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Morphology, Biometry and Ecology of *Arcella excavata* Cunningham, 1919 (Rhizopoda: Arcellinida)

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Summary. Testate amoeba *Arcella excavata* Cunningham, 1919, isolated from the aeration tanks of the Sofia's Wastewater Treatment Plant and its morphology, biometry and ecology have been investigated. Based on the rich live material (120 specimens), a detailed morphological description of *A. excavata* and new data about the cytoplasm and the nuclei of the species are supplied. It was found out that *A. excavata* is a multinuclear species and the nuclei are usually 3 or 4, rarely 5-6 (1-2% only). These new data, as well as the characteristic shape of the shell and its bigger depth, show clearly that *A. excavata* is a separate, well distinguishing species. The size frequency distribution analysis indicates that *A. excavata* is a size-monomorphic species, characterized by a main-size class and a small-size range (all measured individuals have a shell length of 60-70 μm and 98% have a shell breadth of 55-66 μm). The present study shows that *A. excavata* inhabits polluted waters also and this fact does not confirm the conclusion of Snegovaya that it can be used as a bioindicator of the waters with the saprobity β-α. Probably, it is also a freshwater eurybiont as well as the majority of the known species of family *Arcellidae*.

Key words: *Arcella excavata*, biometry, ecology, morphology, Rhizopoda, Testacea.

INTRODUCTION

The genus *Arcella* is one of the most numerous testacean genera. More than 130 taxa of the genus have been described till now. The majorities of them are cosmopolite and inhabit mainly the freshwater pools, moist mosses and rarely soil litter (Deflandre 1928, Chardez 1989).

Besides, some rare species, with a restricted distribution, have been described. The scanty data about their morphology, biometry, distribution and ecological preferences for many of them exist in the literature. Furthermore, the cytoplasm, the types of pseudopodia and nuclei for the majority of these species have not been observed and this causes difficulties in their systematical identification. *Arcella excavata* Cunningham, 1919, is one of these rare and poorly studied species of the genus *Arcella*.

Arcella excavata was observed and described in a small swamp near Durham, N. Carolina (USA) (Cunningham, 1919). The empty shells were observed only. According to the original description the shape of the shell is "somewhat like a quarter-section of cantaloupe, the mouth being situated in the cup. The color is brown to almost black. The sizes of the shell are: length

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55 μm, width 50 μm, total depth 45 μm, depth of depression 25 μm, mouth 15 x 20 μm".

According to Cunningham *A. excavata* is close to *A. curvata* Wailes, 1913, but differs from it by the considerably smaller sizes and by the height of the shell. Later Deflandre (1928) described *A. curvata* as a variety of *A. polypora,* Penard and reported that the sizes of this species range from 125 to 130 μm in diameter.

Deflandre (1928) included *A. excavata* in his monograph of the genus *Arcella* after the description and the figures of Cunningham (1919). Deflandre had a doubt about his independence and noted that: "il serait intéressant de voir si elle est réellement fixée dans la station d'où elle provient et si des exemplaires cultivés ne rétrograderaient pas vers une *Arcella discoides* type or plutôt var. *scutelliformis*, dont les dimensions sont approchantes".

More than forty years after the original description *A. excavata* was not observed in USA and Europe. Štěpánek (1953, 1954) reported the finding of empty shells of *A. excavata* in Moravice River and in aquatic mosses near the Janské Lazni, Krkonoše (Czech Republik). According to Štěpánek the sizes of the observed specimens range as follows: diameter 63-67 μm, depth 35-42 μm, diameter of aperture 16-24 μm, depth of the aperture collar 3 μm. Living specimens have not been observed.

In his monograph Bartoš (1954) also reported *A. excavata* in the Czech Republik (on the data of Štěpánek). He added that it inhabited the sapropele among the aquatic plants. The sizes pointed by Bartoš were borrowed from Štěpánek (1954). Later Geltzer and Korganova (1976), and Geltzer *et al*. (1985) reported the presence of *A. excavata* in Russia also.

Recently Alekperov and Snegovaya (1999, 2000) observed *A. excavata* in four of the studied 5 freshwater pools from Apsheron Peninsula (Azerbaijan). Snegovaya (2001) described *A. excavata* also in a dam of Djeiran-Batan (Azerbaijan). According to Snegovaya (2001) *A. excavata* occurs in comparatively pure water and can be used as a bioindicator of water with saprobity β-α. However, in the above papers no data about the cytoplasm and detailed morphometrical characterization was provided.

During our investigation on the testate amoebae from the aeration tanks of Sofia's Wastewater Treatment Plant we have observed an abundant material of living specimens of *A. excavata* with a high population density. This allows us to make more detailed studies on their morphology, on the variation of his sizes and on the cytoplasm. The results of our studies are the subject of the present paper.

MATERIALS AND METHODS

The material for the present study was collected in July and August 2001 from the aeration tank No 1 of the Sofia's Wastewater Treatment Plant. The water in this aeration tank during the period of the study is characterized by the following chemical parameters: ammonium nitrogen (NH_4-N) - between 8.7 and 13.1 mg N/l; nitrite nitrogen (NO_2-N) - between 0.07 and 0.09 mg N/l; nitrate nitrogen (NO_3-N) - between 0.5 and 0.6 mg N/l; undissolved substances - between 7.0 and 15.1 mg/l.

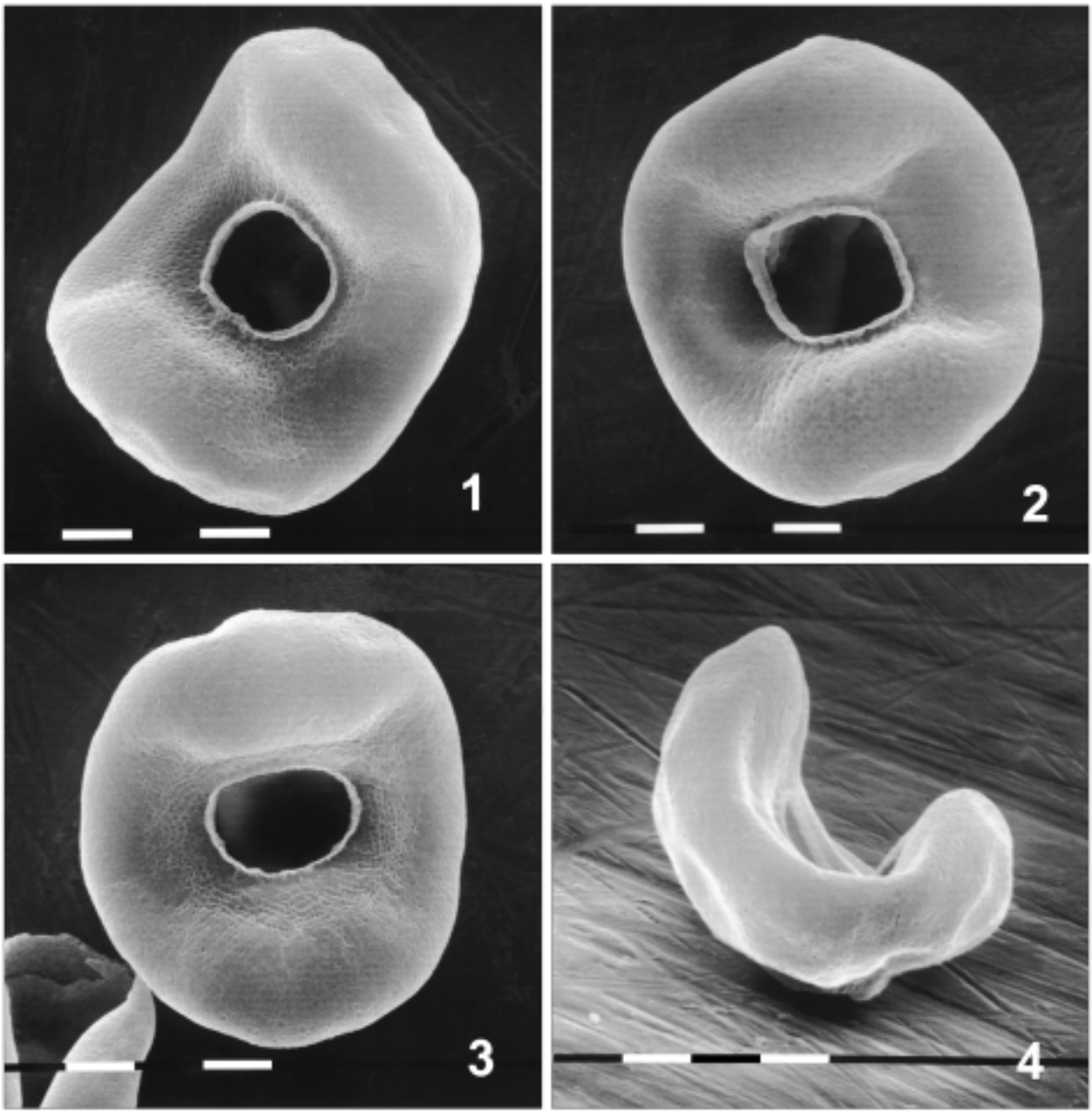
The fresh material was cultivated in a laboratory at room temperature. Thus we were able to observe living specimens, young forms and variability of the shells of *A. excavata*. Some of the observations, measurements and photos were made by optical microscope. For scanning electron microscopy the shells were isolated, cleaned by several transfers through distilled water, mounted directly on stubs and air-dried. The shells were coated evenly with gold in a vacuum coating unit. The microphotographs were obtained by using a Phillips SEM 515, operating at 25 kV.

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. The shell size was measured under light microscope at 400 x magnification.

RESULTS AND DISCUSSION

Morphology of the shell

The shell is colourless or yellowish in young specimens and brown in older specimens, oval or circular in apertural view and croissant-like in lateral view (Figs 1-4, 7-10). The large axis of the shell (length) in almost all observed specimens is in perpendicular direction to the shell's protuberances, when the shape of the shell in apertural view is not circular. Rarely (about 5% of the observed individuals) the large axis of the shell is parallel to the shell's protuberances. The apertural surface is deeply invaginated forming two pronounced protuberances with circular or elliptical aperture at the bottom of invagination. The aperture is bordered by a small lip, of about 3-4 μm (Fig. 5). Štěpánek (1953) notes that small



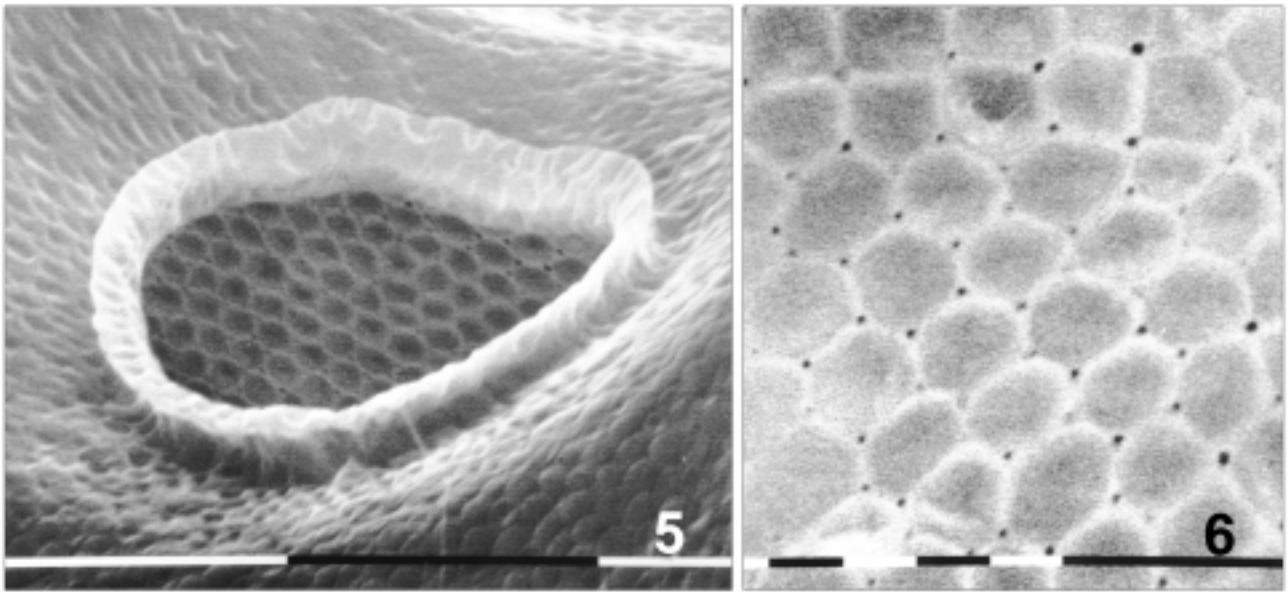
Figs 1-4. SEM photographs of *Arcella excavata*. **1-3 -** apertural views, showing different shell and apertural shape; **4 -** lateral view, showing invagination of apertural surface. Scale bars 10 µm.

pores border the aperture, however in our study we do not observe such pores. The aboral hemispherical region usually has a series of regular depressions (Fig. 7). The shell wall is composed of numerous alveoli in diameter of 1-1.5 µm, made of a proteinaceous material, and arranged in one layer. The shell surface is smooth or irregular and has numerous small pores (Figs 6, 9).

Cytoplasm

The cytoplasm does not quite fill the shell cavity and numerous thin cytoplasmic strands (epipodes) attach it to the inner shell wall (Fig. 7).

The nuclei are 3 or 4, rarely 5-6 (1-2% only) and are large, spherical, between 9.5 and 10.5 ìm in diameter.



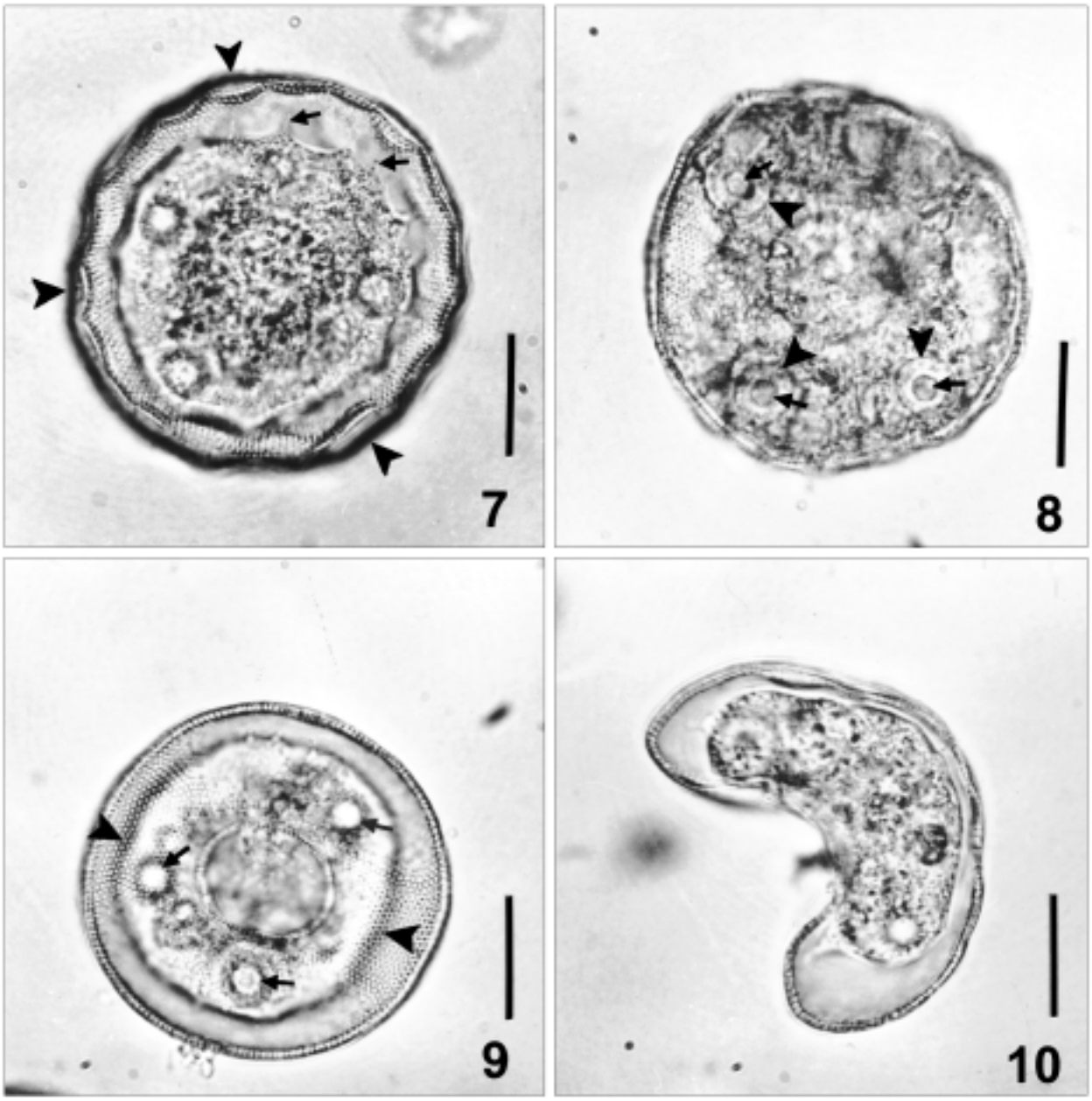
Figs 5-6. *Arcella excavata.* **5** - apertural view to illustrate the small lip, bordering the aperture; **6** -surface of shell showing proteinaceous alveoli and small pores. Scale bars 10 μm (5); 1 μm (6).

Table 1. Morphometric characterization of *Arcella excavata* (measurements in μm).

Character	x	M	SD	SE	CV	Min	Max	n
lenght	64.8	65	2.6	0.2	4.0	60	70	120
breadth	61.1	61	2.8	0.3	4.6	53	70	120
diameter of aperture	19.5	20	0.9	0.1	4.5	18	22	120
depth	40.1	40	1.8	0.2	4.5	36	45	120
apertural invagination	18.9	19	1.7	0.2	9.0	14	25	120

Table 2. Measurements (in μm) of *Arcella excavata* according to different authors.

Authors	Length of shell	Breadth of shell	Depth of shell	Diameter of aperture	Depth of depression	Number of measured specimens
Cunningham 1919	55	50	45	15x20	25	no data
Deflandre 1928	55	50	45	15x20	25	no data
Štěpánek 1953	67	67	35	16	-	1
Štěpánek 1954	63	63	42	24	-	no data
Bartoš 1954	63	63	42	24	-	no data
Present work	60-70	53-70	36-45	18-22	14-25	120

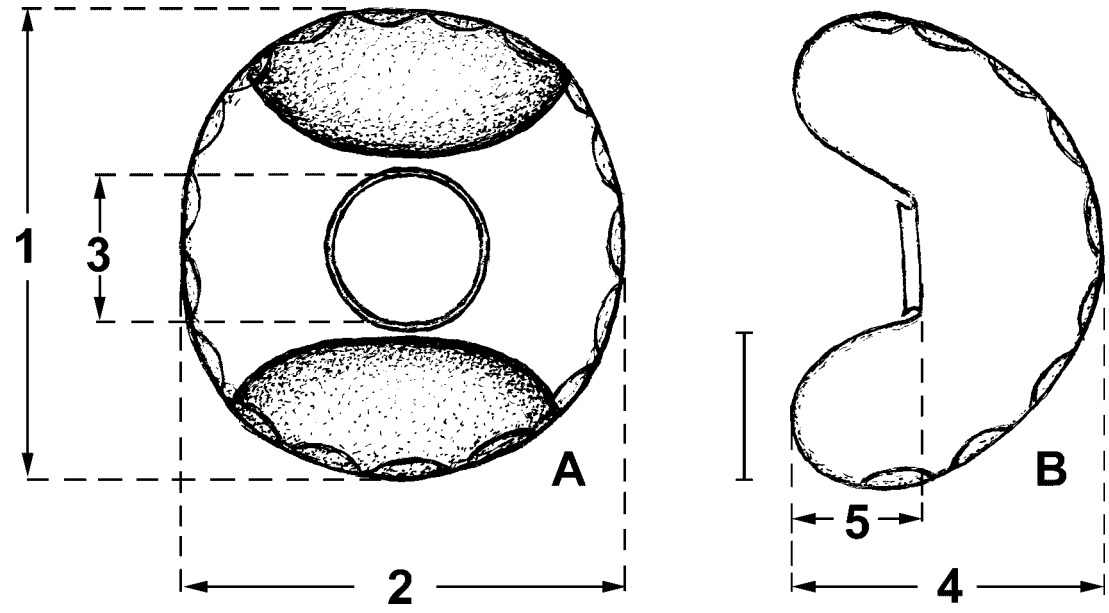


Figs 7-10. Photomicrographs of *Arcella excavata*. **7** - aboral view, showing shell depressions (arrowheads) and thin cytoplasmic strands - epipodes (arrows); **8** - apertural view, showing the nuclei (arrowheads) and the nucleoli (arrows); **9** - apertural view, showing the nuclei (arrows) and the shell structure (arrowheads); **10** - lateral view. Scale bars 20 μm.

They have one central nucleolus (about 5 μm in diameter), easily visible in each of the nuclei (Figs 8, 9). The number of the nuclei, as well as the characteristic shape of the shell and it's bigger depth, show clearly that *A. excavata* is a separate, well distinguished species and it has nothing common with *A. discoides* and *A. discoides* var. *scutelliformis* (as Deflandre, 1928 doubt) or with *A. polypora* var. *curvata* (with that it was compared by Cunningham 1919).

Normally one big contractile vacuole, between 12 and 14 μm in diameter, occurs in the cytoplasm.

The lobopodia are usually 3 or 4, but sometimes they may fuse to form a single fan-like pseudopodium with a ruffled edge.



Figs 11 A, B. Ideal individual of *Arcella excavata*, constructed from median values of all measured specimens; **A** - apertural view: 1 - length, 2 - breadth, 3 - diameter of aperture; **B** - lateral view: 4 - depth, 5 - apertural invagination. Scale bar 20 μm.

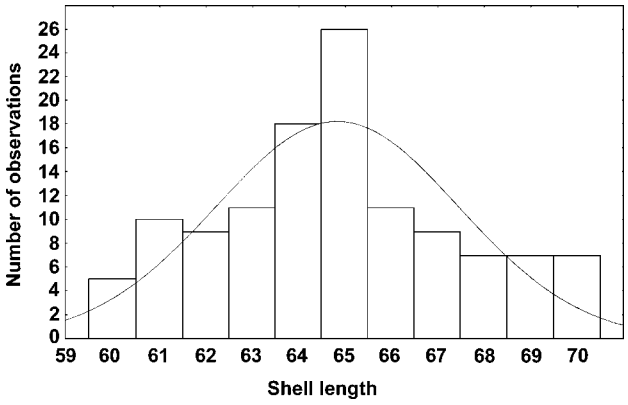


Fig. 12. Histogram showing the size frequency of shell length of *Arcella excavata.*

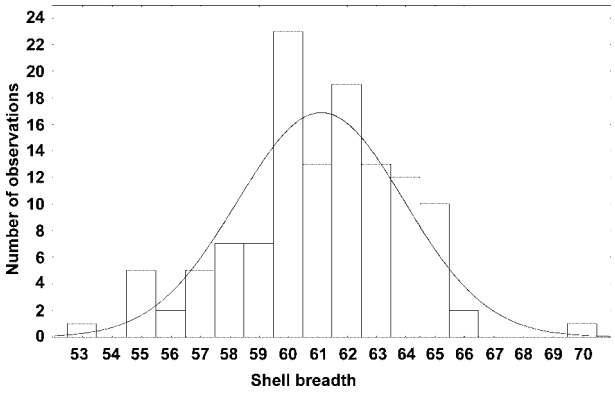


Fig. 13. Histogram showing the size frequency of shell breadth of *Arcella excavata.*

Biometry

Table 1 shows the morphometric characterization of *A. excavata* according to our studies. The ideal individual of this species is constructed from median values of all characters in Fig. 11. Shell measurements with the exception of character (5) are fairly constant and have low variability (CV between 4.0 and 4.5). Our values agree well with those of Štěpánek (1953, 1954) and Bartoš (1954), and are bigger than those of Cunningham (1919) and Deflandre (1928) (Table 2).

Size frequency distribution analysis indicates that *A. excavata* is a size-monomorphic species characterized by a main-size class and a small size range (Figs 12, 13). Figure 12 shows that all measured individuals have a shell length 60-70 μm and more than half of them (55%) are within the limits of 63-66 μm.

The frequency analysis of the shell breadth shows almost the same results (Fig. 13). Ninety-eight percent of all measured individuals have a shell breadth between 55 and 66 μm and about three-fourths of them (75%) are within the limits of 60-65 μm. Only 0.8% has a shell

breadth less than 55 μm and 0.8% has a shell breadth above 66 μm.

Ecology

Since the original descriptions of *A. excavata* there have been scanty data about the ecology of this species. Cunningham (1919) and Deflandre (1928) pointed out that this species occurred in small swamps. According to Štěpánek (1953, 1954) and Bartoš (1954) *A. excavata* inhabits the aquatic mosses and the sapropele among the aquatic plants. Štěpánek (1953) noted that this species is betamesosaprobic. Snegovaya (2001) reported that *A. excavata* occurred in comparatively pure water and could be used as a bioindicator of water with the saprobity β-α.

Our investigation shows that *A. excavata* inhabits also polluted waters of the aeration tanks of Sofia's Wastewater Treatment Plant. This fact does not confirm the conclusion of Snegovaya (2001) that *A. excavata* can be used as a bioindicator of water with the saprobity β-α. Probably *A. excavata* is also a freshwater eurybiont, as well as the majority of the known species of the family *Arcellidae*.

Geographical distribution: Azerbaijan, Bulgaria, Czech Republik, Russia and United States of America.

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