

## Biometry of three rare testate amoebae species (Arcellinida and Euglyphida) from freshwater and moss biotopes in Bulgaria

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### Summary

*Euglypha filifera* var. *cylindracea* Playfair, 1917, *Diffugiella patinata* Schönborn, 1965, and *Cryptodiffugia sacculus* Penard, 1902, collected in different biotopes at the Durankulak Lake and Golden Sands Nature Park in the Northeastern Bulgaria, are redescribed. A detailed morphometrical analysis of variability in natural populations of these testate amoebae is made. The analysis of variation coefficients shows that the most stable characters in the populations are breadth/length ratio, shell breadth and shell length. For all these taxa, high values of correlation coefficients between the length and the breadth of the shell were noted. Diameter of aperture is very often weakly correlated with other parameters. The taxa studied are characterized by a well-expressed main-size class and by a small size range of the characters, and can be defined as size-monomorphic species. *Euglypha filifera* var. *cylindracea* is reported for the first time in the Bulgarian protozoan fauna.

**Key words:** testate amoebae, variability, morphometry

### Introduction

Testate amoebae are a remarkable group of protists which play an important role in aquatic and soil ecosystems. These amoebae represent a significant interest from both theoretical and practical point of view. Therefore, they are subject to extensive and diverse research in many countries. Testacean taxonomy is mainly based on morphological features of external structures such as the shell size, shape, breadth/length ratio etc., which are subject to great natural variability. Many species of testate amoebae show high polymorphism in terms of their size, and are able to form different shapes under variable

environmental conditions, even in the same habitat. Uniformity and precision of the description of species requires the use of accurate biometric analysis which was applied by many authors (Schönborn et al., 1983; Lüfteneger et al., 1988; Lüfteneger and Foissner, 1991; Wanner, 1988, 1999; Foissner and Korganova, 2000; Török, 2001; Todorov, 2002; Todorov and Golemansky, 2003; Bobrov and Mazei, 2004; Yang et al., 2004; Yang and Shen, 2005; Nicholls, 2005, 2006, 2007a, 2007b; Golemansky and Todorov, 2006; Lahr and Lopes, 2006, 2007; Tsyganov and Mazei, 2006, 2007; Zapata and Fernández, 2008; Qin et al., 2008; Todorov et al., 2009; Snegovaya and Alekperov, 2010).

The present paper redescibes three testate amoebae species sampled from different habitats in Bulgaria: *Euglypha filifera* var. *cylindracea* Playfair, 1917 – new testate amoebae to the Bulgarian protozoan fauna, *Diffugiella patinata* Schönborn, 1965 and *Cryptodiffugia sacculus* Penard, 1902. The analysis of literature shows that these testaceans are rare, they are found in single specimens, and detailed biometric data for them are not available so far (Schönborn, 1965; Mazei and Tsyganov, 2006). The aim of the study is to perform a detailed analysis of morphometrical variability in natural populations of these species and to add information about their ecological preference and geographic distribution.

## Material and methods

The material for the present study was collected in different biotopes from Durankulak Lake and Golden Sands Nature Park, from February 2010 to July 2011. The protected area “Durankulak Lake” is situated about 6 km to the south from the border between Bulgaria and Romania, on the Black Sea Coast. It is a shallow (maximum depth 4 m), freshwater eutrophic to hypertrophic lake. The water column in the lake is saturated with oxygen from the surface to the bottom (Georgiev, 1998). Golden Sands Nature Park is a nature park on the Bulgarian Black Sea Coast. According to the World Conservation Union (IUCN), the park is a protected area of V-th category. It is covered with natural deciduous forests and shrubs which occupy 90 % of the territory of the park and contribute to its unique landscape (Nature Park “Zlatni Pyasatsi”, 2012). Description of the investigated samples and found species are presented in Table 1.

Sampling of benthos from the littoral zone of the water basins was accomplished by collecting a small volume (about 10 ml) of surface sediment by submerging a large bore plastic bottle. All benthos samples were stored in 200 ml polycarbonate bottles with water from the collection site. The samples of hydrophyte vegetation and moss were gathered in paper or nylon bags. In the laboratory, the plants stayed for a while in distilled water, after which they were ‘squeezed’ and washed in it. After that the particles of moss and aquatic vegetation were carefully removed and the samples were filtered through a sieve. The remaining fraction was used for establishing the taxonomic composition and counting of testate amoebae. A half of each sample was fixed with 4% formaldehyde. Another half was kept alive for *in vivo* investigation.

For the morphometric characterisation the following parameters were calculated:  $\bar{x}$  – arithmetic mean, M – median, SD – standard deviation, SE – standard error of the arithmetic mean, CV – coefficient of variation in %, Min and Max – minimum and maximum values, n – number of examined individuals (Schönborn et al., 1983). The species were identified and measured with light microscope (LM) “Amplival” using bright field, at 400x magnification. All measurements are in  $\mu\text{m}$ .

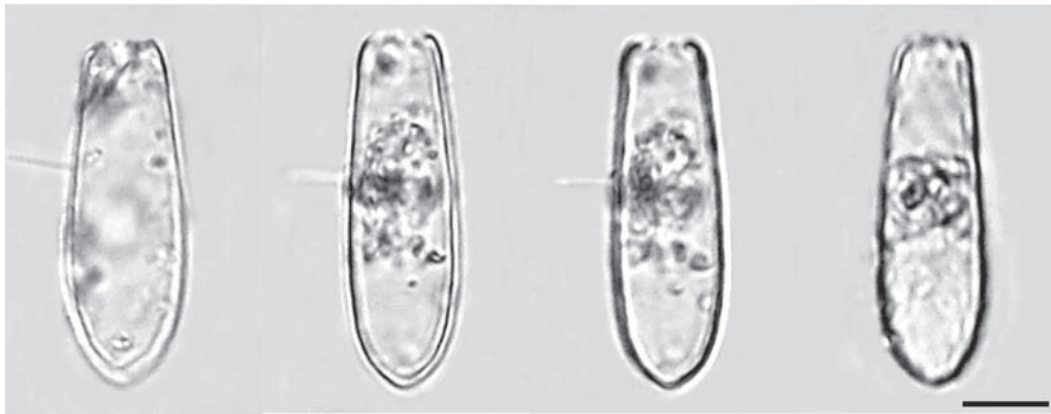
The following abbreviations were used: L – length of shell, B – breadth of shell, D – depth of shell, Da – diameter of aperture, Lsp – length of spines, Nsp – number of spines, Nl – neck length, Nb – neck breadth, B/L – ratio breadth of shell to length of shell and Da/L – ratio diameter of aperture to length of shell. Statistical analysis was performed using the STATISTICA 6.0 software (StatSoft Inc., 1999).

## Results and discussion

### *EUGLYPHA FILIFERA* VAR. *CYLINDRACEA* PLAYFAIR, 1917 (FIG. 1)

Playfair (1917) described this variety from Lismore (Australia) on the basis of its almost cylindrical shell, rounded or slightly pointed behind, with sides almost straight to the aperture. The author indicated the following measurements: L = 65–68, B = 23–24, Da = 9.5–10.0, length of spines 21–23  $\mu\text{m}$ , but not reported data on the depth of the shell. Declootre (1962) included *E. filifera* var. *cylindracea* in his monograph on the genus *Euglypha* based on the measurements and figures of Playfair (1917). Declootre (1962) noted that the shell was more elongated than in type species, and the sides were more straight and less bringing together towards the aperture. Chardez (1969) redescibed *E. filifera* and also indicated existence of the variety *E. filifera* var. *cylindrica*, considering its almost parallel sides, but in this work lacks figures or morphometric data.

Table 2 shows the morphometric characteristics of *E. filifera* var. *cylindracea* obtained from Durankulak Lake. In the samples from Golden Sands Nature Park, this species was found in smaller number of specimens, but organisms were of the same dimensions. By their morphological peculiarities – shell shape and structure, number and structure of the apertural plates, the present specimens are in good agreement with the original description (Playfair, 1917). The specimens observed by us are slightly compressed and maintained their



**Fig. 1.** LM photographs of *Euglypha filifera* var. *cylindracea* – lateral view of different specimens showing general test shape and outline. Scale bar: 10  $\mu$ m.

almost cylindrical shape. It should be noted that this oval cross section is typical for *E. filifera* (Penard, 1902). The dimensions given in Table 2 are smaller than those given by Playfair (1917). Some of the specimens (27 %) have no spines but other features match exactly with the original description of the variety, which allows to treating them respectively (Fig. 1). The length, breadth and depth of the shell, as well as the breadth/length ratio of the shell (B/L), show low or normal variability (CV 2.78 – 5.84 %), whereas diameter of aperture, length and number of spines and the ratio diameter of aperture/length of the shell have high coefficients of variation (12.50 – 73.82 %) (Table 2).

Correlation coefficients between the morphometrical characters of *E. filifera* var. *cylindracea* are represented in Table 3. It shows that depth of

shell is weakly correlated with other parameters and suggests that this shell characteristic changes independently of others. On the contrary, there are high values of correlation coefficients between the length, the breadth of the shell and diameter of aperture.

The ecological preferences of this variety have not been established in detail. Playfair (1917) mentioned only that it occurs in swamps. In our study, it was found in benthic samples from the littoral zone in Durankulak Lake in February 2010, as well as in samples from sediments and *Typha* sp. in a stream and in epiphytic and soil mosses in Golden Sands Nature Park in July 2011 (Table 1). These facts suggest that *E. filifera* var. *cylindracea* is a freshwater (mainly related with the aquatic vegetation) and moss dwelling testacea.

**Table 1.** Location and description of the investigated samples.

Sampling location	Date of collection	Microhabitat	Species
<b>Durankulak Lake</b> 30°40'30"N, 28°33'43"E Altitude 0.09–0.60 m a.s.l.	February 2010	Benthic samples from the littoral zone of the lake, depth from 0.5 to 1 m	<i>Euglypha filifera</i> var. <i>cylindracea</i>
	August 2010	Benthic samples from the littoral zone of the lake, depth from 0.5 to 1 m	<i>Diffugiella patinata</i>
<b>Golden Sands Nature Park</b> 43°18'08"N, 28°02'04"E Mean altitude – 110 m a.s.l.	July 2011	Benthic samples from a stream, near to Kranevo village, depth 0.5 m	<i>Euglypha filifera</i> var. <i>cylindracea</i>
	July 2011	Benthic samples from a stream, near to Directorate of the park, depth 0.5 m	<i>Cryptodiffugia sacculus</i>
	July 2011	Aquatic vegetation ( <i>Typha</i> sp.) from a stream, near to Kranevo village	<i>Euglypha filifera</i> var. <i>cylindracea</i>
	July 2011	Aquatic vegetation ( <i>Phragmites</i> sp.) from a swamp, near to mountain hotel "Academic", depth 0.5 m	<i>Diffugiella patinata</i>
	July 2011	Epiphytic mosses, near to mountain hotel "Academic"	<i>Euglypha filifera</i> var. <i>cylindracea</i>
	July 2011	Soil mosses, near to Directorate of the park	<i>Euglypha filifera</i> var. <i>cylindracea</i>

**Table 2.** Biometric characterization of the investigated testacean species. Abbreviations: see Material and methods.

Characters	x	M	SD	SE	CV	Min	Max	n
<i>Euglypha filifera</i> var. <i>cylindracea</i> *								
L	39.45	40.00	2.18	0.56	5.53	35	42.5	15
B	14.17	14.00	0.43	0.11	3.03	13.5	15	15
D	12.50	12.25	0.73	0.19	5.84	11.25	14	15
Da	6.47	7.00	0.83	0.21	12.83	5.5	7.5	15
Lsp	6.70	8.50	4.25	1.28	63.43	8	10	11
Nsp	2.33	3.00	1.72	0.44	73.82	0	4	15
B/L	0.36	0.36	0.01	0.002	2.78	0.34	0.37	15
Da/L	0.16	0.16	0.02	0.01	12.50	0.14	0.18	15
<i>Diffugiella patinata</i> **								
L	9.87	10.00	1.25	0.32	12.66	8	12	15
	10.42	10.00	0.50	0.10	4.80	10	11	24
B	11.53	12.00	1.30	0.34	11.27	9	13	15
	12.79	13.00	0.88	0.18	6.88	11	14	24
Da	8.47	8.00	1.30	0.34	15.35	7	11	15
	7.71	8.00	0.46	0.09	5.97	7	8	24
B/L	1.16	1.13	0.06	0.02	5.17	1.10	1.25	15
	1.23	1.27	0.08	0.02	6.50	1.10	1.30	24
Da/L	0.87	0.88	0.05	0.06	5.75	0.80	0.92	15
	0.75	0.73	0.04	0.01	5.33	0.70	0.82	24
<i>Cryptodiffugia sacculus</i>								
L	28.85	29.50	1.31	0.29	4.54	26	30	20
B	16.85	17.00	0.88	0.20	5.22	16	18	20
Da	6.25	6.00	0.44	0.10	7.04	6	7	20
NI	4.50	4.50	0.51	0.11	11.33	4	5	20
Nb	7.50	7.50	0.51	0.11	6.80	7	8	20
B/L	0.59	0.59	0.02	0.01	3.39	0.57	0.60	20
Da/L	0.22	0.21	0.01	0.002	4.55	0.20	0.25	20

\* population from Durankulak Lake.

\*\* 1st line: population from Golden Sands Nature Park, 2nd line: population from Durankulak Lake.

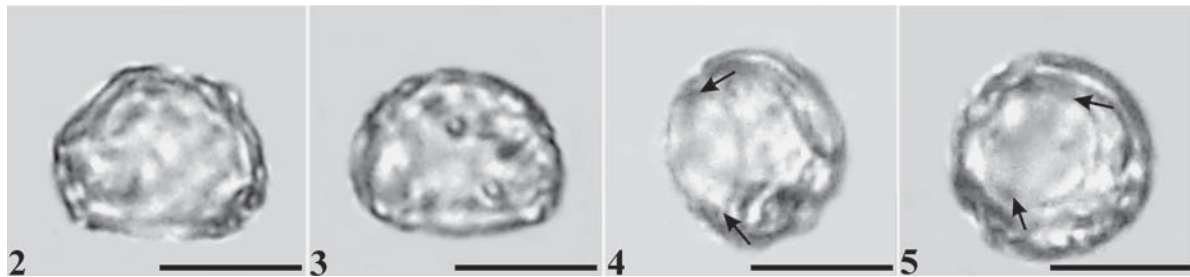
***DIFFLUGIELLA PATINATA* SCHÖNBORN, 1965 (FIGS 2–5)**

Schönborn (1965) described *D. patinata* from Poland and Germany. According to the original description, the shell is bowl-shaped, wider than high. Its upper surface is often rough, covered with foreign elements. The color is yellow to brown, transparent. The aperture is wide and covers almost the whole base of the shell. The sizes of the shell are: L = 9.5–10.2, B = 11.5–13.6, Da = 7.8–10.2  $\mu\text{m}$ , B/L = 1.16–1.36 (Schönborn, 1965).

The specimens found by us display the shell morphology which is in good agreement with

the original description. However, in the present study we established a wider range of dimensions compared to that indicated by Schönborn (1965).

The separate morphometric analysis of the two investigated populations is presented in Table 2. It shows that the median values for the main shell features (length, breadth of shell and diameter of aperture) are equal or close to relevant metrics in the studied populations. However, the coefficients of variation for these parameters differ significantly in both populations (Table 2, Fig. 6). Maximal values of variation coefficients of shell length (12.66%), shell breadth (11.27%) and diameter of aperture



**Figs 2–5.** LM photographs of *Diffugiella patinata*. 2, 3 – lateral view of different specimens showing general test form; 4 – semi-lateral view, showing aperture (arrows); 5 – apertural view (arrows). Scale bar: 10  $\mu\text{m}$ .

(15.35%) are observed in population from Golden Sands Nature Park, while in that from Durankulak Lake these values did not exceed 7%. Despite the fact that above-mentioned shell measurements have different variability, shell breadth/length ratio seems to be constant with low variability (CV is 5.17 and 6.50%, respectively) in both populations (Table 2).

Correlation coefficients between the morphometrical characters are represented in Table 4 and shows that shell length is positively correlated with other parameters.

*D. patinata* is a size-monomorphic species, but there are differences between the two populations. Figure 6 shows that the specimens of the population of the Durankulak Lake are characterized by smaller size range with respect to all characters – the shell length of all individuals measured range between 10 and 11  $\mu\text{m}$ , 75% of specimens have a shell breadth 13–14  $\mu\text{m}$ , and all individuals have a diameter of aperture between 7 and 8  $\mu\text{m}$ . In the population of Golden Sands Nature Park these measurements range, respectively, between 8 and 12, 9 and 13, 7 and 11  $\mu\text{m}$ .

Little information is available about the ecological preference of this species. Data on the ecology of *D. patinata* was provided by Schönborn (1965), who stated that it occurred in samples of *Typha* sp. in the Masurian lakes (Poland) and in Great Shtehlin Lake (Germany). For the first time in Bulgaria, specimens of *Diffugiella patinata* were discovered in

benthic samples and samples of *Phragmites australis* in Srebarna Lake (Davidova, 2005). In the present work, the species were isolated from benthic samples taken in the littoral zone in Durankulak Lake, in August 2010, and from samples of *Phragmites* sp. from a swamp in Golden Sands Nature Park in July 2011.

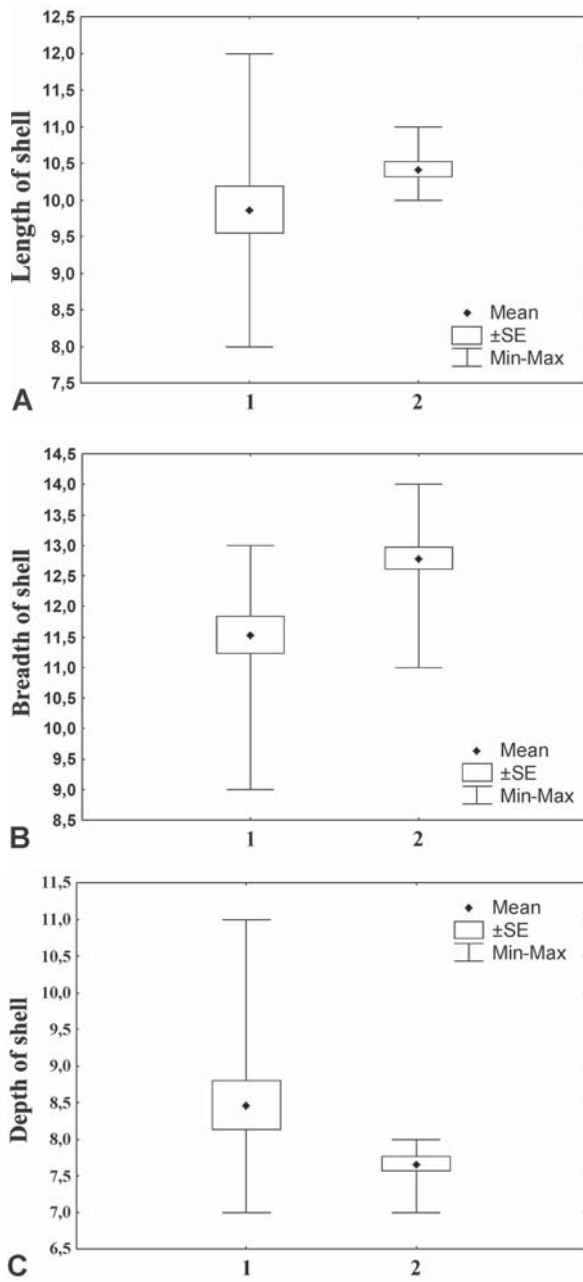
**CRYPTODIFFLUGIA SACCULUS (PENARD, 1902)**  
(FIG. 7), SYN. *DIFFLUGIELLA SACCULUS* (PENARD, 1902)  
DEFLANDRE, 1953

According to the original description of this species (Penard, 1902) the shell is pyriform, with a distinct wide, large neck and circular cross section. It is transparent, colorless or yellowish, covered with more or less xenosomes or with the diatom frustules. The aperture is circular. The length of shell varies between 17 and 26  $\mu\text{m}$ , but data on other dimensions were not indicated. Later, Playfair (1917) found the species in Australia and indicated that the specimens were “very variable in shape”. The author gave the following dimensions: shell length 23–30, shell breadth 17–22, aperture diameter 12–14, neck length 4–8, neck breadth 10–12  $\mu\text{m}$ . Deflandre (1953) studied the genera *Cryptodiffugia* and *Diffugiella* and relying mainly on circular cross-section of the shell of *Cryptodiffugia sacculus*, assigned them to a genus *Diffugiella*. Grospietsch (1964) also confirmed these data and described specimens with measurements: L = 16–30, B =

**Table 3.** Correlation coefficient between morphometric characteristics in *Euglypha filifera* var. *cylindracea*. Abbreviations: see Material and methods.

	L	B	D	Da	Lsp
B	0.910***				
D	0.224 <sup>NS</sup>	0.432 <sup>NS</sup>			
Da	0.775***	0.708**	-0.091 <sup>NS</sup>		
Lsp	-0.656**	-0.644**	-0.009 <sup>NS</sup>	-0.528*	
Nsp	-0.529*	-0.510 <sup>NS</sup>	0.311 <sup>NS</sup>	-0.565*	0.862***

Significant relationship \*\*\*p < 0.001; \*\*p < 0.01; \*p < 0.05; NS – not significant



**Fig. 6.** Box plots representing the shell length (A), shell breadth (B) and shell depth (C) of two *Diffugiella patinata* populations from Golden Sands Nature Park (1) and Durankulak Lake (2).

15–22 µm. Page (1966) constructed a key to the genus *Cryptodiffugia* and included the species as *Cryptodiffugia sacculus*, based on the description of Penard (1902).

Our specimens show morphological characteristics and dimensions which correspond to the species described earlier (Penard, 1902; Playfair, 1917; Deflandre, 1953; Grospietsch, 1964).

**Table 4.** Correlation coefficient between morphometric characteristics in *Diffugiella patinata*\*. Abbreviations: see Material and methods.

	L	B
<b>B</b>	0.872*** 0.401*	
<b>Da</b>	0.922*** 0.418*	0.752** 0.238 <sup>NS</sup>

\* 1st line: population from Golden Sands Nature Park, 2nd line: population from Durankulak Lake. Significant relationship \*\*\*p < 0.001; \*\*p < 0.01; \*p < 0.05; NS – not significant.

The analysis of the variation coefficients shows that the minimal variability is noted for breadth/length shell ratio (3.39%), shell length (4.54%), diameter of aperture/shell length ratio (4.55%) and shell breadth (5.22%) (Table 2). These are the most stable characters in the populations. More variable are the neck breadth (6.80%) and diameter of aperture (7.04%). Maximal variation coefficient is noted for neck length (11.33%). The information in Table 5 illustrates that shell length is positively correlated with the shell breadth, the neck length and the neck breadth.

Penard (1902) described the species from a swamp and indicated that it develops in the presence of different types of algae. Playfair (1917) observed *C. sacculus* in samples of washed water plants (*Myriophyllum* sp., *Elodea* sp.) from a stream. According to Grospietsch (1964), the species inhabits the aquatic vegetation and mosses (including *Sphagnum*), marshes and soils. Davidova (2012) reported that it occurred in sediments from the littoral zone in Ovcharitsa Reservoir. In the present work, the species was found in benthic samples from a stream in Golden Sands Nature Park in July 2011. These facts suggest that *C. sacculus* is a freshwater eurybiont and soil inhabitant.

**Table 5.** Correlation coefficient between morphometric characteristics in *Cryptodiffugia sacculus*. Abbreviations: see Material and methods.

	L	B	Da	NI
<b>B</b>	0.852***			
<b>Da</b>	0.399 <sup>NS</sup>	0.508*		
<b>NI</b>	0.745***	0.762***	0.577**	
<b>Nb</b>	0.745***	0.762***	0.577**	1.000***

Significant relationship \*\*\*p < 0.001; \*\*p < 0.01; \*p < 0.05; NS – not significant.

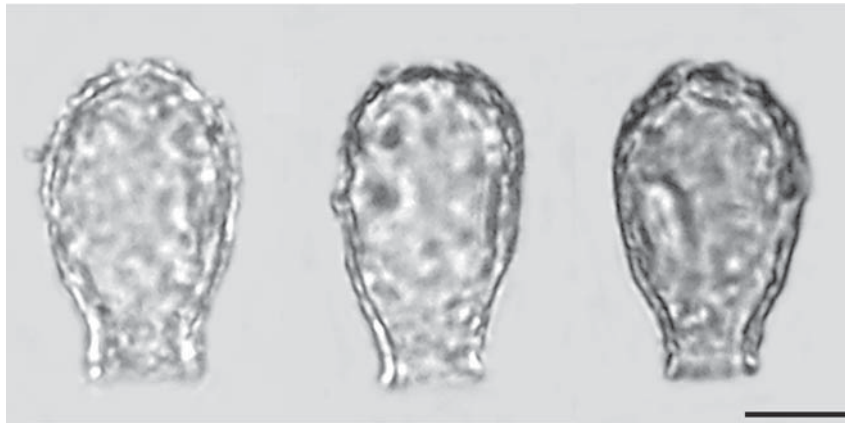


Fig. 7. LM photographs of *Cryptodiffugia sacculus* – lateral view of different specimens showing general test shape and outline. Scale bar: 10  $\mu$ m.

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