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Morphology, Biometry and Ecology of *Nebela bigibbosa* Penard, 1890 (Protozoa: Rhizopoda)

Milcho TODOROV

Institute of Zoology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Summary. Testate amoeba *Nebela bigibbosa* Penard, 1890, isolated from the litter of beech forests in Bulgaria and it's morphology, biometry and ecology have been investigated. Size frequency distribution analysis indicates that *N. bigibbosa* is a size-monomorphic species, characterized by a main-size class and a small size range (95% of all measured individuals have a shell length 145-170 μm and 92% have a shell width 94-110 μm). The investigations of the ecology of *N. bigibbosa* show that it is not a typical inhabitant of *Sphagnum* mosses, but is closely related and frequently found in the litter of deciduous forests (mainly beech forests) and can be used as an indicator for these biotopes.

Key words: biometry, ecology, morphology, *Nebela bigibbosa*, Rhizopoda, Testacea.

INTRODUCTION

The genus *Nebela* is one of the well-studied genera of the testaceans. It includes comparatively large species, which in most cases are clearly differentiated and well distinguished each other. More than 120 taxa of the genus *Nebela* have been described until now. Most of them live in mosses and litter, and rarely occur in other biotopes (soils, littoral, pelagial and benthal of the freshwater pools, etc.).

Nebela bigibbosa is one of the largest and well-distinguished species of the genus *Nebela*. It is characterized by the presence of two large pores on the broad lateral face of the shell. The species was described by Penard (1890) in mosses from the environs of Wiesbaden (Germany). Although the studies of the freshwater and *Sphagnum*-dwelling testaceans were exceptionally intensive in the beginning of 20th century, this species has been reported only from Germany, Ireland, Great Britain, Spitsbergen, Canada and Java till 1960 (Penard 1890, 1903, 1905; Wailes and Penard 1911; Cash *et al.* 1919; Deflandre 1936; Hoogenraad and de Groot 1940; Jung 1942). The fact that the majority of the above authors have rarely found *N. bigibbosa* as single individuals might be the reason of the lack of more biometrical and ecological data about this species. The aim of the present study is to characterise morphologically, biometrically and ecologically *N. bigibbosa* using abundant material, isolated from the litter of beech forests in Bulgaria.

Address for correspondence: Milcho Todorov, Institute of Zoology, Tsar Osvoboditel Blvd. 1, 1000 Sofia, Bulgaria; Fax: (3592) 988-28-97; E-mail: zoology@bulinfo.net

MATERIALS AND METHODS

The material for the present study was collected from the decomposing litter (Ao) of beech forests in Bulgaria. The samples were treated in laboratory immediately after their collecting. The flotation method of Bonnet and Thomas (1958) has been used to isolate the testate amoebae. Isolation was made under the stereomicroscope at 70x magnification. The brown mountain-forest soil is the main soil type of the studied beech forests (pH varies from 4.5 to 6.9). The altitude of the sampling sites ranges from 850 to 1800 m a.s.l. Detailed data about the sampling locations are given in Todorov (2001).

The morphometric characterization of the species and the construction of an ideal individual from the median of the shell measurements were made according to Schönborn *et al*. (1983). The following parameters were calculated: $\bar{x}$ - arithmetic mean; M - median (this value is used to construct the ideal individual); SD - standard deviation; SE - standard error of the arithmetic mean; CV - coefficient of variation in %; Min, Max - minimum and maximum values; n - number of examined individuals. Shell's size was measured under the light microscope at x 400 magnification. Measurements in µm.

For scanning electron microscopy the shells were isolated, cleaned by several transfers through distilled water, mounted on coverslips and air-dried. The shells on coverslips were coated with platinum and examined with a JEOL Superprobe-733 operating at 15 kV.

RESULTS AND DISCUSSION

Description of species *Nebela bigibbosa* Penard, 1890 (Figs 1-8, Table 1, 2)

The shell is colourless or yellowish, laterally compressed (about 2 : 1), pyriform with a broad, slightly convex aperture (Figs 1-4). In broad lateral view, at approximately one third of the body length from the aperture, there are two large pores, located in lateral depressions (Figs 1-4). The shell is composed of oval, circular or elongate shell plates of different sizes (Figs 5-7). The aperture is oval, concave in narrow lateral view, surrounded by a distinct organic collar (Figs 1, 2, 5).

Geographical distribution: British Isles, Bulgaria, Canada, Chile, France, Germany, Greece, Java, Nepal, Philippines, Spitsbergen, Switzerland, Thailand.

References: Penard 1890, 1902, 1903, 1905; Wailes and Penard 1911; Cash *et al*. 1919; Deflandre 1936; Hoogenraad and de Groot 1940; Jung 1942; Bonnet 1965, 1966, 1967, 1974, 1977, 1980, 1981, 1987; Puytorac *et al*. 1972; Ogden and Hedley 1980; Golemansky and Todorov 1985, 1990; Todorov 1993, 1998, 2001; Todorov and Golemansky 1995).

Analysis of characters

Table 1 shows the morphometric characterization of *N. bigibbosa*. The ideal individual of this species is constructed from median values of all characters in Fig. 8. The shell measurements with the exception of character 7 are fairly constant. The coefficients of variation of characters 1 - 6 are rather low and show a remarkable uniformity of *N. bigibbosa*. Only character 7 is more variable. Characters 1 - 4 correspond to the ranges given by Penard (1890), Wailes and Penard (1911), Cash *et al*. (1919), Deflandre (1936), Hoogenraad and de Groot (1940), and Ogden and Hedley (1980), (Table 2).

Size frequency distribution analysis indicates that *N. bigibbosa* is a size-monomorphic species, characterized by a main-size class and a small size range (Figs 9, 10). Figure 9 show that 95% of all measured individuals have a shell length 145-170 µm. More than two thirds of them (71%) are within the limits of 150 - 165µm, since only 2% are less than 145 µm long and only 3% are more than 170 µm long.

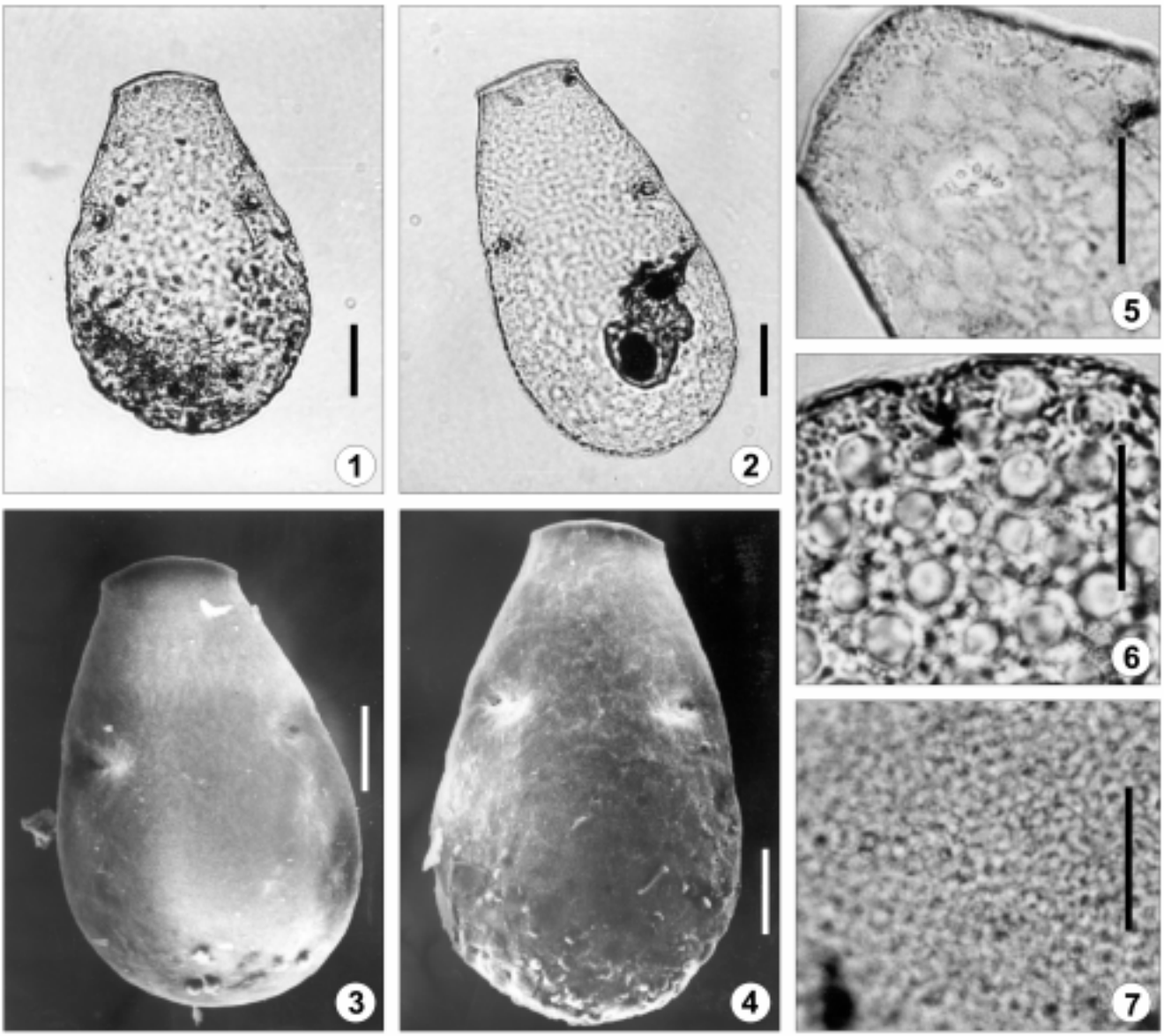
The frequency analysis of the shell width shows almost the same results (Fig. 10). Ninety-two percent of all measured individuals have a shell width between 94 and 110 µm and about two thirds of them (64%) are within the limits 98-106 µm. Only 4% have a shell width less than 94 µm and 4% have a shell width above 110 µm.

A scatter plot of shell length versus shell width of *N. bigibbosa* supports the above conclusion that this species is monomorphic and has a small size range (Fig. 11).

Ecology of *N. bigibbosa*

Although this species was described at the end of the nineteenth century (Penard, 1890) its ecology is not yet fully clarified. Penard described the species from mosses in the environs of Wiesbaden (Germany). Later he, as well as some other authors, found *N. bigibbosa* in *Sphagnum* mosses (Penard 1903, Wailes and Penard 1911, Cash *et al.* 1919). In the monograph of the genus *Nebela* Deflandre (1936) also indicated that *N. bigibbosa* occurs in *Sphagnum* mosses. The majority of these authors found the species in single individuals and described it as rare.

Bonnet (1990) gave comparatively more detailed information about the ecology of *N. bigibbosa*. He



Figs 1-7. *Nebela bigibbosa*. **1, 2** - LM photographs of typical and elongated specimens; **3, 4** - SEM photographs of typical and elongated specimens; **5-7** - views, showing structure of a different shells composed of oval, circular or elongate shell plates. Scale bars: 1-4 - 30 µm; 5-7 - 20 µm

pointed out that this species has a reputation of a *Sphagnum*-dwelling, but it also occurs, although rarely, in some soil habitats as soil mosses, litter, neosols, etc. Bonnet also indicated that the habitats, where *N. bigibbosa* occurs, are characterized by high contents of organic matter, constant high moisture, absence of active Ca and acid reaction of the environment (pH between 3.6 and 6.8).

According to us the reputation of *N. bigibbosa* as a rare *Sphagnum*-dwelling species is due, first of all, to the fact that it is not a typical inhabitant of *Sphagnum* mosses, but occurs there only as an exception. A proof of that is the fact that *N. bigibbosa* was not found by many authors who studied the testacean fauna of the *Sphagnum* mosses (van Oye 1933; Hoogenraad 1934; Harnisch 1938; Hoogenraad and de Groot 1952; Grospietsch 1958; Golemansky 1966, 1967; Schönborn 1966; Moraczewski and Bonnet 1969; Meisterfeld 1973, 1977, 1979; Godeanu 1974; Chardez and Gaspar 1976; Beyens and Chardez 1984; Warner 1987). On the other

Table 1. Morphometric characterization of *N. bigibbosa* (measurements in μm). Broad lateral view: 1 - length, 2 – breadth, 7 - pore diameter; B - narrow lateral view: 3 - breadth, 6 - pore collar aperture distance; C - apertural view: 5, 6 - aperture diameters. See Fig. 8 for character designation

Character	$\bar{x}$	M	SD	SE	CV	Min	Max	n
(1)	157.5	157.0	7.36	0.74	4.7	128	177	100
(2)	101.9	102.0	4.8	0.49	4.7	90	115	100
(3)	51.6	52.0	1.8	0.18	4.2	48	56	100
(4)	40.4	40.0	2.8	0.28	7.0	35	49	100
(5)	20.9	21.0	1.2	0.12	5.8	19	23	100
(6)	59.6	60.0	3.4	0.34	5.7	52	70	100
(7)	3.8	3.5	0.5	0.05	12.4	3	5	100
proportion (2)/(1)	0.65	0.64	0.02	0.002	3.1	0.6	0.7	100

Table 2. Measurements (in μm) of *N. bigibbosa* according to different authors

Authors	Length of shell	Breadth of shell	Depth of shell	Diameter of aperture
Penard, 1890	140-160	100-110	-	-
Wailes and Penard, 1911	135-170	87-110	50-55	34-45
Cash *et al*. 1919	135-170	87-110	50-55	34-45
Deflandre, 1936	135-170	87-110	-	34-45
Hoogenraad and de Groot, 1940	130-170	83-123	-	-
Ogden and Hedley, 1980	153-171	95-115	55-56	38-41
Present work	128-177	90-115	48-56	35-49

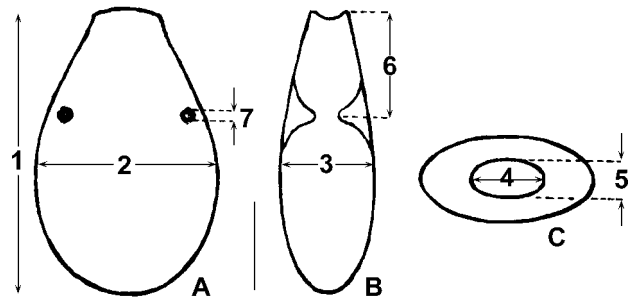


Fig. 8. A-C - Ideal individual of *Nebela bigibbosa* constructed from median values of all measured specimens; A - broad lateral view: 1 - length, 2 - breadth, 7 - pore diameter; B - narrow lateral view: 3 - depth, 6 - pore aperture distance; C - apertural view: 4, 5 - large and small axis of aperture; Scale bar - 50 μm

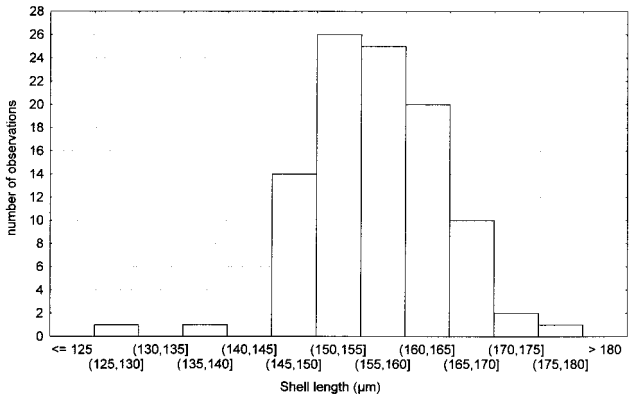


Fig. 9. Histogram showing size frequency of shell length of *N. bigibbosa*

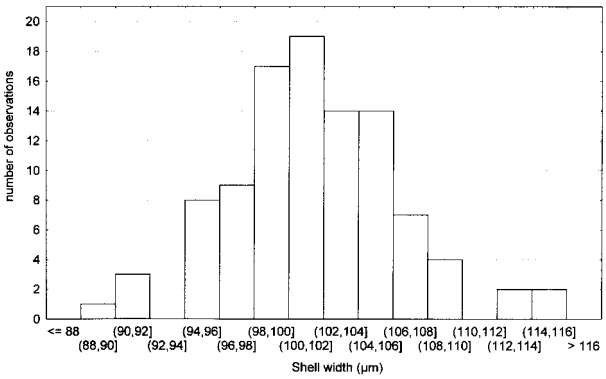


Fig. 10. Histogram showing size frequency of shell width of *N. bigibbosa*

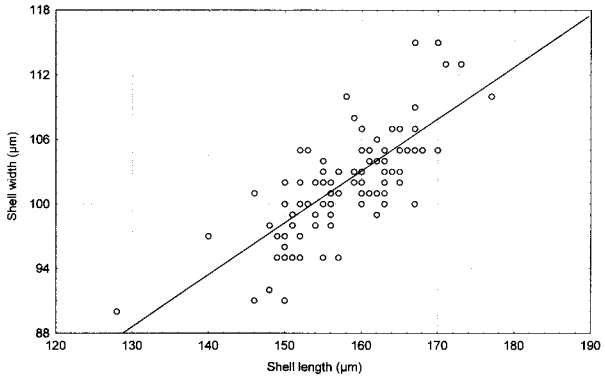


Fig. 11. Scatter plot of shell length versus shell width in *N. bigibbosa*

hand, *N. bigibbosa* has been found many times in the humus and litter of the forest ecosystems of different countries since 1965, when more intensive studies of the soil testate amoebae were begun (Bonnet 1966, 1967, 1977, 1980, 1981, 1987; Todorov 1993, 1998; Todorov and Golemansky 1995).

The results of our investigations show that *N. bigibbosa* is frequent and characteristic species for the litter of beech forests in Bulgaria (Todorov 2001). It was found in 48 of all 73 investigated samples and was constant species for this biotope (frequency of occurrence pF = 65.8%). The other common and constant species found together with *N. bigibbosa* were: *Arcella arenaria, Centropyxis aerophila, C. sylvatica, Cyclopyxis kahli, C. eurystoma, Corythion delamarei, Difflugia lucida, Euglypha laevis, E. rotunda, Heleopera sylvatica, Nebela collaris, N. dentistoma, Plagiopyxis callida, Trinema complanatum, T. enchelys, T. lineare* and *Tracheleuglypha acolla*. Furthermore, *N. bigibbosa* had comparatively high dominance frequency (DF = 31.5%) and was a dominant species in many of the investigated samples.

All these facts suggest that *N. bigibbosa* is not a typical inhabitant of *Sphagnum* mosses but is closely related to the litter of deciduous forests (mainly beech forests) and can be used as an indicator species for these biotopes.

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