA extraction and amplification of the 18S region were performed following the protocol described by De Ley *et al.* (2005). The specimen was placed on a slide with 20 μL of Worm Lysis Buffer (WLB) (5 mL of 0.5 M KCl, 5 mL of 0.1 M Tris HCl pH 8.3, 0.5 mL of 0.25 M $MgCl_2$, 0.225 mL of detergent Nonidet P-40 (NP40), and 0.225 mL of Tween 20), where it was cut into pieces with a sterilized scalpel. The fragments were transferred into an Eppendorf tube containing 2 μL of proteinase K. For amplification (PCR), a solution was prepared containing 18.2 μL of double distilled (DD)H_2O, 2.5 μL of 10× reaction buffer with $MgCl_2$, 0.75 μL of 10 mM deoxyribonucleotide triphosphates (dNTPs) mix, 0.4 μL of 25 μM primer (18S forward 5′-CGCAAATTACCCACTCTC-3′), 0.4 μL of 25 μM primer (18S reverse 5′-AGTCAAATTAAG CCGCAG-3′), 0.25 μL of polymerase (Taq) and 2.5 μL of DNA from the sample. The sequencing procedures were performed following the protocol of the BigDye v.3.1 kit, using an automated sequencer (Applied Biosystems – 3130×/Genetic Analyzer – 16 capillaries).

The *Daptonema matrona* sp. nov. sequence was deposited in GenBank (http://www.ncbi.nlm.nih.gov) under access number EF436228. An additional 24 18S sequences in the databank from other representatives of the family Xyalidae were used in the present study as follows: *Daptonema hirsutum* – AY854223, AM23623, DQ394801, DQ394784; *Daptonema normandicum* – AY854224, DQ394759; *Daptonema oxycerca* – AY854225, DQ394760; *Daptonema procerum* – AF047889; *Daptonema setosum* – AM234045, AY854226, DQ394768, DQ394744; *Daptonema sp*. – AM234624, DQ394782; *Metadesmolaimus* sp. – AJ966491; *Theristus acer* – AJ966505, AM234627, DQ394754, DQ394794; *Theristus agilis* – AY284695, AY284694, AY284693; *Theristus sp*. – DQ394773. *Monhystera riemanni* (AM234622), *Sphaerolaimus hirsutus* (AY593938), and *Spirinia parasitifera* (AM236044) were used as outgroups.

Sequence analysis

The 28 sequences were aligned on BioEdit 5.0.9 (Hall, 1999) using the ClustalW multiple alignment. For this, the gap opening and gap extension costs were 10 and 0.1, respectively (Hall, 2001). The characters were drawn as non-ordinated and with equivalent weights. After the alignment, the extremities of the sequences were cut from the initial and final regions so as to avoid the accumulation of gaps between analysable sites. Aligned sequences were exported as a Nexus file and analysed using maximum parsimony (MP – without weighting; Fitch, 1977), neighbor-joining [NJ – general time reversible (GTR); Saitou & Nei, 1987], and Bayesian inference [BI [nested sets theory (nst) = 6; GTR + Γ]; Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003] in order to gather the most robust evidence possible for MOTU analysis.

For the trees resulting from NJ and from unweighted MP methods, bootstrap and jack-knife branch supports were calculated from 10 000 pseudoreplicates following the rule of branch consistency

equal to or greater than 50% by means of the fast stepwise addition search option. The addition of the operational taxonomic units (OTUs) was random and permutations were carried out using the tree-bisection-reconnection algorithm. All the above-mentioned procedures were performed using the PAUP* v.4.0b10 software program (Swofford, 2000). Bayesian inference analysis was conducted using the MrBayes v. 3.1.1 software program (Huelsenbeck *et al.*, 2001; Ronquist & Huelsenbeck, 2003) in 1 000 000 generations of four Markov chains. The methodological option for using 1 000 000 generations of four Markov chains is based on the fact that at this amount of generations the analysis reached a standard deviation below 0.01, as advised by Huelsenbeck *et al.* (2001) and Ronquist & Huelsenbeck (2003). Subsequent posterior probabilities were obtained from the consensus of the entire set of generations.

RESULTS

MORPHOLOGICAL ANALYSIS

GENUS *DAPTONEMA* COBB (1920)

Diagnosis: The diagnosis of the genus follows Coomans & Eyualem-Abebe (2006).

Cuticle annulated, with lateral field. Somatic setae present. Lip region with ten or 12 seta (these setae may be segmented when long). Cheilostome wide, dome-shaped; stoma funnel-shaped. Ventral gland and secretory–excretory pore absent or obscure. Usually two testes with anterior one on left and posterior one on right side of intestine. Spicules usually about one anal diameter long and strongly bent. Gubernaculum often with lateral guiding pieces and rarely also with dorsocaudal apophysis. Ovary always left of intestine. A post- and prevulval uterine sac may be present. Tail cylindrical in its posterior part, with two (rarely three or four) terminal setae. Most species marine.

List of valid species: The list of 113 valid species of *Daptonema*, according to Lorenzen (1977) and Deprez *et al.* (2005), is presented in alphabetical order (Supporting Information Appendix S1).

***DAPTONEMA MATRONA* SP. NOV.**

Description

Material studied: 11 males; 11 females.

Material type: Material type: Holotype and allotype deposited in the National Museum of Rio de Janeiro. Paratypes slide deposited in the Laboratório de Meiofauna Departamento de Zoologia, Universidade Federal de Pernambuco, Recife, Brazil.

Table 1. Body measurements of holotype and ten male paratypes [means ± standard deviations and (variations) in μm] of ***Daptonema matrona* sp. nov**. See Material and methods for abbreviations

Measurements	Holotype	Paratypes (10 ♂)
L	1341	1209.9 ± 91.3 (1041–13560)
ph	198	195.1 ± 13.2 (171–213)
mbd	60	64.4 ± 5.2 (56.4–72.6)
t	210	192 ± 14.2 (157.5–207)
a	22.35	18.8 ± 1.7 (15.9–22.1)
b	6.8	6.2 ± 0.2 (6–6.6)
c	6.4	6.5 ± 0.2 (6–6.8)
c′	4.8	4.4 ± 0.3 (3.9–4.8)
els	3	3.1 ± 0.2 (3–3.6)
cs	1.8	1.8 ± 0.0
ph bd	50.4	58.9 ± 4.1 (53.4–64.8)
b. cav	10.8	11.8 ± 1.1 (10.2–13.8)
hd	24	24.7 ± 2.1 (21.6–27)
exc. p	42	36.6 ± 13.3 (22.6–59.4)
exc. pbd	39	39.5 ± 3.4 (34.8–43.8)
n. ring	81.6	92.9 ± 8.8 (72–102.6)
n. ringbd	45.6	49.4 ± 3.7 (43.2–56.4)
Amph%	21.8	20 ± 2.6 (17.8–26)
amph pos	13.2	12.8 ± 1.5 (9.6–15.6)
abd	40.2	44.1 ± 2.4 (40.5–48)
spic	27.6 (0.7 abd)	30.3 ± 2.3 (28.2–34.8)
gub	7.2	6.6 ± 0.9 (5.4–7.8)
cylind %	30.7	29.4 ± 6.6 (23.1–37.1)
ts	9	8.6 ± 1.3 (6.6–10.8)

Type specimens: Male holotype MNRJ 337; Female allotype: MNRJ 338; male paratypes 123–132 NM LMZOO-UFPE; female paratypes 133–142 NM LMZOO-UFPE.

Locality: Pina Basin (Recife, Pernambuco, Brazil): estuarine intertidal silt-clay sediments.

Measurements: See Tables 1 and 2.

Etymology: The species' Latin name (matrona = mother family) is based on the method of reproduction by the female i.e. intra-uterine hatching and development.

Holotype: Elongated body, hardly narrowed anteriorly (Figs 1A, 2A); cephalic and pharynx base diameter corresponding to 40 and 84% of the maximum body diameter, respectively. Cuticle transversely striated, striations relatively fine (1.8 μm) (Figs 1B, 3B). Somatic setae short and distributed along the body but more concentrated/numerous in the neck and caudal regions (Fig. 1A). Head rounded with six dis-

Table 2. Body measurements of allotype and ten female paratypes [means ± standard deviations and (variations) in μm] of ***Daptonema matrona* sp. nov**. See Material and methods for abbreviations

Measurements	Allotype	Paratypes (10 ♀)
L	1440	1290.6 ± 109.7 (1122–1524)
ph	213	201.4 ± 12.5 (178.5–222)
mbd	81	88.4 ± 11 (73.2–106.2)
t	246	218.8 ± 16.9 (199.5–253.5)
a	17.8	14.7 ± 1.5 (12–16.7)
b	6.8	6.4 ± 0.3 (6–6.9)
c	5.8	5.89 ± 0.3 (5.5–6.3)
c′	4.7	3.9 ± 0.3 (3.6–4.4)
V%	73.8	74.2 ± 1 (72.5–75.6)
els	3	3.4 ± 0.3 (3.0–3.6)
cs	1.8	2 ± 0.3 (1.8–2.4)
ph bd	65.4	75.4 ± 9.7 (58.5–88.8)
b. cav	13.8	12.5 ± 1.6 (9–14.4)
hd	28.8	31.8 ± 2 (30–36)
exc. p	40.2	32.7 ± 7 (19.8–40.2)
exc. pbd	46.8	47.2 ± 4.1 (41.4–52.8)
n. ring	100.8	98.5 ± 15.6 (74.4–130.2)
n. ringbd	55.2	63.4 ± 7.5 (51–74.4)
Amph%	14.5	11.2 ± 1.5 (9.1–13.8)
amph pos	13.2	14.3 ± 2.3 (11.4–18)
abd	52.8	55.7 ± 5.6 (48–65.4)
v	1063.5	957.4 ± 82 (840–1128)
vbd	72.6	75.2 ± 9.9 (60–89.4)
cylind %	36.2	37 ± 3.7 (33.3–45.4)
ts	8.1	8.2 ± 1.5 (6.6–10.8)

tinct lips, each one with a labial papilla (Fig. 3A). Twelve cephalic setae in six pairs, the longer ones measuring 3 μm (12% of the cephalic diameter) and the shorter ones 1.8 μm (7% of the cephalic diameter) (Fig. 1B; Table 1).One circle of subcephalic setae (Fig. 1B). Buccal cavity conical, with an annular reinforcement delimiting the stoma from the anterior part of the cavity (Fig. 1B, C). Amphidial fovea circular, 21.8% of corresponding body diameter and located 13.2 μm behind anterior end (Figs 1B, 2C, 3C). Secretory–excretory pore at 42 μm and the nerve ring at 81.6 μm from the anterior end (Table 1). Pharynx cylindrical, surrounding stoma (Figs 1C, 2B, D). Cardia with irregular form and partially inserted in the intestine (Fig. 1G). Ventral gland not visualized. Single testis located on the left of the intestine, reaching almost to the base of pharynx (Fig. 1A). Five ejaculatory glands extending 354.5 μm anteriorly to the anal opening (Fig. 1D). Spicules cephalate proximally, 0.7× anal body diameter long and almost straight (Figs 1F, 2E). Gubernaculum without apophysis (Fig. 1F) and with the distal region wing-shaped (Fig. 3D). Four pairs of setae posteriorly to the anal opening, next to the cylindrical region of the tail, on the ventral side (Fig. 1E). Tail conical–cylindrical 4.8× anal body diameter long, the cylindrical region corresponding to 30.7% of the total tail length, with two terminal setae (9 μm) (Fig. 1E; Table 1). Three caudal glands present (Figs 1E, 2F).

Allotype: Female largely similar to male (Fig. 4) but showing sexual dimorphism in size of amphidial fovea, i.e. smaller than in male. Amphidial fovea round, its diameter 14.5% of corresponding body width and located 13.2 μm or 0.46 cephalic diameters from anterior end (Fig. 4B; Table 2). Secretory–excretory pore at 40.2 μm and the nerve ring 100.8 μm behind anterior end (Table 2). Single ovary located left of intestine and extending almost to base of pharynx. A short prevulval uterine sac (spermatheca) in the second third of the body length. Vulva close to anus, i.e. at 73.8% of total body length from anterior end (Fig. 4A). Reproduction apparently by ovoviviparity. Up to 45 eggs as well as first and second stage juveniles observed within the uterus of a single specimen.

Genetic and phylogenetic analyses

Neighbour-joining topology revealed the existence of two large genetic groupings (Fig. 5). The first characterized by the lineage of the genus *Theristus* and *Daptonema normandicum*, the latter of which is genetically closer to *Theristus* agilis. Such a grouping emerged as a genetic sibling lineage to the other grouping, which united *Theristus acer* and *Theristus* sp. The second large genetic grouping revealed the genetic unity of the remaining species of *Daptonema* and a *Metadesmolaimus* sp. *Daptonema matrona* sp. nov. emerged as a genetic sibling lineage of *Daptonema oxycerca* and *Daptonema procerum*. Such a grouping emerged as the genetic sibling lineage to the other remaining group composed of *Daptonema hirsutum*, *Daptonema setosum* + *Daptonema* sp., besides *Metadesmolaimus* sp. (Fig. 5). *Daptonema hirsutum* and *D. setosum* presented identical sequences. *Daptonema oxycerca* + *D. procerum* are genetically close lineages.

Maximum parsimony topology was represented by the consensus of 28 153 equally parsimonious trees. A total of 1416 characters was analysed, 945 of which were constant, 270 were parsimoniously informative, and 201 were parsimoniously non-informative. The length of the consensus tree was 887 steps; retention and consistency indexes were 0.7934 and 0.7193, respectively. The analysis revealed the existence of two monophyletic clades (Fig. 6). The first clade was formed by *Daptonema normandicum* and the *Theristus sp*ecies, in which *Theristus agilis* and *D. normandicum* emerged as an evolutionary sibling lineage

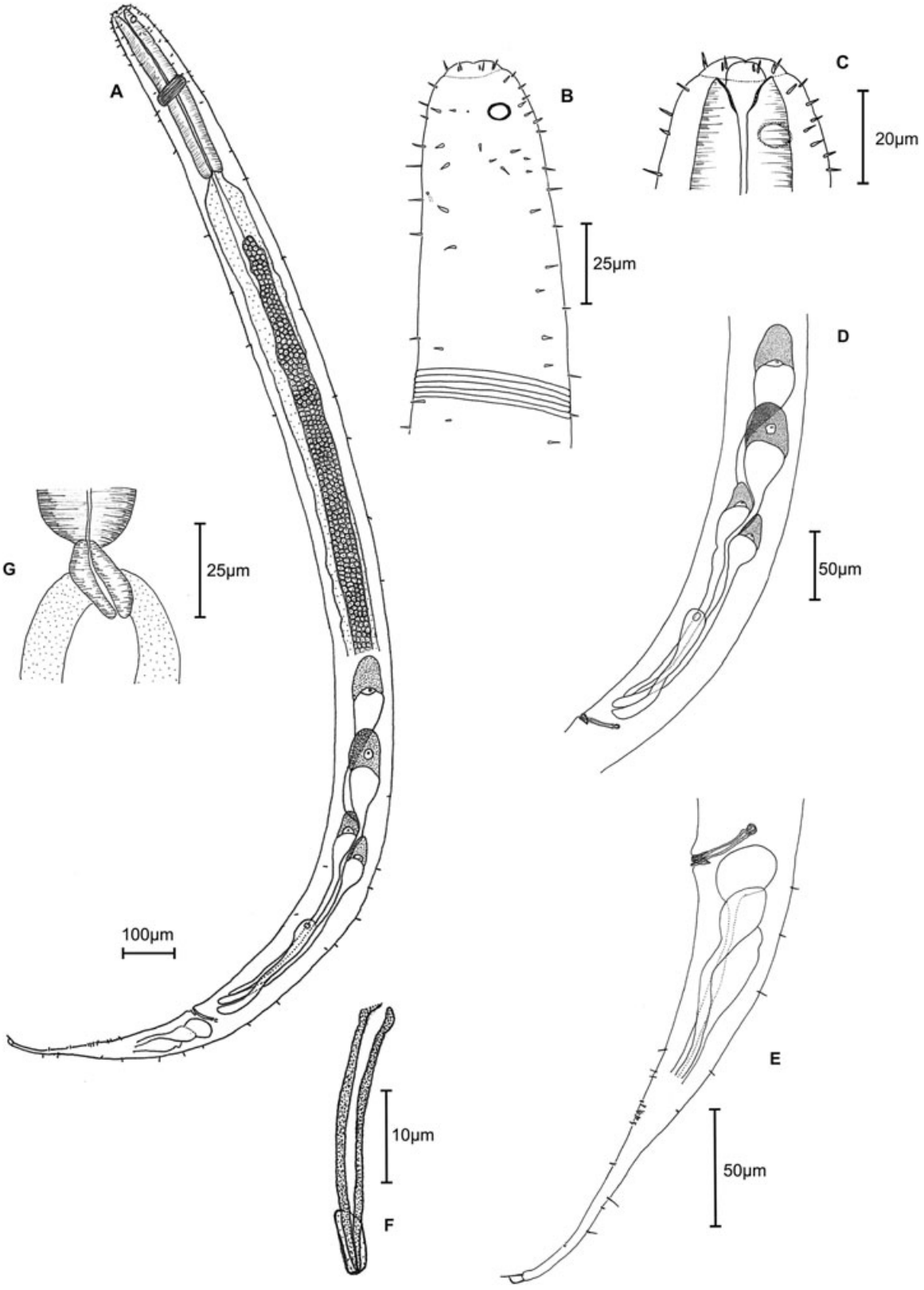

Figure 1. Drawing of ***Daptonema matrona* sp. nov.** holotype: A, habitus; B, cephalic region; C, buccal cavity; D, ejaculatory glands; E, tail; F, copulatory apparatus (paratype); and G, cardia.

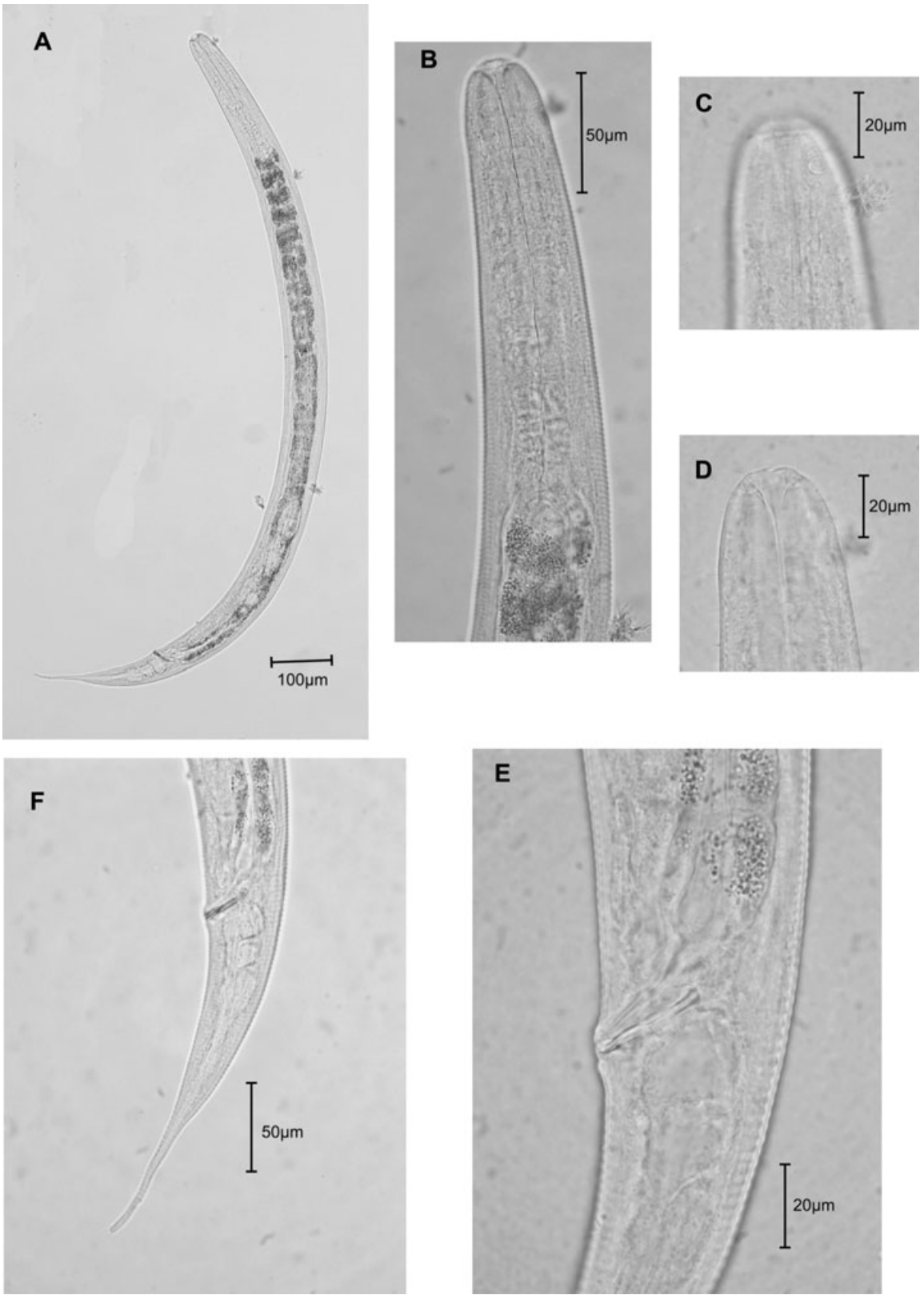

Figure 2. Photographs of ***Daptonema matrona* sp. nov.** holotype: A, habitus; B, anterior region; C, amphid; D, buccal cavity; E, spicule; F, tail.

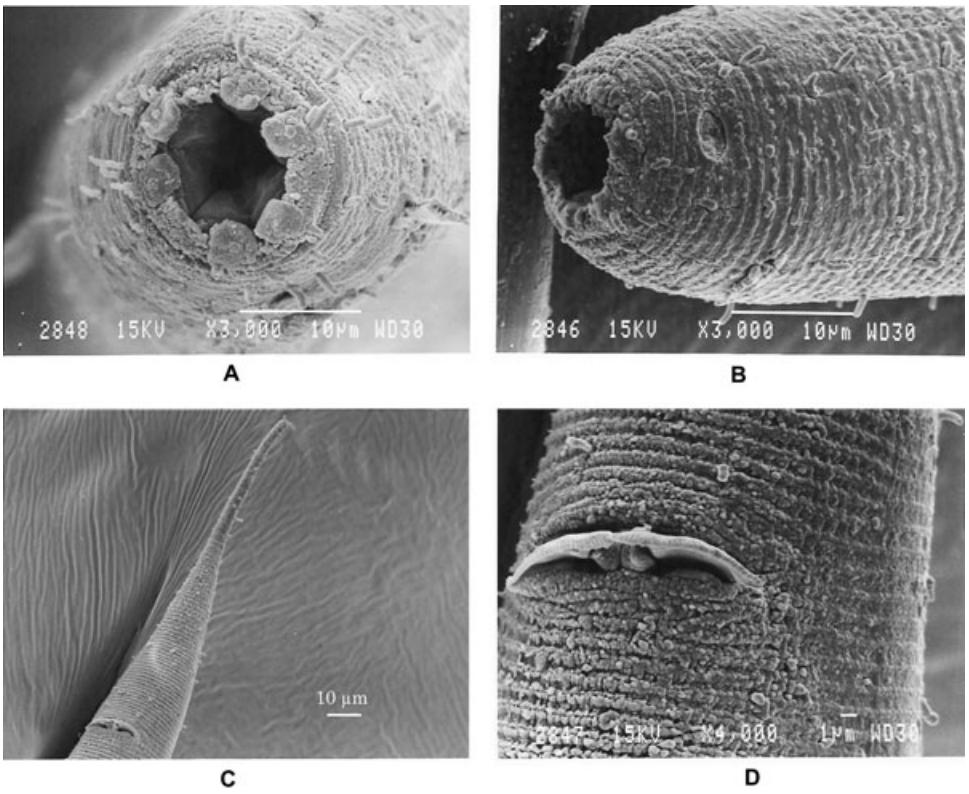

Figure 3. Scanning electron micrographs of ***Daptonema matrona* sp. nov.** male: A, anterior region; B, amphid; C, tail; D, external structure of the gubernuculum.

to the other grouping, which united *T. acer* and *Theristus* sp. The second clade was formed by the other *Daptonema* species, including the new species and *Metadesmolaimus* sp. *Daptonema matrona sp*. nov. was characterized as an evolutionarily distinct branch and a sibling group of the other congeneric species, with the exception of *D. normandicum*. *Daptonema procerum* and *D. oxycerca* were revealed to be sister groups. This evolutionary unit also emerged as a sister group of *D. setosum, Daptonema* sp., *D. hirsutum* + *Metadesmolaimus* sp. (Fig. 6). The analysis also revealed a putative synonymy involving *D. hirsutum* and *D. setosum* as well as a well-supported monophyletic unit (98/97) encompassing diverse species of *Daptonema* and *Metadesmolaimus sp*. (Fig. 6).

Bayesian inference topology resulted from the majority rule consensus of 10 001 trees with a standard deviation of 0.005222, resulting from 1 000 000 generations computed in four Markov chains. The analysis also revealed the existence of two monophyletic groupings (Fig. 7). The first was formed by *D. normandicum* and the *Theristus sp*ecies (like that revealed in the MP topology), in which *T. agilis* and *D. normandicum* emerged as sister taxa to the other grouping, which united *T. acer* and *Theristus* sp. In the second grouping (Fig. 7) formed by the remaining species of *Daptonema* + *Metadesmolaimus* sp., *D. matrona sp*. nov. exhibited the same phylogenetic status as seen in the MP topology, constituting a sibling group of *D. oxycerca, D. setosum, D. procerum, D. hirsutum* + *Metadesmolaimus* sp. *Daptonema procerum* and *D. oxycerca* were also revealed to be a sibling group of *D. setosum, Daptonema sp*., *D. hirsutum* + *Metadesmolaimus* sp. (Fig. 7). The analysis also revealed two polytomies – the first involving *D. hirsutum* and *D. setosum* (as found in the previous analyses) and the second between *D. procerum* and *D. oxycerca* (Fig. 7).

DISCUSSION

DIFFERENTIAL DIAGNOSIS OF *DAPTONEMA MATRONA* SP. NOV.

Despite the large number of described *Daptonema* species (113 spp.), there are few morphological characteristics used as distinctive parameters: setae length, amphidial fovea size and position; copulatory apparatus size and structure; tail shape and length (Warwick *et al.*, 1998). The morphological characteristics were obtained from descriptions contained in Deprez *et al.* (2005).

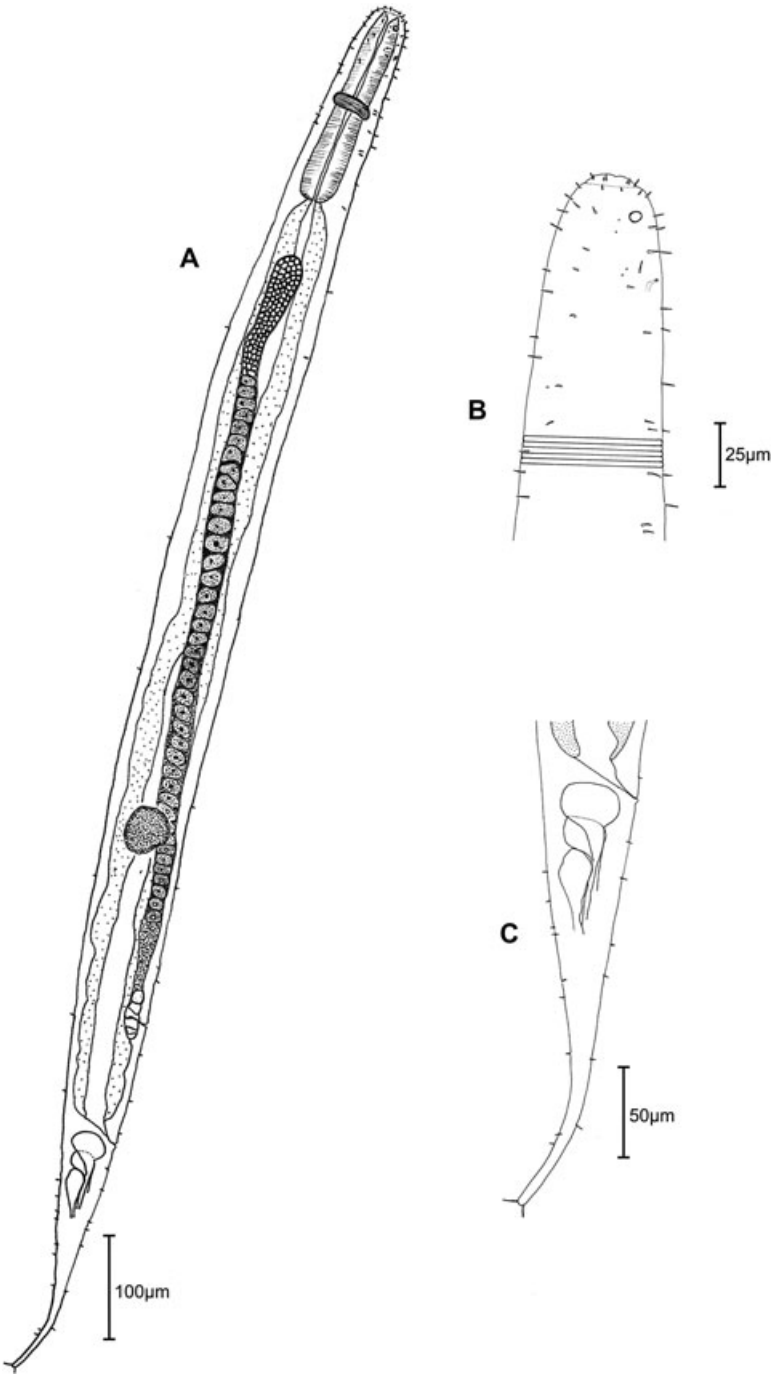

Figure 4. Drawing of ***Daptonema matrona* sp. nov.** allotype: A, habitus; B, cephalic region; C, tail.

Daptonema matrona sp. nov. is distinguished from other species of the genus in that it has a pronounced reduction of the cephalic setae in relation to the cephalic diameter and the straight shape of the spicule vs. curved in the other species, often L-shaped. This characteristic requires an adaptation of the generic diagnosis as follows: spicules usually curved, often L-shaped.

Daptonema planiere Vitiello, 1971 also has small-sized cephalic setae (2.9 µm), but there is a total of ten of these and they are of equal sizes. Furthermore, the proportion of those setae in relation to the cephalic diameter is five to six times greater than the same correlation on the new species. The same arrangement of somatic setae observed on the new species is found on *Daptonema psammoides* Warwick, 1970.

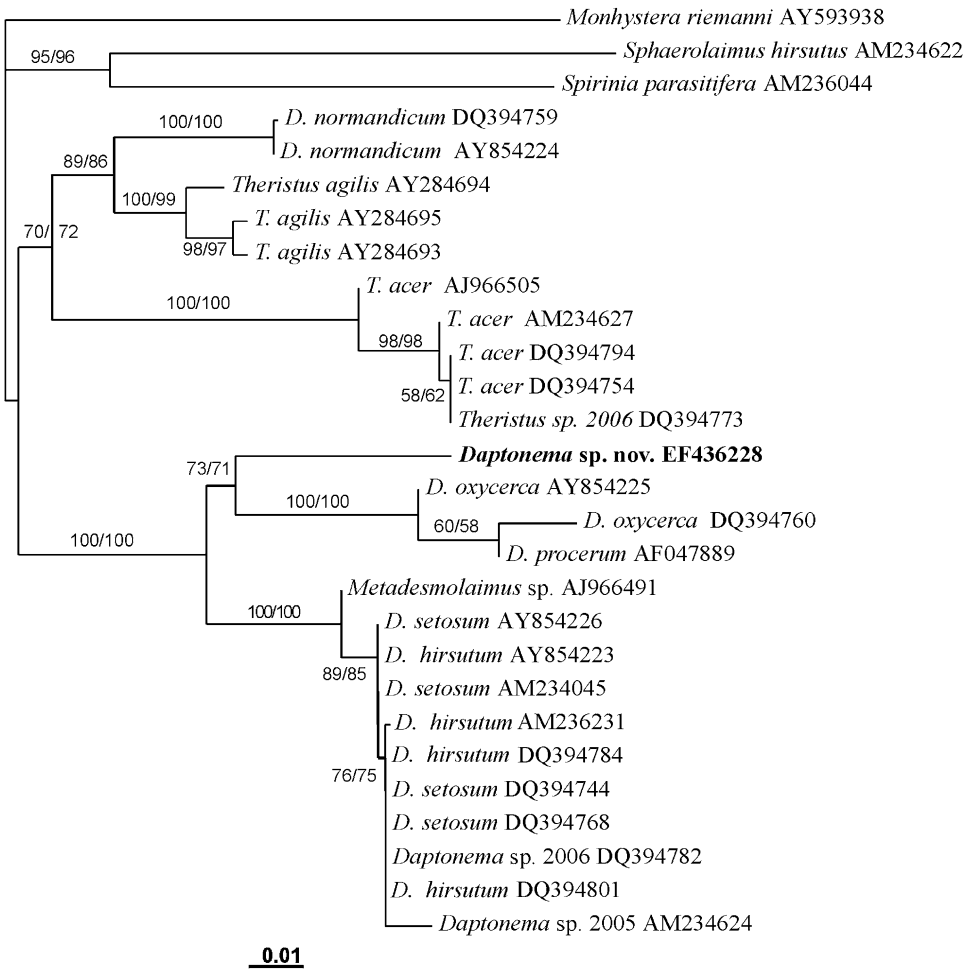

Figure 5. Neighbour-joining topology based on 18S sequences from 25 specimens of Xyalidae and three outgroups (*Monhystera riemanni*, *Sphaerolaimus hirsute*, and *Spirinia parasitifera*). Numbers are bootstrap and jack-knife values, respectively, both with branch support over 50%. Scale bar = 0.01 substitutions per site.

However, the setae are strikingly smaller on the new species. Such a feature can be considered as the first of a total of three major autapomorphies observed in the species.

Daptonema matrona sp. nov. has amphidial fovea situated less than one cephalic diameter from the anterior end, which is a characteristic also seen in *D. oxycerca* De Man, 1888 and *D. procerum* Gerlach, 1951. The new species has the vulva located close to the anal region, which is a feature also seen on *Daptonema calceolatus* De Coninck & Stekhoven, 1933, *Daptonema laxum* Wieser, 1956, *D. hirsutum* Vitiello, 1967, *Daptonema marylinicus* Timm, 1952, *D. oxycerca*, *D. setosum* Butschli, 1874, and *Daptonema trabeculosum* Schneider, 1906. The presence of spermatheca was also seen on *Daptonema arcticus* Steiner, 1916, *Daptonema conicum* Filipjev, 1922, *Daptonema nanum* Lorenzen, 1972, *Daptonema pratti* Murphy & Canaris, 1964, *D. procerum* Gerlach, 1951, *Daptonema proprium* Lorenzen, 1972, *Daptonema sentiens* Cobb, 1914, *D. setosum*, *D. trabeculosum*, and *Daptonema williamsi* Vincx & Coomans, 1983. Despite this shared features regarding the reproductive organ, *D. matrona sp*. nov. is differentiated from all other congeneric species by the potential second major autapomorphy of intra-uterine incubation of its offspring.

Regarding the male reproductive structures, *D. pratti, D. williamsi*, and the new species studied herein are the only species that have ejaculatory glands. Both *D. pratti* and *D. williamsi* have two testes. However, *D. matrona* sp. nov. differs from these other two species by having a single testis as well as in the spicule and gubernaculum shapes. Furthermore, *Daptonema dentatum* Wieser, 1956, *D. planiere*, *Daptonema exutum* Wieser, 1956, and *Daptonema simplex* Allgén, 1959 have the same proportion of the spicule in relation to the anal diameter as *D. matrona sp*. nov. However, these species are dis-

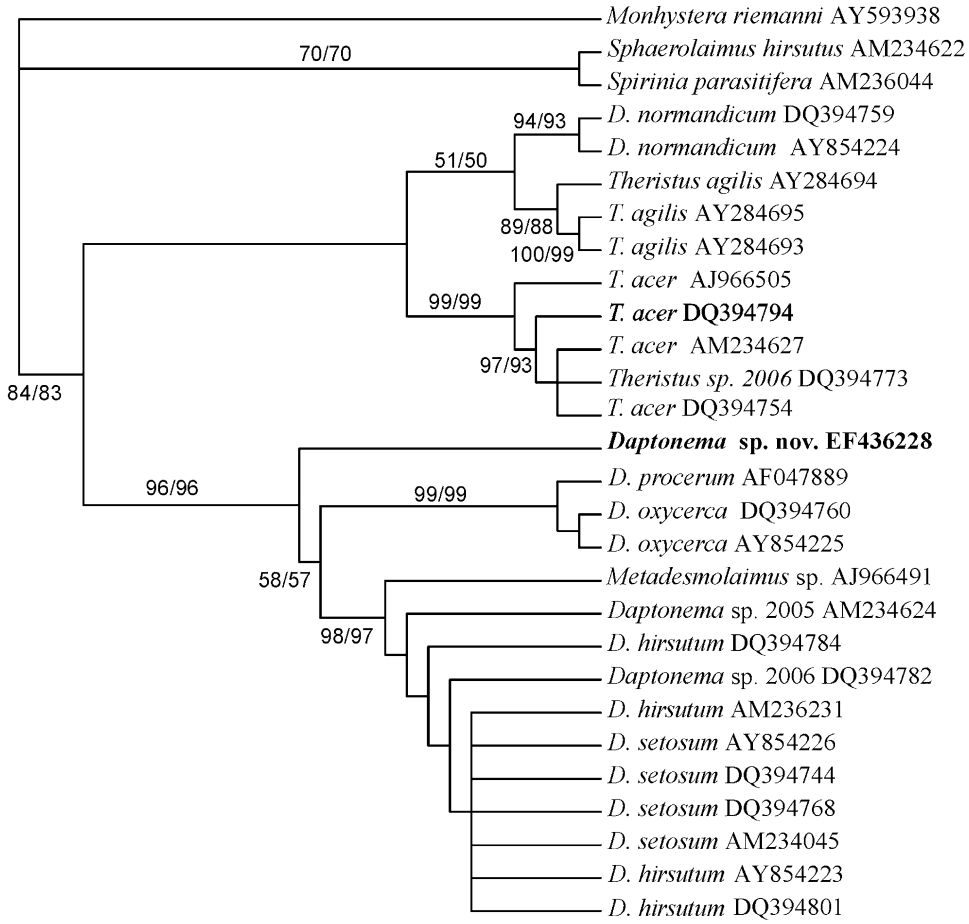

Figure 6. Maximum parsimony (*stricto consensus*) topology based on 18S sequences from 25 specimens of Xyalidae and three outgroups (*Monhystera riemanni*, *Sphaerolaimus hirsute*, and *Spirinia parasitifera*). Numbers are bootstrap and jack-knife values (10 000 replicates), respectively, both with branch support over 50%.

tinguished from the new species by having a curved spicule, by the absence or the shape of the gubernaculum when it is present, as well as other features. The straight shape of the spicule in the new species was the third major autapomorphy observed. Thus, these morphological observations treated together pointed out *D. matrona* as a new taxon for Nematoda.

Genetic and phylogenetic analyses

A large number of studies have proven the usefulness of the 18S rDNA region for phylogenetic studies on the phylum Nematoda (Blaxter *et al.*, 1998; De Ley & Blaxter, 2002, 2004; De Ley *et al.*, 2005; De Ley, 2006; Meldal *et al.*, 2007), especially in species differentiation (Powers, 2004; Bhadury *et al.*, 2006). The results observed herein regarding 18S resolution in phylogenetic approaches corroborate the above-mentioned findings, given the phylogenetic resolution observed amongst the genera and species from Xyalidae (Figs 5–7).

The results related to the phylogenetic treatment of the 18S rDNA sequences reinforced the efficiency of 18S in the identification of unknown species, as mentioned by Blaxter & Floyd (2003) and Tautz *et al.* (2003) and further corroborated by Bhadury *et al.* (2006). Although the MP topology was not conclusive regarding the status of *Daptonema* sp. 2005 and *Daptonema* sp. 2006, NJ and BI analyses suggest that these lineages might be synonymous with *D. setosum* + *D. hirsutum* (Figs 5, 7). Similar results can be observed regarding the genus *Theristus*, as there is solid evidence to indicate that *Theristus* sp. and *T. acer* are also synonymous (Figs 5–7).

The genetic and phylogenetic analyses carried out both exhibited the same topology. Thus, the topologies resulting from the NJ, MP, and BI analyses all support *D. matrona* sp. nov. as a genetically and evolutionarily distinct lineage and a sibling taxon of the remaining *Daptonema* species, with the exception of *D. normandicum*. Yet, satisfactory statistical branch supports were also observed regarding genetic

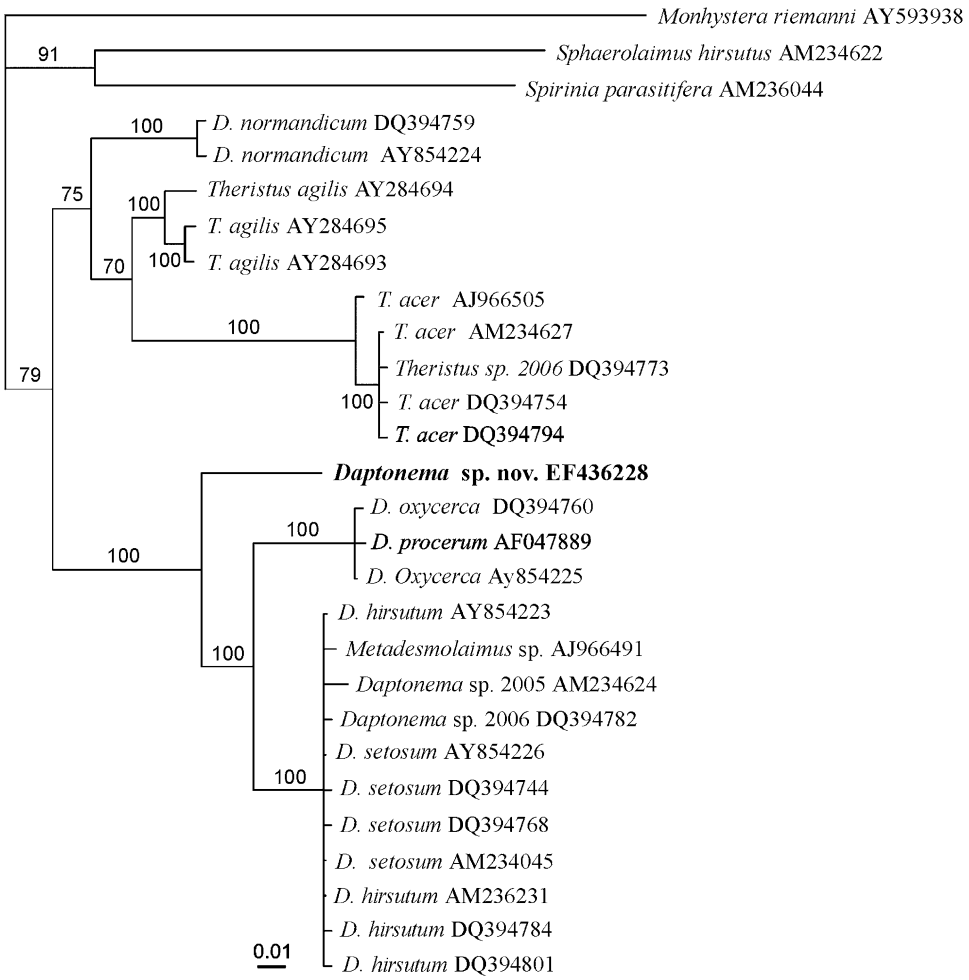

Figure 7. Bayesian inference topology based on 18S sequences from 25 specimens of Xyalidae and three outgroups (*Monhystera riemanni*, *Sphaerolaimus hirsute*, and *Spirinia parasitifera*). The topology results from 10 001 trees (1 000 000 generations/standard deviation of 0.005222).

and evolutionary distinctiveness of *D. matrona* sp. nov. (100, ~ 60, and 100).

Moreover, in the whole analyses, *Metadesmolaimus* sp. appeared to be included within what we believe to be the monophyletic unit of *Daptonema* (Figs 5–7). Such evidence suggests a misidentification of *Metadesmolaimus* sp. This was also observed by Meldal *et al.* (2007), who demonstrated the difficulty in identifying members of Xyalidae using solely morphological features.

The genetic (NJ) and phylogenetic (MP and BI) analyses detected a possible synonymy involving *D. hirsutum* and *D. setosum*. A second BI analysis was carried out with a sump and sumt burnin period equal to 350 (data not shown) and the resulting topology was strictly the same. The most interesting result observed was the newest posterior probabilities obtained in the branch comprising *D. hirsutum* and *D. setosum*. Such results reinforced the hypothesis of synonymy above, given that they were remarkably low, ranging from 2 to 12. This additional evidence also reinforced the previous procedures applied for BI. Vitiello (1967) reported the similarity between the two species and stated that they are distinguished from one another by just morphometry (length of the cephalic setae, tail, and spicule). However, Sharma (1985) stated that such features are insufficient to separate them into two species and that they should be treated as intraspecific variations. Therefore, the analyses carried out herein suggest *D. setosum* to be a junior synonym of *D. hirsutum*.

According to the NJ and MP analyses (Figs 5, 6), *D. oxycerca* and *D. procerum* are closely related lineages from genetic and evolutionary standpoints. The BI analysis, however, indicates that the two species might be synonymous. The morphological characters of *D. oxycerca* and *D. procerum* corroborate the first hypothesis, as there is considerable similarity

between the species in the proportion of the cephalic setae in relation to the cephalic diameter, in the position of the amphidial fovea in relation to the anterior extremity, in the length of the tail, spicule length, and in the position of the vulva. However, the differences are not only from morphometrics but are also of a structural nature (shape of the gubernaculum; Warwick *et al.*, 1998). This discontinuity between genetic and phylogenetic data and the differences in structural anatomy for a reproductive organ (gubernaculum) might reflect a recent evolutionary phenomenon not widely detected through the molecular evidence of a conserved region, but strikingly evident in a copulatory structure. Based on that, we suggest the use a fast evolving genomic region (i.e. cytochrome *b* or COI) in order to clarify the taxonomic controversy observed between *D. oxycerca* and *D. procerum*.

The data from the phylogenetic analyses (MP and BI; Figs 6, 7) indicate that *Daptonema* may not be a strictly natural group because of the putative paraphyly detected and further supported by the closer phylogenetic relationship of *Daptonema normandicum* with the *Theristus* clade. The neighbor-joining topology appears to support this paraphyly, as the same relationships (in this case genetic) were observed. However, based on morphological characters, *Daptonema* appeared to be a polyphyletic group given that the genus appeared to be closely related to nine other genera as follows: *Stylotheristus*, *Theristus*, *Filipjeva*, *Paramonystera*, *Zygonemella*, *Promonystera*, *Linhystera*, *Amphimonystera*, and *Ammotheristus* (Nicholas & Trueman, 2002). In more specific terms, *D. normandicum* may be a species of *Theristus,* given the greater genetic and phylogenetic similarity. As per previous papers on the taxonomy of Xyalidae (Wieser, 1956; Lorenzen, 1977; Warwick *et al.*, 1998; Nicholas & Trueman, 2002; Meldal *et al.*, 2007), our results have reinforced the close relationship of these genera, but equally demonstrated the need for a profound taxonomic–systematic revision of both taxa by total evidence means (molecular and morphological).

It is important to point out that the employment of molecular methods does not impoverish the field of systematics, as has been affirmed, but should be treated as part of the data for analyses of the relationships amongst taxa (Blaxter & Floyd, 2003). The difficulties encountered in the nematode taxonomy reinforce the need for synergy between traditional morphological diagnoses observed through microscopy and molecular analyses (Bhadury *et al.*, 2006). Such conduct aims to minimize the overabundance of synonyms and dubious names resulting from past taxonomic practices (Dayrat, 2005) and gives new impetus to the discovery of biodiversity (Tautz *et al.*, 2003).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Valid species of *Daptonema* according to the Lorenzen (1977) and Deprez *et al.* (2005).

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