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Descriptions of *Deladenus albizicus* n. sp. and *D. processus* n. sp. (Nematoda: Hexatylina) from Haryana, India

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Abstract: Two different nematodes were isolated from the bark of *Albizia lebbeck* trees; one from insect infested and another from noninfested, healthy tree. Based on the biological, morphological, and molecular evidences, the nematodes are described as *Deladenus albizicus* n. sp. and *D. processus* n. sp. (Nematoda: Hexatylina). *Deladenus albizicus* n. sp., isolated from insect-infested tree, multiplied on the fungus *Nigrospora oryzae*. Myceliophagous females of this nematode reproduced by parthenogenesis and spermathecae were indistinct. Infective females, readily produced in the cultures, are dorsally curved. Only one type of males containing small-sized sperms in their genital tracts were produced in the culture. Myceliophagous females: L = 0.75 to 1.71 mm, a = 32.3 to 50.8, b = 9.3 to 11.2, b' = 5.2 to 7.3, c = 27.2 to 35.6, V = 91.0 to 93.3, c' = 2.0 to 2.9, stylet = 11 to 12 μm, excretory pore in the region of median pharyngeal bulb, 43 to 47 μm anterior to hemizonid. *Deladenus processus* n. sp., isolated from bark of healthy *A. lebbeck* tree, was cultured on *Alternaria alternata*. Myceliophagous females reproduced by amphimixis and their spermathecae contained rounded sperms. Infective females were never produced, even in old cultures. Myceliophagous females: L = 0.76 to 0.99 mm, a = 34 to 49, b = 13.3 to 17.7, b' = 3.8 to 5.8, c = 19.6 to 22.8, V = 92.2 to 93.5, c' = 2.7 to 3.5, stylet = 6 to 7 μm, excretory pore in the proximity of hemizonid, tail conoid, tapering from both sides to a long pointed central process. It is proposed to classify *Deladenus* species in three groups: *durus*, *siricidicola*, and *laricis* groups based on female and spermatogonia dimorphism, mode of reproduction, and insect parasitism.

Key words: Albizia lebbeck, *Alternaria alternata*, *Beddingia*, biology, *COI*, *D2D3*, *Deladenus processus* n. sp., *D. albizicus* n. sp., entomoparasitic nematode, *ITS*, *Nigrospora oryzae*, *Phaenopsitylenchus*, taxonomy.

Researchers became interested in studying *Deladenus* species ever since Bedding and Akhurst (1974) showed the biocontrol potential of *Deladenus siricidicola* Bedding, 1968 for the management of *Sirex noctilio* Fabricius. *D. siricidicola* and related species have life history involving profound female and spermatogonia dimorphism associated with mycophagous and entomophagous generations—both generations reproducing by amphimixis. Blinova and Korenchenko (1986) proposed the genus *Beddingia* for these species and limited *Deladenus* for the species in which only myceliophagous forms were known. They also erected a new genus *Phaenopsitylenchus* in which myceliophagous females reproduced by parthenogenesis and entomoparasitic generation by amphimixis. They also proposed family Phaenopsitylenchidae to accommodate *Phaenopsitylenchus* and *Beddingia*. Chitambar (1991) synonymized *Beddingia* and Siddiqi (2000) *Phaenopsitylenchus* with *Deladenus*. However, Poinar et al. (2002) while describing a genus *Elaeolenchus* considered *Phaenopsitylenchus* as a valid genus. They also recognized *Beddingia* and proposed a different family Beddingiidae for this genus. Bedding and Iede (2005) preferred to use *Beddingia siricidicola* than *D. siricidicola*. However, recently described species with dimorphic females have been assigned to the genus *Deladenus* (Nasira et al., 2013; Yu et al., 2013, 2014).

Molecular data are presently available for *D. proximus* Bedding, 1974, *D. siricidicola* and *D. prorsus* Yu et al., 2013 (Yu et al., 2009, 2012, 2013; Morris et al., 2013). All these species have dimorphic females with amphimixis mode of reproduction in both the generations.

Here we present morphological and molecular accounts of two species of *Deladenus sensu lato* that were collected from *Albizia lebbeck* (L.) Willd trees growing at CCS Haryana Agricultural University Campus, Hisar, India.

Materials and Methods

Isolation, culturing, and developmental biology of nematodes: Bark of *A. lebbeck* (along with frass) was collected from the vicinity of holes made by insect borers in the plant trunk. It was incubated in water for 24 hr. Nematodes were then extracted by modified Baermann Funnel Technique. *Deladenus* species (described as *Deladenus albizicus* n. sp. in this paper) present in the samples were handpicked and used for further studies.

For raising culture of these nematodes, fungi growing on the bark were inoculated into several test tubes containing Potato Dextrose Agar (PDA) under aseptic conditions. Pure culture of different fungi growing in these tubes was established by transferring a bit of hyphae from these tubes to newer PDA tubes after 3 d of inoculation. Freshly isolated nematodes were inoculated in tubes in which fungus mat had completely covered the PDA slant. These tubes were daily checked for nematode multiplication under a stereozoom microscope for 15 d. Nematodes were subsequently reared on the fungus (identified as *Nigrospora oryzae* (Berk. and Br.) Petch) on which they had multiplied.

For studying the development of infective stages, 10 gravid females collected from the culture tubes were

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inoculated in fresh culture tubes of *N. oryzae* and incubated at 15°C, 20°C, 25°C, and room temperature. There were five replicates for each temperature. After 30 d, 5-ml water was added in each tube. After 10 min, water was gently rinsed and poured in to a counting dish for recording number myceliophagous and infective females, males and juveniles.

For studying mode of reproduction, ten culture tubes were inoculated with fourth stage female juveniles, each tube receiving a single nematode. Tubes were incubated at 25°C and examined after 15 d for the presence of different stages of nematodes.

Another population of *Deladenus* was collected from tree not attacked by insect borers. Bark was sampled from near the cracks at a height of 2 m and was processed for nematode recovery and multiplication as described above. Protocol, as described above, was followed for studying production of infective females and mode of reproduction. However, this nematode multiplied on *Alternaria alternata* (Fr.) Keissler and the same fungus was used as host for further studies. Also gravid females were inoculated in tubes of *A. alternata* cultured on PDA containing 1% lactic acid to record the occurrence of infective stage, if any. This population has been described as *Deladenus processus* n. sp. below.

Taxonomic studies: For conducting taxonomic studies, nematodes were killed and fixed in hot 4% formalin and processed to anhydrous glycerin by slow method.

Molecular characterization and phylogenetic analysis: For molecular analysis, nematode samples were lysed in 10 µl worm lysis buffer—30 mM Tris (pH 8), 8 mM EDTA, 100 mM NaCl, 0.7% NP40, 0.7% Tween 20, Proteinase K 100 µg/ml added just before use (Ahringer, 2006). Two microliter of this supernatant was used for PCR amplification of the *ITS*, *COI*, and *D2D3* markers using the primers listed in Table 1. Reaction conditions for the amplification of *COI* and *LSU* were the same as those in Ye et al. (2007). For the *ITS* gene, the thermal cycling program used by Subbotin et al. (2001, 2006) was followed. The PCR product was checked on a gel and purified from the gel using QIAquick Gel Extraction Kit (Qiagen, Valencia, CA) and cloned into PCR2.1 TA cloning vector (Invitrogen, Carlsbad, CA) was transformed into *Escherichia coli*, and clones were screened using a blue–white screening using TOPO TA Cloning Kit (Invitrogen). The positive clones were verified by PCR prior to extraction of plasmids (Qiagen Miniprep Kit, Qiagen). To ensure good sequence quality, at least two clones were sequenced for each specimen in forward and reverse directions. The plasmids were sent for sequencing using M13 forward and reverse primers supplied with the TOPO TA Cloning Kit.

The raw sequences were manually checked and edited for quality using MEGA6, aligned, and a consensus sequence was generated for each gene using Bioeditsoftware (Tom Hall; Ibis Biosciences, Carlsbad, CA). The evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013). The sequences were aligned using ClustalW, and evolutionary history was inferred by Maximum Likelihood method using *COI* and *ITS* sequences (Hasegawa et al., 1985; Saitou and Nei, 1987). The bootstrap consensus tree inferred from 1000 replicates (Felsenstein, 1985) was taken to represent the evolutionary history of the nematodes. *Drosophila* parasite, *Howardula aoronymphium*, was used as the out-group.

The sequences have been deposited in the NCBI GenBank with accession numbers KM403370-KM403374.

Results and Discussion

Biology: Nematodes isolated from infected-infested plants multiplied only on *N. oryzae*. After 30 d of inoculation of 10 gravid females/tube, 24,273, 25,440, 30,610, and 32,390 nematodes were present in a tube incubated at 15°C, 20°C, 25° C, and room temperature, respectively. Infective females constituted 12.1%, 14.3%, 19.3%, and 20.0% of total population with almost equal number of males at these temperatures, respectively. Microscopic examination of extracted nematodes revealed no spermatheca in mycophagous females, and only small-sized sperms were found in both males and infective females.

In culture tubes of *N. oryzae* receiving single fourth stage female juvenile each, nematode populations comprising myceliophagous and infective females, males and juveniles were observed after 15 d of inoculation. On an average there were 28.3 mycophagous females, 3.2 infective females, 1.6 males, and 408.5 juveniles in a tube. Since female nematode could develop and reproduce in isolation, it is inferred that nematode reproduces by parthenogenesis. Presence of comparatively fewer numbers of infective females indicates that they were either produced by aging females of first

Table 1. PCR amplification primers used in the study.

Primer name	Sequence	Gene	Reference
D2D3 F	ACAAGTACCGTGAGGGAAAGTTG	*LSU*	Subbotin et al. (2006)
D2D3 R	TCGGAAGGAACCAGCTACTA	*LSU*	Subbotin et al. (2006)
TW81 F	GTTTCCGTAGGTGAACCTGC	*ITS*	Subbotin et al. (2001)
AB28 R	ATATGCTTAAGTTCAGCGGGT	*ITS*	Subbotin et al. (2001)
LCO1490	GGTCAACAAATCATAAAGATATTGG	*COI*	Folmer et al. (1994)
HC02198	TAAACTTCAGGGTGACCAAAAAATCA	*COI*	Folmer et al. (1994)

generation or by second generation mycophagous females. Detailed biology of both these species will be discussed in a separate paper.

Nematodes isolated from healthy 'Siras tree' multiplied on *A. alternata* only. Reproduction took place at all the temperatures tested. However, in no case infective female could be recovered from thousands of specimen that developed. No infective female was even developed in culture tubes wherein PDA was supplemented with 1% lactic acid.

Single female juvenile inoculated in culture tubes developed to adulthood but failed to reproduce. This shows that the mode of reproduction in this species is amphimixis.

Description

Deladenus albizicus n. sp.
(Fig. 1 A–F)

Measurements

Holotype female: L = 0.89 mm, a = 38, b =9.9, b' =5.8, c = 34, V = 91.0, c' = 2.2, stylet = 11 μm.

Paratype females (15): L = 0.91 ± 0.07 (0.75 to 1.71) mm, a = 41.5 ± 5.6 (32.3 to 50.8), b = 10.0 ± 0.6 (9.3 to 11.2), b' = 6.2 ± 0.8 (5.2 to 7.3), c = 31.2 ± 2.6 (27.2 to 35.6), V = 92.1 ± 0.6 (91.0 to 93.3), c' = 2.4 ± 0.32 (2.0 to 2.9), stylet = 11 to 12 μm, tail = 29.0 ± 3.0 (25 to 33) μm.

Paratype males (11): L = 0.73 ± 0.04 (0.65 to 0.80) mm, a = 42.2 ± 5.6 (34.8 to 53.7), b = 8.1 ± 0.4 (7.5 to 8.7), b' = 5.2 ± 0.6 (4.2 to 6.4), c = 27.3 ± 2.1 (24.7 to 31.4), c' = 2.6 ± 0.3 (2.2 to 3.3), stylet = 11 to 12 μm, tail = 27.0 ± 2.1 (29 to 35) μm, spicules = 15 to 16 μm.

Paratype infective females (11): L = 0.64 ± 0.05 (0.51 to 0.73) mm, a = 38.8 ± 6.4 (29 to 51), b = 6.6 ± 0.9 (5.2 to 8.5), b' = 2.4 ± 0.2 (2.2 to 2.7), c = 25.8 ± 2.1 (22.9 to 28.4), V = 91.2 ± 0.7 (90.3 to 92.6), c' = 2.7 ± 0.3 (2.3 to 3.0), stylet = 12 to 14 μm, tail = 24.9 ± 2.0 (22 to 30) μm.

Female: Body slightly ventrally curved on fixation. Lip region low, slightly marked off from rest of boy. Cuticle annulated, lateral fields nearly one-fourth of body width, with seven to eight closely spaced lateral lines. Excretory pore 59.1 ± 2.77 (56 to 62) μm from anterior end, in the region of median bulb, excretory duct not cuticularized, poorly discernible in some

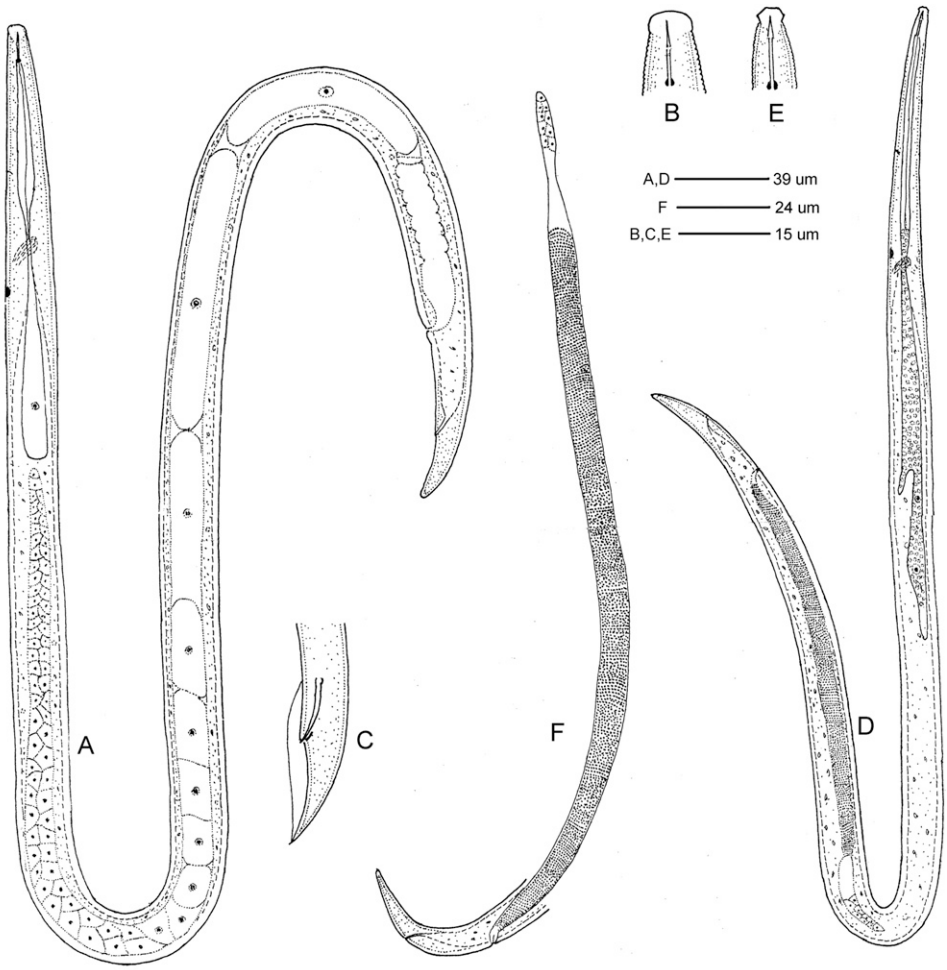

Fig. 1. *Deladenus albizicus* n. sp.: A and B Female: A. Entire. B. Head region. C. Male posterior regions. D–G. Infective female: D. Entire. E. Head region. F. Posterior region and genital tract.

paratypes. Hemizonid posterior to nerve ring, 45.6 ± 1.60 (43 to 47) μm behind excretory pore. Deirids distinct, 1 to 13 μm posterior to hemizonid. Stylet weak, with small, posteriorly directed knobs. Opening of dorsal pharyngeal gland 1 to 2 μm from stylet base. Median pharyngeal bulb only slightly swollen with median chamber, basal bulb 41 to 56 × 12 to 15 μm in size, overlapping intestine mainly laterally, with distinct dorsal gland nucleus. Genital tract outstretched may reach up to median pharyngeal bulb. Oocytes arranged in 1 to 2 to multiple rows in zone of multiplication and in a single row in growth zone. Eggs near vulva elongated-oval, 81 to 112 × 10 to 13 μm in size. Spermatheca indistinct, crustaformeria made up 8 to 12 columns of cells. Vagina almost straight, vulval lips nonprotuberant. Postuterine sac absent. Vulva–anus distance 1.3 to 2.0 times the tail length. Tail conoid, tapering from both sides to a broadly rounded terminus.

Male: Similar to female except for reproductive system. Stylet and pharynx well developed. Testis outstretched, spermatocytes arranged in multiple rows, sperm very small and rounded in distal region of genital tract. Spicules tylenchoid, slightly ventrally curved with pointed tips, 15 to 16 μm in size. Gubernaculum trough shaped. Tail conoid ending in a pointed to mucronate tip, enveloped by caudal alae.

Infective female: Body dorsally curved, "C"-shaped pon fixation. Lip region narrow, conoid. Lateral field narrow, 1/4 to 1/3 of body width, with two lateral lines. Stylet 12 to 14 μm long; anterior conical region short triangular, 3 to 4 μm long; shaft 9 to 10 μm long with wide lumen; and well-developed basal knobs. Opening of dorsal pharyngeal gland 3 to 4 μm from base of stylet. Pharynx slightly swollen in middle, basal pharyngeal bulb enormously developed with dorsal and ventral gland nuclei. Excretory pore near middle of median pharyngeal bulb, 56 to 61 μm from anterior end, and 40 to 55 μm anterior to hemizonid. Ovary with 8 to 12 ovarian cells arranged in 1 to 2 rows; remaining part of genital tract filled with minute, rounded sperm as seen in males. Postuterine sac absent. Tail elongated conoid, tapering from both the sides, and ending in narrowly rounded to digitate terminus.

Differential diagnosis: D. albizicus n. sp. differs from *D. laricis* (Blinova and Korenchenko, 1986) Siddiqi, 2000, the only other species in the genus that has dimorphic females and mycophagous females reproducing by parthenogenesis. The new species, however, differs from the latter species in having differently shaped lip region, stylet and tail, position of excretory pore in myceliophagous females, shape of spicules in males, and shape of lip region and tail in infective females (lip region high; stylet knobs inverted "Y" shaped; excretory pore behind nerve ring in myceliophagous females; spicules robust in males; lip region broad; and tail short cylindrical in infective females of *D. laricis*).

Type host and locality: Collected from the bark of *A. lebbeck* infested with bark borers and other insects at CCS Haryana Agricultural University Campus, Hisar, India (N 29°08.723′, E 75°42.275′).

Type material: Holotype mounted on slide *D. albizicus* n. sp./1; paratypes on slides *D. albizicus* n. sp./2 to 7. Two paratype females, two paratype males, and two paratype infective females deposited with National Nematode Collection of India (NNCI), Division of Nematology, Indian Agricultural Research Institute, New Delhi, India. Holotype and rest of paratypes with Department of Nematology, CCS Haryana Agricultural University, Hisar, India.

Deladenus processus n. sp.
(Fig. 2 A–E)

Measurements

Holotype female: L = 0.86 mm, a = 46, b = 13.7, b' = 4.0, c = 19.6, V = 92.6, c' = 3.5, stylet = 7 μm.

Paratype females (12): L = 0.89 ± 0.08 (0.76 to 0.99) mm, a = 42.7 ± 5.46 (34 to 49), b = 15.3 ± 1.55 (13.3 to 17.7), b' = 4.49 ± 0.79 (3.8 to 5.8), c = 21.1 ± 1.08 (19.6 to 22.8), V = 92.7 ± 0.36 (92.2 to 93.5), c' = 3.3 ± 0.32 (2.7 to 3.5), stylet = 6 to 7 μm, tail = 40.8 ± 4.23 (35 to 44) μm.

Paratype males (12): L = 0.71 ± 0.06 (0.55 to 0.78) mm, a = 45.2 ± 5.88 (35 to 57), b = 12.4 ± 0.84 (10.9 to 13.6), b' = 4.1 ± 0.29 (3.6 to 4.4), c = 22.6 ± 1.69 (18.2 to 25.0), c' = 2.76 ± 0.24 (2.2 to 3.1), stylet = 6 to 7 μm, tail = 31.5 ± 1.96 (29 to 35) μm, spicules = 15 to 16 μm.

Female: Body slightly ventrally curved on fixation. Lip region low, slightly marked off from rest of boy. Cuticle annulated, lateral fields nearly one-fourth of body width, with six lateral lines, inner two lines more widely spaced than others. Excretory pore 114 ± 7.39 (100 to 124) μm from anterior end, in the vicinity of nerve ring, excretory duct cuticularized. Hemizonid just anterior excretory pore. Deirids distinct, 0 to 8 μm posterior to hemizonid. Stylet small with rounded knobs. Opening of dorsal pharyngeal gland 1 to 2 μm from stylet base. Median pharyngeal bulb hardly discernible, median corporeal chamber absent, basal bulb elongated, overlapping intestine, with distinct dorsal gland nucleus. Genital tract outstretched, may reach up to median pharyngeal bulb. Oocytes arranged in a single row. Spermatheca distinct, elongated-oval, 35 to 45 × 17 to 25 μm in size, with large rounded sperm. Crustaformeria made up 8 to 12 columns of cells. Vagina almost straight, vulval lips protuberant. Postuterine sac absent. Vulva–anus distance 0.46 to 0.0.72 times the tail length. Tail conoid, tapering from both sides to a long pointed central process.

Male: Similar to female except for reproductive system. Stylet and pharynx well developed. Testis outstretched, spermatocytes arranged in multiple rows, sperms large, rounded in distal region of genital tract.

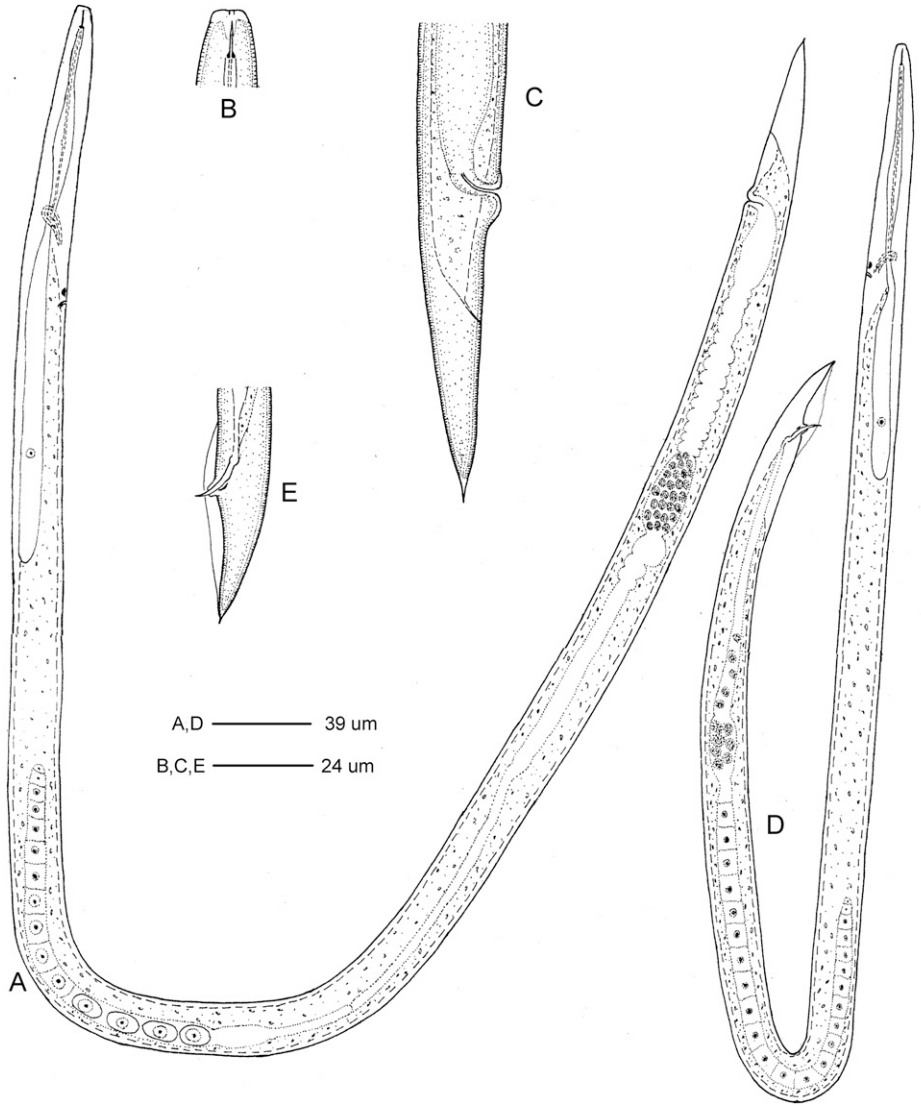

Fig. 2. *Deladenus processus* n. sp. A–C. Female: A. Entire. B. Head region. C. Posterior region. D–E. Male: D. Entire, E. Tail.

Spicules tylenchoid, distinctly cephalated and with pointed tips, 15 to 16 µm long. Gubernaculum trough shaped, 3 to 4 µm long. Tail conoid with a mucronate terminus, enveloped by caudal alae.

Differential diagnosis: D. processus n. sp. comes close to *D. aridus* Andrassy, 1957, *D. megacondylus* (Mulvey, 1969) Sumenkova, 1975, *D. durus* (Cobb, 1922) Thorne, 1941, *D. obesus* Thorne, 1941, *D. parvus* Zell, 1985, and *D. saccatus* Andrassy, 1954 in having only mycophagous generation in life cycle and excretory pore lying posterior to hemizonid. It differs from *D. saccatus* and *D. megacondylus* in lacking postvulval uterine sac and from *D. parvus* in position of vulva and tail shape (V = 80 to 85, tail tip with tubular appendix in *D. parvus*). New species differs from *D. aridus* and *D. obesus* in number of lateral lines and tail shape (lateral lines usually four, tail tip arcuate in *D. aridus* and bluntly rounded in *D. obesus*). *D. processus* n. sp. comes more close to *D. durus* in having differently shaped tail, value of c', absence of corpus median chamber, presence of sperms in spermatheca in females, and common occurrence of males with shorter spicules (tail usually broadly to narrowly rounded, c' = 1.6 to 2.8, spermatheca without sperm, corpus median chamber present, males very rare, spicules = 24 µm in *D. durus*).

Type host and locality: Collected from the bark of *A. lebbeck* at CCS Haryana Agricultural University Campus, Hisar, India (N 29°08.723′, E 75°42.275′).

Type material: Holotype mounted on slide *D. processus* n. sp./1; paratypes on slides *D. processus* n. sp./2 to 7. Two paratype females and two paratype males deposited with NNCI, Division of Nematology, Indian Agricultural Research Institute, New Delhi, India. Holotype and rest of paratypes with Department of Nematology, CCS Haryana Agricultural University, Hisar, India.

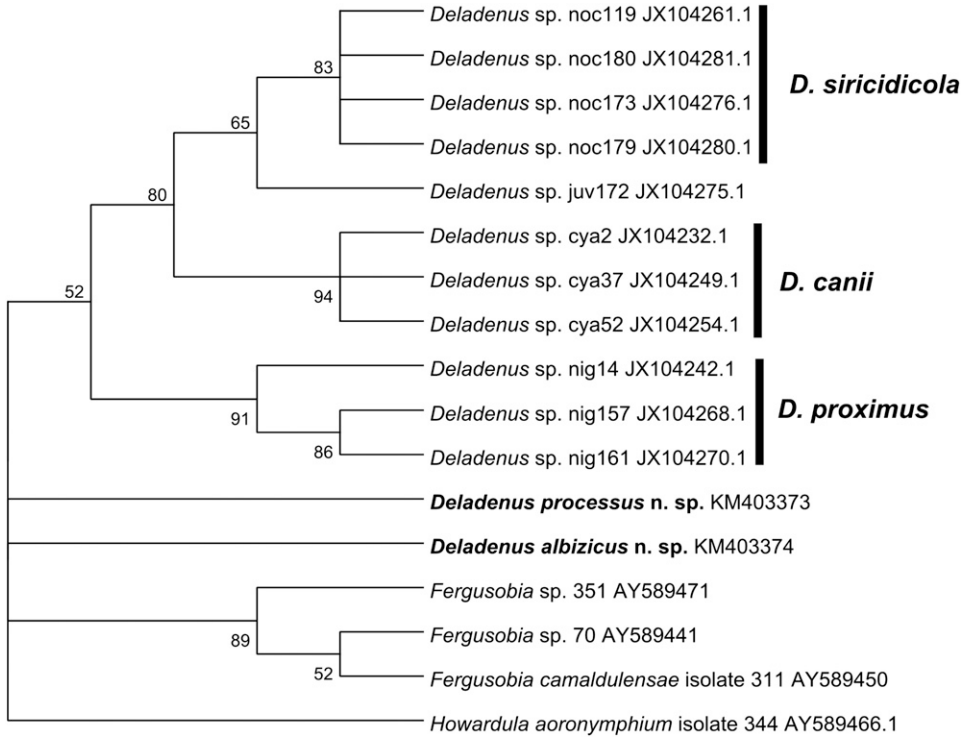

FIG. 3. Evolutionary relationships of *Deladenus albizicus* n. sp. and *D. processus* n. sp. using the mitochondrial *cox*1 sequences. The evolutionary history was inferred by using the Maximum Likelihood method based on the Hasegawa–Kishino–Yano model. DNA sequences of the related taxa were taken for comparison from the NCBI GenBank and from Morris et al. (2013). The bootstrap consensus tree inferred from 1,000 replicates is taken to represent the evolutionary history of the analyzed taxa. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying neighbour-joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (five categories [+*G*, parameter = 0.2494]). The analysis involved 17 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 317 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

Molecular characterization: Phylogenetic analysis of the mitochondrial *COI* sequence from both the nematodes and *ITS* sequence from nematode growing on *N. oryzae* (*D. albizicus* n. sp) showed that the sequences were highly divergent from other *Deladenus* species, and the sequences represented novel taxa. The phylogenetic analysis shows that these two nematodes did not group with other described species of *Deladenus* but branched out separately from all other sequences tested (Figs. 3,4). The dataset gave similar results across all tree reconstruction algorithms. Molecular phylogenetic analysis support the morphological and biological observations that that these two species represent novel species of *Deladenus*. The *ITS* region could not be amplified from nematode growing on *A. alternaria* (*D. processus* n. sp.) even after repeated trials. The tree based on D2D3 expansion region of the 28s rRNA gene was not able to resolve any phylogenetic differences, as has been found earlier (Morris et al., 2013).

These two species represent different groups of *Deladenus*—*D. processus* n. sp. has only myceliophagous generation in life cycle and females are monomorphic. *D. albizicus* n. sp. has dimorphic females: myceliophagous and entomoparasitic, but unlike other species of *Deladenus* having dimorphic females, it reproduces by parthenogenesis and infective females are dorsally curved.

Genus *Deladenus* presently contains species that can be classified in three groups: Group 1—*durus* group for species having only mycophagous generation and reproducing by amphimixis or parthenogenesis; Group 2—*siricidicola* group for *D. canii* Bedding, 1974, *D. cocophilus* Nasira et al., 2013, *D. imperialis* Bedding, 1974, *D. nevexii* Bedding, 1974, *D. proximus*, *D. prorsus*, *D. rudyi* Bedding, 1974, *D. siricidicola*, *D. valveus* Yu et al., 2014, and *D. wilsoni* Bedding, 1968. These species have amphimictic mycophagous and entomoparasitic generations, distinct female and spermatogonia dimorphism, and ventrally curved infective females; Group 3—*laricis* group containing *D. laricis* and *D. albizicus* n. sp. They have dimorphic parthenogenetic mycophagous and amphimictic infective females, no dimorphism of spermatogonia, and dorsally curved infective females. In fact, Blinova and Korenchenko (1986) splitted the genus *Deladenus* into *Deladenus* and *Beddingia* and also proposed a new genus *Phaenopsitylenchus* for these

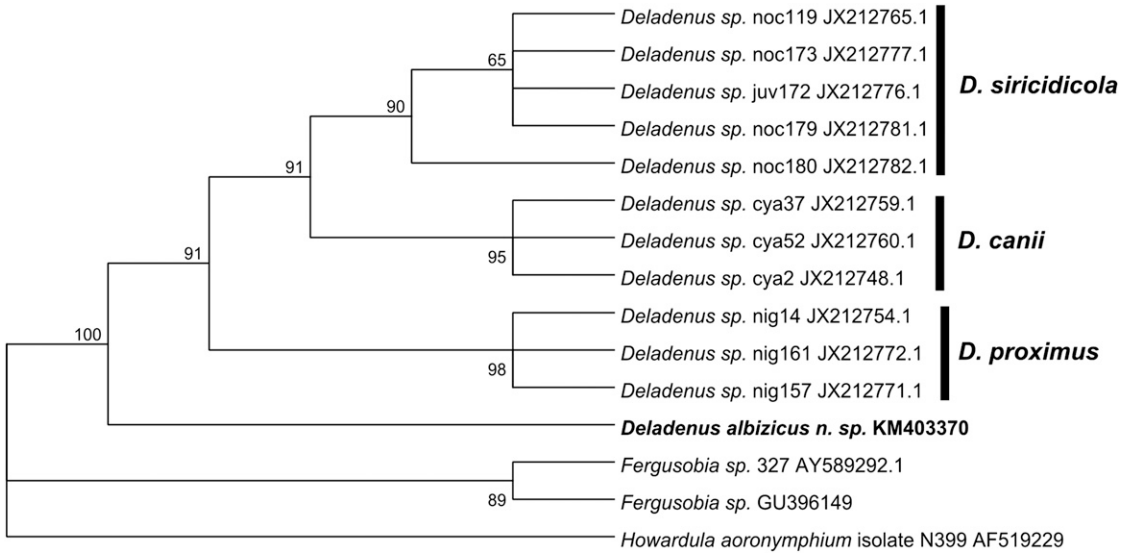

Fig. 4. Evolutionary relationships of *Deladenus albizicus* n. sp. using ITS sequences. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model. DNA sequences of the related taxa were taken for comparison from the NCBI GenBank and from Morris et al. (2013). The tree with the highest log likelihood (−2319.2222) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained by applying the neighbour-joining algorithm to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach. The analysis involved 15 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 506 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

groups. These three genera are hard to distinguish from one another on the basis of morphology of mycophagous females. Though there are definite differences in the biology and molecular data of species that can be assigned to these genera but such information is available only for a very few species. Till substantial information becomes available on the biology and molecular data of more species; genera *Beddingia* and *Phaenopsitylenchus* are here regarded as junior synonym of *Deladenus* representing species groups only.

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