

## *Deladenus valveus* n. sp. (Nematoda: Neotylenchidae) in Dunnage Wood from South Korea Intercepted in Ningbo, China

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**Abstract:** *Deladenus valveus* n. sp., isolated from packaging wood originated from South Korea and intercepted in Ningbo, P.R. China, is described and illustrated. Both mycetophagous and infective forms were recovered and are described. The new species *D. valveus* n. sp. resembles other *Deladenus* species in which the excretory pore is situated anterior to the hemizonid: in mycetophagous females, the excretory pore is 59 to 74 µm from the anterior end and 37 to 54 µm anterior to the hemizonid. The new species is characterized by the presence of a distinct valve at the esophago-intestinal junction in mycetophagous females and by a degenerate esophagus in mycetophagous males, both of which characters are reported for the first time in a species of *Deladenus*.

**Key words:** *Deladenus*, morphology, morphometrics, new species, taxonomy.

*Deladenus* Thorne, 1941 comprises two groups of species: one consisting of species with mycetophagous forms only (fungal feeding), and the other with both mycetophagous and infective forms (insect parasites). There is enhanced interest in studying insect parasitic species as potential biological control agents for managing insect pests in forests, partly because *D. siricidicola* Bedding 1968 is used successfully for controlling European wood wasps (*Sirex noctilio*), a devastating pest of pines in several countries in the Southern hemisphere. This nematode species was recently discovered in European wood wasps, a recently reported invasive species in Ontario, Canada, and in neighboring states of the United States (Yu et al., 2009, William et al., 2012). In addition, *D. proximus* Bedding 1974 was discovered in *S. nigricolis* in Ontario (Yu et al., 2012). Therefore, an improved understanding of the biodiversity, biology, and taxonomy of the species of this genus is particularly important.

With the recent descriptions of *D. prorsus* Yu, Gu, and Ye 2013 isolated from wooden packaging from Malaysia intercepted in Ningbo, China, and of *D. cocophilus* Nasira, Shahina & Firoza 2013 from coconut fruits in Pakistan, the group of *Deladenus* species that have both infective and mycetophagous forms consists of 10 nominal species, the other eight being *D. canii* Bedding, 1974, *D. imperialis* Bedding, 1974, *D. nevexii* Bedding, 1974, *D. proximus* Bedding, 1974, *D. rudyi* Bedding, 1974, *D. siricidicola*, *D. wilsoni* Bedding, 1968, and *D. minimus* Chizhov and Sturhan, 1998. Descriptions of all of the above include mycetophagous females, males, and infective females described, except for *D. minimus*, for which mycetophagous females, the form most important for diagnostics, were not described.

Countries that are signatories to the International Standards for Phytosanitary Measures (ISPM) are required to heat-treat or fumigate wood packaging to kill all organisms therein before export. This is in

compliance with ISPM Article 15, which is administrated by the International Plant Protection Convention (IPPC). Unfortunately, invasive species continue to be found and intercepted in wood dunnage, pallets, crating, and other wood packaging material at many ports worldwide. *Deladenus prorsus* was the first species from this genus to be reported from wood packaging. The present study records another species of *Deladenus* from this niche intercepted in Ningbo, but in this case originating from South Korea. The objective of this study was to describe and illustrate this new species as *D. valveus* n. sp.

### MATERIALS AND METHODS

Samples were taken by sawing pieces from wood packaging (dunnage) from lot number 365-3043 were broken into pieces less than 1 cm wide. Nematodes were extracted for 24 hours by a Baermann-funnel technique. The nematodes were heat killed, and fixed in TAF (8% formalin and 2% triethanolamine in distilled water). The fixed nematodes were shipped to the senior author for morphological studies. Nematode specimens fixed in TAF were processed to anhydrous glycerin following the method of Seinhorst (1959). Two distinct forms, the presumptive mycetophagous and infective forms, were separated under the microscope and were studied morphologically. Specimens were examined using a Leica DM5500 B compound microscope with differential interference contrast. Pictures were taken with a Leica DFC 420 digital camera. Measurements were made from the images using a Leica micro-application system and drawings were prepared using a drawing tube.

### DESCRIPTION

*Deladenus valveus*\* n. sp.  
(Figs. 1–4)

**Measurements:** Morphometrics of the holotype, paratype, mycetophagous females, males, and infective females are given in Table 1.

\* The species epithet indicates the unusual valve at the esophago-intestinal junction of the mycetophagous females.

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FIG. 1. *Deladenus valveus* n. sp. A. Head and esophagus of mycetophagous female. B. Vulva and tail of mycetophagous female. C. Reproductive system of mycetophagous female. D. Reproductive system of infective female. E. Head region of the infective female. F. Vulva and tail of infective female. G. Esophago-intestinal valve of mycetophagous female. H. Body of mycetophagous female. I. Body of infective female. J. Tail of mycetophagous male.

*Mycetophagous female*: The body is medium-sized, vermiform, and obese, especially near the vulva. The body is straight when relaxed. The cuticle is finely annulated; the rings are 1  $\mu\text{m}$  apart. Lateral fields are narrow, occupying about one-third of the body width, each with eight fine lines at midbody. The lip region is low and not offset from the body contour. The stylet is 9- to 13- $\mu\text{m}$  long, delicate with very small base-knobs, with three guide rings; the shaft is slightly longer than the cone. The esophagus is of the Hexatylinia-type (Geraert,

2006): the esophageal corpus is cylindrical to fusiform, slightly swollen in the middle region; a metacarpus and associated pump are absent; and the esophageal gland region is nonmuscular. Both the dorsal gland and subventral glands overlap the anterior end of the intestine. The dorsal gland opening is immediately posterior to the base of stylet; and the subventral gland openings are in the slightly swollen midregion of the corpus. The excretory pore is located 37 to 54  $\mu\text{m}$  anterior to the hemizonid and 59 to 74  $\mu\text{m}$  from the anterior end. The

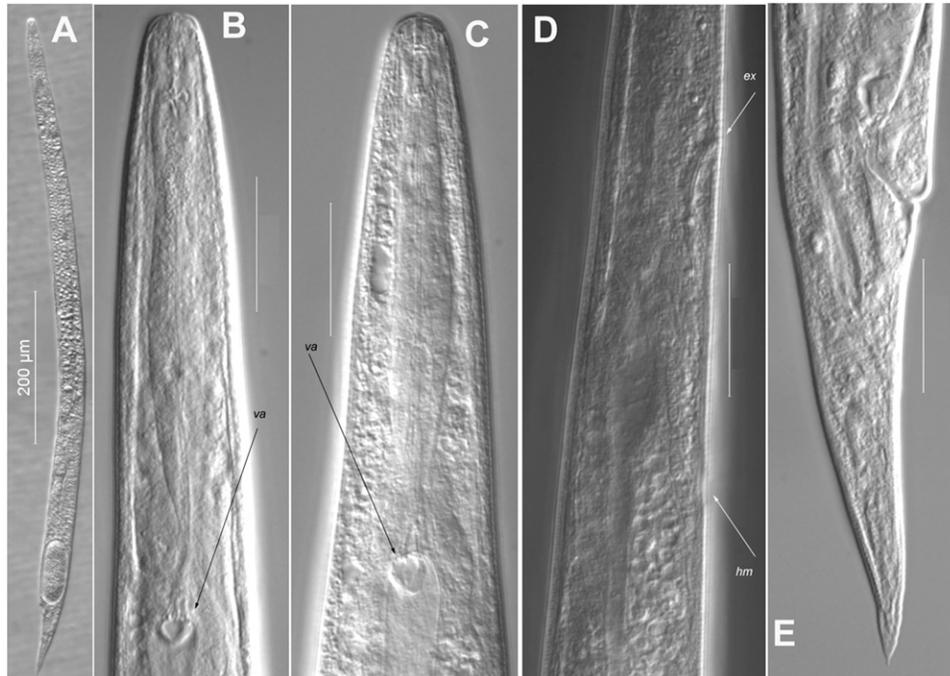


FIG. 2. Light micrographs of mycetophagous females of *Deladenus valveus* n. sp. Scale bar length is 20  $\mu\text{m}$  unless specified otherwise. A. Whole body. B,C. Head and neck of two individuals, showing esophago-intestinal valve (va). D. Excretory pore (ex) and hemizonid (hm). E. Vulva and tail region.

hemizonid is located 96 to 128  $\mu\text{m}$  from the anterior end. The esophageal and intestinal junction is located at or immediately posterior to the nerve ring, with an oval to round valve about 5 to 6  $\mu\text{m}$  in diam., which appears

nonmuscular, and looks like a sclerotized extension from the esophagus into the intestine lumen; it is suspended inside the lumen and detached from the intestine walls. The female reproduction system is well

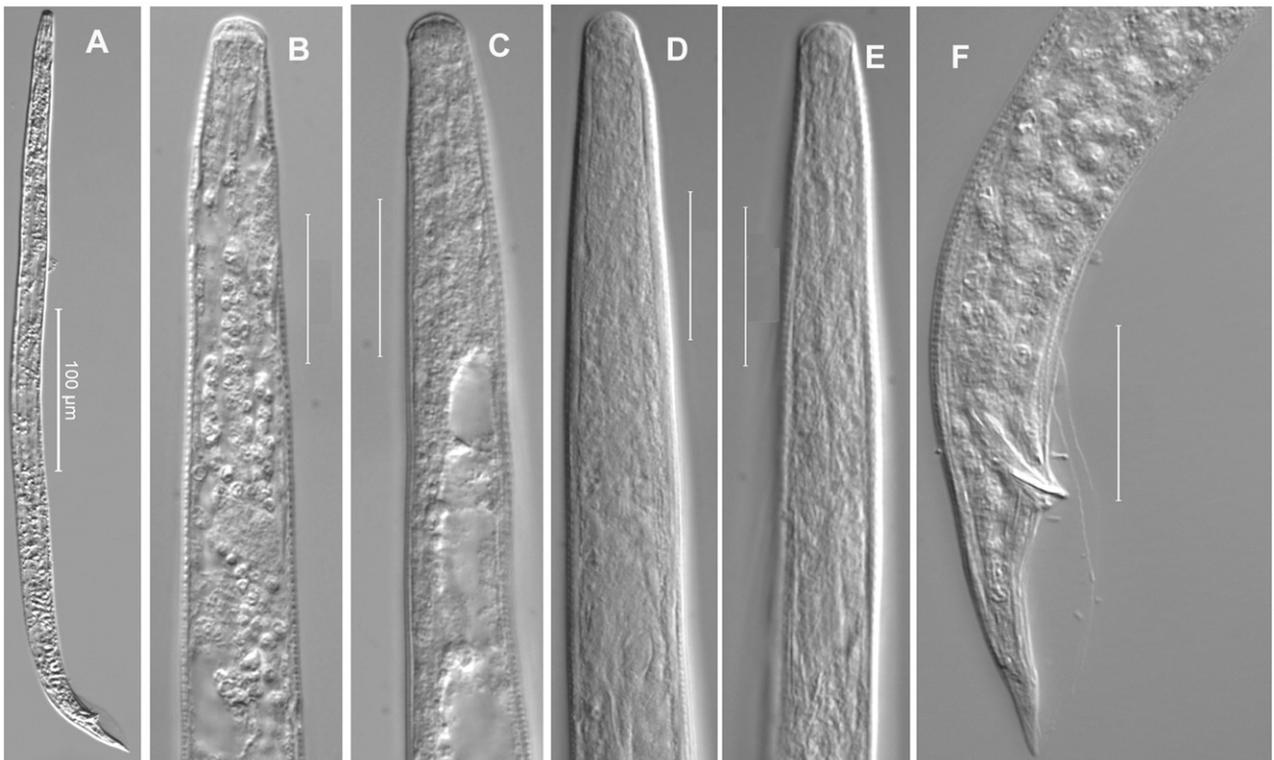


FIG. 3. Light micrographs of mycetophagous males of *Deladenus valveus* n. sp. Scale bar is 20  $\mu\text{m}$  unless specified otherwise. A. Whole body. B-E. Head and neck of four individuals. F. Tail.

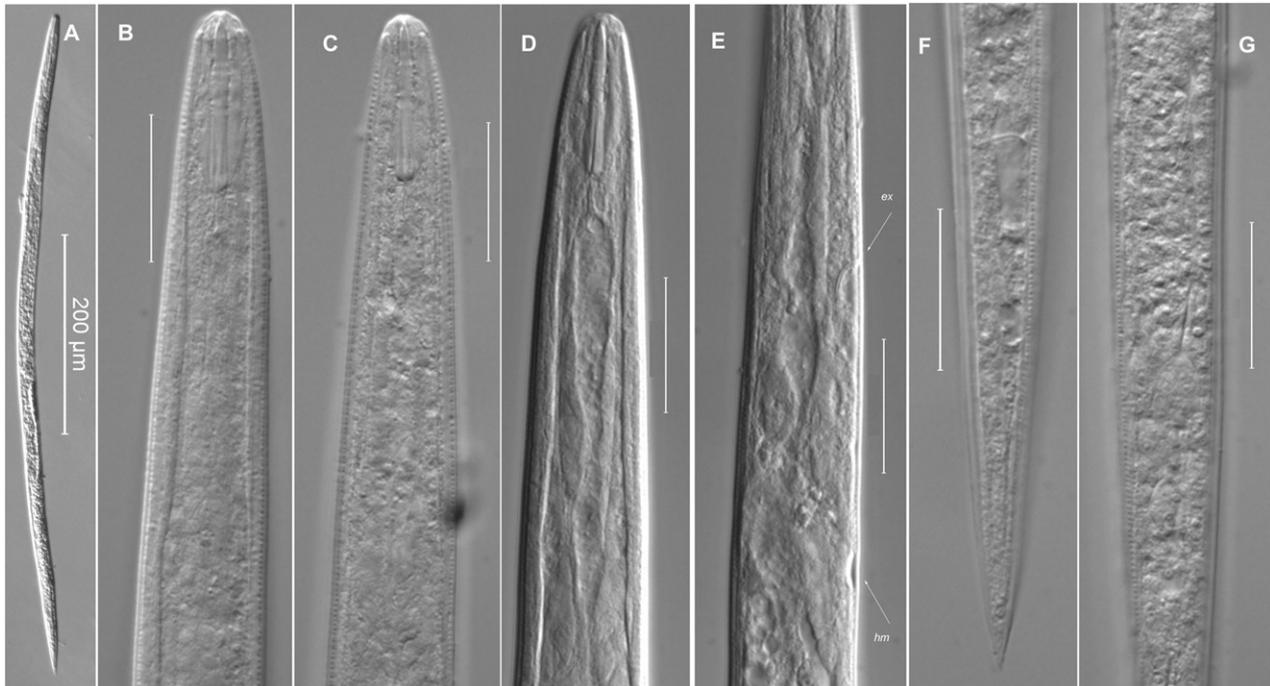


FIG. 4. Light micrographs of infective females of *Deladenus valveus* n. sp. Scale bar is 20 µm unless specified otherwise. A. Whole body. B–D. Head and neck of three individuals. E. Excretory pore and hemizonid (ex) and hemizonid (hm). F. Tail. G. Vulva region.

developed. The ovary is prodelphic without a post-uterine sac; it is outstretched, overlaps the dorsal gland in some females, and has multiple rows of oocytes, an oviduct of seven cells, and a spermatheca that is variable

in shape, ranging from a circular to ovoid to an elongated bag, and filled with small sperm cells. The uterus comprises six cells and terminates in a deep vagina and a vulva that is wide, well developed, and with slightly

TABLE 1. Morphometrics of *Deladenus valveus* n.sp. All measurements are in µm and in the form: mean ± s.d. (range).

Character	Mycetophagous			Infective Female Paratype
	Female Holotype	Paratypes	Male Paratype	
n	–	40	5	10
L	681	647 ± 101 (509-970)	483 ± 47 (457-504)	683 ± 36 (551-727)
a	21.1	18.5 ± 3.2 (17.2-29.9)	27.6 ± 2.2 (24.7-30.0)	27.8 ± 3.1 (24.7-31.5)
b	7.6	8.3 ± 0.9 (7.1-12.4)	–	6.2 ± 0.8 (5.5-7.3)
c	16.9	16.4 ± 5.5 (13.6-20.8)	14.4 ± 0.9 (13.4-15.8)	12.4 ± 2.1 (11.3-14.8)
V	89.9	90.1 ± 1.5 (88.3-92.1)	–	75.7 ± 1.4 (74.3-77.2)
G <sub>1</sub> or T	67.8	69.6 ± 3.6 (60.1-74.7)	55.4 ± 2.4 (49.8-58.2)	24.5 ± 2.5 (22.5-26.9)
Body width	32	46 ± 2.7 (30-49)	21 ± 0.5 (18-23)	22 ± 2.9 (21-25)
Stylet length	12	11 ± 1.7 (9-13)	–	22 ± 1.7 (20-24)
Excretory pore from the anterior end	64	61 ± 9.3 (59-74)	59 ± 5.5 (53-69)	71 ± 6.6 (57-78)
Excretory pore anterior to the hemizonid	40	46 ± 12.6 (37-54)	40 ± 9.3 (31-49)	44 ± 5.5 (39-49)
Tail length	40	41 ± 2.6 (38-47)	36 ± 3.4 (30-43)	48 ± 6.5 (41-55)
Spicule length	–	–	25 ± 2.3 (20-27)	–

protruding lips. The intestinal lumen is wide. The rectum is wide, occupying about half the body width. The tail gradually narrows to a pointed terminus. Phasmids were not observed.

*Mycetophagous male:* The body is thinner than that of mycetophagous females and is always straight when relaxed, the lateral fields narrow and with four lines at midbody. The lip region is barely offset from the body. The stylet and esophageal region is greatly reduced and not clearly observed by light microscopy. The testis is outstretched, comprising a single row of germ cells; the vesicular seminalis is long, the vas deferens is short, and the sperm cells are round and about 3  $\mu\text{m}$  in diam. The spicules and gubernaculum are typical tylenchoid, i.e., each spicule is divided into three sections: the head (manubrium), shaft (calomus), and blade (lamina). The bursa is peloderan and well developed. The tail narrows to a sharp, pointed terminus.

*Infective female:* This form is markedly different from the mycetophagous female. The body is slimmer than that of the mycetophagous female, and it has fine annulations. The lateral fields consist of four lines. Lips appear fused. The stylet is strong, 20- to 24- $\mu\text{m}$  long; the cone, shaft, and knobs are amalgamated; and it is accompanied by three guide rings. The esophago-intestinal junction is near the level of the nerve ring. The dorsal gland opening is about 6  $\mu\text{m}$  behind the stylet base. The dorsal gland is massive, whereas the subventral glands vary in size and have openings near the level of the duct for the excretory pore. The intestine lumen is not easily observed. The female reproductive system is small and immature. The ovary has a single row of cells, the oviduct is narrow, and the spermatheca is elongate, filled with sperm cells of different shapes ranging from circular to elongate; the uterus is short and reduced, the vagina narrow and shallow, and the vulva is small and comprising a short, narrow slit. A short post-uterine sac is present and about 20- $\mu\text{m}$  long. The anus is not easily observed and is located halfway between the vulva and posterior end. The tail narrows to a sharply pointed terminus.

*Diagnosis and relationships:* *Deladenus valveus* n. sp. is distinguished from all other species of the genus by the presence vs. absence of a distinct esophago-intestinal valve in the mycetophagous females and by a degenerate vs. a well-formed esophagus in the mycetophagous males. This species clearly belongs to the group of *Deladenus* species in which the excretory pore is anterior to the hemizonid in the mycetophagous forms, and the species is most similar to *D. prorsus* in that the excretory pore is close to the anterior end.

Attempts at PCR amplification and sequencing of DNA from fixed specimens were unsuccessful, so it was not possible to obtain molecular characters informative of phylogenetic relationships.

*Type habitat and locality:* The type specimens were isolated from dunnage exported from South Korea and

intercepted by the Ningbo Entry-exit Inspection and Quarantine Bureau, China, in 2012.

*Type specimens:* Holotype mycetophagous female, 10 paratype mycetophagous females, 5 paratype mycetophagous males, and 10 paratype infective females of *D. valveus* n. sp. were deposited in the Canadian National Collection of Nematodes, Ottawa, ON, Canada, under the accession number T528. Three paratype mycetophagous females were deposited in each of the following collections: USDA Nematode Collection, Beltsville, MD; Ningbo Entry-exit Inspection and Quarantine Bureau Nematode Collection, Ningbo, China; University of California Riverside Nematode Collection, Riverside, CA.

#### DISCUSSION

This is the first species of *Deladenus* reported to have a distinct esophago-intestinal valve in mycetophagous females. Such a structure is distinct from the void plug present in *D. canii* (Geraert, 2006). The structure of the valve in *D. valveus* is distinct and nonmuscular, and shapes vary. Because this character is unique among *Deladenus* species, its taxonomic significance remains to be determined. The new species is also the first described to have a degenerate esophagus in mycetophagous males. A degenerate esophagus in males is not uncommon in Tylenchomorpha, although it most frequently occurs in Criconematoidea and Tylenchoidea (Geraert, 2006). The males of *D. valveus* n. sp. are therefore predicted to lack the ability to feed.

The location of the excretory pore in the mycetophagous form varies among species of *Deladenus*. The identification of species in the genus thus relies on the relative position of the excretory pore to the hemizonid: all species with known infective stages have their excretory pore anterior to the hemizonid, whereas in most other species of *Deladenus* this configuration is reversed (Chitambar, 1991). The distances between the two structures vary greatly, ranging from 1 to 100  $\mu\text{m}$  and are therefore used to discriminate among *Deladenus* species. The relative position of the excretory pore to the anterior end of the nematodes also varies greatly, from 30 to 100  $\mu\text{m}$ , and can be used to identify several other species. A combination of the above two metrics can diagnose the remaining species of *Deladenus*.

The classification of *Deladenus* at the family rank, i.e., in Neotylenchidae (Thorne, 1941, 1949), remains questionable as those species with a known infective stage share characteristics uniquely circumscribing Allantonematidae, namely in that infective females have a stylet of 15  $\mu\text{m}$  or longer with or without knobs, the esophagus glands are elongated and lobe-like, the vulva is small and inconspicuous, and the vagina is short (Blinova and Korentchenko, 1986). However, the alternative placement of the genus in the family Allantonematidae

(Jairajpuri and Siddiqi, 1969; Siddiqi, 1986, 2000; Fortuner and Raski, 1987) is also disputed, because clearly the mycetophagous forms share some characteristics of Neotylenchidae, i.e., a fusiform and non-muscular esophagus, a median bulb lacking a valve, and the absence of a basal bulb. Unfortunately, we were unable to isolate DNA from our TAF-fixed specimens of *D. valveus* n. sp. Both *Deladenus* species for which molecular data are presently available (*D. proximus* and *D. siricidicola*) make up a monophyletic group that is sister to molecularly characterized Allantonematidae, which includes species of *Bradynema* (zur Strassen, 1892) and *Howardula* Cobb, 1921 (Ye et al., 2007; Yu et al., 2009; 2012). It is likely that *D. valveus* n. sp. also belongs to this clade including these two species of *Deladenus*, given the similarities among these three species: namely, an excretory pore anterior to the hemizonid and the presence of infective stages. More studies will be necessary to resolve relationships of the two groups of species within *Deladenus* and among the families of Sphaerularioidea.

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