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New Data to the Shell Ultrastructure and the Biometry of the Marine Interstitial Testate Amoebae (Rhizopoda: Testaceafilosia)

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Summary. The data about the shell ultrastructure of 6 interstitial testate amoebae: *Chardezia caudata*, *Psammonobiotus balticus*, *P. golemanskyi*, *Corythionella minima*, *C. pontica* and *Micramphora pontica* is presented for the first time. A biometric analysis of all of them, found in rich populations in the North-Eastern Black Sea littoral, is also accomplished. On the basis of the present investigations some morphometric descriptions and taxonomic diagnoses of the studied interstitial testate amoebae are supplemented and corrected. The summarized data on their geographical distribution so far in the World Ocean is given, too.

Key words: biometry, distribution, shell ultrastructure, taxonomy, testate amoebae.

INTRODUCTION

The marine interstitial testate amoebae form a specific taxocenose in the underground waters of the marine sand supralittoral. They became a subject of more detailed investigations only in the last four decades. The morphological descriptions and taxonomic diagnoses of most of the taxa reported so far have been performed mainly by light microscopy. Because of that, the information about some essential details of the shell morphology, the structure and the character of the idiosomes and xenosomes, forming the shells, is scanty and incomplete. As a result the taxonomic status of some of the established taxa and their phylogenetic relations with close genera and species are still unclear.

Eighty psammobiotic interstitial testate amoebae from the marine sand supralittoral have been established so far, but more detailed information about their structure and ultra-morphology has been given only for 26 of them (Golemansky and Ogden 1980; Golemansky and Coûteaux 1982; Ogden and Coûteaux 1986, 1989; Anderson *et al.* 1996; Golemansky and Todorov 1996, 2004). Further investigations on the ultra-morphology of other taxa of this group (especially of genera and species with problematic systematic status) are necessary, so that, the taxonomic problems with the marine interstitial testate amoebae and their relations with the freshwater testate amoebae can be solved and explained.

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The purpose of this article is to present the results of our last investigations on the shell ultra-morphology of 6 interstitial testate amoebae that have not been studied so far by scanning electron microscopy (SEM). Because of the presence of rich populations of some of them in our samples, a biometric analysis of their morphological variability also has been accomplished. Some corrections and supplements to the taxonomic diagnoses and descriptions of the studied interstitial testate amoebae have been made on the basis of the obtained results.

MATERIALS AND METHODS

The materials were collected from several localities in sandy beaches of the Bulgarian Black Sea Coast during the period from April to October 2005. Samples were taken from the level of the underground water in holes with a depth of 0.20-1.00 m, which were excavated in the sand at different distances from the waterline. Samples were kept live until the moment of their study.

The biometric characterization of the studied species was made by the method of Schönborn *et al.* (1983). The following parameters were calculated: arithmetic mean ($\overline{X}$); median (M); standard deviation (SD); standard error of the mean (SE); coefficient of variation in % (CV); extreme values (Min and Max); number of examined individuals (n). Size measurements in micrometers (μm) of shells were made by light microscopy at 400× magnification.

For scanning electron microscopy (SEM) the shells were isolated, cleaned through several washes with distilled water, mounted directly on stubs and air-dried. The shells were coated evenly with gold in a vacuum coating unit. Microphotographs were obtained by using a Phillips SEM 515, operated at 25 kV.

RESULTS AND DISCUSSION

Chardezia caudata Golemansky, 1970 (Figs 1-4, Table 1)

Shell morphology. The shell is elongate-elliptical, plagiostomic, colourless and translucent by light microscopy. The aboral end of the shell is prolonged in a hallow spine, reaching to 1/3 of its total length. The cross section of the shell is circular. In lateral view the shell is slightly curved, narrowed at the anterior end, and forms a brief neck. The ventral wall is almost flat and the dorsal wall is blown up. After the neck the shell is enlarged in a funnel-like circular collar around the aperture (Figs 1, 2).

According to the original description of the shell by light microscopy it is proteinaceous, smooth, without idiosomes or xenosomes (Golemansky 1970a). Our SEM investigations of the shell structure show that it is formed of many ellipsoidal or oval idiosomes (2.5-3.0 μm length and 1.2-1.3 μm width) which are randomly arranged to overlap each other (Figs 3, 4). By its shell ultra-structure *C. caudata* is close to the genus *Corythionella*, but by its shell form, cross section and the presence of the caudal spine it is similar to the genus *Pseudocorythion.* The periphery of the collar rim is formed of smaller elliptic idiosomes, united together by organic cement like that of *Corytionella.* Undoubtedly, the genus *Chardezia* is a member of the family Psammonobiotidae, close to the genera *Pseudocorythion* and *Corythionella*.

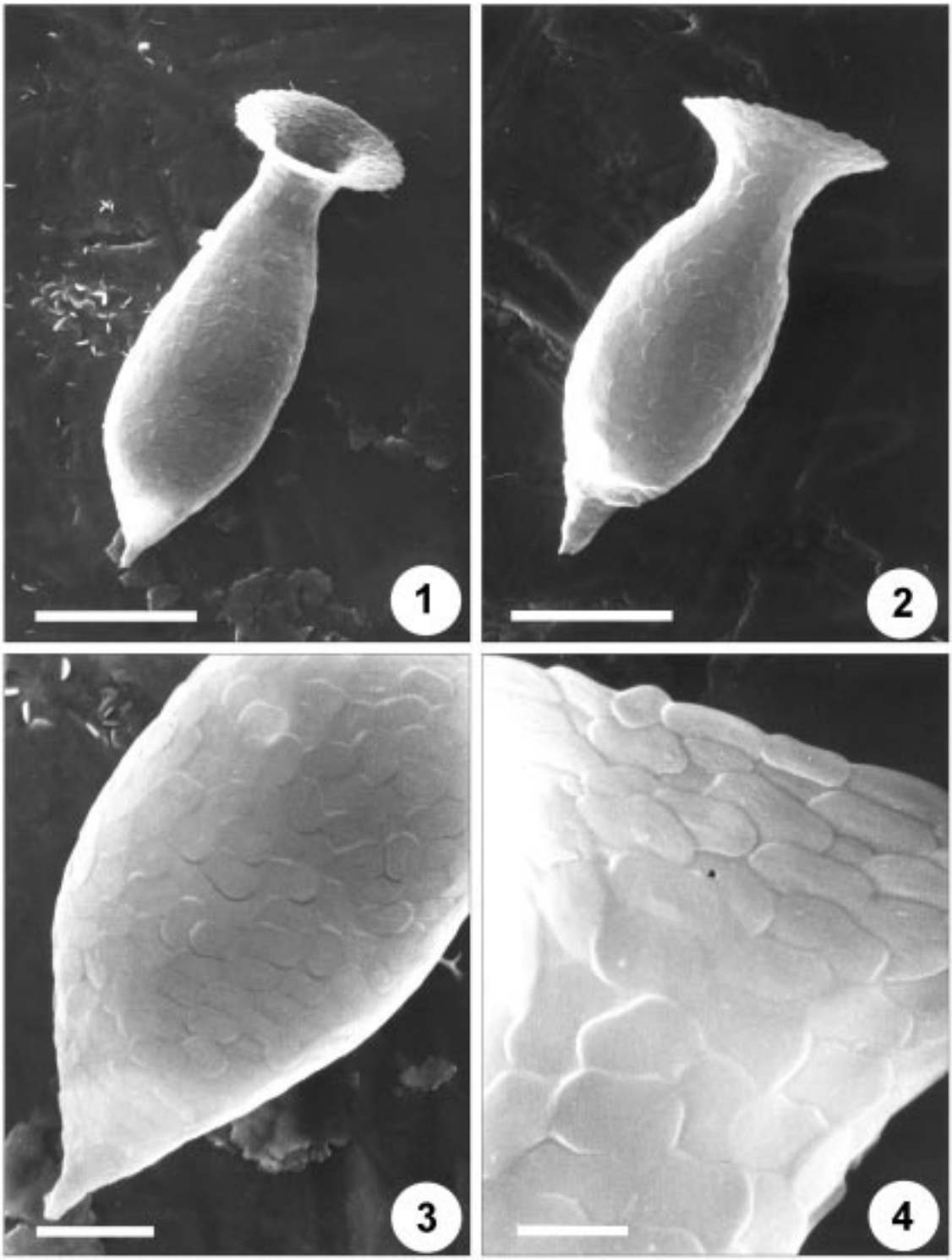
Biometry. Coefficients of variation of the measured taxonomical characters (except the length of spine) are less or near to 10% and show that shell parameters are moderately variable. The length of spine is more variable (CV=22.78) and range between 5 and 14 μm. This variability of the spine length is the main reason for variability in measurements of the total shell length in *C. caudata* (between 40 and 51 μm). As a whole our values correspond well to the original description (Golemansky 1970a).

Analysis of the size frequency distribution indicates that *C. caudata* is a size-monomorphic species (Figs 5A, B). The presence of comparatively well-expressed main-size class of the shell length (46-48 μm), as well as of the shell diameter (13-14 μm), and the lack of subsidiary peaks (bell-shaped curves) indicate a normal distribution (Figs 5A, B). The average length and diameter of *C. caudata* shells amounted to 46.3 ± 3.3 ($\overline{X}$ ± SD; n=35) and 13.1 ± 0.7 ($\overline{X}$ ± SD; n=35), respectively. These arithmetical means agree well with the main size classes of both, shell length and shell diameter, and testify to the monomorphism of the species.

Geographical distribution. So far *Chardezia caudata* was sporadically known from the underground waters of a few sandy beaches of the Black Sea (Golemansky 1970a, 1974), Mediterranean and Aegean Seas (Golemansky 1976a, 1982), the Pacific (Golemansky and Todorov 1996) and the Atlantic (Golemansky 2000). Undoubtedly, *C. caudata* has a cosmopolitan distribution in the World Ocean, but it is often imperceptible by light microscopy because of the perfect transparency of its shell and the small dimensions.

Psammonobiotus balticus Golemansky, 1973 (Fig. 6, Table 1)

Shell morphology. In ventral view the shell is ovoid, colourless and translucent. It has two distinct parts: a



Figs 1-4. SEM photographs of *Chardezia caudata*. **1, 2** - lateral view of two specimens; **3** - posterior end of the shell; **4** - shell structure of the neck. Scale bars: 15 µm (1, 2); 5 µm (3), 2 µm (4).

Table 1. Biometric characterization of the investigated testacean species. $\bar{x}$ - arithmetic mean; M - median; SD - standard deviation; SE - standard error of the mean; CV - coefficient of variation in %; Min - minimum; Max - maximum; n - number of examined individuals (measurements in µm).

Character	$\bar{x}$	M	SD	SE	CV	Min	Max	N
Chardezia caudata								
Total length	46.28	47	3.32	0.56	7.17	40	51	35
Diameter of shell	13.11	13.5	0.69	0.12	5.22	11.5	14	35
Diameter of aperture	6.16	6	0.62	0.1	10.06	5	7	35
Diameter of collar	17.42	17.5	1.24	0.21	7.12	15	20	35
Length of spine	9.7	10	2.21	0.37	22.78	5	14	35
Psammonobiotus balticus								
Length	22.14	22	1.62	0.35	7.31	20	26	21
Width	17.04	17	0.86	0.2	5.04	15.5	19	21
Height	19.52	19.5	1.17	0.25	5.99	18	22	21
Diameter of aperture	8.5	8.5	0.89	0.2	10.47	7	10	21
Length / Width	1.29	1.29	0.05	0.01	3.87	1.22	1.39	21
Width / Height	0.87	0.87	0.02	0.01	2.29	0.83	0.92	21
Psammonobiotus golemanskyi								
Length	31.25	31.5	1.98	0.25	6.33	28	36	62
Width	19.93	20	1.28	0.17	6.42	17	22	62
Height	18.31	18	1.16	0.15	6.33	16	20	62
Diameter of aperture	8.17	8	0.61	0.08	7.46	6.5	9.5	62
Large axis of collar	22.00	22	1.29	0.16	5.86	20	25	62
Small axis of collar	18.00	18	1.40	0.17	7.77	15	21	62
Length / Width	1.57	1.56	0.09	0.01	5.73	1.4	1.79	62
Width / Height	1.08	1.1	0.07	0.01	6.48	0.9	1.25	62
Corythionella minima								
Length	50.1	50	3.24	0.48	6.46	44	60	47
Width	23.09	23	1.85	0.27	8.01	18	27	47
Height	14.72	15	0.64	0.1	4.34	13.5	16	47
Diameter of aperture	9.97	10	0.67	0.1	6.72	8	11	47
Large axis of collar	23.84	24	1.28	0.18	5.37	21	26	47
Small axis of collar	21.74	22	1.29	0.19	5.93	20	24	47
Length / Width	2.18	2.18	0.25	0.04	11.46	1.74	2.94	47
Corythionella pontica								
Length	96.11	98	6.79	1.04	7.06	80	105	43
Width	38.79	39	1.72	0.26	4.43	35	43	43
Height	26.53	27	0.63	0.10	2.37	25	28	43
Diameter of aperture	19.95	20	0.99	0.15	4.96	17	22	43
Large axis of collar	46.86	48	3.21	0.49	6.85	39	52	43
Small axis of collar	39.25	40	1.51	0.23	3.84	35	42	43
Length / Width	2.47	2.47	0.13	0.02	5.26	2.16	2.71	43
Micramphora pontica								
Total length	23.35	23	3.04	0.55	13.01	20	32	31
Diameter of shell	16.83	16	2.06	0.37	12.24	15	22	31
Diameter of aperture	8	8	0.84	0.15	10.5	7	10	31
Diameter of collar	18.79	19	1.94	0.35	10.32	16	24	31
Length of collar	9	8	2.77	0.5	30.77	7	17	31

rounded central part and a peak elongated in the form of a crescent overlaying the aperture. The aperture is round or oval, disposed in a slight invagination of the ventral wall. In lateral view the shell is like a helmet with a round basis and an elongated overlaying peak (Golemansky 1973).

The SEM investigations show that the central part of the shell is composed of a proteinaceous matrix, covered with irregular flat plates (Fig. 6). Some larger plates can be observed on the dorsal part of the shell. The elongated peak is composed mainly of organic cement and its periphery is thin and flexible (Fig. 6).

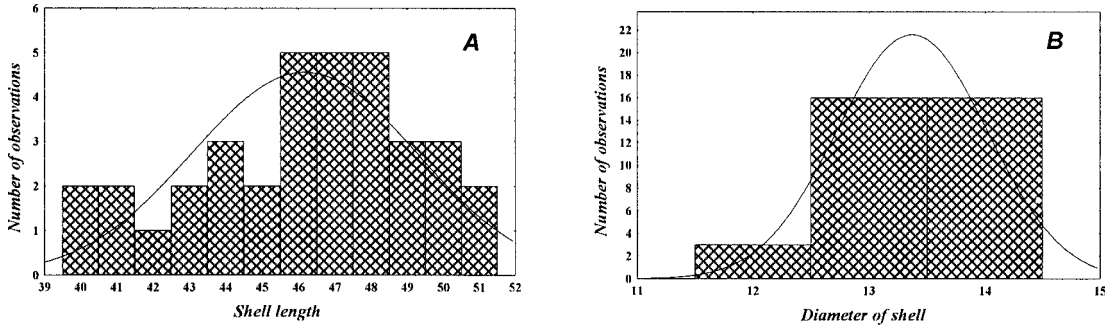


Fig. 5. Histograms showing the size frequency of the shell length (A) and the shell width (B) of *Chardezia caudata*.

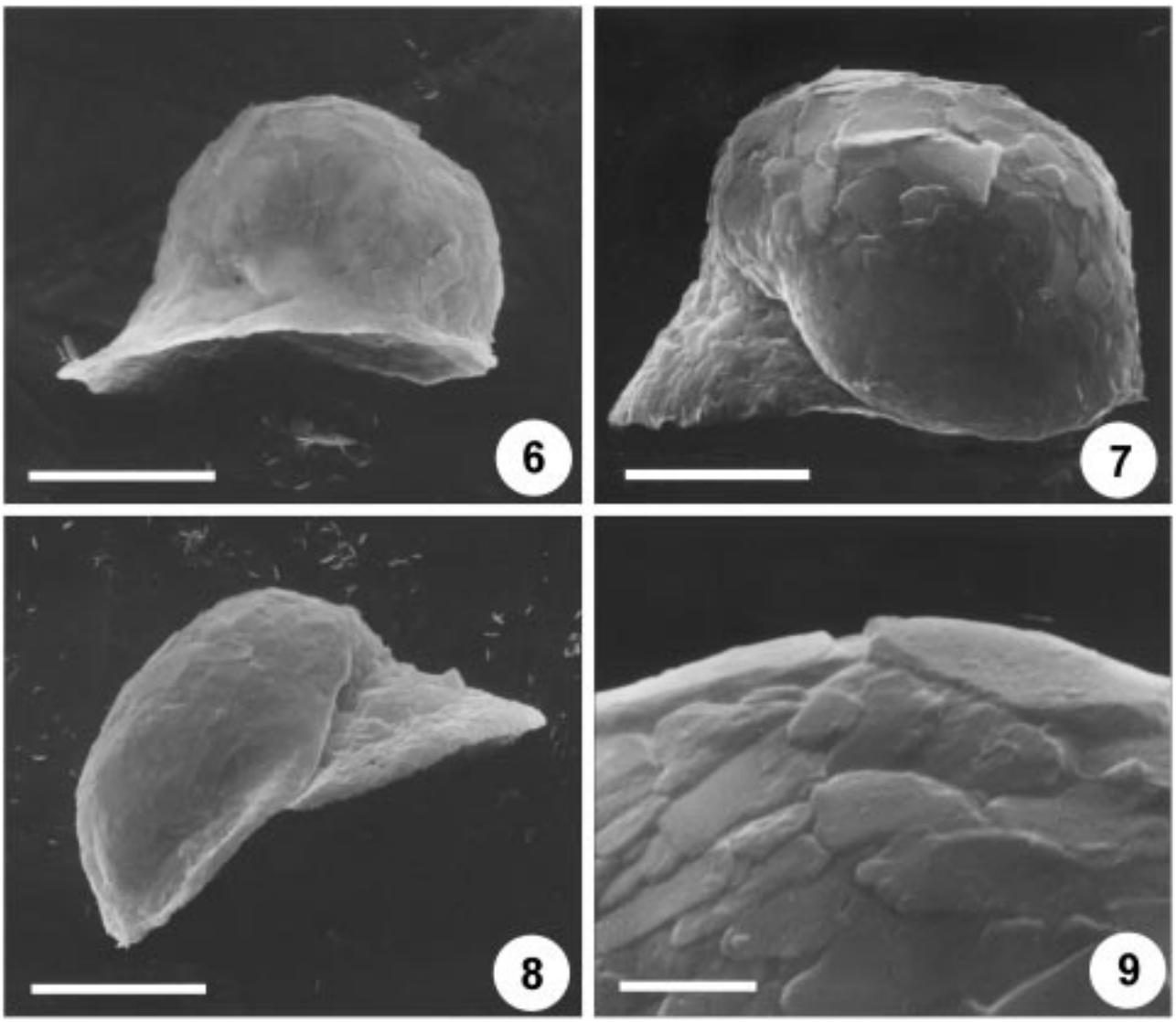


Fig. 6. *Psammonobiotus balticus* - lateral view. Scale bar: 10 µm.
Figs 7-9. SEM photographs of *Psammonobiotus golemanskyi*. **7, 8** - lateral view of two specimens, showing the invagination of the neck; **9** - detail of the dorsal structure of the shell, composed of flattened plates. Scale bars: 10 µm (6-8); 3 µm (9).

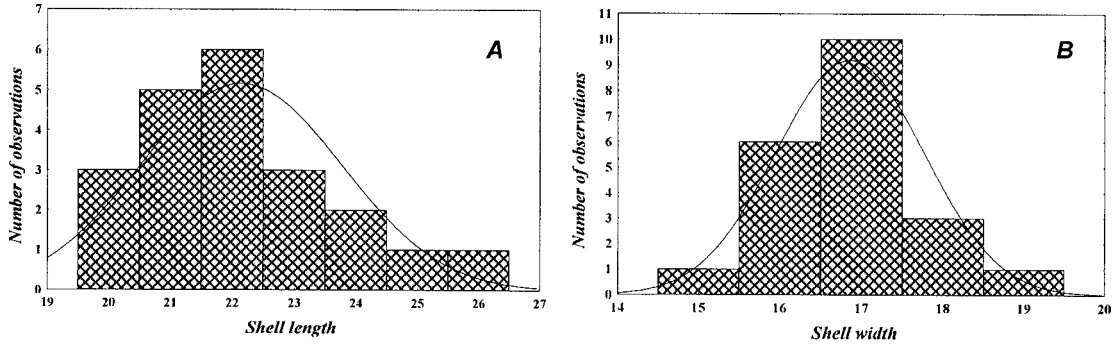


Fig. 10. Histograms showing the size frequency of the shell length (A) and the shell width (B) of *Psammonobiotus balticus.*

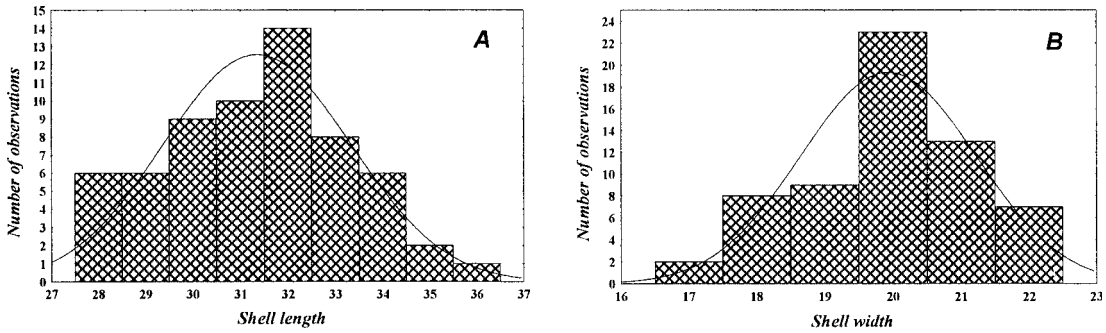


Fig. 11. Histograms showing the size frequency of the shell length (A) and the shell width (B) of *Psammonobiotus golemanskyi*.

Biometry. Most of the measured characters (shell length, width and height) are fairly constant and have low variability (CV between 5.04 and 7.31). Only the aperture diameter is more variable (CV=10.47). Obtained values correspond to the original description of species (Golemansky 1973).

The analysis of the size frequency distribution indicates that *P. balticus* is a size-monomorphic species, characterized by a main-size class and a small size range (Figs 10A, B). All measured specimens of our population have a shell length 20-26 µm and shell width 15.5-19 µm. The presence of comparatively well-expressed main-size class of the shell length (21-22 µm), as well as of the shell width (16-17 µm), and the lack of the subsidiary peaks (bell-shaped curves) indicate a normal distribution (Figs 10A, B). The average length and width of *P. balticus* shells were 22.1 ± 1.62 ($\overline{X}$ ± SD; n=21) and 17.0 ± 0.86 ($\overline{X}$ ± SD; n=21), respectively. These arithmetical means agree well with the main size classes of the shell length and width, and testify to the monomorphism of the species.

Geographical distribution. *P. balticus* is a widely spread psammobiotic testate amoeba with a cosmopolitan distribution. It has been observed in the following seas and oceans: the Baltic Sea (Golemansky 1973), the Black Sea (Golemansky 1974), the North and Mediterranean Seas (Chardez 1977, 1986; Golemansky 2000), the Aegean and Marmara Seas (Golemansky 1982,

1998a, c), the Pacific and Indian Oceans (Sudzuki 1976, 1979, 1986), the Atlantic (Chardez and Thomas 1980, Golemansky 1992).

Psammonobiotus golemanskyi Chardez, 1972 (Figs 7-9, Table 1)

Shell morphology. According to the light microscopic description of Chardez (1972) the shell of *P. golemanskyi* is plagiostomic, with a flat ventral wall and a rounded dorsal wall. In lateral view there is a clearly visible point at the posterior end of the shell (Figs 7, 8). The aperture is in the center of a well-invaginated neck and is surrounded by a large funnel-like collar. The collar is in the same plane with the ventral wall of the test (Figs 7, 8). The shell and the collar are laterally flattened.

Psammonobiotus golemanskyi and *P. balticus* are morphologically very close and it is difficult to distinguish them in ventral view. But in lateral view *P. golemanskyi* differs well from *P. balticus* by its invaginated neck, the characteristic posterior point and the laterally flattened shell and collar.

The shell of *P.golemanskyi* is composed of organic cement, covered with irregularly disposed flattened plates, like *P. balticus*. At the dorsal edge some bigger overlapping plates are visible (Fig. 9).

Biometry. All measured characters of the species are fairly constant and have low variability (CV between 5.73 and 7.77). Our values are somewhat larger than those of the original description of species (Chardez 1972).

The size frequency distributions yield bell-shaped curves (normal distribution) and indicate that *P. golemanskyi* is a size-monomorphic species, characterized by well-expressed main-size class (31-32 µm shell length and 20-21 µm shell width) and by a small size range. Figures 11A, B show that in all measured specimens the shell length and the shell width range in close limits of 28-36 µm and 17- 22 µm, respectively.

Geographical distribution. *Psammonobiotus golemanskyi* has a cosmopolitan distribution in the underground waters of different marine sandy beaches. After the first observation from the North Sea (Chardez 1972, 1977) it was reported from the Pacific (Sudzuki 1977a, b, 1980, 1981b, 1985, 1986; Golemansky 1979), the Indian Ocean (Sudzuki 1979), the Atlantic (Chardez and Thomas 1980, Sudzuki 1983, Golemansky 1992), the Mediterranean, Aegean, Black and Marmara Seas (Chardez 1989); Golemansky 1982, 1994, 1998a, b). During the present investigation, a rich population of *P. golemanskyi* was also observed in the Black Sea (Varna). The biometry of the species was made on the observed Black Sea population.

Corythionella minima Golemansky, 1970 (Figs 12-13, Table 1)

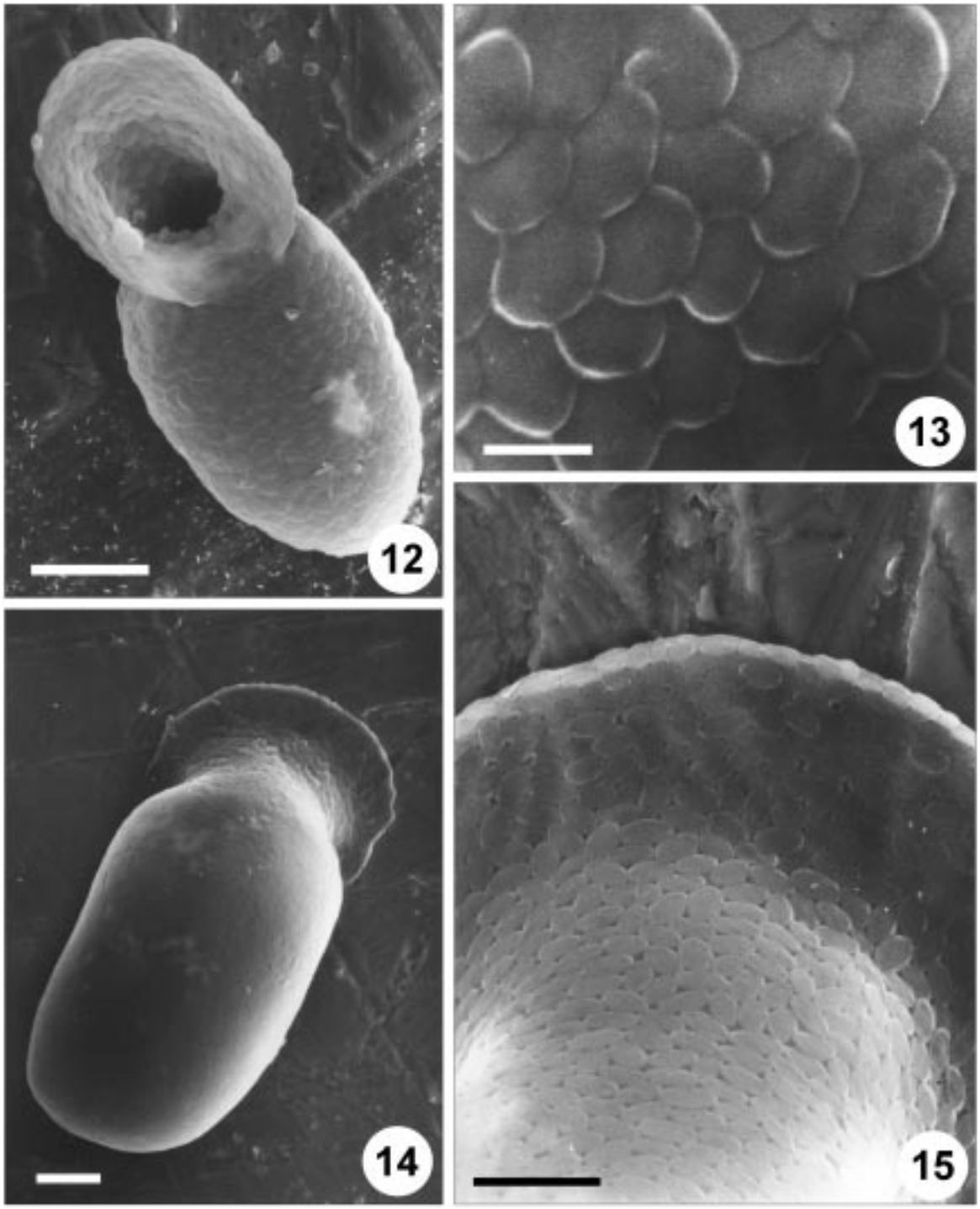
Shell morphology. According to the original description, made by light microscopy, the shell of *C. minima* is elongate-elliptical, plagiostomic, narrowed at the anterior end and lancet-arched at the posterior end. The shell is dorso-ventrally flattened, colourless and translucent. The aperture is round, oblique as regards the longitudinal axis. In the region of the aperture the shell is enlarged and forms a large funnel-like collar. In lateral view the collar is funnel-like, but in ventral view it is like a large round or oval disc around the aperture (Golemansky 1970b). Sometimes a little spine is observed at the posterior end of the shell.

The ultrastructural investigation shows that the shell is composed of numerous oval or elliptic idiosomes (2-2.5 × 1.5-2.0 µm), randomly arranged or overlapping each other (Figs 12, 13). The neck and the collar are composed of the same plates as those in the shell. The rim of the flexible collar is composed of organic cement and small elliptic plates.

Data about the ultra-structure of *C. minima* have been given by Ogden and Coûteaux (1989), but they related it to *C. pontica*. Those authors studied only two specimens with body length of 39 and 45 - typical dimensions for *C. minima*. However, it is important to remember that the shell dimensions of *C. pontica* are twice bigger (length: 61-105 µm) and they are never smaller than 60 µm. (See also the biometric data about two species in Table 1).

Biometry. The coefficients of variation of all characters are less than 10% and show that shell measurements of the Black Sea population of *C. minima* are moderately variable. The present values correspond well to those of the original description of species (Golemansky 1970a).

The analysis of the size frequency distribution indicates that *C. minima* is a size-polymorphic species, characterized by increasing size range (shell length 44-60 µm, shell width 18-27 µm) and by reduced main size-class in favour of subsidiary classes (Figs 16A, B). The average length and width of *C. minima* shells amounted to 50.1 ± 3.24 ($\overline{X}$ ± SD; n=47) and 23.1 ± 1.85 ($\overline{X}$ ± SD; n=47), respectively. These arithmetical means do not agree with the main size classes of both, shell length and width, and testify to the polymorphism of the species.



Figs 12, 13. SEM photographs of *Corythionella minima*. **12** - ventral view; **13** - shell structure, showing the overlaping silicious plates (idiosomes) on the ventral shell surface. Scale bars: 10 µm (12); 2.5 µm (13).
Figs 14, 15. SEM photographs of *Corythionella pontica*. **14** - dorsal view; **15** - shell structure, showing the irregularly arranged elliptic plates (idiosomes) on the dorsal shell surface and on the collar. Scale bars: 10 µm (14); 5 µm (15).

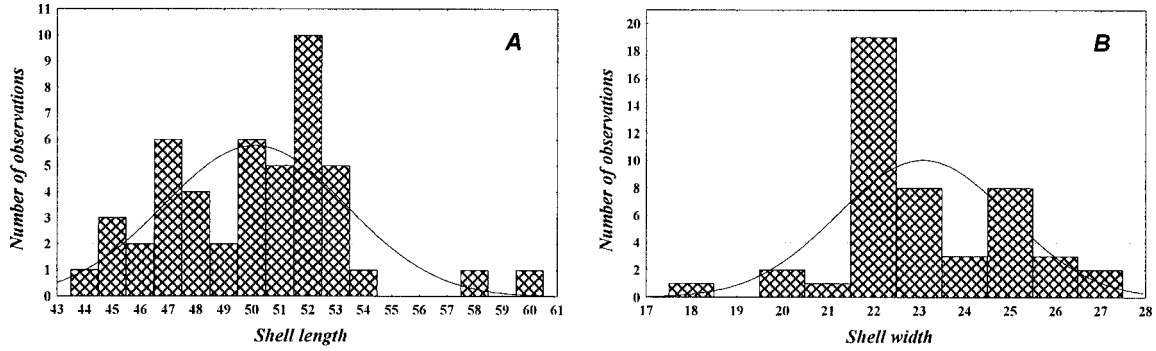


Fig. 16. Histograms showing the size frequency of the shell length (A) and the shell width (B) of *Corythionella minima.*

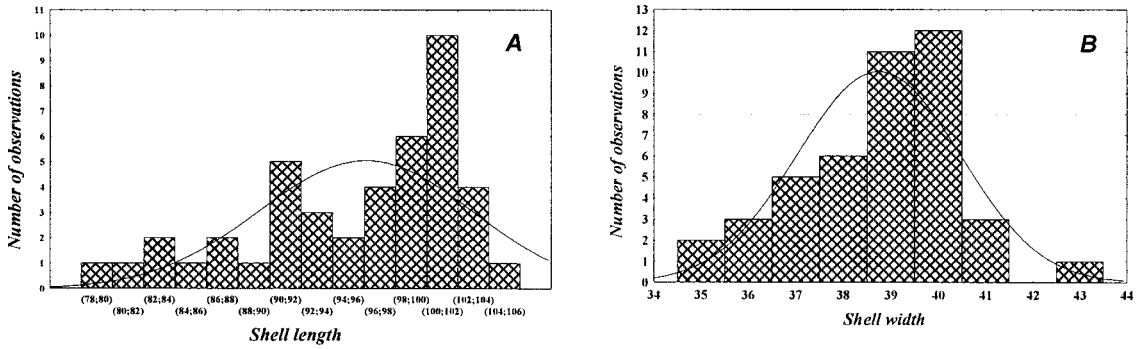


Fig. 17. Histograms showing the size frequency of the shell length (A) and the shell width (B) of *Corythionella pontica.*

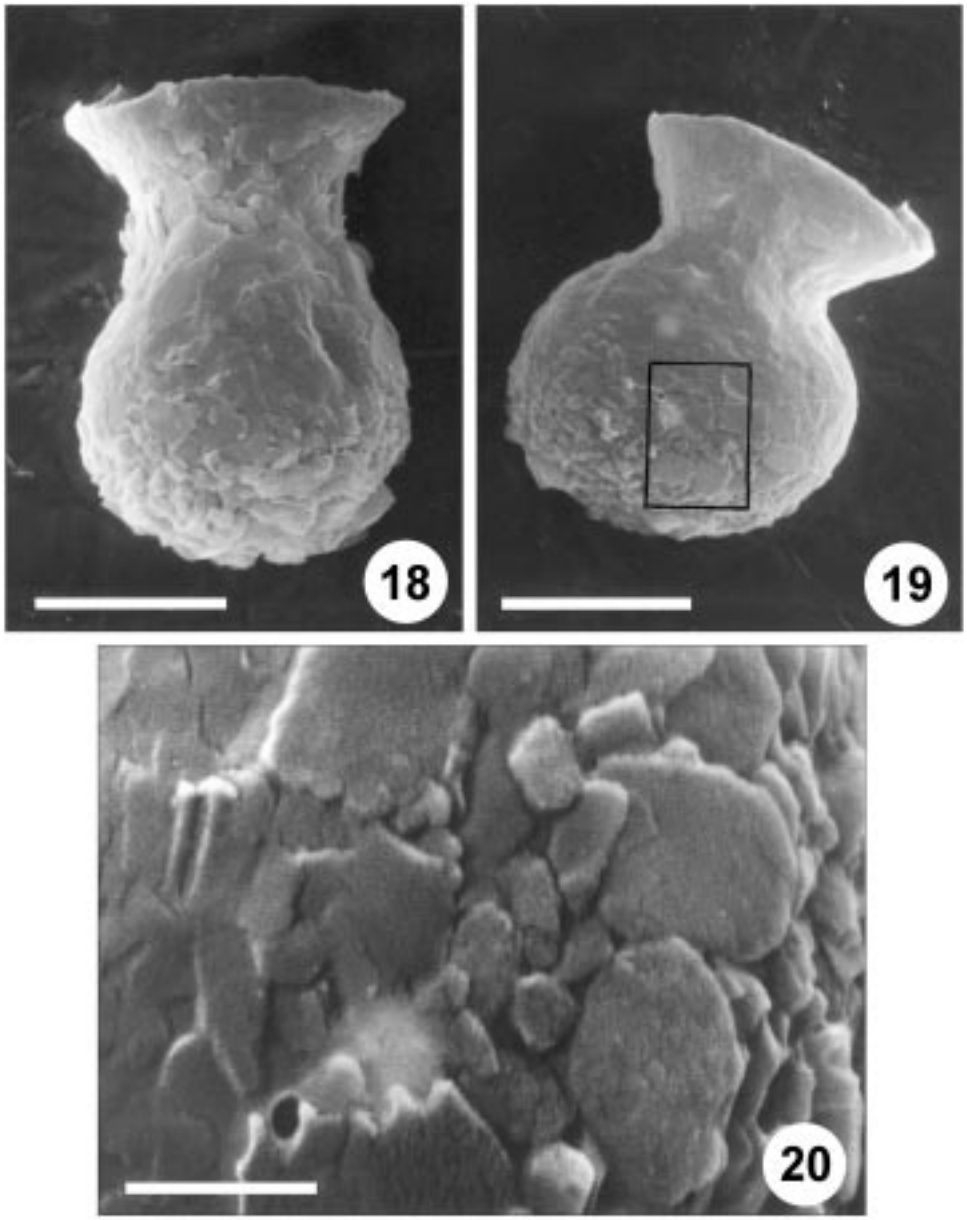
Geographical distribution. Firstly described from the sand supralittoral of the Caribbean Sea by Golemansky (1970b), *C. minima* was later observed in more than 27 different localities in all studied seas and oceans. It has a cosmopolitan distribution and it is a frequent habitant of the marine interstitial habitats (Golemansky 1980).

Corythionella pontica Golemansky, 1970 (Figs 14-15, Table 1)

Shell morphology. In ventral and dorsal view the test is elliptic, slowly narrowed in the region of the aperture, colourless and translucent, dorso-ventrally flattened (Fig. 14). Aborally it is rounded, rarely narrow, without any spine or spike. In lateral view the shell is plagiostomic, with a large collar surrounding the aperture. Usually the collar is circular, rarely large-elliptic, with a diameter of the test.

The shell and the funnel-like collar are formed of elliptic or oval idiosomes. As distinct from *C. minima*, the body and collar plates are irregularly arranged and the organic cement matrix is visible between them (Fig. 15). The flexible periphery of the collar is also formed of smaller plates, embedded within organic cement and forming a thin organic rim (Fig. 15).

Biometry. The coefficients of variation of all characters are low (CV between 2.37 and 7.06) and show that the shell measurements of the Black Sea population of *C. pontica* are moderately variable. The species shows a great diversity only in shell length (CV=7.06). However, shell width, height, and diameter of aperture are



Figs 18-20. SEM photographs of *Micramphora pontica*. **18, 19** - lateral view of two specimens, showing the large funnel-like collar and the neck of the shell; **20** - detail of the shell structure of the specimen shown in fig. 19. Scale bars: 10 µm (18, 19); 2 µm (20).

fairly constant and have low variability (CV between 2.37 and 4.96). Our values correspond to those of the original description (Golemansky 1970c).

Analysis of the size frequency distribution indicates that *C. pontica* is a size-polymorphic species by the shell length and a size-monomorphic species by the shell width (Fig. 17). Figure 17A shows that the specimens of our population are characterized by big size range of the shell length (80-105 µm). The curve of the size classes shows one well-expressed main-size class (100-102 µm) and another not well-expressed subsidiary size class (90-92 µm). Figure 17B shows that the specimens of our population are constant by the shell width - all specimens measured are in limits of 35-43 µm and more than 75% of them have a shell width 37-40 µm. The average length and width of *C. pontica* shells amounted to 96.1 ± 7.06

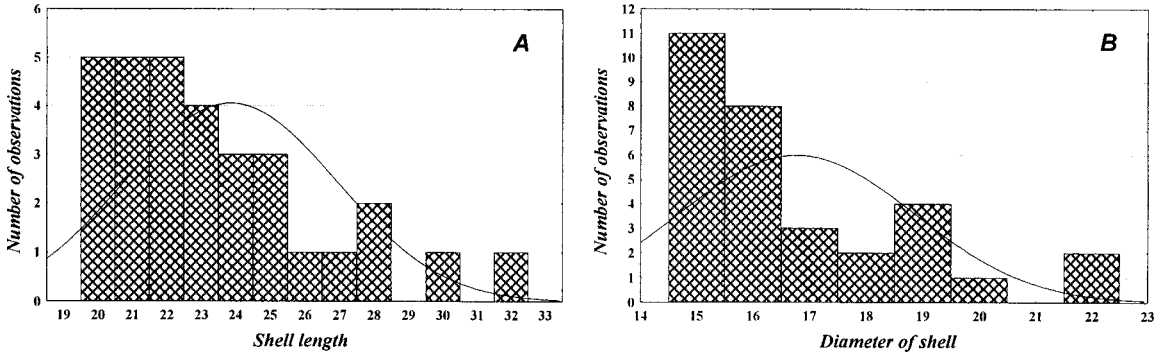


Fig. 21. Histograms showing the size frequency of the shell length (A) and the shell diameter (B) of *Micramphora pontica.*

($\overline{X}$ ± SD; n=43) and 38.8 ± 4.43 ($\overline{X}$ ± SD; n=43), respectively. These arithmetical means confirm the polymorphism of *C. pontica* by the shell length, and the monomorphism of the species by the shell width.

Geographical distribution. *C. pontica* is one of the biggest marine interstitial testate amoebae and it is easy to be observed by light microscopy. It seems that the species has a cosmopolitan distribution, but till now it has been observed only in 10 localities in the Black Sea (Golemansky 1970c, 1980), the Mediterranean Sea (Laminger 1973, the Atlantic (Golemansky 1976b), the Pacific (Sudzuki 1976, 1977b, 1981b), the Marmara Sea (Golemansky 1998c) and the North Sea (Golemansky 2002).

Micramphora pontica Valkanov, 1970 (Figs 18-20, Table 1)

Shell morphology. The shell is oval, with circular cross section, colourless and translucent. At the anterior end the shell is enlarged and forms a large funnel-like collar with a round aperture at the bottom. The diameter of the collar is equal to or bigger than the shell diameter. The aboral end of the shell is rounded.

According to Valkanov (1970) the shell is chitinoid and "the only visible by immersion structural elements are some rows of irregularly disposed pimples between the neck and the funnel-like collar". Our investigations show that the shell of *M. pontica* is covered with polygonal organic plates, closely arranged on the organic matrix and overlapping (Figs 18-20). Bigger organic plates, as well as some mineral xenosomes can be observed on the bases of the shell and in the region of the neck. The rim of the collar is formed of organic cement with smaller plates and it is flexible (Figs 18,19).

Biometry. Coefficients of variation of all measured characters are more to 10% and show that shell measurements of *M. pontica* are rather variable. The species shows a great diversity in length of col and in total length, which have high variability (CV=30.77 and 13.01, respectively). As a whole our values correspond well to the original description (Valkanov 1970).

Micramphora pontica show nearly a normal distribution of size classes, but there are some subsidiary peaks (Figs 21A, B). Also, this species is characterized by increasing size range (20-32 mm shell length and 15-22 µm diameter of shell). These features indicate that *M. pontica* is not well-defined monomorphic species. The average length and diameter of *M. pontica* shells amounted to 23.3 ± 3.04 ($\overline{X}$ ± SD; n=31) and 16.8 ± 2.06 ($\overline{X}$ ± SD; n=31), respectively. These arithmetical means do not agree well with the main size classes of both, shell length and shell diameter, and put a doubt about the monomorphism of the species.

Geographical distribution. *M. pontica* has a cosmopolitan distribution. So far it has been found in more than 39 localities of all studied seas and oceans (Golemansky 1980). It is an eurybiotic species, living at the salinity from 1.5 ‰ to 37.0 ‰.

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