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# *Nebela golemanskyi* sp. nov., a New Sphagnicolous Testate Amoeba from Bulgaria (Amoebozoa: Arcellinida, Nebelidae)

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**Summary.** A new sphagnicolous species of the genus *Nebela* isolated from wet *Sphagnum* mosses gathered at Vitosha Mountain (Bulgaria) is described. Shell ultrastructure and morphological variability of *Nebela golemanskyi* sp. nov. are investigated using scanning electron microscopy. The newly described species is characterized by its almost uncompressed shell and by its large, rounded, voluminous and hollow structural elements (idiosomes). The analysis of the variation coefficients shows that the studied population is too homogeneous and all measured morphological characters are feebly variable (CV range from 3.28% to 5.02%). Size frequency distribution analysis indicates that *N. golemanskyi* is size-polymorphic species, characterized by not well-expressed main-size class in favour of many subsidiary classes. A biometrical and morphological comparison between *N. golemanskyi* and *N. speciosa* is made.

**Key words:** Testate amoebae, Amoebozoa, Arcellinida, *Nebela golemanskyi* sp. nov., morphology, biometry.

## INTRODUCTION

The testate amoebae's genus *Nebela* Leidy, 1874 has been extensively studied by many authors. It includes comparatively large and widespread species, inhabiting mainly *Sphagnum* peatlands, wet mosses, forest litter and rarely freshwater pools. Since, most of the nebelids had been described in the end of 19th and in the beginning of 20th century their descriptions were based mainly on light microscopy investigations. This left some species poorly described and without any information about their ultramorphology and biometry. The increasing use of advanced equipment and methods such as scanning electron microscopy and molecular-genetic methods led to the revelation of additional morphological, ultrastructural and genetic peculiarities of the studied testate amoebae and many new species have been discovered and described as a result of that. Also these findings are considerable contribution to the clarification of the taxonomy and phylogeny of testate amoebae, and for the solution of the problems with the close-related and incorrectly described species (Ogden 1983, 1984; Anderson 1987; Lüftenegger and Foissner 1991; Wylezich *et al*. 2002; Nikolaev *et al*. 2005; Lara *et al*. 2007, 2008; Todorov *et al*. 2009).

In the present paper we report, an unidentified sphagnicolous species of the genus *Nebela* observed during investigations on the nebelids from Bulgaria (herein described as *N. golemanskyi* sp. nov.). This new spe-

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cies was found in association along with many other nebelids, such as *N. speciosa*, *N. galeata*, *N. penardiana*, *N. bohemica*, *Argynnia dentistoma* and *A. vitraea*.

The aim of the present study was to describe morphologically and biometrically *N. golemanskyi* sp. nov. and to compare this species with the close-related species *N. speciosa* Deflandre, 1936.

## MATERIAL AND METHODS

The material for the present study was extracted from wet *Sphagnum* mosses gathered in the vicinity of "Aleko" hut, Vitosha Mountain, Bulgaria (42°36′ N, 23°17′ E, 1850 m a.s.l.) on 04 June, 2009.

The shell dimensions of 24 specimens were measured with optical microscope "Amplival" (Zeiss-Jena) using 40 × objective and 10 × oculars lens. The isolated specimens were placed in a drop of glycerol to achieve optimal orientation for the measurement.

The biometric description was made according to Schönborn *et al*. (1983). The following basic statistics were calculated: arithmetic mean; median (M); standard deviation (SD); standard error of mean (SE); coefficient of variation in % (CV); extreme values (Min and Max). Frequency distribution analysis was carried out in order to describe variation of characters. Statistical analysis was performed using the computer program STATISTICA, version 6.0 (StatSoft 1999).

For scanning electron microscopy (SEM) specimens were isolated by searching through small isolates of material in a petri dish. Specimens were extracted using a glass micropipette, washed several times in distilled water, and then individual shells were positioned with a single-hair brush onto a small drop of Araldite on a previously cleaned standard aluminium stub and air-dried. The shells were coated evenly with gold in a vacuum coating unit. The photomicrographs were obtained using a JEOL JSM-5510, operating at 10 kV.

## RESULTS

### *Nebela golemanskyi* sp. nov. (Figs 1–12; Table 1)

**Description:** The shell is large, colourless and pyriform, with a distinct short neck about one fourth of the body length, and a rounded aboral region (Figs 1, 2, 7, 8). It is almost uncompressed laterally, has no lateral margins and lateral pores. The shell has a comparatively thick wall and is composed of characteristic voluminous, globular or rarely elongated, rounded and hollow siliceous plates with an average diameter of about 10–15 µm (Figs 1, 2, 5, 6, 9, 10). Occasionally small diatom frustules scattered sparsely over the surface are incorporated amongst the plates (Figs 1, 2). There are usually large parts between plates interspersed with well visible small beads of organic cement (Fig. 6). The aperture is roughly circular to oval, truncate but not convex and bordered by a thin collar of organic cement (Figs 3–5).

**Biometry:** Biometrical characterization of *N. golemanskyi* is given in Table 1. The analysis of the variation coefficients shows that the studied population is too homogeneous and all measured morphological characters are feebly variable (CV range from 3.28% to 5.02%). The shell length, shell depth and large axis of aperture are the most stable characters (CV between 3.28% and 3.46%), whereas the shell width and the length of neck have maximal variation coefficients (4.83% and 5.02%, respectively).

Analysis of the size frequency distribution indicates that *N. golemanskyi* is size-polymorphic species, characterized by not well-expressed main-size class in favour of many subsidiary classes (the bell-shaped curves are flattened). Average length, width and depth of shells amounted to 209.1 ± 6.94 (n = 24), 118.2 ± 5.71 (n = 24) and 94.8 ± 3.28 (n = 24), respectively. These arithmetical means do not agree with the main-size classes of the characters, and testify to the polymorphism of the species.

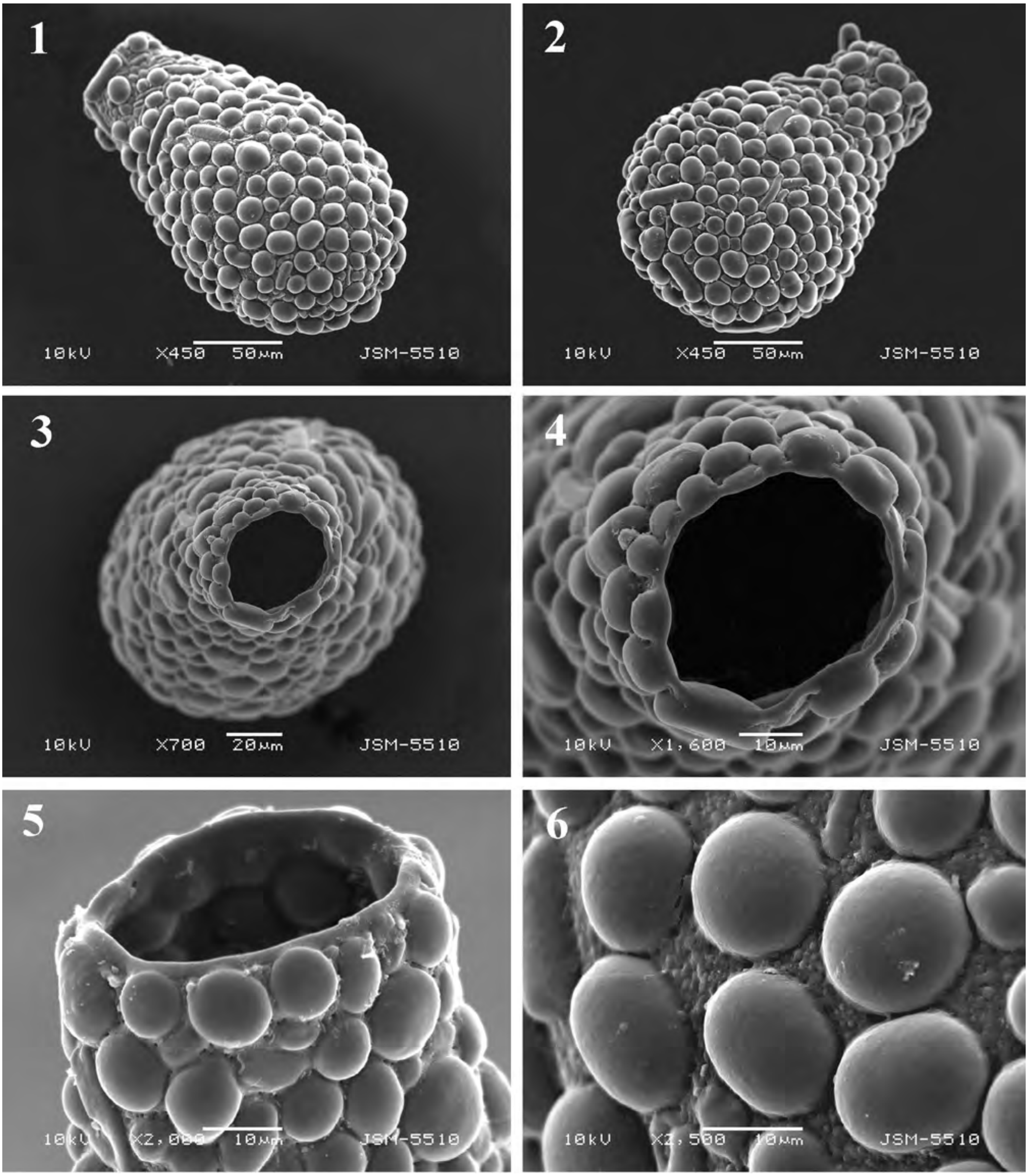
**Ecology:** *N. golemenskyi* inhabits wet *Sphagnum* peatlands (1800–2000 m a.s.l.) in the Vitosha Mountain (Bulgaria). It was found in associations with the next dominant species: *Hyalosphenia papilio*, *N. galeata*, *N. speciosa*, *N. penardiana*, *Argynnia dentistoma*, *A. vitraea*, *Q. symmetrica* and *Cyphoderia ampulla.* The habitats, where *N. golemanskyi* occurs, are characterized by constant high moisture, high contents of organic matter and acid reaction of the environment (pH between 4.9 and 5.6).

**Type locality:** Wet *Sphagnum* mosses in the vicinity of "Aleko" hut, Vitosha Mountain, Bulgaria (42°36′ N lat., 23°17′ E long., 1850 m a.s.l.).

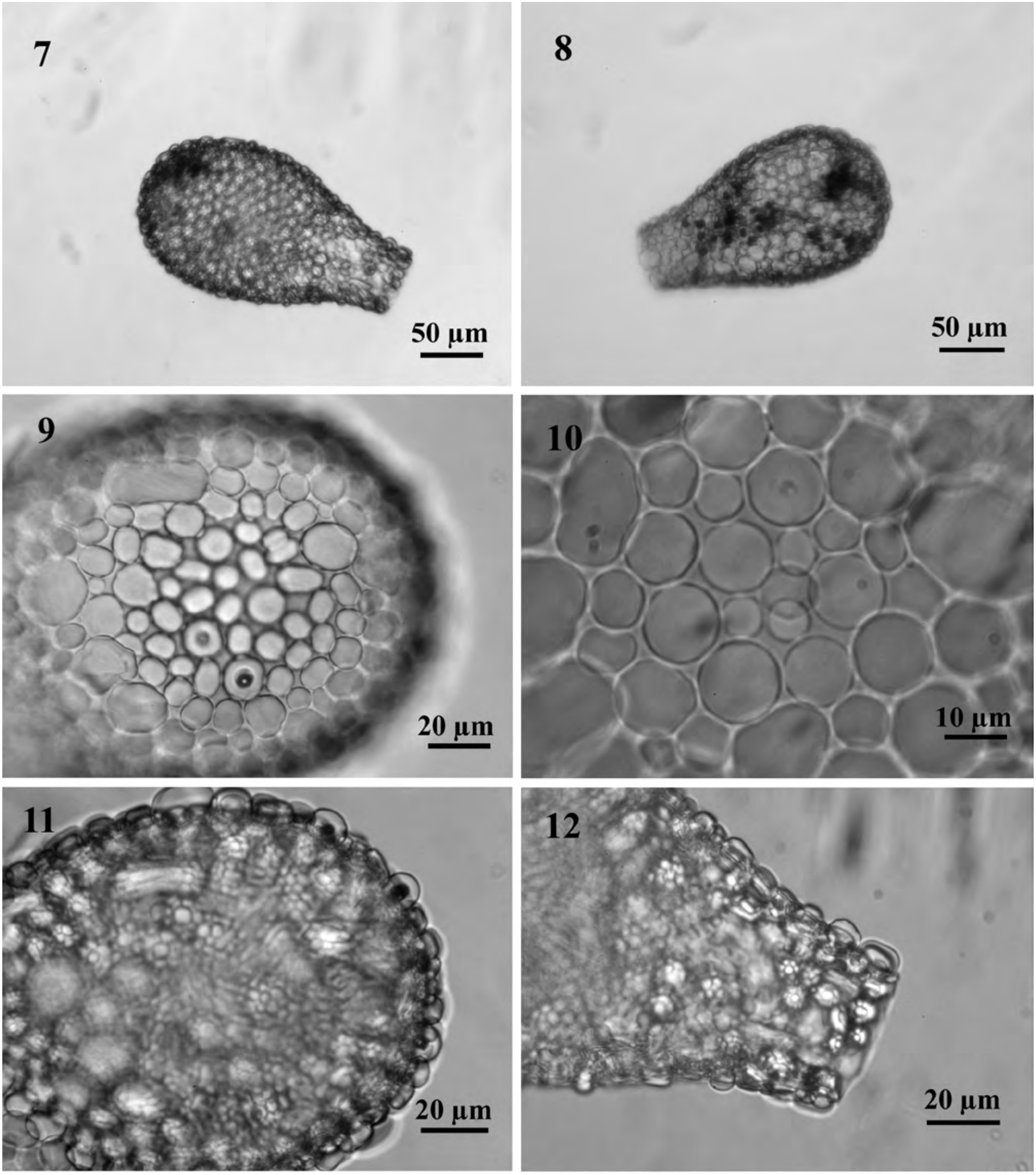
**Material from type locality:** Retained by the author in samples №№ 017-019/2009, collected on 04 June, 2009.

**Type specimen:** The type specimen and paratypes mounted in Canada balsam on a glass slide, are retained in the collection of Dr. M. Todorov, Institute of Zoology, BAS, Sofia; preparations №№ T-001/2009, P-001/2009 and P-002/2009.

**Etymology:** This species is named after my teacher, the Bulgarian protozoologist Prof. DSc. Vassil Golemansky, in recognition of his contribution to the study of testate amoebae.




**Figs 1–6.** SEM micrographs of *N. golemanskyi*. **1–2** – broad lateral view of two specimens showing the general shape of the shell; **3–4** – apertural views showing shell and aperture outline, and lack of lateral margins on the shell; **5** – 2/3 lateral view of the aperture to show thin apertural collar; **6** – detail of shell surface showing voluminous and rounded shell plates interspersed with small beads of organic cement.




**Figs 7–12.** LM micrographs of *N. golemanskyi*. **7**–**8** – lateral view of two specimens showing shell's shape; **9**–**10** – view of shell structure when the focal distance is adjusted to the external surface of the plates; **11**–**12** – views of shell structure when the focal distance is adjusted to the internal surface of the plates.

**Table 1.** Biometrical characterization of *Nebela golemanskyi* sp. nov.: M – median, SD – standard deviation, SE – standard error of the mean, CV – coefficient of variation in %, Min – minimum, Max – maximum, n – number of individuals examined (measurements in μm).

| Characters | Mean | M | SD | SE | CV | Min | Max | n |
|---|---|---|---|---|---|---|---|---|
| Length | 209.1 | 210 | 6.94 | 1.42 | 3.32 | 198 | 219 | 24 |
| Width | 118.2 | 118 | 5.71 | 1.17 | 4.83 | 111 | 132 | 24 |
| Depth | 94.8 | 94.5 | 3.28 | 0.67 | 3.46 | 89 | 100 | 24 |
| Large axis of aperture | 38.1 | 38 | 1.25 | 0.25 | 3.28 | 36 | 40 | 24 |
| Small axis of aperture | 31.1 | 31 | 1.25 | 0.25 | 4.02 | 29 | 33 | 24 |
| Length of neck | 45.4 | 46 | 2.28 | 0.46 | 5.02 | 40 | 48 | 24 |
| Ratio Width/Length | 0.56 | 0.56 | 0.02 | 0.005 | 3.57 | 0.52 | 0.61 | 24 |
| Ratio Depth/Width | 0.8 | 0.8 | 0.03 | 0.006 | 3.75 | 0.76 | 0.88 | 24 |

## DISCUSSION

Some known nebelid species from the genera *Argynnia, Physochila* and *Nebela* have thickened shell plates similar to that of the newly described species, but *N. golemanskyi* has specific features as shell shape, size and structure which are different from the other species and cannot be easily mistaken for them.

*N. golemanskyi* can be clearly distinguished from the species of the genus *Physochila* (e.g. *Ph. griseola*) by: (i) absence of apertural collar which is typical for this genus and represents a turned-over continuation of the shell's neck; (ii) presence of a thin collar of organic cement surrounding the aperture; (iii) larger dimensions.

The differences between *N. golemanskyi* and the species of the genus *Argynnia* (e.g. *A. dentistoma* and its larger varieties) are as follows: (i) presence of a thin collar of organic cement surrounding the aperture (such collar is always absent in *Argynnia'* species and the aperture is surrounded by a siliceous plates, which give it a rough and indented outline); (ii) nearly uncompressed and (iii) more elongated shell (*Argynnia'* species are compressed and more rounded).

The main shell structure and the presence of an thin collar of organic cement surrounding the aperture in both, *N. golemanskyi* and the species of the genus *Nebela* make this species most closer related to genus *Nebela*. Moreover, the structure of the organic cement in *N. golemanskyi* is similar to that of some *Nebela*'s species (e.g. *N. galeata*, *N. speciosa*, *N. penardiana*, *N. carinata*), and the cement is visible as small beads interspersed shell plates (Ogden and Hedley 1980, Plates 35D, 39D, Ogden 1984, Figs 19, 21; pers. observations). After considering these similarities we have decided to include *N. golemanskyi* in the genus *Nebela*.

Two of the *Nebela'* species *N. galeata* and *N. speciosa* are most similar to *N. golemanskyi* in regard to their shell size and shell structure. While the first one *N. galeata* is clearly distinguished by its lateral compression and a presence of a distinct thickened lateral margin in comparison to the *N. golemanskyi*, the second one *N. speciosa* is more similar to *N. golemanskyi*. That was the main reason for us to perform more detailed comparison between these two species. The differences between *N. golemanskyi* and *N. speciosa* can clearly be seen using scanning electron microscopy. Our observation shows the following differences between the two species:

1. The shell of *N. golemanskyi* is composed of characteristic voluminous, globular or rarely elongated, rounded and hollow plates, usually with large parts between them interspersed with small beads of organic cement. The shell plates of *N. speciosa* are smaller and more compressed and they are situated closer to each other.
2. The shell of *N. golemanskyi* has no lateral margins and is nearly uncompressed, while the shell of *N. speciosa* is always compressed and has small lateral margins.
3. The shell of *N. golemanskyi* is comparatively shorter and is not elongated like the *N. speciosa*'s shell. The ratio width/length is higher (0.56 ± 0.02 (n = 24) and 0.45 ± 0.03 (n = 100), respectively); in the newly described species.
4. The aperture of *N. golemanskyi* is roughly circular to oval. It is not convex and is bordered by a thin collar of organic cement. In contrast *N. speciosa*'s aperture is regular oval and is slightly convex in broad view and concave in lateral view, and is surrounded by a thick collar of organic cement.

It is interesting to note that *N. golemanskyi* shows the following peculiarity. When it is observed under LM microscope an optical illusion can be seen by changing the focal distance to the object. It gives the impression that the shell is constructed by two layers of plates. While the external shell layer is composed of larger round plates, the inner layer is built of much smaller and numerous round plates. We think that this illusion is caused by the refraction, because the shell structural elements in *N. golemanskyi* are too voluminous. When the focal distance is adjusted to the external surface of the plates we could see their real size and location (Figs 9, 10). But, when the microscope is focused to the internal surface of the shell, the structural elements are shown as an inverted magnifier, which moves away the objects. In this case the structural elements become too small on the opposite side of the shell and make impression of the presence of second layer, which is composed by comparatively smaller plates (Figs 11, 12).

Meanwhile, the same peculiarity was observed on *N. galeata* as well (Deflandre 1936, p. 210). In his paper the author reported that the shells of some specimens, which have been isolated from two widely separated populations, are composed by two layers. He indicated that the external layer is composed by thickened, non-imbricated and rounded shell plates, which could be easily seen. In contrast, the inner layer is composed by significantly smaller plates which could not be so easily distinguished.

During our own LM observation on the shell structure in nebelids we have seen the same peculiarity (illusion for the presence of two shell layers) in certain specimens of *N. speciosa*.

There were two main views in the past about the origin of the shell plates of the nebelids. Some of the earlier scientists (Leidy 1879, Taranek 1882) believed that the shell of the nebelids is constructed by endogenous plates which are produced in the cytoplasm of testate amoebae. But since the nebelids are predators and their shell structure significantly vary within the group, other scientists (Penard 1902, Deflandre 1936) assumed that they use the plates from their prey for building the shell. Furthermore, the same authors suggest that nebelids have the ability to process the captured plates additionally in their own cytoplasm.

Recent studies prove the last view and at the present time the scientists agree that nebelids do not secrete their own shell plates but derive them by predation from the cytoplasm of smaller testate amoebae (mainly Euglyphida), where they are stored as reserve plates prior to division (Ogden 1979, Ogden and Hedley 1980, Meisterfeld 2000, pers. observations). Thus, many nebelids show considerable diversity with respect to the used material for shell construction (i.e. available prey testate amoebae). Shell plates of smaller testate amoebae, such as *Euglypha*, *Trinema*, *Assulina*, *Corythion*, *Quadrulella*, etc. are usually found unmodified on the shell surface of many nebelids (*N. collaris*, *N. lageniformis*, *N. militaris*, *N. tubulata*, *N. tubulosa*, etc.). However, in some species these plates are thickened, most likely as a result of their additional treatment in the cytoplasm of the nebelids and by accumulation of silicon on their surface (e.g. *N. galeata*, *N. speciosa*, *A. dentistoma*, etc.) (Deflandre 1936, p. 209; pers. observations). Some results from experimental cultures prove the above theory of the shell construction in nebelids. MacKinlay (1936) observed that *N. collaris* produced a membranous shell devoid of shell plates when it was grown in culture without available prey testate amoebae. Ogden (1989) showed that *H. petricola*, which is a close species to the nebelids, also constructs its shell depending on the material available in the culture (different pray testate amoebae, such as *Tr. dentata* and *E. rotunda*, or additional artificial building material).

The results of our study show that the shell of *N. golemanskyi* is constructed by voluminous and massive plates which are considerably different from the shell plates of their possible prey. We suppose that newly described species *N. golemanskyi*, together with some other nebelids (e.g. *N. galeata*, *N. speciosa*) are capable of modifying the shell plates of the captured prey. These species may represent an intermediate clone between the nebelids, which use in shell construction almost untreated plates from their prey (e.g. *N. collaris*, *N. lageniformis*, *N. militaris*, *N. tubulata*, *N. tubulosa*, etc.) and the representatives of the family Lesquereusiidae, which build their shells of endogenous plates, produced by the organisms themselves.

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