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Testate amoebae as predators of nematodes

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Abstract Testacea were observed ingesting nematodes in the litter horizons of native forests in New Zealand. Nematodes were most commonly attacked from the tail end but some specimens were attacked at mid-body. Nematodes with damaged tails were recovered in greatest numbers from the highest, wettest site sampled. *Nebela (Apodera) vas* (Certes, 1989) and *Difflugia* sp. (possibly *lanceolata* Penard, 1890) were the predators; both species were redescribed using light and scanning electron microscopy and morphometry. Most damaged nematodes were *Ironus* sp. but *Clarkus, Tobrilus, Iotonchus, Cobbonchus, Dorylaimus* and *Plectus* were also attacked. Literature on testacean feeding strategies was reviewed briefly.

Key words Testate amoebae · Nematodes · Predation · Soil · Litter · *Nebela (Apodera) vas* · *Difflugia lanceolata*

Introduction

Although the effect of the nematode/protozoa/bacterial predation or grazing chain has been studied because of its importance in the cycling of plant nutrients (Coleman et al. 1984; Schönborn 1992), the only reports of protozoan predation on nematodes concern ciliates and naked amoebae (Small 1988). In this note we report predation by testate amoebae on nematodes in forest litter.

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Materials and methods

Litter and soil samples were collected in November–December 1990 from four unmilled native forest sites at the Orongorongo Valley Field Station, southern North Island, New Zealand under *Nothofagus menziesii*-dominant forest at 800 m altitude on Station Ridge (near site d of Moeed and Meads 1985); *N. menziesii*-dominant forest at 680 m altitude on the track to Station Ridge; *N. truncata*-dominant forest at 160 m altitude (near site c of Moeed and Meads 1985); and mixed broadleaf forest at 100 m altitude (near site a of Moeed and Meads 1985).

The average annual rainfall at 100 m altitude is 2500 mm and the mean monthly air temperatures are 6–26 °C; as orographic cloud frequently envelopes the forests from about 550 m, sites above this level are cooler and wetter. Moeed and Meads (1985) have given monthly litter moisture and temperature data for various sites.

Samples for nematodes were extracted using the Whitehead and Hemming tray method (Southey 1986), counted live, fixed by the addition of boiling fixative (100 ml 40% formaldehyde: 10 ml glacial acetic acid: 390 ml distilled water; Southey 1986), processed to glycerol, and mounted on glass slides.

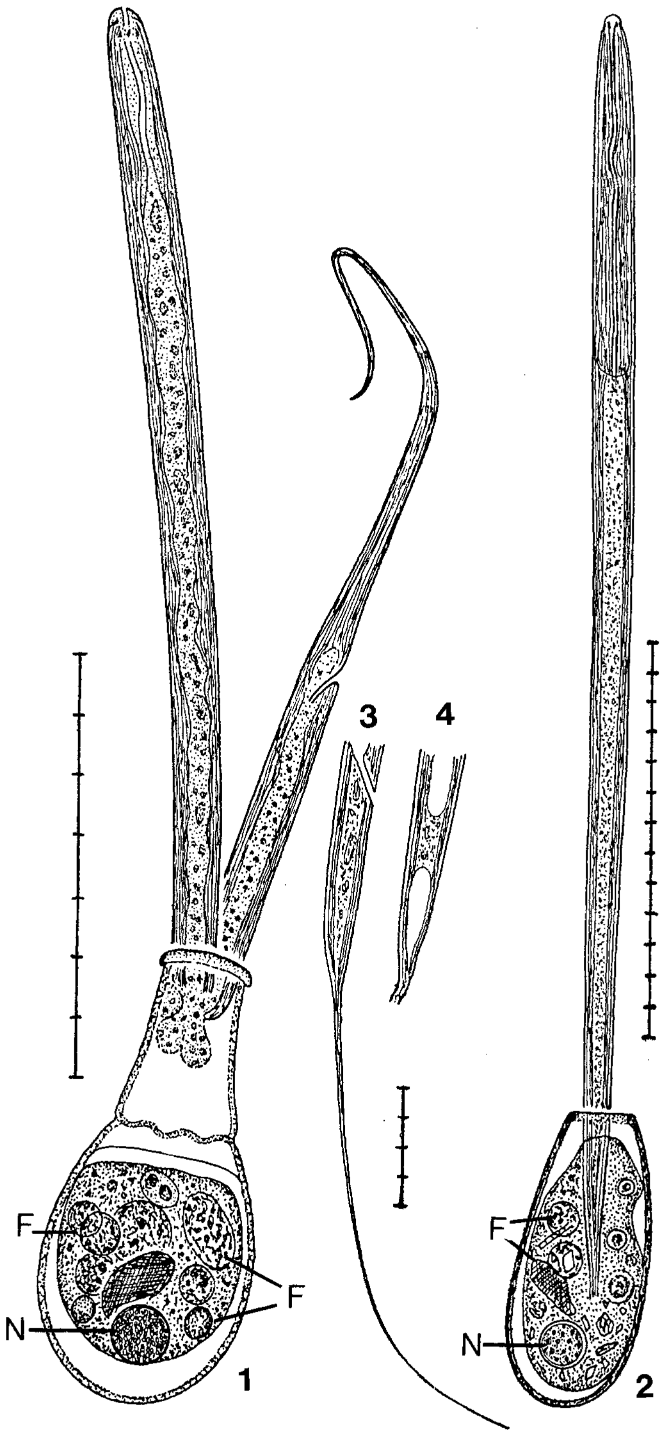
Samples for identification of testate amoebae were collected on 1 May 1993 from site d at 800 m altitude on Station Ridge. Single specimens were isolated by a micropipette and processed as described by Schönborn et al. (1983).

Results

Predation by testate amoebae on nematodes

When counting the live extracts, we observed several nematodes being ingested by testate amoebae (Figs. 1–14). Most observations involved a protozoan engulfing the tail of a nematode (Figs. 2, 6, 10); in one case ingestion had proceeded to mid-body length and in another a lateral attack had been successful (Figs. 1, 5).

Several attempts were made to recover and preserve testacea actively feeding on nematodes but the physical links were weak and separation occurred. However, fixed bulk extracts contained a few intact predator/prey couples (Figs. 1, 2, 5, 6, 10). A total of 115 mounted nematodes were found to have tails damaged in a way similar to those observed to be damaged by testacea (Table 1). Seven nem-



Figs. 1–4 Feeding of testate amoebae on nematodes. The cytoplasm of the amoebae is shrunken because of the preparation procedure used; it contains a nucleus (*N*) and many food vacuoles (*F*) with humus particles, fungal hyphae, and unidentified materials. **1** *Nebela vas* feeding on *Ironus* sp. The nematode is ruptured near the middle of its length and drawn into the test orifice. **2** *Difflugia lanceolata* feeding on *Ironus* sp. The nematode is attacked at the tail and drawn into the cytoplasm whereby it is apparently lysed. **3, 4** Tail region of normal and testacean-attacked *Ironus* sp. Scale bar division 20 μm

atode genera were affected but the greatest incidence was in *Ironus* sp. (Figs. 1–8, 10, 11). Apparent damage to *Cobbonchus* sp. is illustrated by Figs. 9, 12–14. The greatest number of occurrences was at the highest, wettest site and only at that site were any damaged specimens found below the superficial litter layers.

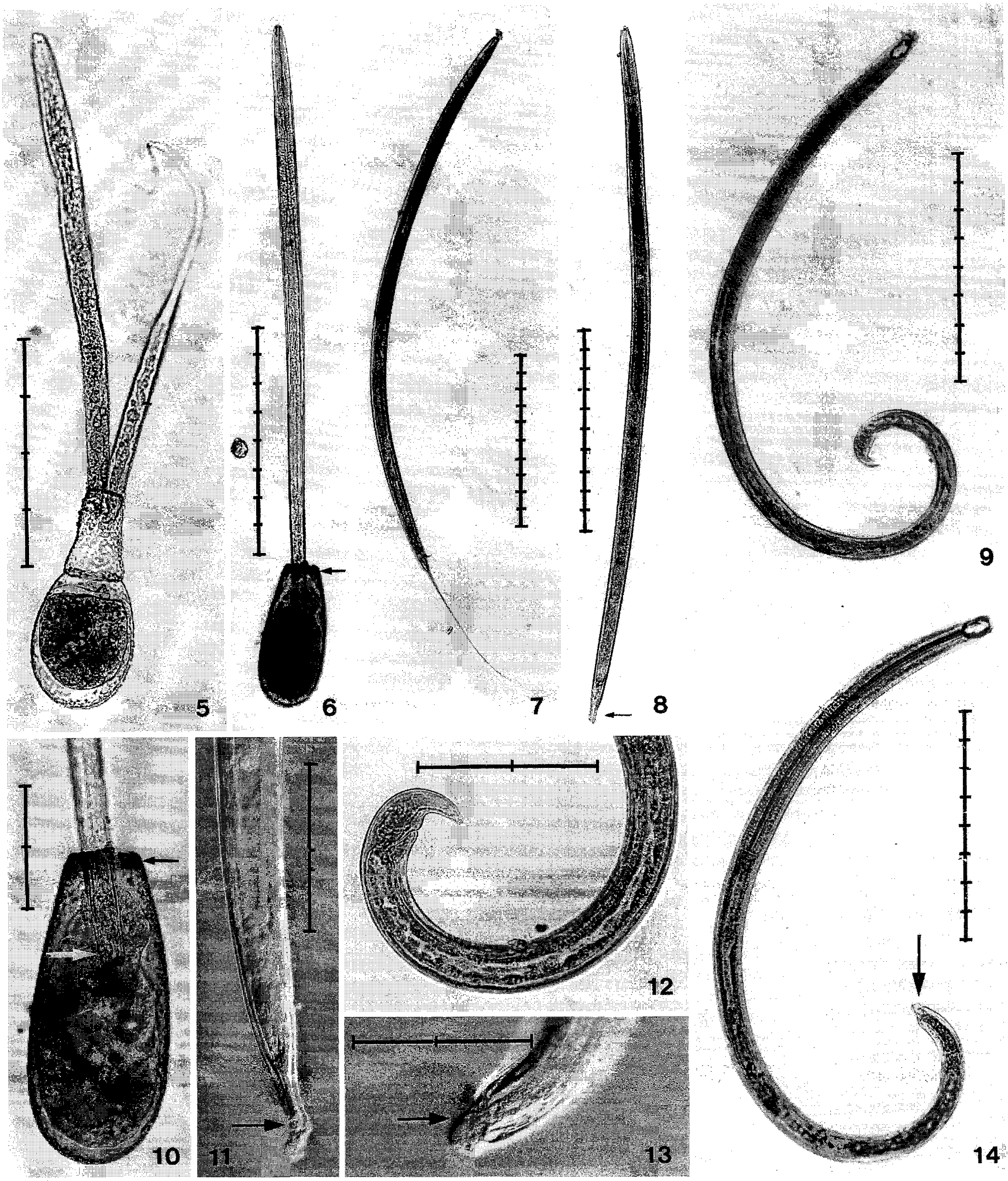
Description and identification of the testate amoebae

Two testacean species were observed feeding on nematodes (Figs. 1, 2); they were identified as *Nebela vas* and *Difflugia lanceolata.* However, identification of testate amoebae is fraught with problems. It is thus necessary to give detailed descriptions of our material, for the benefit of future ecologists and taxonomists.

Nebela (Apodera) vas (Certes, 1889)

Description (Figs. 1, 5, 15–18, Table 2). Shell yellowish or hyaline, lageniform, length: width proportion highly variable, viz., 1 : 1.5–2.0; likewise proportion of inflated posterior part (fundus) to cylindroid apertural part (neck) rather variable, viz., 1 : 1.5–1.7. Organisms thus slender to broad drumstick-shaped, with neck length slightly less to distinctly more than one-third of shell length. Neck orientated more or less strongly obliquely to fundus in about 5% of specimens (Fig. 15). Shell distinctly flattened and slightly curved laterally (Figs. 16, 17). Ground substance of shell unstructured, usually completely covered by circular and elliptical platelets obviously derived from other testate amoebae, such as *Trinema* and *Corythion*, often arranged in very regular and beautiful patterns (Fig. 18). Oral aperture distinctly elliptical, well-defined by yellowish, slightly thickened, structureless rim 2–4 μm high (Figs. 1, 17, 18).

Remarks. This large and beautiful species was very abundant and associated with several other Gondwanian testaceans, such as *Nebela martiali.* It has also been recorded from New Zealand by other authors (Penard 1911; Deflandre 1936; Hoogenraad and de Groot 1948; van Oye 1956), and our observations agree well with theirs. *Nebela vas* is a bryophilic species restricted to Gondwanian fragments in the southern hemisphere (Deflandre 1936; Grospietsch 1971; Hoogenraad and de Groot 1979). Most of our specimens fit well with the common form observed, by Certes (1889), Penard (1911), Deflandre (1936), Hoogenraad and de Groot (1948), and van Oye (1956). About 5% of our material matches *N. vas obliqua* Grospietsch, 1971 (having the neck obliquely attached to the fundus), *N. vas longicollis* Grospietsch 1971 (having rather a long neck), and *N. vas recticollis* Jung, 1942 (having a nearly cylindrical neck and a less inflated fundus). Because in our material there were many transitions between the normal form and the other shapes, we consider that such forms are within the normal variability of this species (cf. Deflandre 1936).



Figs. 5–14 Feeding of testate amoebae on nematodes. The cytoplasm of the amoebae is shrunken because of the preparation procedure used. **5** *Nebela vas* feeding on *Ironus* sp. (cf. Fig. 1). The nematode is ruptured near the middle of its length and drawn into the test orifice. **6, 10** *Difflugia lanceolata* feeding on *Ironus* sp. (cf. Fig. 2). The nematode is attacked at the tail (Fig. 6) and drawn into the cytoplasm (Fig. 10, *white arrow*) whereby it is apparently lysed. Note the distinct rim of organic cement (*black arrow*) around the test orifice (pseudostome) of the testate amoeba. **7, 8, 11** A normal and two testacean-attacked *Ironus* sp. whose tails are distinctly shortened (*arrows*). **9, 12–14** Normal (**9, 12**) and testacean-attacked (**13, 14**) *Cobbonchus* sp. whose tails appear nibbled (*arrows*). Scale bar division 40 μm

Table 1 Nematode genera with specimens apparently damaged by predation by testate amoebae at four beech (*Nothofagus* spp.) and broadleaf forest sites. The number of damaged specimens is given, together with the total number of that genus observed in that horizon. Site details include altitude and organic horizon

	Beech				Broadleaf (100 m) L+F
	N. menziesii (800 m)		*N. menziesii* (680 m) L+F	*N. truncata* (160 m) L+F	
	L+F	Oh			
Ironus (Ironoidea)	74 (76)	8 (60)	– (–)	– (–)	2 (2)
Clarkus (Mononchoidea)	6 (17)	– (–)	2 (15)	– (–)	2 (5)
Tobrilus (Tripyloidea)	3 (8)	– (1)	– (–)	– (–)	– (–)
Iotonchus (Mononchoidea)	1 (16)	– (–)	2 (9)	– (–)	– (–)
Cobbonchus (Mononchoidea)	– (–)	2 (8)	– (–)	– (–)	– (–)
Dorylaimus (Dorylaimoidea)	1 (1)	1 (6)	6 (32)	– (–)	4 (29)
Plectus (Plectoidea)	1 (1)	– (–)	– (8)	– (9)	– (–)

Table 2 Morphometric characteristics of *Nebela (Apodera) vas* from *Nothofagus* spp. forest. Data are based on 23 randomly selected, empty shells. All measurements are given in µm. CV is given as % (*M* median, *Min* minimum, *Max* maximum)

Character	$\bar{X}$	M	SD	CV	Min	Max
Length (total)	165.0	168	13.5	8.2	132	184
Length (fundus)	104.0	104	6.7	6.5	88	120
Width of fundus (broader side)	92.3	93	7.0	7.6	79	110
Width at base of neck (broader side)	52.6	52	4.1	7.9	44	60
Width at pseudostome (broader side)	33.7	34	2.1	6.4	30	38
Width of fundus (narrower side)	60.5	64	7.9	13.1	44	72
Width at pseudostome (narrower side)	25.3	25	3.4	13.3	20	32

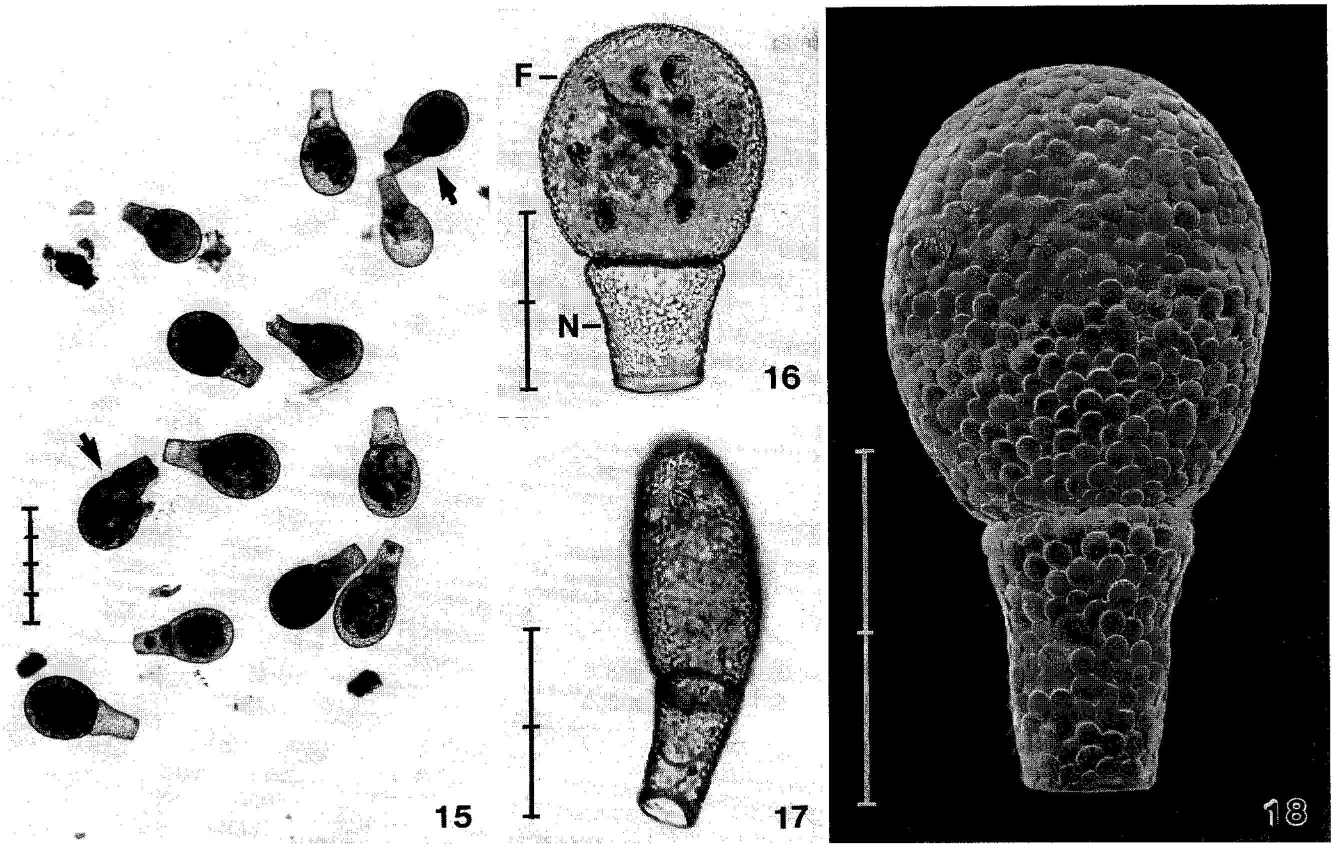
Apodera Loeblich and Tappan, 1961 is, depending on the criteria used by specialists, a genus closely related to *Nebela* or, in our view, a subgenus of *Nebela*.

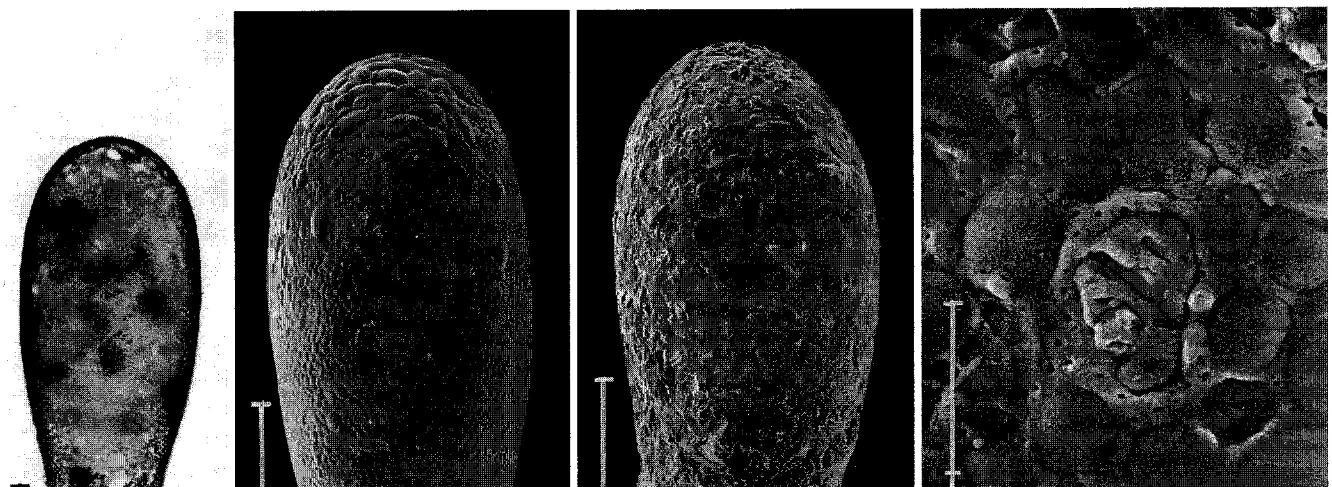
Difflugia lanceolata Penard, 1890?

Description (Figs. 2, 6, 10, 19–22, Table 3). Shell yellowish or hyaline, pouch-shaped, tapering from widest diameter situated about two-thirds of body length from aperture, rounded aborally and evenly angled towards aperture; rarely slightly curved (Fig. 21) or indented near apertural region to produce inconspicuous neck (Figs. 19, 20). Shell composed of small to medium flattish pieces of quartz and many roundish platelets clearly derived from other testate amoebae such as *Trinema, Corythion,* and *Euglypha* (Figs. 20–22); surface always very smooth, appearing as though polished (Figs. 19, 20). Organic cement reticulate, apparently composed of tightly spaced or fused rings (Fig. 22). Shell orifice (pseudostome) circular, well-defined by organic cement appearing as yellowish ring under light microscope (Figs. 6, 10, 19). Nucleus with many tiny nucleoli.

Remarks. This species was very rate, only seven specimens being found. Species determination in the genus *Difflugia* is extremely difficult. However, it is clear that our population belongs to the "superspecies" *D. oblonga* Ehrenberg, to which Štěpánek (1952) and Patterson et al. (1985) assign, among others, *D. lanceolata* and *D. lacustris.* This was, however, criticized by Ogden and Ellison (1988) who found distinct differences in the organic cement which binds the shell particles. Our identification is primarily based on two remarks by Ogden (1983), viz. that "the cell surface of *D. lanceolata* appears polished, a feature that often permits easy identification" and that "the organic rim surrounding the aperture is almost unique to *D. lanceolata*". Both features are displayed by our specimens (Figs. 10, 19, 20). Likewise, the reticulate pattern of the organic cement is very similar to that described by Ogden (1983) for that species. However, the size of our specimens is closer to *D. oblonga oblonga* and *D. lacustris*, because the reported size range of *D. lanceolata* is smaller, viz., 114–160×48–92 µm (Penard 1890; Golemansky 1966; Ogden 1983).

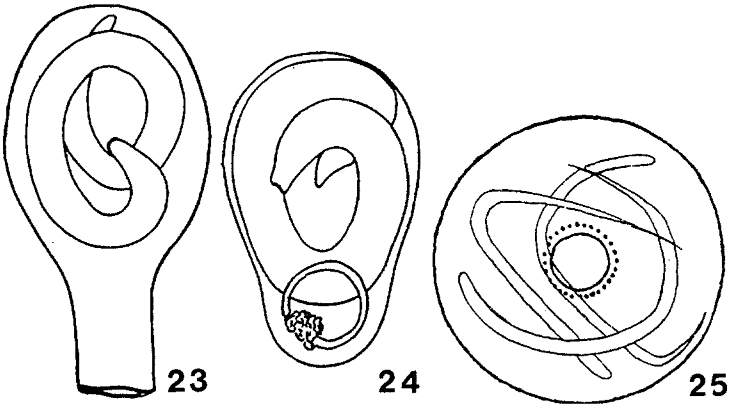
Difflugia lanceolata is an aquatic species, although there are some reports from soil and dry mosses, all without figures and thus questionable. One of the reviewers suggested that our organism might be a new species because of its terrestrial habitat, larger size, and the brownish organic cement around the shell aperture. We agree, however, that more specimens must be analyzed before such a decision is made. For the moment it seems wise to affiliate our population with *D. lanceolata* Penard which is the species closest to our material.

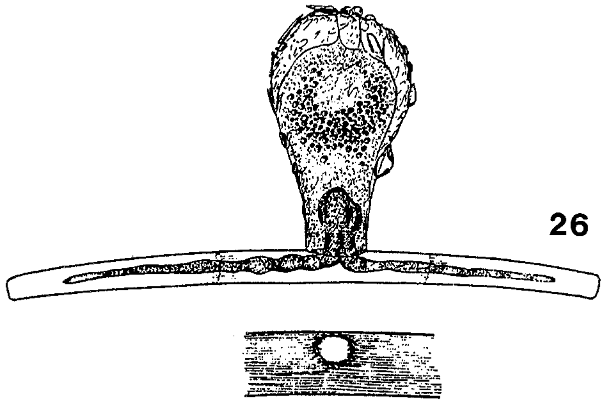




Figs. 15–18 Light and scanning electron micrographs of shells of *Nebela* (*Apodera*) *vas.* **15** A collection of specimens showing variability of shape and size. *Arrows* mark shells with neck orientated obliquely to fundus. **16, 17** Broad and narrow side view of typical specimen. The shell is distinctly flattened and consists of an inflated fundus (*F*) and a cylindroid neck (*N*). **18** The shell is composed of circular and ellipsoid platelets derived from devoured testate amoebae, very likely *Trinema* and *Corythion.* Scale bar division 40 µm

Figs. 19–22 Light and scanning electron micrographs of shells of *Difflugia lanceolata.* **19, 20** The same specimen, showing the test orifice surrounded by a brownish rim of organic cement which contains, like the rest of the shell, ellipsoid platelets from devoured testate amoebae, possibly *Trinema* and *Corythion.* **21** A slightly curved shell composed mainly of flattish pieces of quartz. **22** High magnification of shell surface showing foreign shell platelets (mainly from *Trinema*) embedded in reticulate organic cement. Scale bar division 40 µm (**19–21**) and 4 µm (**22**)





Figs. 23–25 Empty shells of testate amoebae (*Nebela lageniformis, Trinema enchelys, Arcella arenaria*) containing nematodes (from Varga 1959, 1960). Varga observed these specimens in forest soil litter and in the moss of a gutter and suggested that nematodes preyed on the amoebae

Figs. 26–27 *Difflugia rubescens* ingesting the contents of *Closterium* sp. (Desmidiaceae) by perforation lysis of the cell wall (from Hoogenraad and de Groot 1941)

Discussion

The nematodes examined were recovered by an active extraction method so not only were nematodes significantly damaged in the field excluded from observation but also any significant damage that was observed had occurred during the 24-h extraction process. It is not known how many prey had been destroyed by predation; only damaged specimens were recorded.

Testaceans have not previously been reported preying on nematodes. They have, however, a diverse food spectrum (reviews by Foissner 1987; Laminger and Bucher 1984; Schönborn 1966), although most are probably polyphagous and/or microphagous (feeding on bacteria). Others feed on fungal hyphae and spores, on humus particles, or on other protozoans, including smaller testaceans with a store of reserve platelets that are used for the construction of their own tests (Figs. 18, 20, 22). Both *Nebela* spp. and *Difflugia* spp. are well-known predators. It was thus not surprising to observe that they also feed on micrometazoans, although such predation has not previously been reported for either soil or freshwater testaceans. However, it may occur more frequently but remain unrecognized since, as we observed, the physical links between the predator and the prey are easily separated.

Testate amoebae have evolved a wide range of feeding strategies (reviews by Grospietsch 1965; Schönborn 1966) and we illustrate some in Figs. 23–27. Usually, however, the food is touched and encircled by the pseudopodia before it is drawn into the shell. More rare and complicated modes include perforation lysis (Figs. 26, 27; see also Chardez 1985 who reports that *Hyalosphenia platystoma* feeds by this mode on *Euglypha filifera*) and food-bundle formation, i.e., aggregates of humus particles collected during optimal periods around the pseudostome and digested during less favourable periods (Schönborn et al. 1987). The two modes observed in this study, viz., attacking prey in mid-body or at the end, have also been described for *Netzelia tuberculata* feeding on filaments of the green alga *Spirogyra* (Anderson 1989). In fact, these modes of ingestion are similar. Further, ciliates may take up filaments of cyanobacteria in the same ways (Peck 1985).

While *Ironus* and *Dorylaimus* with filiform tails may well survive damage or malformation, the outcome in *Clarkus, Iotonchus,* and *Plectus,* where even slight damage led to exposure of somatic musculature, may be loss of body fluids, infection, and death.

In view of these observations, records of "deformed tails" in soil nematodes may be reviewed to assess the possible association with predation [e.g., the occurrence of 1% malformed tails in females of *Thornenema wickeni* (Dorylaimoidea) collected from Wicken Fen (Yeates 1970) where testacea are abundant (Stout 1971)].

The occurrence of both large filiform-tailed nematodes and testacea in open-textured, moist habitats may sometimes be simple co-existence rather than an indication of predator/prey interaction.

The range of food habits of testacea and the relative mobilities of nematodes and testacea in a given habitat need to be assessed before the impact of predation by testacea on soil nematodes can be evaluated. Also, some

Table 3 Morphometric characteristics of *Difflugia lanceolata*. Data are based on seven empty shells (see Table 2 for further explanations)

Character	$\bar{X}$	M	SD	CV	Min	Max
Length	184.0	184	15.8	8.6	176	208
Width	87.4	88	6.3	7.2	80	96
Width at pseudostome	48.1	48	3.8	8.0	44	56
Pseudostome (diameter)	34.7	35	3.0	8.6	32	40

nematodes may feed on testacea. Varga (1959, 1960) and W. Foissner (unpublished data) observed soil nematodes within the shell of various testaceans (Figs. 23–25), indicating predation by nematodes on testate amoebae, and Varga (1959) observed a live nematode ingesting cytoplasm within the test of a *Centropyxis aerophila.*

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