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 calvescens 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. calvescens 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. calvescens and C. hirta.

Keywords – biological control, Brazil, Clidemia hirta, Costa Rica, description, Ditylenchus drepanocercus, Miconia calvescens, 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 calvescens DC. and Tibouchina herbacea (DC.) Cogn. (Cronk & Fuller, 1995; DeWalt et al., 2004). Miconia calvescens is the worst weed among these ecosystem invaders. Pacific island native forest ecosystems, particularly 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
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* Goody, 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 (Kilgore 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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Host</th>
<th>Locality and date of collection</th>
<th>Population code/collector’s number</th>
<th>Morphological study</th>
<th>Molecular study</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. drepanocercus</em></td>
<td><em>Miconia calvescens</em> DC. (Bonpl.) D.Don</td>
<td>Guaraciaba, MG, Brazil; 16.04.2004</td>
<td>CA46, CD930, RWB740</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>D. gallaeformans</em> sp. n.</td>
<td><em>Clidemia capitellata</em> (Bonpl.) D.Don</td>
<td>Guaraciaba, MG, Brazil; 16.04.2004</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><em>D. gallaeformans</em> sp. n.</td>
<td><em>Leandra lacunosa</em> Cogn.</td>
<td>Reserva Biológica do Tripuí, Ouro Preto, MG, Brazil; 20.10.2004</td>
<td>CA131, RWB735</td>
<td>+</td>
<td>JQ429783</td>
</tr>
<tr>
<td><em>D. gallaeformans</em> sp. n.</td>
<td><em>M. albicans</em> Triana</td>
<td>Guaraciaba, MG, Brazil; 16.12.2004</td>
<td>CA45, RBW736</td>
<td>+</td>
<td>JQ429777, JQ429776</td>
</tr>
<tr>
<td><em>D. gallaeformans</em> sp. n.</td>
<td><em>M. albicans</em></td>
<td>Arboreto/Dendrologia, Campus of UFV, MG, Brazil; 24.08.2005</td>
<td>CA121, CA129</td>
<td>–</td>
<td>JQ429775, JQ429776</td>
</tr>
<tr>
<td><em>D. gallaeformans</em> sp. n.</td>
<td><em>M. calvencens</em></td>
<td>Turrialba, Costa Rica, 19.04.2004</td>
<td>CA124</td>
<td>+**</td>
<td>JQ429780</td>
</tr>
<tr>
<td><em>D. gallaeformans</em> sp. n.</td>
<td><em>M. calvencens</em></td>
<td>Cristais, Viçosa, MG, Brazil; 16.04.2004</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><em>D. gallaeformans</em> sp. n.</td>
<td><em>M. coralline</em> Spring</td>
<td>Reserva Biológica do Tripuí, Ouro Preto, MG, Brazil; 20.10.2004</td>
<td>CA47, RWB734</td>
<td>+</td>
<td>JQ429779</td>
</tr>
<tr>
<td><em>D. gallaeformans</em> sp. n.</td>
<td><em>M. ibaguensis</em> (Bonpl.) Triana</td>
<td>Cristais, Viçosa, MG, Brazil; 16.12.2004</td>
<td>CA126, RBW739</td>
<td>+</td>
<td>JQ429782</td>
</tr>
<tr>
<td><em>D. gallaeformans</em> sp. n.</td>
<td><em>M. ibaguensis</em></td>
<td>Guaraciaba, MG, Brazil; 16.12.2004</td>
<td>CA122</td>
<td>–</td>
<td>JQ429781, JQ429771</td>
</tr>
<tr>
<td><em>D. gallaeformans</em> sp. n.</td>
<td><em>M. latecrenata</em> (DC.) Naudin</td>
<td>Arboreto/Dendrologia, Campus of UFV, MG, Brazil; 24.08.2005</td>
<td>CA128</td>
<td>+</td>
<td>JQ429784</td>
</tr>
<tr>
<td><em>D. gallaeformans</em> sp. n.</td>
<td><em>M. mendoncae</em> Cogn.</td>
<td>Guaraciaba, MG, Brazil; 16.12.2004</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

*No data.
**See Dietrich (2006).
100%. Dehydrated specimens were critical point-dried in a Tousimis autosamdri-810 critical point drier, mounted on aluminium stabs, 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 μl ddH2O, 2 μl 10× PCR buffer and 2 μl Proteinase K (600 μg 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 15 000 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′-GCTTGCTCTCAAAGATTAAGCC-3′) (Blaxter et al., 1998) and reverse R18Ty11 (5′-GTCTCAAGAAATTCCA CCTCTC-3′) (Chizhov et al., 2006); and ii) forward F18Ty12 (5′-CAGCCGCGGTAAATTCGAC-3′) and reverse R18Ty12 (5′-CGGTGTGTACAAAGGGCAGG-3′) (Chizhov et al., 2006). The ITS1-5.8S-ITS2 rDNA gene region was amplified using a set of primers: forward TW81 (5′-GTTTCCGTAGGTGACACTG-3′) and reverse 5.8SM5 (5′-GGCGCAATGTGCATTCGA-3′) or the specific reverse D_gall (5′-TCAGCCCAAGCC AGACAAGTCTAGT-3′) (Chizhov et al., 2006). For several samples, only the ITS1 rDNA 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 genes 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′-TCAGCCCAAGCC AGACAAGTCTAGT-3′) or the specific reverse D_gall (5′-TGGCACACTCTTTGGACTGATGCT-3′) primers for diagnosis of D. drepanocercus or D. galleformans 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 μl 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 (Holtermann 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 invariant 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 × 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...
Ditylenchus gallaeformans sp. n. from Brazil and Costa Rica

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 overrun 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 necro-
sis was seen on gall tissue and they appeared to become
gradually senescent together with neighbouring healthy
tissue. Artificial inoculation studies (data not shown) in-
dicated 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 forma-
tions 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 cav-
ities where numerous trichomes were formed and the ne-
matodes concentrated. After repeated and detailed micro-
scopic 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 devel-
oped 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 lat-
eral 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 quad-
rangular outline. Stylet delicate, knobs rounded, well de-
volved, 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 nu-
cleii not seen. Nerve ring surrounding isthmus, hemizonid
located from excretory pore level to 3 μm anteriorly. Ex-
cretory pore 97 ± 11.8 (67-109) μm from anterior end.
Female reproductive system with characteristics of Dity-
lenchus, reproductive tract prodelphic, 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) × 24 ± 1.3 (18-25) μm in size.
Tail conical, regularly tapering towards a variable tip, fre-
quently 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 follow-
ing 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.
Ditylenchus gallaeformans sp. n. from Brazil and Costa Rica

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 μm; B-D = 10 μm; E = 20 μm.)
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.
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 ± s.d. (range).

<table>
<thead>
<tr>
<th>Hosts character</th>
<th>Miconia <em>ibaguensis</em></th>
<th>M. <em>ibaguensis</em> capitellata</th>
<th>Clidemia <em>lacunos</em></th>
<th>Leandra <em>lacunos</em></th>
<th>M. <em>albicans</em></th>
<th>M. <em>calvescens</em></th>
<th>M. <em>corallina</em></th>
<th>M. <em>latercrenata</em></th>
<th>M. <em>mendoncae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>locality</td>
<td>Cristais, Viçosa</td>
<td>Cristais, Viçosa</td>
<td>Guaraciaba</td>
<td>Guaraciaba</td>
<td>Ouro Preto</td>
<td>Cristais, Viçosa</td>
<td>Ouro Preto</td>
<td>Arboréto, Viçosa</td>
<td>Guaraciaba</td>
</tr>
<tr>
<td>n</td>
<td>–</td>
<td>20</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>L</td>
<td>614.0</td>
<td>607 ± 14.9 (590-639)</td>
<td>612 ± 34.3 (540-663)</td>
<td>689 ± 31.1 (626-761)</td>
<td>509 ± 37.7 (442-578)</td>
<td>572 ± 43.4 (509-668)</td>
<td>592 ± 36.2 (528-651)</td>
<td>627 ± 47.5 (524-698)</td>
<td>574 ± 35.8 (516-614)</td>
</tr>
<tr>
<td>a</td>
<td>32.0</td>
<td>33.8 ± 3.9 (26.4-42.7)</td>
<td>29.2 ± 3.8 (24.0-38.1)</td>
<td>26.6 ± 2.0 (24.7-31.1)</td>
<td>34.4 ± 12.0 (23.3-67.2)</td>
<td>33.8 ± 11.1 (22.6-71.2)</td>
<td>29.9 ± 2.8 (22.0-30.5)</td>
<td>30.0 ± 3.9 (23.3-38.0)</td>
<td>31.4 ± 3.3 (27.8-38.9)</td>
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<tr>
<td>c</td>
<td>14.3</td>
<td>14.2 ± 0.7 (10.1-16.5)</td>
<td>14.0 ± 3.0 (9.9-22.1)</td>
<td>15.2 ± 1.6 (12.2-18.6)</td>
<td>13.6 ± 2.5 (7.3-16.5)</td>
<td>14.6 ± 2.6 (7.5-19.5)</td>
<td>14.3 ± 3.3 (8.0-24.0)</td>
<td>13.2 ± 4.0 (7-20)</td>
<td>14.6 ± 1.9 (11.8-29.6)</td>
</tr>
<tr>
<td>c’</td>
<td>3.7</td>
<td>4.1 ± 0.3 (3.3-4.4)</td>
<td>4.1 ± 0.8 (2.3-5.2)</td>
<td>3.7 ± 0.3 (3.3-4.5)</td>
<td>3.8 ± 0.9 (2.8-5.1)</td>
<td>3.8 ± 0.6 (3.0-5.6)</td>
<td>3.5 ± 0.7 (2.1-5.0)</td>
<td>4.5 ± 0.9 (3.3-4.6)</td>
<td>3.5 ± 0.6 (2.4-4.8)</td>
</tr>
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<td>V</td>
<td>74.3</td>
<td>74.6 ± 1.1 (72.7-77.3)</td>
<td>74.6 ± 0.7 (72.8-75.8)</td>
<td>75.4 ± 0.8 (74.3-76.6)</td>
<td>72.3 ± 1.0 (70.0-74.0)</td>
<td>74.4 ± 1.6 (71.4-77.4)</td>
<td>74.2 ± 1.4 (71.5-76.3)</td>
<td>74.4 ± 1.9 (68.0-75.7)</td>
<td>75.0 ± 0.7 (73.7-76.1)</td>
</tr>
<tr>
<td>Stylet</td>
<td>7.7</td>
<td>7.7 ± 0.4 (6.5-8.3)</td>
<td>8.0 ± 0.5 (7.7-9.0)</td>
<td>8.3 ± 0.6 (7.7-9.0)</td>
<td>7.5 ± 0.4 (6.4-7.7)</td>
<td>7.5 ± 0.6 (5.9-8.4)</td>
<td>8.3 ± 0.5 (7.7-9.0)</td>
<td>7.7 ± 0.7 (6.3-8.9)</td>
<td>7.7 ± 0.8 (6.4-9.0)</td>
</tr>
<tr>
<td>DGO</td>
<td>–</td>
<td>2.1 ± 0.6 (1.5-2.6)</td>
<td>1.5 ± 0.5 (1.0-2.6)</td>
<td>1.3 ± 0.5 (0.3-2.6)</td>
<td>1.4 ± 0.6 (0.6-2.6)</td>
<td>1.0 ± 0.4 (0.5-1.9)</td>
<td>1.5 ± 0.5 (0.9-2.6)</td>
<td>1.8 ± 0.9 (1.0-4.0)</td>
<td>1.4 ± 0.6 (0.3-2.6)</td>
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<tr>
<td>PUS%VA</td>
<td>15.4</td>
<td>16.7 ± 1.6 (12.7-19.0)</td>
<td>15.6 ± 2.1 (12.7-19.8)</td>
<td>16.1 ± 1.6 (12.5-19.1)</td>
<td>15.8 ± 2.1 (12.2-20.0)</td>
<td>15.0 ± 2.6 (9.9-20.1)</td>
<td>15.0 ± 2.6 (13.1-19.8)</td>
<td>15.2 ± 2.9 (9.2-19.1)</td>
<td>15.2 ± 2.4 (15.9-26.1)</td>
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<td>PUS/VBD</td>
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<td>1.7 ± 0.2 (1.3-1.9)</td>
<td>1.3 ± 0.2 (1.0-1.5)</td>
<td>1.3 ± 0.2 (1.1-1.8)</td>
<td>1.3 ± 0.2 (1.0-1.9)</td>
<td>1.4 ± 0.3 (1.0-1.7)</td>
<td>1.2 ± 0.3 (0.6-1.6)</td>
<td>1.3 ± 0.3 (0.7-1.9)</td>
<td>1.6 ± 0.2 (1.4-1.9)</td>
</tr>
<tr>
<td>Median bulb height</td>
<td>16.6</td>
<td>14.5 ± 1.3 (12.8-16.6)</td>
<td>13.3 ± 1.4 (11.5-16.6)</td>
<td>13.8 ± 1.1 (11.5-16.0)</td>
<td>12.2 ± 1.4 (10.2-15.4)</td>
<td>13.6 ± 1.4 (10.9-15.8)</td>
<td>14.4 ± 1.2 (12.8-16.6)</td>
<td>14.1 ± 2.0 (9.9-16.8)</td>
<td>13.7 ± 1.5 (11.5-16.6)</td>
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<tr>
<td>Median bulb diam.</td>
<td>7.7</td>
<td>7.7 ± 0.6 (6.4-9.0)</td>
<td>7.9 ± 0.7 (6.4-9.0)</td>
<td>8.4 ± 0.6 (7.7-9.0)</td>
<td>6.8 ± 0.6 (6.4-7.7)</td>
<td>7.1 ± 0.5 (5.9-7.9)</td>
<td>8.2 ± 0.7 (7.0-9.0)</td>
<td>8.0 ± 1.0 (6.9-9.9)</td>
<td>7.6 ± 0.7 (6.4-9.0)</td>
</tr>
<tr>
<td>Head to cardia</td>
<td>139.0</td>
<td>141 ± 6.5 (128-152)</td>
<td>138 ± 6.2 (126-152)</td>
<td>143 ± 5.0 (130-149)</td>
<td>128 ± 6.8 (117-138)</td>
<td>133 ± 4.4 (128-140)</td>
<td>138 ± 8.7 (129-150)</td>
<td>149 ± 9.6 (133-168)</td>
<td>127 ± 8.5 (104-139)</td>
</tr>
<tr>
<td>Anal body diam.</td>
<td>–</td>
<td>–</td>
<td>11.0 ± 1.2 (9.0-12.8)</td>
<td>12.1 ± 0.8 (10.2-13.4)</td>
<td>10.4 ± 1.9 (6.4-15.4)</td>
<td>10.4 ± 1.6 (7.9-14.1)</td>
<td>12.5 ± 1.3 (10.2-14.1)</td>
<td>11.5 ± 2.4 (7.5-15.8)</td>
<td>11.4 ± 1.3 (8.3-12.8)</td>
</tr>
<tr>
<td>Tail length</td>
<td>42.9</td>
<td>43.5 ± 4.7 (38.4-60.2)</td>
<td>45.5 ± 10.1 (38.4-52.5)</td>
<td>45.7 ± 4.0 (30.7-78.1)</td>
<td>39.3 ± 12.0 (30.7-74.2)</td>
<td>40.0 ± 10.4 (24.3-70.4)</td>
<td>43.3 ± 10.2 (35.0-101.9)</td>
<td>52.9 ± 21.2 (31.3-45.4)</td>
<td>39.8 ± 4.3 (31.3-45.4)</td>
</tr>
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</table>
**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.

<table>
<thead>
<tr>
<th>Character</th>
<th><em>M. ibaguensis</em> Cristais, Viçosa</th>
<th><em>Clidemia capitellata</em> Guaraciaba</th>
<th><em>Leandra lacunos</em> Ouro Preto</th>
<th><em>M. albicans</em> Cristais, Viçosa</th>
<th><em>M. calversons</em> Ouro Preto</th>
<th><em>M. corallina</em> Arboreto, Viçosa</th>
<th><em>M. latecrenata</em> Guaraciaba</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paratypes</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>L</td>
<td>554 ± 36.0</td>
<td>548 ± 32</td>
<td>603 ± 34.7</td>
<td>478 ± 24.8</td>
<td>558 ± 40.4</td>
<td>543 ± 26</td>
<td>587 ± 31.3</td>
</tr>
<tr>
<td>a</td>
<td>29.1 ± 5.0</td>
<td>31.2 ± 3.6</td>
<td>34 ± 4</td>
<td>27.9 ± 2.8</td>
<td>30.9 ± 3.2</td>
<td>27.0 ± 2.4</td>
<td>33.7 ± 7.0</td>
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<td></td>
<td>(21.9-36.2)</td>
<td>(25.0-35.7)</td>
<td>(27.8-39.6)</td>
<td>(23.9-32.6)</td>
<td>(27.2-38.9)</td>
<td>(23.3-33.0)</td>
<td>(27.0-55.9)</td>
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<td>c</td>
<td>26.3 ± 7.0</td>
<td>27.7 ± 6.6</td>
<td>27.8 ± 7.7</td>
<td>22.9 ± 2.6</td>
<td>26.1 ± 4.4</td>
<td>27.3 ± 6.9</td>
<td>30.4 ± 5.9</td>
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<td>(17.8-37.6)</td>
<td>(18.8-41.5)</td>
<td>(16.5-43.6)</td>
<td>(17.4-27.0)</td>
<td>(19-38)</td>
<td>(16-41)</td>
<td>(21.2-43.0)</td>
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<tr>
<td>c'</td>
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<td>1.9 ± 0.5</td>
<td>2.1 ± 0.5</td>
<td>2.0 ± 0.4</td>
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<td>(1.0-2.5)</td>
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<td>(1.6-3.6)</td>
<td>(1.4-2.7)</td>
<td>(1.4-2.3)</td>
<td>(1.2-2.6)</td>
<td>(1.5-2.6)</td>
</tr>
<tr>
<td>Stylet</td>
<td>7.5 ± 0.4</td>
<td>7.7 ± 0.4</td>
<td>8.3 ± 0.6</td>
<td>7.3 ± 0.8</td>
<td>7.3 ± 0.7</td>
<td>7.9 ± 0.6</td>
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<td>(5.9-7.9)</td>
<td>(6.4-9.0)</td>
<td>(6.9-8.4)</td>
</tr>
<tr>
<td>DGO</td>
<td>2.0 ± 0.6</td>
<td>1.7 ± 0.6</td>
<td>1.2 ± 0.4</td>
<td>1.4 ± 0.6</td>
<td>1.1 ± 0.2</td>
<td>1.7 ± 0.7</td>
<td>1.8 ± 0.5</td>
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<tr>
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<td>(1.0-2.6)</td>
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<td>(0.9-2.6)</td>
<td>(0.3-2.6)</td>
<td>(0.6-1.3)</td>
<td>(1.0-3.5)</td>
<td>(1.0-2.6)</td>
</tr>
<tr>
<td>Median bulb</td>
<td>14.0 ± 2.1</td>
<td>12.8 ± 1.5</td>
<td>13.6 ± 1.5</td>
<td>13.3 ± 2.8</td>
<td>13.4 ± 1.9</td>
<td>13.3 ± 1.6</td>
<td>14.1 ± 1.7</td>
</tr>
<tr>
<td>height</td>
<td>(10.2-20.5)</td>
<td>(10.2-14.1)</td>
<td>(11.5-15.4)</td>
<td>(10.2-15.4)</td>
<td>(10-17)</td>
<td>(11.5-15.4)</td>
<td>(11.5-17.8)</td>
</tr>
<tr>
<td>Median bulb</td>
<td>8.4 ± 1.1</td>
<td>7.5 ± 0.8</td>
<td>7.6 ± 0.6</td>
<td>7.7 ± 1.7</td>
<td>7.5 ± 1.1</td>
<td>8.3 ± 0.8</td>
<td>7.7 ± 0.9</td>
</tr>
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<td>diam.</td>
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<td>(6-4.9)</td>
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<td>(7-7.10)</td>
<td>(6.4-9.0)</td>
</tr>
<tr>
<td>Head to car dia</td>
<td>136 ± 8.9</td>
<td>134 ± 6.4</td>
<td>140 ± 9.8</td>
<td>135 ± 11</td>
<td>134 ± 25.6</td>
<td>132 ± 12.9</td>
<td>140 ± 15.8</td>
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<tr>
<td></td>
<td>(114-150)</td>
<td>(123-142)</td>
<td>(119-152)</td>
<td>(114-155)</td>
<td>(50-175)</td>
<td>(95-147)</td>
<td>(101-173)</td>
</tr>
<tr>
<td>Bursa</td>
<td>48.7 ± 5.8</td>
<td>49.9 ± 3.9</td>
<td>49.3 ± 3.6</td>
<td>42.0 ± 3.8</td>
<td>45.8 ± 3.2</td>
<td>48.0 ± 4.5</td>
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<td>(41-55)</td>
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<td>Spicules</td>
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<td>18.6 ± 2.1</td>
<td>17.9 ± 0.8</td>
<td>16.8 ± 1.4</td>
<td>18.2 ± 2.2</td>
<td>18.6 ± 1.5</td>
<td>19.6 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>(19.4-23.7)</td>
<td>(16.6-25.6)</td>
<td>(16.6-19.2)</td>
<td>(14-19.2)</td>
<td>(13.8-21.8)</td>
<td>(15.4-21.8)</td>
<td>(16.8-20.3)</td>
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<tr>
<td>Gubernaculum</td>
<td>5.4 ± 0.9</td>
<td>5.4 ± 0.8</td>
<td>5.3 ± 1.0</td>
<td>5.2 ± 1.1</td>
<td>4.9 ± 0.5</td>
<td>5.7 ± 1.0</td>
<td>5.3 ± 0.8</td>
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<td>(3.8-7.0)</td>
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<td>(3.8-6.4)</td>
</tr>
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<td>Tail length</td>
<td>22.2 ± 4.9</td>
<td>20.7 ± 4.7</td>
<td>23.2 ± 6.2</td>
<td>21.1 ± 2.7</td>
<td>19.7 ± 2.5</td>
<td>21.1 ± 5.2</td>
<td>19.9 ± 3.5</td>
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<td>(14.1-32.0)</td>
<td>(14.2-29.4)</td>
<td>(14.1-37.1)</td>
<td>(17.3-28.2)</td>
<td>(15.4-23.7)</td>
<td>(12.8-33.3)</td>
<td>(12.9-26.9)</td>
</tr>
</tbody>
</table>
Ditylenchus gallaeformans sp. n. from Brazil and Costa Rica

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 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. 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.
Ditylenchus gallaeformans sp. n. from Brazil and Costa Rica

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 Anguiniidae. 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 Anguiniidae 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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**References**

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