

Ditylenchus gallaeformans sp. n. (Tylenchida: Anguinidae) – a neotropical nematode with biocontrol potential against weedy Melastomataceae

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Summary – *Ditylenchus gallaeformans* sp. n. was found on several hosts at numerous locations in Brazil and Costa Rica. In its native habitats it attacks several genera in the Melastomataceae, including two species ranked as among the worst invasive weeds of Pacific island forests, namely *Miconia calvenscens* and *Clidemia hirta*. The new species causes a severe disease on infected plants involving the formation of gall-like structures on infected leaves, inflorescences and stems, and may cause significant impact on its hosts. Morphological study using light and scanning electron microscopy and analysis of the partial 18S rRNA, the D2-D3 expansion fragments of 28S rRNA and the ITS rRNA gene sequences showed little variations between populations from different hosts or geographical origins. The molecular study revealed that the new species is related to *D. drepanocercus*, which was recently found in association with *M. calvenscens* but causing angular leaf spots on this host. *Ditylenchus gallaeformans* sp. n. is distinguished from *D. drepanocercus* by having a bursa reaching the tail tip (*vs* covering around 50% of tail in *D. drepanocercus*) and a conical tail, regularly tapering towards a variable tip (*vs* tail with a distinctive apical falciform appendage in both sexes in *D. drepanocercus*). PCR with species-specific primers was developed for diagnostics of both *Ditylenchus* species. *Ditylenchus gallaeformans* sp. n. deserves further investigation as a potential biocontrol agent against *M. calvenscens* and *C. hirta*.

Keywords – biological control, Brazil, *Clidemia hirta*, Costa Rica, description, *Ditylenchus drepanocercus*, *Miconia calvenscens*, molecular, morphology, morphometrics, phylogeny, taxonomy.

Three neotropical plant species in the Melastomataceae are presently of special relevance as invaders in the Pacific, namely: *Clidemia hirta* (L.) D. Don, *Miconia calvenscens* DC. and *Tibouchina herbacea* (DC.) Cogn. (Cronk & Fuller, 1995; DeWalt *et al.*, 2004). *Miconia calvenscens* is the worst weed among these ecosystem invaders. Pacific island native forest ecosystems, particu-

larly those of French Polynesia, the Hawaiian Islands and Australia, have been experiencing invasions by this previously obscure shrub or small tree species since its introduction in the mid-20th century (Medeiros *et al.*, 1997; Meyer, 1998). Mechanical removal and chemical control have been successful in some parts of Hawaii in preventing its expansion into new areas, but a more complete and

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permanent control is likely to be reached only through the classical introduction of biocontrol agents collected in the centre of origin (Medeiros *et al.*, 1997).

In 1995 a cooperative work involving Brazilian and American organisations was started, aimed at collecting and investigating potential biocontrol agents of *M. calvescens* and other invasive Melastomataceae from Brazil and the neotropical region. The potential biocontrol agent list included: a phytoplasma (Seixas *et al.*, 2002) causing witches' broom; a plant-parasitic nematode, *Ditylenchus drepanocercus* Goodey, 1953, causing angular leaf spots (Seixas *et al.*, 2004a); an oomycete, *Pythium* sp., causing root rot; as well as numerous fungi (Seixas *et al.*, 2007; Alves *et al.*, 2010) and some insect species collected in Brazil (Picanço *et al.*, 2005; Burckhardt *et al.*, 2006) and Costa Rica (Buckhardt *et al.*, 2005). Until recently, only one natural enemy, the fungus *Colletotrichum gloeosporioides* f. sp. *miconiae*, has been released for classical biological control of *M. calvescens*, being introduced into the Hawaiian Islands in 1997 (Killgore *et al.*, 1999) and French Polynesia in 2001 (Meyer *et al.*, 2008).

In June 2004, while examining plant abnormalities and galls supposedly caused by eriophyid mites in *Miconia* spp. in Costa Rica and Mexico, an entomologist, Alec McClay (McClay Ecoscience), discovered several nematode specimens associated with dissected galls. Further detailed examination of plant samples revealed that nematode galls were also rather common in Brazil on various species of *Miconia* and other Melastomataceae. The nematode specimens were identified as representatives of the genus *Ditylenchus* Filipjev, 1936, but they seemed to be different from *D. drepanocercus*, which was previously recorded as attacking *M. calvescens* in the neotropics (Seixas *et al.*, 2004a, b) and causing a different kind of symptom (angular leaf spots). This publication includes a description of a new species of *Ditylenchus* found in association with *M. calvescens*. Only systematic aspects are presented in this publication: other aspects, including life-cycle, gall development, impact on host plant, host range and epidemiology are in preparation and will be published separately.

Materials and methods

NEMATODE POPULATIONS AND SAMPLING

Leaves and inflorescences infected with nematodes were collected from plants at an easily accessible location in the vicinity of the campus of the Federal University

of Viçosa (Chácara Cristais, municipality of Viçosa, state of Minas Gerais, Brazil). Specimens belonging to other populations were also collected from members of the Melastomataceae in several municipalities of Minas Gerais State (MG) and from one site in Costa Rica. Some samples were submitted to morphometric and molecular analyses (Table 1). Although galls and foliage distortions caused by *Ditylenchus* were observed on *M. calvescens* in Brazil, another species of *Miconia*, *M. ibaguensis* (Bonpl.) Triana, was found to be infected by even larger populations of this nematode. This species became a convenient source of nematodes throughout the year and for collecting the material used in the description of the new species and in inoculation experiments. Additionally, *D. drepanocercus* was collected again from *M. calvescens* in Brazil and compared with the new species.

NEMATODE EXTRACTION, FIXATION AND MOUNTING

Galled tissues were separated from leaves, cut into small pieces and placed in a 250 ml flask filled with tap water. The material was kept for 24-48 h under continuous aeration provided by an aquarium pump. The resulting suspension was poured through an 840 μm pore sieve to remove leaf debris and through a 37 μm pore sieve to extract the nematodes from the suspension. Nematodes were killed in a water bath (55°C for 4 min) and fixed in TAF (triethanolamine formaldehyde) for 48 h. Nematode specimens were mounted in glycerin on permanent slides by following Seinhorst's (1959) method.

LIGHT AND SCANNING ELECTRON MICROSCOPY

Nematode specimens were studied with an Olympus BX51 equipped with a Nomarski differential interference contrast and a drawing tube. Light photographs of females and males were taken with a digital camera (Olympus E-volt 330) attached to the microscope.

For SEM observations, adult males and females were obtained from infected *M. ibaguensis*. Specimens were heat-relaxed with hot water (65°C) and immediately fixed with a 5% buffered (pH 7.0) formalin solution for 24 h. After primary fixation in a 5% formalin solution nematodes were rinsed with several changes of 0.1 M phosphate buffer and then transferred to a beam capsule, followed by a post-fixation period of 4 h in a 4% OsO₄ solution. Post-fixed specimens were rinsed with several changes of cold 0.1 M phosphate buffer within a 15 min period and then dehydrated through a series of absolute ethanol aqueous dilutions from 20% through

Table 1. Populations of *Ditylenchus gallaeformans* sp. n. and *D. drepanocercus* used in the present study.

Species	Host	Locality and date of collection	Population code/ collector's number	Morpho-logical study	PCR with specific primers	Molecular study		
						ITS rRNA	D2D3 of 28S rRNA	GenBank accession numbers
<i>D. drepanocercus</i>	<i>Miconia calvescens</i> DC.	Cristais, Viçosa, MG, Brazil; 16.04.2004	CA46, CD930, RWB740	-*	+	JQ429774	JQ429772, JQ429773	JQ429768
<i>D. gallaeformans</i> sp. n.	<i>Clidemia capitellata</i> (Bonpl.) D. Don	Guaraciaba, MG, Brazil; 16.04.2004	-	+	-	-	-	-
<i>D. gallaeformans</i> sp. n.	<i>Leandra lacunosa</i> Cogn.	Reserva Biológica do Tripuí, Ouro Preto, MG, Brazil; 20.10.2004	CA131, RWB735	+	+	JQ429783	JQ429770	-
<i>D. gallaeformans</i> sp. n.	<i>M. albicans</i> (Sw) Triana	Guaraciaba, MG, Brazil; 16.12.2004	CA45, RWB736	+	+	JQ429777, JQ429778	JQ429769	JQ429767
<i>D. gallaeformans</i> sp. n.	<i>M. albicans</i>	Arboreto/Dendrologia, Campus of UFV, MG, Brazil; 24.08.2005	CA121, CA129	-	+	JQ429775, JQ429776	-	-
<i>D. gallaeformans</i> sp. n.	<i>M. calvescens</i>	Turrialba, Costa Rica, 19.04.2004	CA124	+**	+	JQ429780	-	-
<i>D. gallaeformans</i> sp. n.	<i>M. calvescens</i>	Cristais, Viçosa, MG, Brazil; 16.04.2004	-	+	-	-	-	-
<i>D. gallaeformans</i> sp. n.	<i>M. coralline</i> Spring	Reserva Biológica do Tripuí, Ouro Preto, MG, Brazil; 20.10.2004	CA47, RWB734	+	-	JQ429779	-	-
<i>D. gallaeformans</i> sp. n.	<i>M. ibaguensis</i> (Bonpl.) Triana	Cristais, Viçosa, MG, Brazil; 16.12.2004	CA126, RWB739	+	+	JQ429782	-	-
<i>D. gallaeformans</i> sp. n.	<i>M. ibaguensis</i>	Guaraciaba, MG, Brazil; 16.12.2004	CA122	-	-	JQ429781	JQ429771	-
<i>D. gallaeformans</i> sp. n.	<i>M. latecrenata</i> (DC.) Naudin	Arboreto/Dendrologia, Campus of UFV, MG, Brazil; 24.08.2005	CA128	+	+	JQ429784	-	-
<i>D. gallaeformans</i> sp. n.	<i>M. mendoncae</i> Cogn.	Guaraciaba, MG, Brazil; 16.12.2004	-	+	-	-	-	-

* No data.

** See Dietrich (2006).

100%. Dehydrated specimens were critical point-dried in a Tousimis autosamdri-810 critical point drier, mounted on aluminium stubs, coated with a 25 nm layer of gold palladium and observed with a XL30 Phillips SEM at 10 Kv.

DNA EXTRACTION, PCR, CLONING AND SEQUENCING

Several nematode individuals from sampled populations (Table 1) were transferred to a series of separate Eppendorf tubes, each containing 16 μ l ddH₂O, 2 μ l 10 \times PCR buffer and 2 μ l Proteinase K (600 μ g ml⁻¹) (Promega) and crushed with an ultrasonic homogeniser. The tubes were incubated at 65°C (1 h) and then at 95°C (15 min). After incubation, the tubes were centrifuged for 2 min at 15000 *g* and kept at -20°C until use. Detailed protocols for PCR, cloning and automated sequencing were as described in Tanha Maafi *et al.* (2003). The 18S rRNA gene was amplified using two sets of primers (two overlapping fragments): *i*) forward G18SU (5'-GCTTGTCTCAAAGATTAAGCC-3') (Blaxter *et al.*, 1998) and reverse R18Ty11 (5'-GGTCCAAGAATTTCA CCTCTC-3') (Chizhov *et al.*, 2006); and *ii*) forward F18Ty12 (5'-CAGCCGCGGTAATTCCAGC-3') and reverse R18Ty12 (5'-CGGTGTGTACAAAGGGCAGG-3') (Chizhov *et al.*, 2006). The ITS1-5.8S-ITS2 rRNA gene region was amplified using a set of primers: forward TW81 (5'-GTTTCCGTAGGTGAACCTGC-3') and reverse AB28 (5'-ATATGCTTAAGTTCAGCGGGT-3') (Subbotin *et al.*, 2005). For several samples, only the ITS1 rRNA gene region was amplified with forward TW81 and reverse 5.8SM5 (5'-GGCGCAATGTGCATTCGA-3') primers as described by Zheng *et al.* (2000). The D2-D3 expansion segments of 28S rRNA gene were amplified using forward D2Ty1B (5'-GAGAGAGTTAAANAGBAC GTG-3') and reverse D3B (5'-TCGGAAGGAACCAGCT ACTA-3') primers. The newly obtained sequences were deposited in GenBank under the accession numbers indicated in Table 1.

PCR WITH SPECIES-SPECIFIC PRIMERS

Several *Ditylenchus* samples were used to test two species-specific primers. The PCR mixture was prepared as described in Tanha Maafi *et al.* (2003). The universal forward TW81 primer was used in PCR with combinations of the specific reverse D_drep (5'-TCAGCCAAGCC AGACAAGTCAGT-3') or the specific reverse D_gall (5'-TGGCACACTCTTGACTGATGCT-3') primers for di-

agnosis of *D. drepanocercus* or *D. gallaeformans* sp. n., respectively. The PCR amplification profile consisted of 4 min at 94°C; 30 cycles of 1 min at 94°C, 45 s at 57°C and 45 s at 72°C, followed by a final step of 10 min at 72°C. Four μ l of the PCR product was run on a 1.4% TAE buffered agarose gel, stained and photographed.

SEQUENCE AND PHYLOGENETIC ANALYSIS

The newly obtained sequences for 18S rRNA and D2-D3 of 28S rRNA genes were aligned using ClustalX 1.83 with default parameters with corresponding published gene sequences of Anguinidae and several outgroups (Holterman *et al.*, 2006; Subbotin *et al.*, 2006; Meldal *et al.*, 2007; Davies *et al.*, 2009; Zhao *et al.*, 2011), respectively. The best fit model of DNA evolution was obtained using the program MrModeltest 2.2 (Nylander, 2002) with the Akaike Information Criterion in conjunction with PAUP*. Sequence datasets were analysed with Bayesian inference (BI) using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001) with the general time-reversible (GTR) model of nucleotide substitution including a proportion of invariable sites (I) and a gamma distribution (G) of among-site-rate heterogeneity with six rate categories. The analysis was initiated with random starting trees and was run with four chains for 1.0 \times 10⁶ generations. The Markov chains were sampled at intervals of 100 generations. After discarding burn-in samples and evaluating convergence, the remaining samples were retained for further analysis. The topologies were used to generate a 50% majority rule consensus tree.

Results

SYMPTOMS OF INFECTION

Upon closer examination, symptoms caused by eriophyid mite attack, although involving foliage distortions as in infection by the novel nematode species, were recognised as being clearly distinct. Distorted tissues were always blister-like and accompanied by velvety erynose. Larviform eriophyid mites were always found within the erynose but attempts to extract nematodes from such tissues yielded no *Ditylenchus* individuals. Symptoms caused by the new gall-forming *Ditylenchus* were similar for all host plant species. Nematodes induced severe foliage and inflorescence distortions mostly associated with gall-like formation of various sizes (Fig. 1B-F). On leaves, symptoms were amphigenous on the lamina and were also

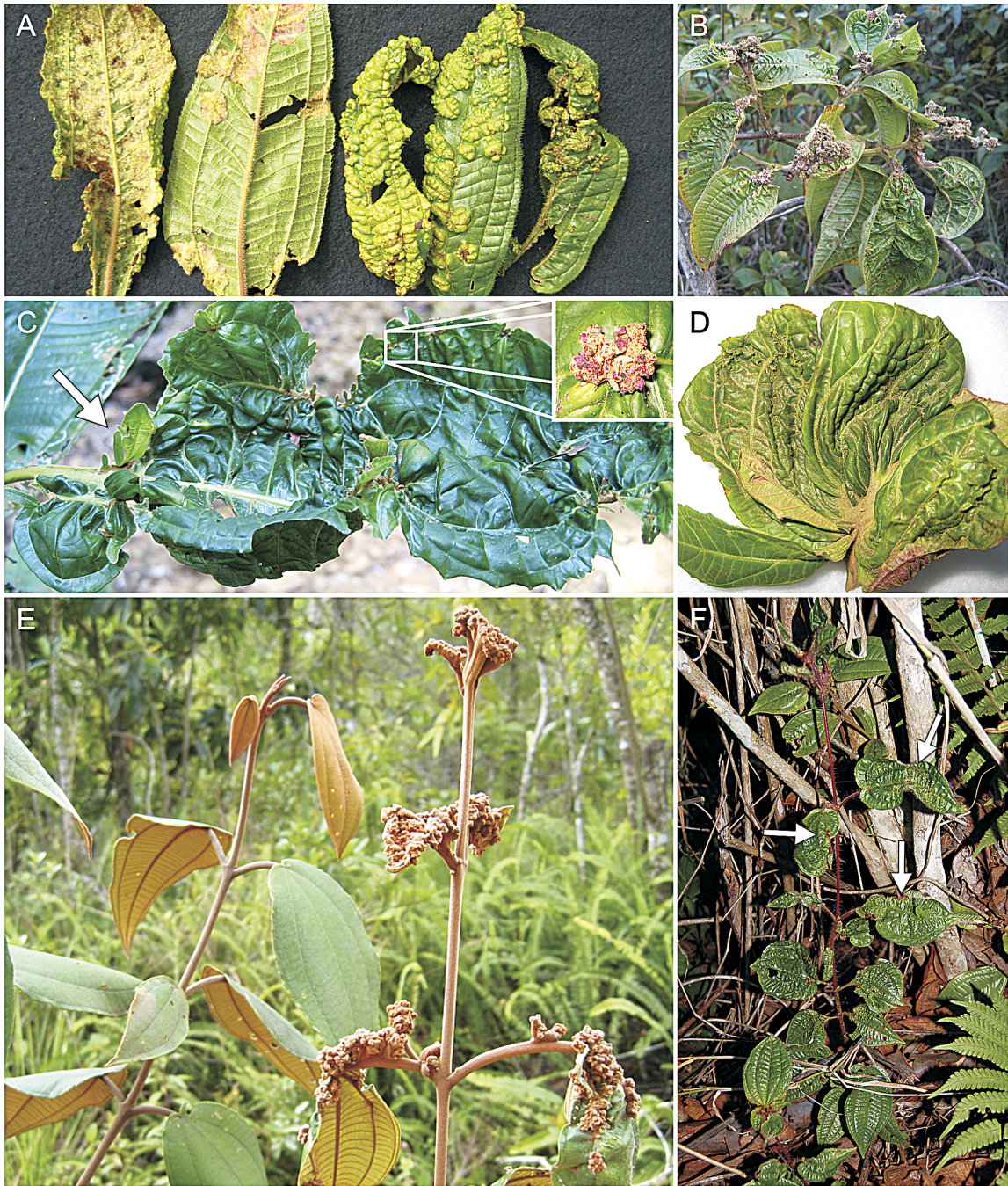


Fig. 1. Field symptoms. A: Intense velvety erynose and pronounced blister-like leaf distortion on *Miconia ibaguensis* caused by an unidentified eriophyid mite (nematodes absent from erynose); B: Abundant galls on inflorescences and leaves on *M. ibaguensis* caused by *Ditylenchus gallaeformans* sp. n.; C, D: Foliar deformation resulting from parasitism of *D. gallaeformans* sp. n. on *M. calvescens* (on C note miniature upward oriented leaves (arrowed) and detail of gall (inset); on D note drastic deformation of leaf due to nematode attack rendering it cabbage-shaped); E: Healthy branch of *M. corallina* (left) besides a branch overrudden by abundant leaf-galling (right); F: *Clidemia hirta* individual plant-bearing galls and foliage distortion as the result of *D. gallaeformans* sp. n. infection.

seen on veins and petioles close to the stems. Complete distortion of the apical bud, followed by the formation of groups of galls or larger galls up to 5 cm diam., was also common. Additionally, few to numerous miniature leaves arising vertically from infected areas of the lamina were often observed (Fig. 1C). Infection of inflorescences led to the formation of galls instead of fruits. On some hosts, such as *Clidemia capitellata* (Bonpl.) D. Don and *Miconia latecrenata* (DC.) Naudin, galls on stems were also commonly observed and sometimes were even more abundant than on other organs. No evidence of immediate necrosis was seen on gall tissue and they appeared to become gradually senescent together with neighbouring healthy tissue. Artificial inoculation studies (data not shown) indicated that there is a latent period of approximately 30 days from inoculation to onset of gall formation. Galls emerged mostly next to leaf veins and were always on expanding young leaves. Infection points appeared firstly as small pale dots representing areas where dense formations of hyaline trichomes were formed. Galls developed from these points and increased gradually in size. Galls were usually formed by the disorganised growth of young foliar tissues leading to the formation of galleries and cavities where numerous trichomes were formed and the nematodes concentrated. After repeated and detailed microscopic examinations of plant sections of infected tissues of different hosts and different pathogenesis stages, no evidence was found of the presence of nematodes inside the plant tissues. Nematode individuals were only found abundantly in the deep crevices and open-ended crypts of galls. A preliminary report of a study of the gall development was recently published (Santin *et al.*, 2009) and a histopathological analysis will be published separately in detail showing that this species is an ectoparasite of melastomes forming populations that congregate on galls.

***Ditylenchus gallaeformans** sp. n.**
 = *Ditylenchus gallaeformans* apud
 Santin *et al.*, 2009
 (Figs 2-5)

MEASUREMENTS

See Tables 2 and 3.

* Specific epithet referring to the galls formed by this nematode on infected hosts.

DESCRIPTION

Female

Body posture, after fixation and mounting in glycerin, straight to slightly arcuate. Cuticular annulation and lateral field weakly expressed, annuli 1-2 μm wide; lateral field with four lines. Under light microscope, four lines almost indistinct, seemingly equidistant. Lateral field only observed in a few females and males, lines as faint as transverse annuli. Head low, not offset, 5.1 μm broad and 1.7 μm high, framework not sclerotised. Head annuli indistinct under LM. Under SEM, face view with quadrangular outline. Stylet delicate, knobs rounded, well developed, 1.5-2.6 μm wide, conical part about half stylet length. Pharynx with subcylindrical procorpus, median bulb oval, 51 ± 2.2 (47-55) μm from anterior end, with central sclerotised thickenings of lumen. Isthmus narrow, cylindrical, 1.5-1.8 times as long as procorpus. Basal bulb pyriform, offset, sometimes slightly overlapping intestine, large dorsal nucleus often distinct, subventral gland nuclei not seen. Nerve ring surrounding isthmus, hemizonid located from excretory pore level to 3 μm anteriorly. Excretory pore 97 ± 11.8 (67-109) μm from anterior end. Female reproductive system with characteristics of *Ditylenchus*, reproductive tract prodelpic, ovary outstretched, oocytes mainly in single row, uterine quadricolumellar followed by an elongated spermatheca packed with large rounded sperm, vulva a transverse slit, vagina somewhat oblique to body axis, reaching more than halfway across body. Post-uterine sac covering almost twice (1.4-1.8) vulval body diam. and extending one-fifth to one-seventh of vulva-anus distance (PUS%VA). Eggs (paratypes; n = 50) 54 ± 5.3 (49-61) \times 24 ± 1.3 (18-25) μm in size. Tail conical, regularly tapering towards a variable tip, frequently regularly pointed to minutely rounded, sometimes exhibiting annulations at posterior end.

Male

Similar to female but usually shorter. Spicules short, simple, curved ventrally, gubernaculum simple, short. Testis usually reflexed at anterior end. In lateral view, bursa totally covering tail, beginning before proximal end of spicule and extending to tail tip.

Second-stage juveniles

Second-stage juvenile paratypes (n = 20) with following dimensions: L = 215 ± 25.2 (184-295) μm ; stylet length = 7.2 ± 0.5 (6.4-7.8) μm ; tail length = 20.7 ± 2.5 (16.0-25.6) μm .

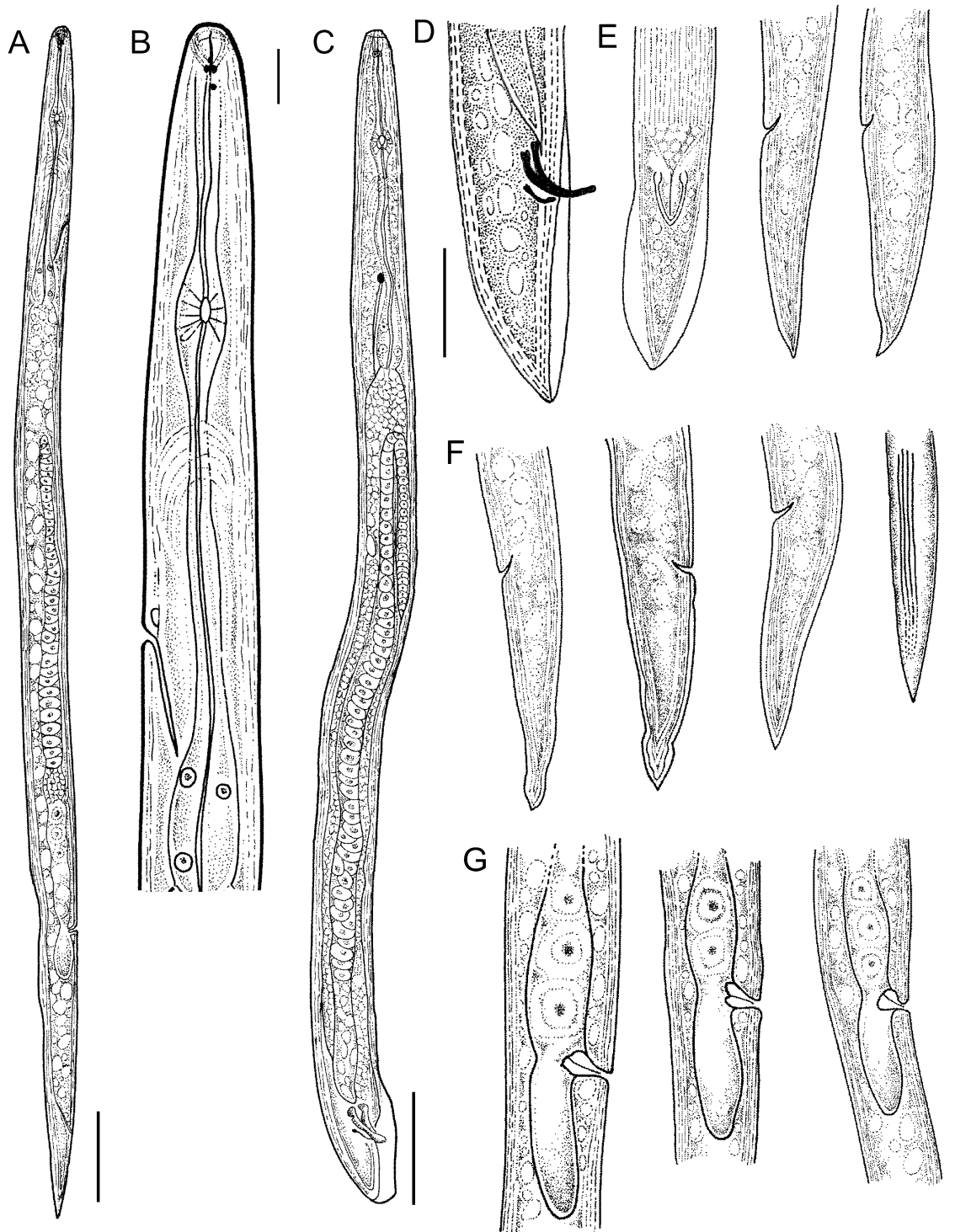


Fig. 2. *Ditylenchus gallaeformans* sp. n. A: Entire female body; B: Anterior end of female; C: Entire male body; D: Posterior end of male; E: Posterior ends of (from left to right): one male and two females; F: Posterior female end; G: Female body at vulva level. (Scale bars: A, C, E-G = 50 µm; B = 10 µm; D = 20 µm.)

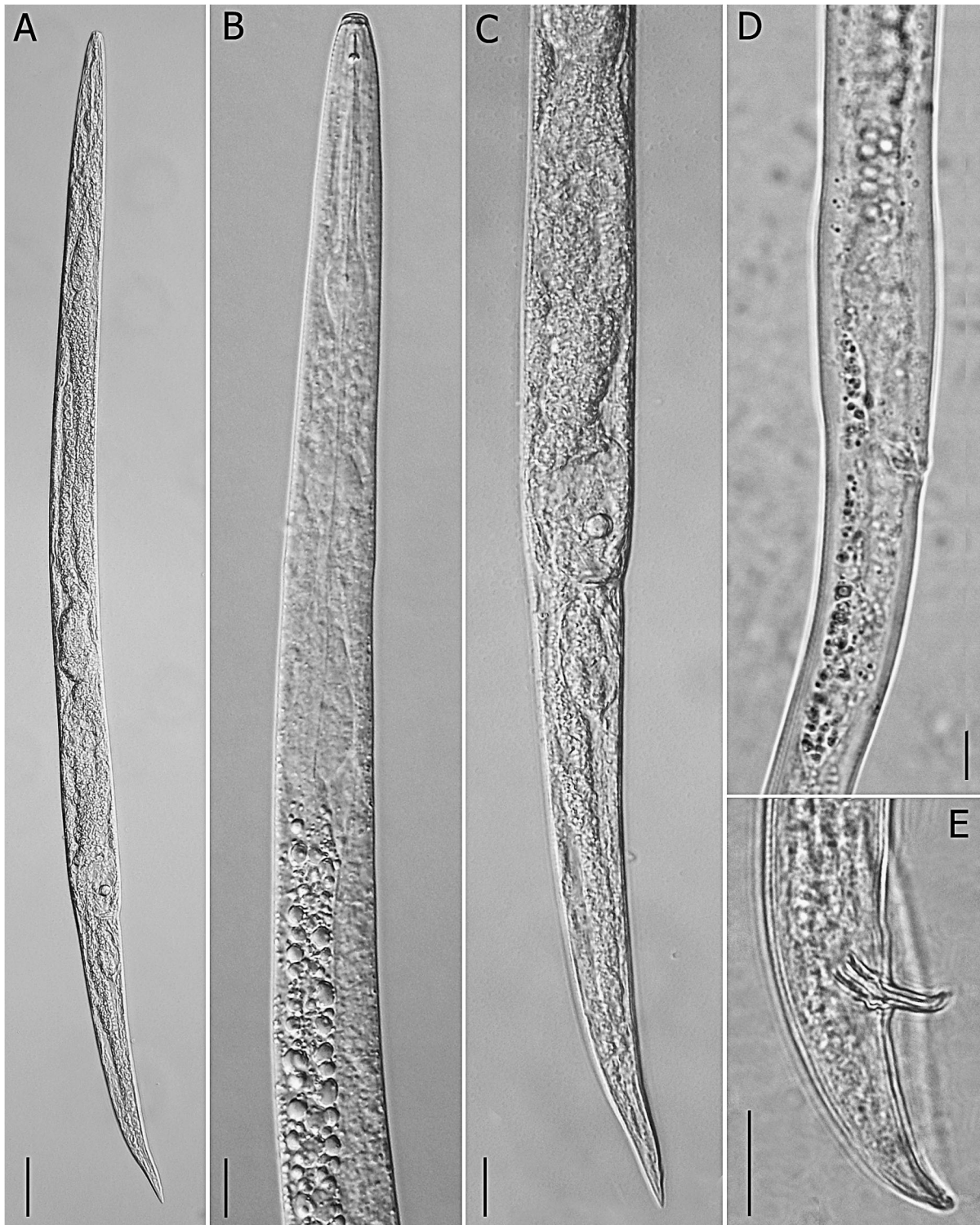


Fig. 3. Light photomicrographs of *Ditylenchus gallaeformans* sp. n. A: Entire female; B: Anterior end of female; C: Posterior end of female; D: Vulval region with post-uterine sac; E: Posterior end of male. (Scale bars: A = 25 μ m; B-D = 10 μ m; E = 20 μ m.)

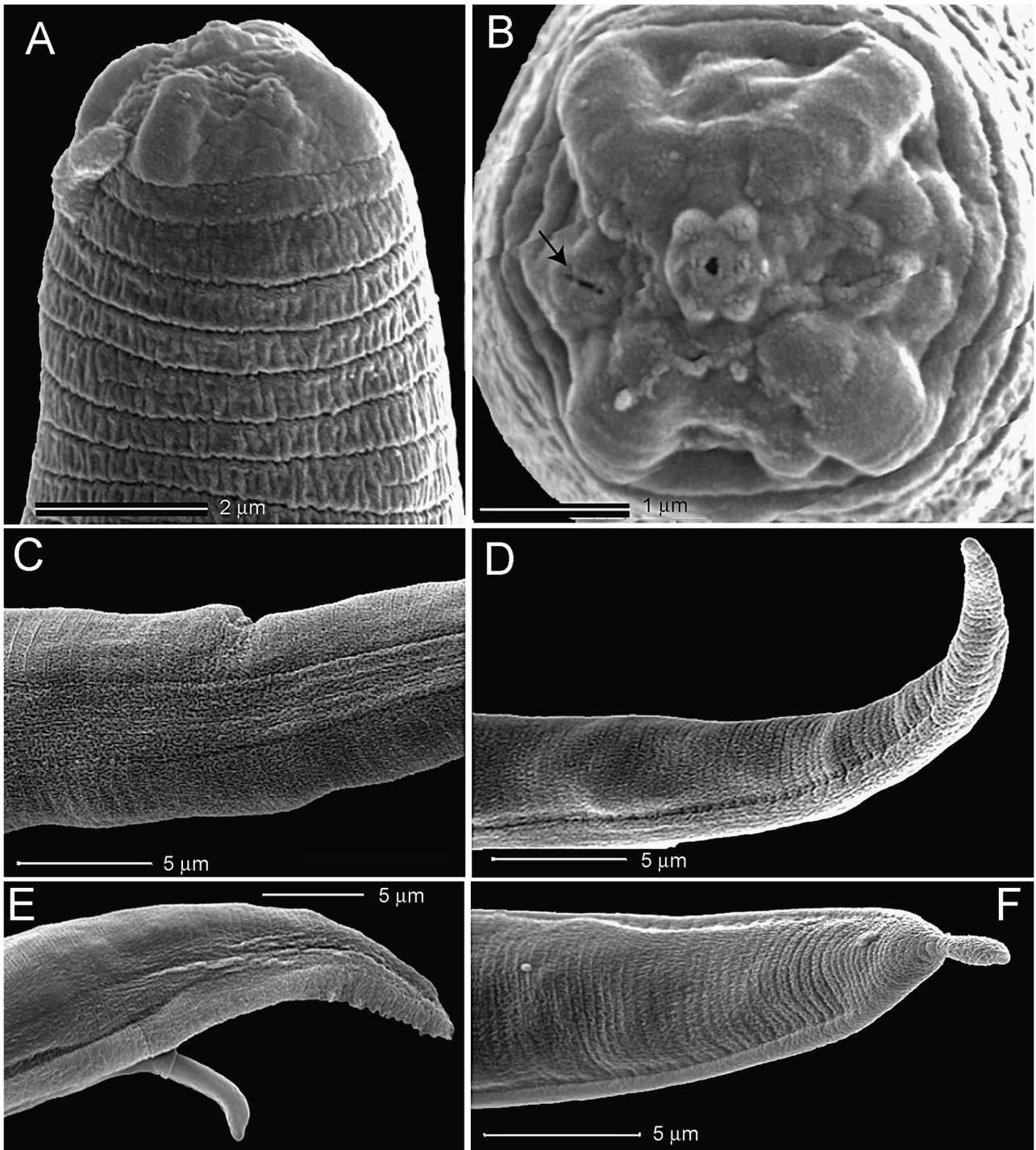


Fig. 4. Scanning electron photomicrographs of *Ditylenchus gallaeformans* sp. n. A: Sublateral view of female showing head annulation; B: Face view showing labial plate and position of amphids (arrow); C: Lateral view showing lateral field at vulval region; D: Tail of a female showing end of lateral field; E: Lateral view of male showing bursa and protruding spicule; F: Ventral view of tail.

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                *      20      *      40      *      60
D. gallaeformans sp.n.: GTTTCCGTAGGTGAACCTGCTGCCGGATCATTAACGATCATACCAAACCAACATTAGTTGT : 61
D. drepanocercus      : .....T.....A.TT...A...-G...A.A : 60

                *      80      *      100     *      120
D. gallaeformans sp.n.: GTGTGTGTGATAGAGCTCTA---TGGCTGTGATTCTCTATCCTAAAAATGAGTGGAAAGTG : 118
D. drepanocercus      : .C...TG.GA..T.A...CATT.....G...C..TCG..A.-----CG..C.C... : 115

                *      140     *      160     *      180
D. gallaeformans sp.n.: CTGCTTATGCCGAATTCTCCTACTCCAATTCAGCATCAGTCCAAGAGTGTGCCATGGCAG : 179
D. drepanocercus      : .A.TG.G.-.AA..G-----.....AT...T...T.G.....A... : 159

                *      200     *      220     *      240
D. gallaeformans sp.n.: TGTGCACAGTGCTATTTGTGTGATTTCCCATCGAATAGCATGCTTAGTCTTGGGGCAAAA : 240
D. drepanocercus      : .....G.....GA.....AG.....GC..TC...TG..C.GT....AA.....C : 220

                *      260     *      280     *      300
D. gallaeformans sp.n.: AACGGCTGCGTTGGCTTCTAAGTCTCTTTGAGCGGTTGTGCTA--TTGTTC--CCTCCAA : 297
D. drepanocercus      : .....C.....T...GG.....A.....G..C.ACC.....GTTT.TT.G : 281

                *      320     *      340     *      360
D. gallaeformans sp.n.: CTCTGTAAG-----CGTGTAGCAGAGCTGTTTTTCTTGGGGGGATAAGTTGGCTACGTC : 351
D. drepanocercus      : T.G..CT..ACTGACTT..C.G..TTG...A.GAATGA.CAA..TCTGTG.....T... : 342

                *      380     *      400     *      420
D. gallaeformans sp.n.: CGTGGCCGCGATGAAGAGAGACGGCAGCTGGTCTTTAGTGATCGCGAGAATTAATGAGCAC : 412
D. drepanocercus      : .....T..T.....TT.....C-.....T..... : 402

                *      440     *      460     *      480
D. gallaeformans sp.n.: AGGCTGTTGTGCCCAACAACCAACCCATTTTGCATTTTGTGAAGCTAGAAATTT : 473
D. drepanocercus      : T...T.G.....C..AG.....A.....ATGCTGG.A..... : 461

                *      500     *      520     *      540
D. gallaeformans sp.n.: CTAGCCTTATCGGTGGATCACTCGGTTCGTGGATCGATGAAGAACGCAGCCAACTGCGATA : 534
D. drepanocercus      : .....T.....G..... : 522

                *      560     *      580     *      600     *
D. gallaeformans sp.n.: AATAGTGTGAACTGCAGATATTTTGAACACTAAAGCTTCGAATGCACATTGCGCCATTGGA : 595
D. drepanocercus      : .G.....G..C..... : 583

                620      *      640      *      660      *
D. gallaeformans sp.n.: TTTTTATCCTTTGGTACATCTGGCTCAGGGTCGTATATACAAAACAAGTGCT--TTGTTTG : 654
D. drepanocercus      : .....T....C...T.....A.....G.A...AC.GAC... : 644

                680      *      700      *      720      *
D. gallaeformans sp.n.: TTGTTGAGTGCTATATTACGGTCCACGGCGGGTACACGTGCGTTAGTGGGTGCTTTT- : 714
D. drepanocercus      : ..AGAG....TATA.....C.TT---A..T..GTGT...T---.AC.....AA : 698

                740      *      760      *      780      *
D. gallaeformans sp.n.: CCATCTGCGGTATACCTTGC-TGAAAATGGATCGGATGCTTCTTTCCAGTCCTGCTGAT : 774
D. drepanocercus      : T...G.C...CAGCTG.C.TG.TTTTGA...GT.A...TAGACCG.TG.-.T..T..G : 758

                800      *      820      *      840      *
D. gallaeformans sp.n.: CGTGCTTTTGAATAGTGTGTGATTGCAGTTTCGATTGTGACCTATGTTGGTTTACTTGAA : 835
D. drepanocercus      : .AA..T...C..C.G.-A.A.--.TT.CC.TTG..T..GGGCGCT.CC..C.----.G. : 812

                860      *      880      *      900      *
D. gallaeformans sp.n.: GTGCCTTAGTTGCTTATTTTATTTAAGGCGTTCACCATTTGTGGGTAGCTTTCGACTGCAT : 896
D. drepanocercus      : ..TG..CGAC...-----.....GC....A.G.TT...TTT. : 849

                920      *      940      *      960
D. gallaeformans sp.n.: TCGCTATTCCGACCTGAGCTCAGTTGTGACTACCCGCTGAACCTAAGCATAT : 948
D. drepanocercus      : :----- : 895

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Fig. 5. Sequence alignment of the ITS1-5.8S-ITS2 rRNA gene for *Ditylenchus gallaeformans* sp. n. from *Miconia corallina* and *D. drepanocercus*. The 18S, 5.8S and 28S rRNA gene sequences are marked in bold. The positions of three universal primers are underlined and the position of two species-specific primers are underlined and marked by grey in the sequence alignment.

Table 2. Morphometrics of adult females of *Ditylenchus gallaeformans* sp. n. from various host plants of the Melastomataceae from the state of Minas Gerais, Brazil. All measurements are in μm and in the form: mean \pm s.d. (range).

Hosts locality character	<i>Miconia ibaguensis</i>		<i>M. ibaguensis</i>		<i>Clidemia capitellata</i>		<i>Leandra lacunosa</i>		<i>M. albicans</i>		<i>M. calvescens</i>		<i>M. corallina</i>		<i>M. latecrenata</i>		<i>M. mendoncae</i>		
	Cristais, Viçosa	Cristais, Viçosa	Cristais, Viçosa	Cristais, Viçosa	Guaraciaba	Ouro Preto	Guaraciaba	Ouro Preto	Guaraciaba	Ouro Preto	Cristais, Viçosa	Cristais, Viçosa	Ouro Preto	Arboreto, Viçosa	Arboreto, Viçosa	Guaraciaba	Guaraciaba		
	Holotype		Paratypes		-		-		-		-		-		-		-		
n	-	20	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	
L	614.0	607 \pm 14.9 (590-639)	612 \pm 34.3 (540-663)	689 \pm 31.1 (626-761)	509 \pm 37.7 (442-578)	572 \pm 43.4 (509-668)	592 \pm 36.2 (528-651)	627 \pm 47.5 (524-698)	574 \pm 35.8 (516-614)										
a	32.0	33.8 \pm 3.9 (26.4-42.7)	29.2 \pm 3.8 (24.0-38.1)	26.6 \pm 2.0 (24.7-31.1)	34.4 \pm 12.0 (23.3-67.2)	33.8 \pm 11.1 (22.6-71.2)	25.9 \pm 2.8 (22.0-30.5)	30.0 \pm 3.9 (23.3-38.0)	31.4 \pm 3.3 (27.8-38.9)										
c	14.3	14.2 \pm 0.7 (10.1-16.5)	14.0 \pm 3.0 (9.9-22.1)	15.2 \pm 1.6 (12.2-18.6)	13.6 \pm 2.5 (7.3-16.5)	14.6 \pm 2.6 (7.5-19.5)	14.3 \pm 3.3 (8.0-24.0)	13.2 \pm 4.0 (7-20)	14.6 \pm 1.9 (11.8-29.6)										
c'	3.7	4.1 \pm 0.3 (3.3-4.4)	4.1 \pm 0.8 (2.3-5.2)	3.7 \pm 0.3 (3.3-4.5)	3.8 \pm 0.9 (2.8-5.1)	3.8 \pm 0.6 (3.0-5.6)	3.5 \pm 0.7 (2.1-5.0)	4.5 \pm 0.9 (3.3-6.4)	3.5 \pm 0.6 (2.4-4.8)										
V	74.3	74.6 \pm 1.1 (72.7-77.3)	74.6 \pm 0.7 (72.8-75.8)	75.4 \pm 0.8 (74.3-76.6)	72.3 \pm 1.0 (70.0-74.0)	74.4 \pm 1.6 (71.4-77.4)	74.2 \pm 1.4 (71.5-76.3)	74.4 \pm 1.9 (68.0-75.7)	75.0 \pm 0.7 (73.7-76.1)										
Stylet	7.7	7.7 \pm 0.4 (6.5-8.3)	8.0 \pm 0.5 (7.7-9.0)	8.3 \pm 0.6 (7.7-9.0)	7.5 \pm 0.4 (6.4-7.7)	7.5 \pm 0.6 (5.9-8.4)	8.3 \pm 0.5 (7.7-9.0)	7.7 \pm 0.7 (6.3-8.9)	7.7 \pm 0.8 (6.4-9.0)										
DGO	-	2.1 \pm 0.6 (1.5-2.6)	1.5 \pm 0.5 (1.0-2.6)	1.3 \pm 0.5 (0.3-2.6)	1.4 \pm 0.6 (0.6-2.6)	1.0 \pm 0.4 (0.5-1.9)	1.5 \pm 0.5 (0.9-2.6)	1.8 \pm 0.9 (1.0-4.0)	1.4 \pm 0.6 (0.3-2.6)										
PUS%VA	15.4	16.7 \pm 1.6 (12.7-19.0)	15.6 \pm 2.1 (12.7-19.8)	16.1 \pm 1.6 (12.5-19.1)	15.8 \pm 2.1 (12.2-20.0)	15.0 \pm 2.6 (9.9-20.1)	16.5 \pm 2.4 (13.1-19.8)	15.2 \pm 2.9 (9.2-19.1)	19.4 \pm 2.4 (15.9-26.1)										
PUS/VBD	1.6	1.7 \pm 0.2 (1.3-1.9)	1.3 \pm 0.2 (1.0-1.5)	1.3 \pm 0.2 (1.1-1.8)	1.3 \pm 0.2 (1.0-1.9)	1.4 \pm 0.3 (1.0-1.7)	1.2 \pm 0.3 (0.6-1.6)	1.3 \pm 0.3 (0.7-1.9)	1.6 \pm 0.2 (1.4-1.9)										
Median bulb height	16.6	14.5 \pm 1.3 (12.8-16.6)	13.3 \pm 1.4 (11.5-16.6)	13.8 \pm 1.1 (11.5-16.0)	12.2 \pm 1.4 (10.2-15.4)	13.6 \pm 1.4 (10.9-15.8)	14.4 \pm 1.2 (12.8-16.6)	14.1 \pm 2.0 (9.9-16.8)	13.7 \pm 1.5 (11.5-16.6)										
Median bulb diam.	7.7	7.7 \pm 0.6 (6.4-9.0)	7.9 \pm 0.7 (6.4-9.0)	8.4 \pm 0.6 (7.7-9.0)	6.8 \pm 0.6 (6.4-7.7)	7.1 \pm 0.5 (5.9-7.9)	8.2 \pm 0.7 (7.0-9.0)	8.0 \pm 1.0 (6.9-9.9)	7.6 \pm 0.7 (6.4-9.0)										
Head to cardia	139.0	141 \pm 6.5 (128-152)	138 \pm 6.2 (126-152)	143 \pm 5.0 (130-149)	128 \pm 6.8 (117-138)	133 \pm 4.4 (128-140)	138 \pm 8.7 (129-150)	149 \pm 9.6 (133-168)	127 \pm 8.5 (104-139)										
Anal body diam.	-	-	11.0 \pm 1.2 (9.0-12.8)	12.1 \pm 0.8 (10.2-13.4)	10.4 \pm 1.9 (6.4-15.4)	10.4 \pm 1.6 (7.9-14.1)	12.5 \pm 1.3 (10.2-14.1)	11.5 \pm 2.4 (7.5-15.8)	11.4 \pm 1.3 (8.3-12.8)										
Tail length	42.9	43.5 \pm 4.7 (38.4-60.2)	45.5 \pm 10.1 (25.6-64.0)	45.7 \pm 4.0 (38.4-52.5)	39.3 \pm 12.0 (30.7-78.1)	40.0 \pm 10.4 (30.7-74.2)	43.3 \pm 10.2 (24.3-70.4)	52.9 \pm 21.2 (35.0-101.9)	39.8 \pm 4.3 (31.3-45.4)										

Table 3. Morphometrics of adult males of *Ditylenchus gallaeformans* sp. n. from various host plants of the Melastomataceae from the state of Minas Gerais, Brazil.

Character	<i>M. ibaguensis</i>		<i>Clidemia capitellata</i>		<i>Leandra lacunosa</i>		<i>M. albicans</i>		<i>M. calvescens</i>		<i>M. corallina</i>		<i>M. latecrenata</i>		<i>M. mendoncae</i>		
	Cristais, Viçosa		Guaraciaba		Ouro Preto		Guaraciaba		Cristais, Viçosa		Ouro Preto		Arboreto, Viçosa		Guaraciaba		
	Paratypes		-		-		-		-		-		-		-		
n	20	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	
L	554 ± 36.0 (503-614)	548 ± 32 (491-602)	603 ± 34.7 (528-675)	478 ± 24.8 (442-516)	558 ± 40.4 (489-638)	543 ± 26 (504-614)	587 ± 31.3 (549-658)	530 ± 31.5 (479-589)									
a	29.1 ± 5.0 (21.9-36.2)	31.2 ± 3.6 (25.0-35.7)	34 ± 4 (27.8-39.6)	27.9 ± 2.8 (23.9-32.6)	30.9 ± 3.2 (27.2-38.9)	27.0 ± 2.4 (23.3-33.0)	33.7 ± 7.0 (27.0-55.9)	30.9 ± 3.2 (27.2-38.9)									
c	26.3 ± 7.0 (17.8-37.6)	27.7 ± 6.6 (18.8-41.5)	27.8 ± 7.7 (16.5-43.6)	22.9 ± 2.6 (17.4-27.0)	26.1 ± 4.4 (19-38)	27.3 ± 6.9 (16-41)	30.4 ± 5.9 (21.2-43.0)	26.1 ± 4.4 (19.2-38.4)									
c'	1.8 ± 0.4 (1.0-2.5)	1.9 ± 0.5 (1.2-3.0)	2.1 ± 0.5 (1.6-3.6)	2.0 ± 0.4 (1.4-2.7)	1.9 ± 0.2 (1.4-2.3)	1.9 ± 0.4 (1.2-2.6)	2.1 ± 0.3 (1.5-2.6)	1.9 ± 0.2 (1.4-2.3)									
Stylet	7.5 ± 0.4 (6.4-8.3)	7.7 ± 0.4 (6.4-8.3)	8.3 ± 0.6 (7.7-9.0)	7.3 ± 0.8 (5.1-7.7)	7.3 ± 0.7 (5.9-7.9)	7.9 ± 0.6 (6.4-9.0)	7.8 ± 0.5 (6.9-8.4)	8.0 ± 0.5 (7.7-9.0)									
DGO	2.0 ± 0.6 (1.0-2.6)	1.7 ± 0.6 (1.0-2.6)	1.2 ± 0.4 (0.9-2.6)	1.4 ± 0.6 (0.3-2.6)	1.1 ± 0.2 (0.6-1.3)	1.7 ± 0.7 (1.0-3.5)	1.8 ± 0.5 (1.0-2.6)	1.0 ± 0.2 (0.6-1.3)									
Median bulb height	14.0 ± 2.1 (10.2-20.5)	12.8 ± 1.5 (10.2-14.1)	13.6 ± 1.5 (11.5-15.4)	13.3 ± 2.8 (10.2-15.4)	13.4 ± 1.9 (10-17)	13.3 ± 1.6 (11.5-15.4)	14.1 ± 1.7 (11.5-17.8)	14.2 ± 1.4 (11.5-16.6)									
Median bulb diam.	8.4 ± 1.1 (7.0-11.5)	7.5 ± 0.8 (6.4-9.0)	7.6 ± 0.6 (6.4-9.0)	7.7 ± 1.7 (6.4-12.8)	7.5 ± 1.1 (5.0-8.9)	8.3 ± 0.8 (7.7-10.2)	7.7 ± 0.9 (6.4-9.9)	7.7 ± 0.6 (6.4-8.3)									
Head to cardia	136 ± 8.9 (114-150)	134 ± 6.4 (123-142)	140 ± 9.8 (119-152)	135 ± 11 (114-155)	134 ± 25.6 (50-175)	132 ± 12.9 (95-147)	140 ± 15.8 (101-173)	132 ± 3.3 (126-139)									
Bursa	48.7 ± 5.8 (42.2-62.7)	49.9 ± 3.9 (43.5-57.6)	49.3 ± 3.6 (41-55)	42.0 ± 3.8 (35.8-48.0)	45.8 ± 3.2 (41.5-51.4)	48.0 ± 4.5 (39.7-57.6)	51.5 ± 6.2 (40.0-64.3)	43.1 ± 5.2 (33.3-51.2)									
Spicules	21.2 ± 1.2 (19.4-23.7)	18.6 ± 2.1 (16.6-25.6)	17.9 ± 0.8 (16.6-19.2)	16.8 ± 1.4 (14.1-19.2)	18.2 ± 2.2 (13.8-21.8)	18.6 ± 1.5 (15.4-21.8)	19.6 ± 1.3 (16.8-20.3)	17.9 ± 1.1 (15.4-19.2)									
Gubernaculum	5.4 ± 0.9 (3.8-7.0)	5.4 ± 0.8 (3.8-6.4)	5.3 ± 1.0 (3.8-7.0)	5.2 ± 1.1 (2.6-6.4)	4.9 ± 0.5 (3.8-5.8)	5.7 ± 1.0 (3.8-6.4)	5.3 ± 0.8 (3.8-6.4)	5.4 ± 0.8 (3.8-6.4)									
Tail length	22.2 ± 4.9 (14.1-32.0)	20.7 ± 4.7 (14.2-29.4)	23.2 ± 6.2 (14.1-37.1)	21.1 ± 2.7 (17.3-28.2)	19.7 ± 2.5 (15.4-23.7)	21.1 ± 5.2 (12.8-33.3)	19.9 ± 3.5 (12.9-26.9)	20.8 ± 3.4 (12.8-27.0)									

NOTE

Biometric data for either sex did not vary significantly between different populations collected from various hosts and geographic origins (Tables 2, 3). The largest females and males were found in *Leandra lacunosa* and the smallest in *M. albicans*. For these samples, ratio a ranged from 26 to 34, but other character variations were minimal. The vulval position was consistent ($V = 72-75$). Additionally, a nematode population obtained from *M. mendoncae* had a larger PUS%VA (19.4) compared to other samples. None of these differences can be considered as sufficient to justify separate taxonomic status for any sample.

This publication formerly proposes a binomen which was previously mentioned in the literature in a conference abstract (Santin *et al.*, 2009) but without a complete and formal description.

TYPE HOST AND LOCALITY

Miconia ibaguensis (Bonpl.) Triana leaves and inflorescences collected in Cristais, Viçosa, Minas Gerais State, Brazil, by R.W. Barreto (RWB739) on 16 December 2004.

OTHER HOSTS AND LOCALITIES

Initially, six species of *Miconia*, *C. capitellata*, *C. hirta* and *Leandra lacunosa* Cogn. were found to be infected with the nematode in the state of Minas Gerais, Brazil. Nematode infection was reported on *M. calvescens* from Costa Rica (Turrialba) (Table 1). Later, when the survey was expanded, numerous additional geographical and host plant records of this species were recorded in Brazil (Oliveira *et al.*, unpubl.) and Costa Rica (Dietrich, 2006).

TYPE MATERIAL

Holotype, female and male paratypes, mounted on glass slides deposited at the United States Department of Agriculture Nematode Collection (accession numbers: T-6132-T-6138; seven slides, each with a male and female), Beltsville, MD, USA, and University of California Riverside Nematode Collection (30722-30727; six slides, each with a male and female), Riverside, CA, USA.

DIAGNOSIS AND RELATIONSHIPS

Ditylenchus gallaeformans sp. n. is characterised by straight to slightly arcuate body 442-698 μm in length, lip region not offset, presence of four incisures, slender stylet

6.4-9.0 μm in length, basal bulb slightly overlapping the intestine, post-uterine sac 1.4-1.8 vulval body diam. long and conical tail with regularly pointed to minutely rounded terminus.

Ditylenchus gallaeformans sp. n. is most similar to *D. drepanocercus*, a species previously described in association with angular leaf spot on *M. calvescens* (Seixas *et al.*, 2004a). The latter species has a distinctive falciform appendage on the apex of the tail in both sexes, which allows for its easy separation from *D. gallaeformans* sp. n. Additionally, the male bursa of *D. drepanocercus* covers 50% of tail whereas in *D. gallaeformans* sp. n. it covers the entire tail (Brzeski, 1991; Seixas *et al.*, 2004a).

Ditylenchus gallaeformans sp. n. differs from other plant-parasitic *Ditylenchus* (*D. angustus* (Butler, 1913) Filipjev, 1936, *D. dipsaci* (Kühn) Filipjev, 1936 and *D. dryadis* Anderson & Mulvey, 1980) as follows: from *D. angustus* by having a shorter body and a shorter stylet ($L = 0.6$ (0.59-0.64) vs 0.8-1.2 mm; stylet = 7.7 (6.5-8.3) vs 10-11 μm) and also by the absence of a mucronate tail vs mucronate in *D. angustus*; from *D. dipsaci* by having a shorter body and stylet ($L = 0.6$ (0.59-0.64) vs 1.0-1.3 mm; stylet = 7.7 (6.5-8.3) vs 10-12 μm), and lower values for V and PUS%VA ($V = 74.6$ (72.7-77.3) vs 76-86; PUS%VA = 16.7 (12.7-19.0) vs 40-70); from *D. dryadis* by having four vs 6-8 incisures in the lateral field, and lower V and PUS%VA ($V = 74.6$ (72.7-77.3) vs 80-83; PUS%VA = 16.7 (12.7-19.0) vs 61-86). Additionally, *D. gallaeformans* sp. n. has the bursa extending over the entire tail whereas it extends to less than 100% in *D. angustus*, and covers 40-70% of the tail length in *D. dipsaci* and 64-76% in *D. dryadis*.

Although the presence of a bursa covering the entire tail in *D. gallaeformans* sp. n. is a very distinctive feature, it is not exclusive to this species. There are four other species of *Ditylenchus* having males with a bursa extending over almost the whole length of tail, namely: *D. cyperi* Husain & Khan, 1967, *D. nanus* Siddiqi, 1963, *D. mirus* Siddiqi, 1963 and *D. virtudesae* Tobar-Jimenez, 1964 (Brzeski, 1991; Siddiqi, 2000). The new species can be distinguished from *D. cyperi* by four vs five incisures in the lateral field, higher ratio a (31.2 (25.0-35.7) vs 18-29 in females of *D. cyperi*) and lower PUS%VA (16.7 (12.7-19.0) vs 50); from *D. nanus* by four vs six incisures, lower PUS%VA (16.7 (12.7-19.0) vs 60-70) and longer spicules (18.6 (16.6-25.6) vs 14-15 μm); from *D. mirus* by having a pointed to slightly rounded tail tip vs broadly rounded and lower V value of 74.6 (72.7-77.3) vs 83-85; and from *D. virtudesae* by number of four vs six incisures, a pointed

to slightly rounded tail tip vs broadly rounded and lower V value of 74.6 (72.7-77.3) vs 79.9-81.7.

MOLECULAR CHARACTERISATION AND DIAGNOSTICS OF *DITYLENCHUS GALLAEFORMANS* SP. N. AND *D. DREPANOCERCUS*

Sizes of PCR products amplified by TW81 and AB28 primers were ca 946-948 and 895 bp for *D. gallaeformans* sp. n. and *D. drepanocercus*, respectively. The sequence alignment showing the differences in the ITS rRNA region between these species is given in Figure 5. Ten obtained sequences from different populations and PCR cloned products of *D. gallaeformans* sp. n. differed from each other by three nucleotides and two insertions/deletions (TG in ITS1 and TTTA in ITS2) only. Comparison of ITS rRNA gene sequences for *D. gallaeformans* and *D. drepanocercus* with those belonging to the *Ditylenchus dipsaci* species complex (Subbotin *et al.*, 2005) revealed a substantial divergence between these groups (data not shown).

In our study, we designed specific primers for both *Ditylenchus* species. The results of PCR with these primers are given in Figure 6. The combination of the universal TW81 primer with the species-specific D_gall primer yielded an amplicon of ca 173 bp in length for all *D. gallaeformans* sp. n. samples and the combination of the universal TW81 primer with the species-specific D_drep primer yielded an amplicon of 313 bp in length for a *D. drepanocercus* sample (Fig. 6).

PHYLOGENETIC RELATIONSHIPS OF *DITYLENCHUS GALLAEFORMANS* SP. N. WITH OTHER ANGUINIDAE

The alignment of partly sequenced 18S rRNA gene was 709 bp in length and contained 19 taxa including three outgroup taxa. Phylogenetics analysis of the 18S rRNA gene sequences using BI revealed close relationships of *D. gallaeformans* sp. n. and *D. drepanocercus*. These *Ditylenchus* species formed a highly supported clade (posterior probability = 99) in the majority consensus BI tree (Fig. 7).

The alignment of D2-D3 expansion segments of 28S rRNA gene was 695 bp in length and contained 18 taxa, including two outgroup taxa. The phylogenetic relationships of *Ditylenchus* with other Anguinidae is presented in Figure 8. *Ditylenchus gallaeformans* sp. n. and *D. drepanocercus* clustered together with the highest posterior probability. Phylogenetic analysis of rRNA gene sequences revealed that these species are neither closely

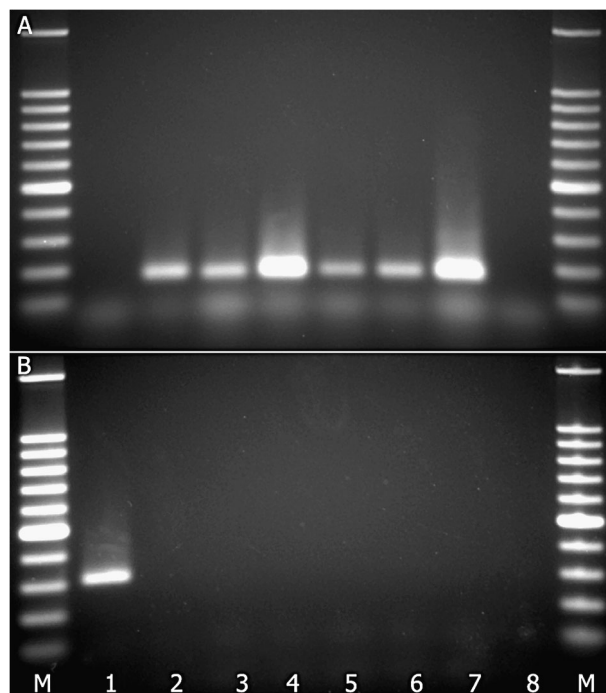


Fig. 6. Gel with specific amplicons obtained from PCR with species-specific primers. A: PCR with *Ditylenchus gallaeformans* sp. n. specific primer (TW81 + D_gall primers); B: PCR with *D. drepanocercus* species-specific primer (TW81 + D_drep primers). Lanes: M: 100 bp DNA ladder (Promega); 1: *D. drepanocercus*; 2-7: *D. gallaeformans* sp. n. (2-CA45; 3-CA124; 4-CA131; 5-CA121; 6-CA129; 7-CA128); 8: control without DNA.

related to the *D. dipsaci* species complex or to *D. destructor*.

Thus, the BI analysis of the rRNA gene sequences showed that *Ditylenchus* might be a paraphyletic taxon including several evolutionary independent lineages. The molecular groupings are congruent with those proposed by Fortuner (1982), Sumenkova (1982) and Siddiqi (2000), who divided the genus into several morphological and biological species groups. The first main group constitutes plant-parasitic species of *Ditylenchus* with the representatives of Anguininae, which have almost lost their primitive trait of fungal feeding. The other group, named as the *D. triformis* group by Siddiqi (2000), included *D. destructor* Thorne, 1945 and unidentified (possibly fungal-feeding *Ditylenchus*). Identity of the sample identified in the tree as *D. brevicauda* (Micoletzky, 1925) Filipjev, 1936 might still be doubtful as Fortuner (1982) and Brzeski (1991) considered this species as imperfectly described and regarded it as *species inquirenda*.

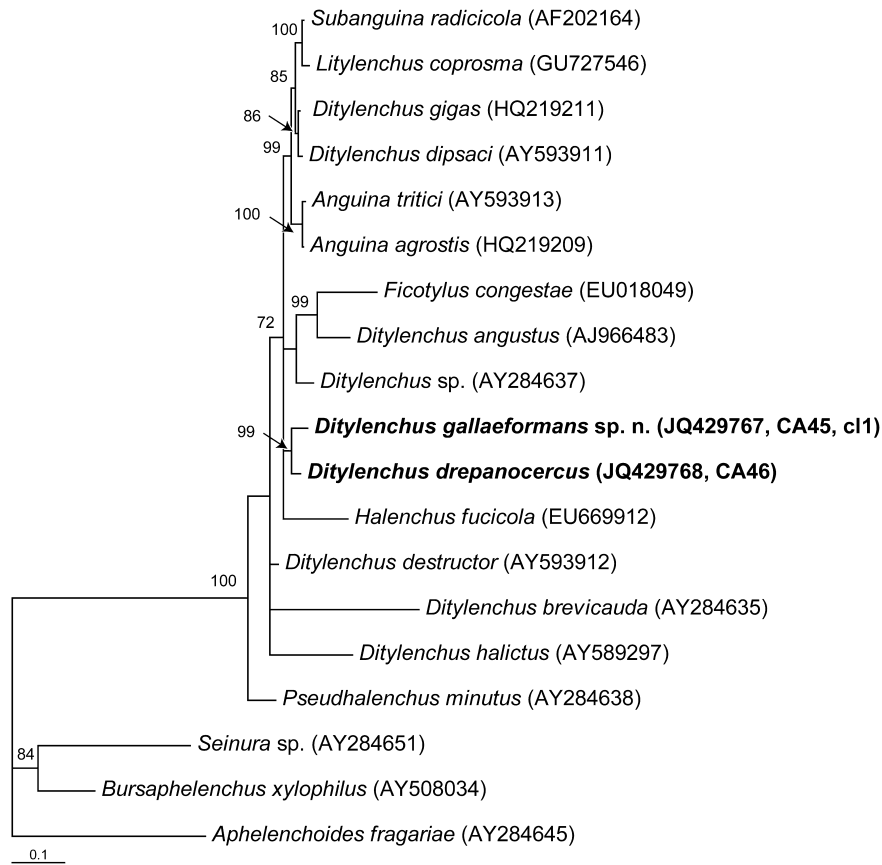


Fig. 7. The 50% majority rule consensus tree from Bayesian analysis generated from the partial 18S rRNA gene sequence dataset using the GTR + I + G model and showing the relationships of *Ditylenchus gallaeformans* sp. n. and *D. drepanocercus* with other Anguinidae. Posterior probabilities more than 70% are given for appropriate clades.

On the other hand, we cannot exclude the possibility that the observed paraphyly could be the result of the use of an inappropriate model of evolution for rRNA or possible mistakes in identification. The phylogenetic hypothesis of *Ditylenchus* as a monophyletic taxon should be carefully tested in the future using several genetic markers and additional species of *Ditylenchus*.

BIOLOGICAL CONTROL POTENTIAL

Only a few nematode species have been investigated so far as potential biocontrol agents of weeds. All of these nematodes belong to the Anguinidae and are parasites of aerial plant parts, producing deformities and galls on their hosts (Parker, 1991). Such nematodes belong to three genera: *Orrina* Brzeski, 1981, *Mesoanguina* Chizhov & Subbotin, 1985 and *Ditylenchus*. The nematode species and their target weeds are: *O. phyllobius* (Thorne, 1934)

Brzeski, 1981 for *Solanum elaeagnifolium* Cav. (Robinson *et al.*, 1978; Skinner *et al.*, 1980; Parker, 1986); *M. picridis* (Kirjanova, 1944) Chizhov & Subbotin, 1985 for *Acroptilon repens* (L.) DC. (Watson, 1986); *M. amsinckiae* (Steiner & Scott, 1935) Chizhov & Subbotin, 1985 for *Amsinckia* spp. (Pantone, 1987) and *D. drepanocercus*, for *M. calvescens* (Seixas *et al.*, 2004a, b).

Ditylenchus gallaeformans sp. n. is regarded as having good potential for use as a classical biological control agent against the weeds *M. calvescens* and *C. hirta* in the Pacific islands. These two important weed species of the Melastomataceae were found to be infected by this nematode in natural habitats and infection sometimes led to significant damage on both weeds in the field, also in laboratory tests (Santin, 2008). The nematode is already considered as being of particular interest for use as a classical biological control agent against *M. calvescens* and also *C. hirta* as these two very important weed species

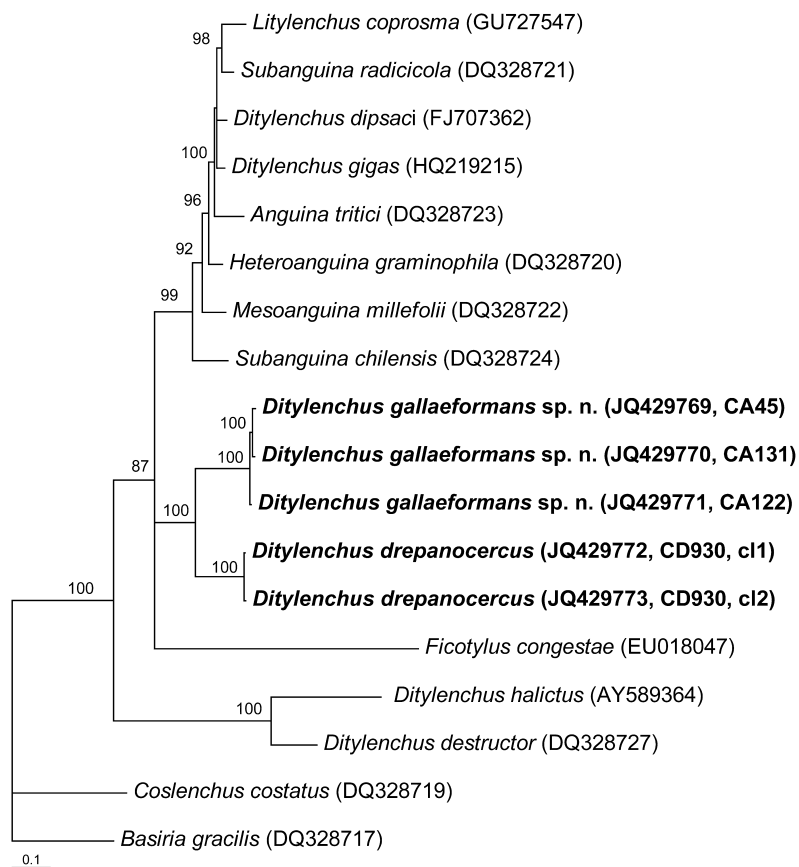


Fig. 8. The 50% majority rule consensus tree from Bayesian analysis generated from the D2-D3 expansion segments of 28S rRNA gene sequence dataset using the GTR + I + G model and showing the relationships of *Ditylenchus gallaeformans* sp. n. and *D. drepanocercus* with other Anguinidae. Posterior probabilities more than 70% are given for appropriate clades.

have been observed to be attacked by *D. gallaeformans* and significantly damaged both in the field and in tests under controlled conditions (Santin, 2008). Although the host-range of the new nematode species is relatively wide within the Melastomataceae (but restricted to this family) (Dietrich, 2006; Santin, 2008), in the case of Hawaii, in particular, this is of little concern as there are no native Melastomataceae in Hawaii nor economically important exotic cultivated melastomes. The biocontrol potential of this new species has been extensively investigated by Dietrich (2006) and Santin (2008) and results of this research will be published separately.

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