FIRST REPORT OF *DITYLENCHUS GALLAEFORMANS* (TYLENCHIDA: ANGUINIDAE) INDUCING GALLS ON *CLIDEMIA FENDLERI* (MELASTOMATACEAE) FROM VENEZUELA

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ABSTRACT

Morales-Montero, P., S. Flores, S. A. Subbotin and E. San-Blas. 2013. First report of *Ditylenchus gallaeformans* (Tylenchida: Anguinidae) inducing galls on *Clidemia fendleri* (Melastomataceae) from Venezuela. Nematropica 43:241-246.

Ditylenchus gallaeformans was found in a tropical rainforest in the central part of Cordillera de la Costa from Venezuela as a parasite of *Clidemia fendleri* (Melastomataceae) causing deformations and galls on inflorescences and abaxial surface of the leaves. Morphological, morphometrical and molecular characterization of this population is provided. The PCR-D2-D3-28S-RFLP diagnostic profile for *D. gallaeformans* generated by six restriction enzymes is presented. *Clidemia fendleri* being an endemic plant of Venezuela is a new host of *D. gallaeformans*. This finding is a first record of this nematode in Venezuela.

Key words: nematode, parasite-host interaction, RFLP, rainforest

RESUMEN

Morales-Montero, P., S. Flores, S. A. Subbotin and E. San-Blas. 2013. Primer reporte de *Ditylenchus gallaeformans* (Tylenchida: Anguinidae) induciendo agallas en *Clidemia fendleri* (Melastomataceae) en Venezuela. Nematropica 43:241-246.

Ditylenchus gallaeformans (Anguinoidea: Anguinidae) fue encontrado en un bosque tropical lluvioso de la parte central de la Cordillera de la Costa de Venezuela, parasitando *Clidemia fendleri* (Melastomataceae) e induciendo agallas en inflorescencias y en la cara abaxial de las hojas. La caracterización morfológica, morfométrica y molecular es proporcionada. El perfil diagnóstico PCR-D2-D3-28S-RFLP para *D. gallaeformans*, generado por el uso de seis enzimas de restricción, también se presenta. *C. fendleri* es una planta endémica de Venezuela, por lo tanto, se la considera como un nuevo hospedador para *D. gallaeformans*. Por otro lado, este hallazgo representa el primer reporte de este nematodo para Venezuela.

Palabras clave: nematodo, interacción parasito-hospedador, RFLP, bosque lluvioso.

INTRODUCTION

The genus *Ditylenchus* Filipjev, 1936 (Tylenchida, Anguinoidea, Anguinidae) contains nearly 60 species, some of them considered as agricultural important plant parasites (Siddiqi, 2000). Although several *Ditylenchus* species are reported from Central and South America, only one species of this genus, *D. dipsaci* parasitizing onion and garlic, has been found in Venezuela (Crozzoli, 2002). In 2009, a neotropical nematode *Ditylenchus gallaeformans* (Oliveira *et al*., 2013). attacking several species of invasive melastomatacean weeds was found in Brazil and Costa Rica (Santin *et al*., 2009) and later the description of this species was published by Oliveira *et al.* (2013). The nematode induces formation of gall-like structures on the foliage and inflorescences. The host range includes nine Melastomataceae species (*Miconia ibaguensis*, *M. albicans*, *M. calvescens*, *M. coralline*, *M. latecrenata*, *M. mendoncae*, *Clidemia capitellata*, *C. hirta*, and *Leandra lacunosa*).

During our nematological survey, a new population of *D. gallaeformans* was isolated from a Venezuelan tropical rainforest. This nematode parasitizes *Clidemia fendleri* Cogn. plants and induces moderate galls on

the inflorescences and the abaxial surface of the leaves. *Clidemia fendleri* is an endemic plant of Venezuela and, thus, it should be considered as a new host for this nematode.

MATERIAL AND METHODS

Nematode Isolation and Morphological Study

Leaves and inflorescences of *C. fendleri* showing the typical galls induced by *D. gallaeformans* were isolated from the edge of a pathway which crosses a tropical rainforest located at Instituto Venezolano de Investigaciones Científicas (10°23'45.23''N; 66°59'17.7''W); at 1700 mamsl. The galls were cut off from the healthy material and finely divided in a Petri dish with Ringer´s solution. After a few hours, some of the nematodes were collected from the dishes and observed alive, the rest were heat killed at 60°C and fixed with TAF fixative (Courtney *et al.*, 1955). Fixed nematodes were put in a Syracuse glass with 5% glycerol and left for 15 days until water evaporated and pure glycerol remained. The Syracuse glasses were partially covered to allow a slow dehydration of the samples. All measurement and pictures were done using a Leica DM2500 compound microscope fitted with a differential interference contrast system.

Molecular Characterization

DNA was extracted from several dried specimens using the proteinase K protocol. Detailed protocols for PCR, cloning and sequencing of nematode samples were as described by Tanha Maafi *et al.* (2003). The forward D2A (5'-ACAAGTACCGTGAGGGAAAGTTG - 3') and the reverse D3B (5'- TCGGAAGGAACCAGCTACTA - 3') primers were used for amplification of the D2-D3 expansion segments of the 28S rRNA gene (Subbotin *et al.*, 2006). The newly obtained sequence was deposited in GenBank under the accession number KF494346. The new sequence was aligned using ClustalX 1.83 with default parameters and compared with corresponding published gene sequences of *D. gallaeformans* (Oliveira et al. 2013).

Several µl of D2-D3-28S rDNA PCR product were digested by one of the following restriction enzymes: *Alu*I, *Ava*I, *Bsh*1236I, *Bse*NI, *Bsu*RI or *Mva*I (Fermentas) in the buffer stipulated by the manufacturer. The digested DNA was run on a 1.3% TAE buffered agarose gel, stained with ethidium bromide, visualized on a UV transilluminator and photographed. Two *Ditylenchus* samples were also tested in duplex PCR with species-specific primers for *D. gallaeformans* and *D. drepanocercus*. The PCR mixture was prepared as described in Tanha Maafi *et al*. (2003). The universal forward TW81 (5' -GTTTCCGTAGGTGAACCTGC - 3') primer was used in PCR with combinations of the specific reverse D_drep (5' - TCAGCCAAGCCAGACAAGTCAGT - 3') and the specific reverse D_gall (5' - TGGCACACTCTTGGACTGATGCT – 3') primers for diagnosis of *D. drepanocercus* and *D. gallaeformans* (Oliveira *et al*. 2013), respectively.

RESULTS AND DISCUSSION

Description

Morphometrics of adult stage *D. gallaeformans* are presented in Table 1.

Female

Body slender. Lateral fields are sometimes visible in light microscopy but the number of incisures is not distinguishable. Vulva a transverse slit, lips continuous with body wall. Reproductive tract prodelphic. Ovary sometimes exceeding basal bulb, outstretched or reflected (Fig. 1A), oocytes arranged in a single row, spermatheca, uterine quadricolumella and post uterine sac are distinct (Fig 1C). Tail short and conoid. Eggs are oval (Fig 1B), their length average about 2.6 times longer than body diameter and 3 times longer than wide.

Male

Body slender, slightly curved ventrally at posterior end, almost straight shaped when heat-killed. Cuticle finely striated. Lateral fields are sometimes visible in light microscopy but the number of incisures is not distinguishable. Head truncate to slightly round, continuous with body, slightly sclerotized. Stylet with rounded basal knobs. Pharynx with subcylindrical procorpus, median bulb muscular, oval-shaped and very distinct, valvated in the anterior one-third, isthmus surrounded by a nerve ring. Basal bulb distinct, offset from intestine, cardia not observed. Excretory pore always posterior to nerve ring, ranging from 48% to 65% of the length from anterior body end to pharynx base. Testis outstretched or reflexed at anterior end. Bursa with two lobes extending to tail tip (Fig. 1E, D). Spicules paired, curved with distinctive hook-like curvature in the tip.

The description and measurements of the new population of *D. gallaeformans* fit almost all the morphological and morphometrical characteristics provided by Oliveira *et al.*, (2013), except for minor differences: average index "PUS%VA" for females is slightly larger (21.3 ± 7 (10.2 – 38.6) vs 16.7 ± 1.6 (12.7-19.0)), average index a for males is larger (40.5 ± 5.1 (31.8 – 50.7) vs 29.1 ± 5.0 (21.9 – 36.2)) and average index c´ for males is also larger (3.2 ± 0.4 (2.0 – 3.6) vs 1.8 ± 0.4 (1.0 -2.5)). We also noticed that the ovaries of many older females were reflexed (Fig. 1).

Symptoms

The symptoms induced by *D. gaellaformans* in *C. fendleri* (Fig 2) were not very severe, as has been reported by Oliveira *et al*. (2013) for other plants. Parasitism of *D. gallaeformans* on *C. fendleri* seems to be more intense in inflorescences rather than leaves. Galls were never longer than 2 cm in dia (Fig 2) and some leaves have several galls merged together.

Molecular Characterization

The D2-D3 segment of 28S rRNA gene sequence obtained from *D. gallaeformans* of Venezuela showed 100% similarity with the GenBank sequence JQ429771 from this species from Brazil. Amplification of the sequence using D2A and D3B primers from the *D. gallaeformans* samples yielded a single fragment of ca 780 bp in length. The molecular diagnostic profile for *D. gallaeformans* generated by six restriction enzymes is presented in Fig. 3. The results of multiplex PCR with species-specific primers for *D. gallaeformans* and *D. drepanocercus* are given in Fig. 4. The PCR in two samples (lanes 1 and 2) yielded an amplicon of ca 173 bp in length, which corresponded to a specific product for *D. gallaeformans*.

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Table 1: Morphometrics of females and males of *Ditylenchus gallaeformans* parasitizing *Clidemia fendleri*. All measurements are in µm and in the form: mean ± s.d. (range).

Character	Females (Venezuelan population)	Females (Type population, Oliveira *et al*. (2013))	Males (Venezuelan population)	Males (Type population, Oliveira *et al*. (2013))
N	25	20	25	20
L	683 ± 59.2 (590 - 802)	607 ± 14.9 (590 - 639)	608 ± 44.4 (536 - 676)	554 ± 36.0 (503 - 614)
Width	18.6 ± 3.1 (13.1 - 24.6)	-	14.8 ± 2.2 (10.6 - 18.6)	-
a	38.0 ± 5.6 (29.7 - 49.8)	33.8 ± 3.9 (26.4 - 42.7)	40.5 ± 5.1 (31.8 - 50.7)	29.1 ± 5.0 (21.9 - 36.2)
c	15.6 ± 2.4 (11.9 - 22.5)	14.2 ± 0.7 (10.1 - 16.5)	19.3 ± 2.4 (16.5 - 26.6)	26.3 ± 7.0 (17.8 - 37.6)
c´	4.9 ± 1.5 (3.0 - 10.5)	4.1 ± 0.3 (3.3 - 4.4)	3.2 ± 0.4 (2.0 - 3.6)	1.8 ± 0.4 (1.0 - 2.5)
Stylet	7.4 ± 0.4 (6.0 - 8.1)	7.7 ± 0.4 (6.5 - 8.3)	7.1 ± 0.7 (5.7 - 8.1)	7.5 ± 0.4 (6.4 - 8.3)
Median bulb height	12.7 ± 1.2 (10.2 - 14.2)	14.5 ± 1.3 (12.8 - 16.6)	12.2 ± 1.2 (10.2 - 14.2)	14.0 ± 2.1 (10.2 - 20.5)
Median bulb diameter	8.0 ± 1.0 (6.3 - 10.0)	7.7 ± 0.6 (6.4-9.0)	7.3 ± 0.6 (6.2 - 8.6)	8.4 ± 1.1 (7.0 - 11.5)
Head to cardia	147 ± 12.3 (116 - 172)	141 ± 6.5 (128 - 152)	145 ± 14.0 (95 - 163)	136 ± 8.9 (114 - 150)
V	76.0 ± 2.0 (69.4 - 78.7)	74.6 ± 1.1 (72.7 - 77.3)	-	-
Post uterine sac (PUS)	23.4 ± 6.2 (12.4 - 40.2)	-	-	-
PUS%VA	21.3 ± 7.1 (10.2 - 38.6)	16.7 ± 1.6 (12.7 - 19.0)	-	-
PUS/VBD	1.6 ± 0.5 (0.7 - 3.2)	1.7 ± 0.2 (1.3 - 1.9)	-	-
Bursa	-	-	45.9 ± 11.2 (24.2 - 84.2)	
Spicules	-	-	17.9 ± 1.4 (15.2 - 21.1)	
Gubernaculum	-	-	5.8 ± 0.7 (4.0 - 7.0)	
AWD	9.7 ± 1.8 (3.9 - 13.4)	-	11.5 ± 1.5 (8.8 - 14.7)	
Tail length	43.9 ± 6.9 (27.9 - 56.3)	43.5 ± 4.7 (38.4 - 60.2)	28.6 ± 3.3 (22.2 - 34.7)	
Eggs length (n=8)	47.6 ± 13.8 (74.4 - 35.8)	-	-	-
Eggs width (n=8)	14.6 ± 1.4 (16.4 - 11.8)	-	-	-

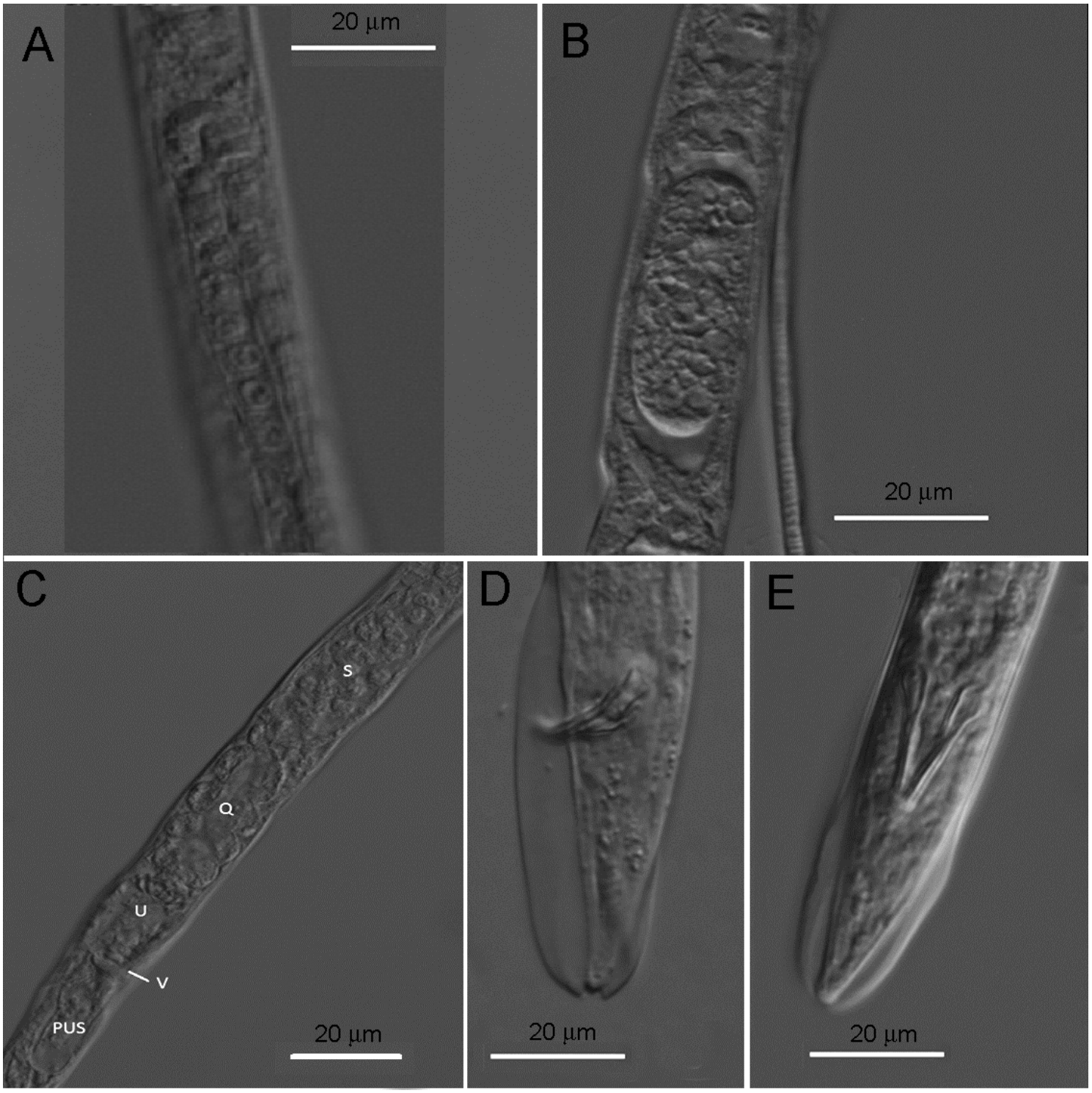

Fig. 1. Microphotographs of *Ditylenchus gallaeformans* females and males. A) Reflexed ovary; B) Egg within a female body; C) Reproductive tract of female: s - spermatheca, q - quadricolumella, u - uterus, v - vulva, pus - post uterine sac; D, E) Bursa.

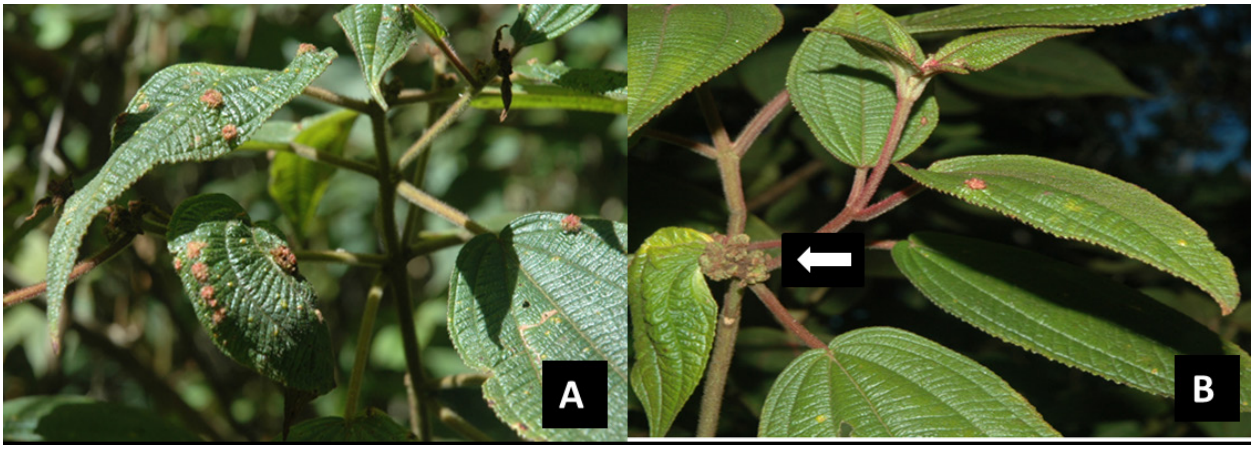

Fig. 2. *Clademia fendleri* infected by *Ditylenchus gallaeformans*. A) Foliar galls; B) severe gall formation at inflorescence (arrow).

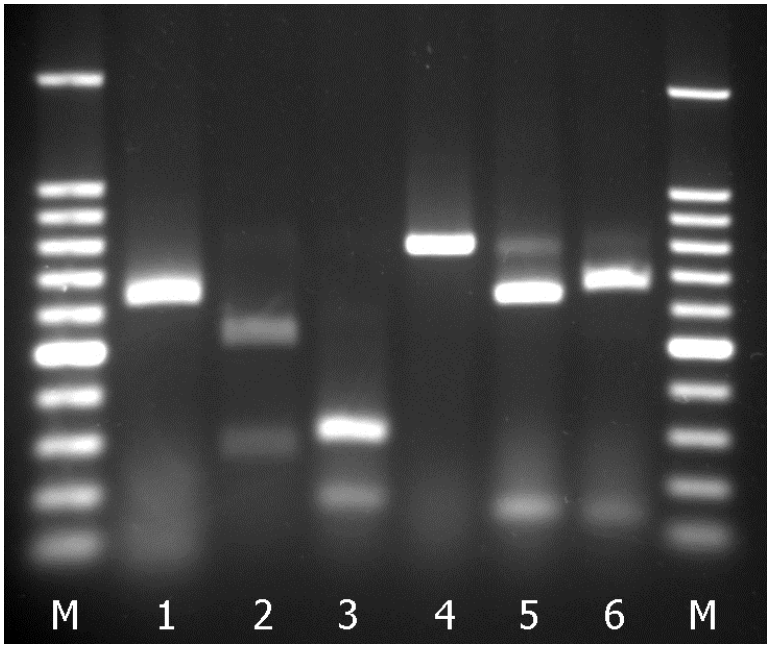

Fig. 3. Diagnostic PCR-D2-D3-28S-RFLP profile for *Ditylenchus gallaeformans*. Lanes: M = 100 bp DNA marker (Promega), 1 - *Alu*I, 2 - *Ava*I, 3 - *Bsh*1236I, 4 - *BseN*I, 5 – *BsuR*I, 6 - *Mva*I.

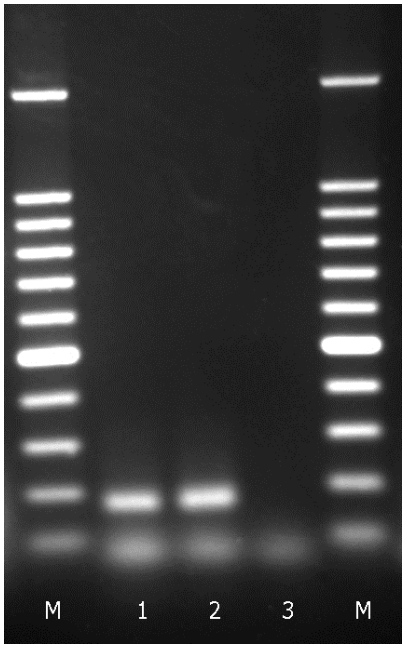

Fig. 4. Gel with specific amplicon obtained from multiplex PCR (TW81 + D_gall +D_drep primers) with species-specific primers for *Ditylenchus gallaeformans* and *D. drepanocercus*. Lanes: M: 100 bp DNA marker (Promega); 1, 2: *D. gallaeformans*; 3: control without DNA.