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Protistology

Morphology and biometry of *Arcella intermedia* (Deflandre, 1928) comb. nov. from Russia and a review of hemispheric species of the genus *Arcella* (Testcealobosea, Arcellinida)

Andrei Tsyganov [1] and Yuri Mazei [2]

[1] *Department of Hydrobiology, M.V. Lomonosov State University, Moscow, Russia*
[2] *Department of Zoology and Ecology, V.G. Belinsky State Pedagogical University, Penza, Russia*

Summary

Morphology and biometry of the testate amoeba *Arcella intermedia* (Deflandre, 1928) comb. nov. (formerly *Arcella hemisphaerica intermedia undulata* Deflandre, 1928) isolated from different microbiotopes in Russia were investigated. Size frequency distribution analysis showed that *A. intermedia* is a size-monomorphic species characterised by a main size and a small size range. Comparison of *A. intermedia* and two testate amoebae with a similar hemispheric shell, *A. gibbosa* and *A. hemisphaerica*, demonstrated them to be three clearly distinct morphospecies.

Key words: *Arcella intermedia*, testate amoebae, morphology; morphometry, Rhizopoda

Introduction

Arcella Ehrenberg, 1832 is one of the largest testacean genera. More than 130 taxa of this genus have been described (Deflandre, 1928; Decloitre, 1976). Most of them are cosmopolitan, inhabiting freshwater pools, moist mosses and, rarely, soil litter (Deflandre, 1928; Bartos, 1954; Chardez, 1989). Many *Arcella* species are difficult to distinguish due to lack of morphological and morphometric data.

The aim of the present study was to characterize morphologically and biometrically *Arcella intermedia* (Deflandre, 1928) comb. nov. using abundant material from different biotopes in Russia and to compare this

species with the recently redescribed *A. gibbosa* Penard, 1980 (Tsyganov and Mazei, 2006) and other hemispheric species of the genus *Arcella*.

Material and Methods

The material was collected in 2003 in various microbiotopes of lake Krugloe (Sura river basin, Penza Region, Russia). In 1960-70ies, chemical weapons were destroyed in this lake, and now the amount of the toxic element arsenic (As) in its water and sediments is over 10 times higher than in undisturbed neighbouring habitats. Moreover, the water is rather acid (pH 4.4-4.8).

Three populations were studied morphometrically: population P1 from the moss *Fontinalis hypnoides* sampled on May 27, population P2 from same microbiotope sampled on June 14, population P3 from coarse detritus sediments sampled on July 22.

Testate amoebae were isolated under a stereomicroscope at 100× magnification. The shell size was measured under a light microscope at 200× magnification. Measurements are given in μm.

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: x - arithmetic mean; M - median (the value 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 individuals examined. Frequency distribution and correlation analyses were carried out in order to describe variation of characters. To compare two populations, Kolmogorov-Smirnov criterion was used. Statistical analysis was performed with the aid of STATISTICA 5.5A software (StatSoft 1999). For scanning electron microscopy the shells were isolated, cleaned by several transfers through distilled water, mounted on coverslips and air-dried. Then they were coated with gold and examined with JSM T-330 scanning electron microscope operating at 25 kV.

Results

Description of *Arcella intermedia* (Deflandre, 1928) comb. nov. (formerly *Arcella hemisphaerica intermedia undulata*). The shell is colourless, light yellow or brown, circular in apertural view, domed or hemispherical with an implicit basal border in lateral view. The apertural surface and the basal border are smooth, but the aboral hemisphere has a series of regular depressions. The aperture is circular with or without a distinct collar (Fig. 1).

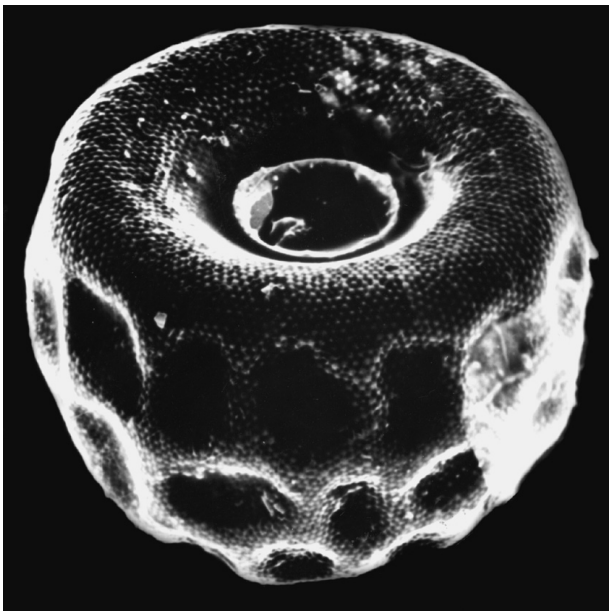

Fig. 1. Shell of *Arcella hemisphaerica* under scanning electron microscopy: ventro-lateral view.

Biometric characterization of 91 shells was carried out (Table 1). General shell morphology and basic morphometric characters taken into the account are shown in Fig. 2. The measurements obtained in the present survey correspond well with the values given for Arcella hemisphaerica Perty, 1852 by other authors, although their references to the shell size are restricted to the shell diameter, shell height and aperture diameter.

Morphometric comparison of populations. Morphometric characteristics of the three populations are given in Table 1 and Fig. 3. The ideal individual of *Arcella intermedia* constructed from median values of all the characters, as well as extreme specimens, are represented in Fig. 2. Median values of the basic characters (shell diameter, shell height, aperture diameter and length of preapertural cavity) are different across the populations (Table 3). Individuals from moss (P1, P2) and detritus (P3) differ significantly in shell diameter and aperture diameter. Other shell measurements (shell height and length of preapertural cavity) give a continuous series of transition forms in the range of limits.

Variability of characters. The analysis of the variation coefficients (Table 2) shows that variability is minimal for shell diameter (4.2-8.9% in different populations, on the average 5.2 ± 2.2), internal diameter of preapertural cavity (6.1-8.5%, 7.4 ± 0.8), aperture diameter (6.6-9.3%, 7.8 ± 1.0), and, consequently, their ratios. These are the most stable characters in the populations. Shell height is more variable (6.3-12.1%, 8.6 ± 2.4). Maximal coefficient of variation is marked

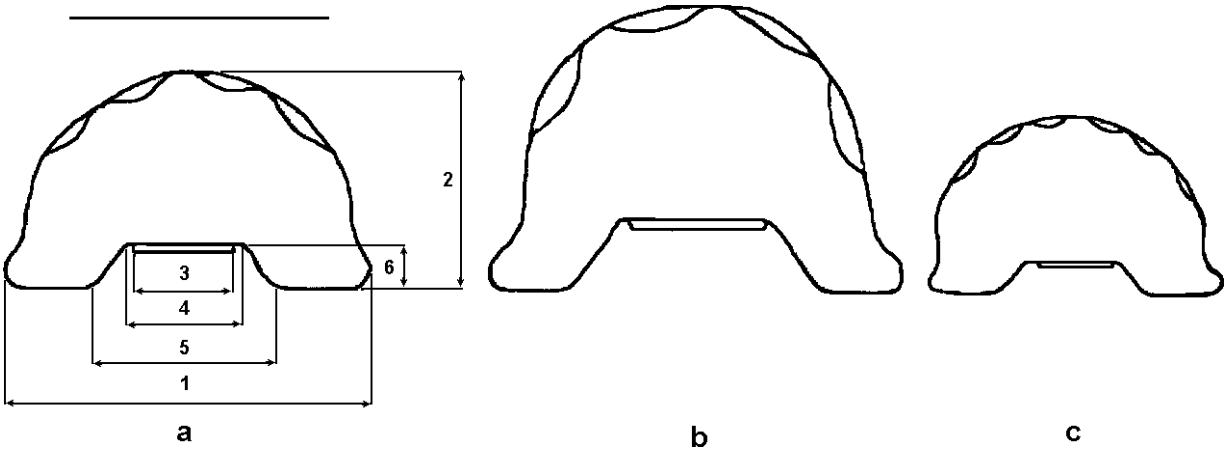

Fig. 2. Basic morphometric characters of *Arcella hemisphaerica*; 1 - shell diameter, 2 - shell height, 3 - aperture diameter, 4 - internal diameter of preapertural cavity, 5 - external diameter of preapertural cavity, 6 - length of preapertural cavity; **a** - ideal individual, **b** and **c** - extreme individuals. Scale bar: 50 μm.

for external diameter of preapertural cavity (8.4-15.2%, 11.0 ± 2.8) and length of preapertural cavity (11.5-21.1%, 15.6 ± 3.5).

Frequency distribution of characters. Frequency distribution analysis indicates that *Arcella intermedia* is a size-monomorphic species by all characters (Fig. 4). For example, in 92% of all the individuals measured shell diameter falls in the range of 60-75 μm; moreover, in 80% it falls in the range of 65-75 μm. Only in 2% of the amoebae shell diameter is less than 60 μm and only in 5% it is over 75 μm. An equally demonstrative example is frequency distribution of shell height: it is between 35-50 μm in 96.5% of all the individuals measured; in about 50% it is 40-45 μm. Only 2% of the amoebae have a shell height less than 35 μm and 1% has a shell height above 50 μm.

Frequency distribution of shell height and aperture diameter are characterized by an approximately zero level of skewness, which indicates that amoebae with average measurements are predominant in populations (Table 4). At the same time, skewness of frequency distribution of other characters has nonzero values, showing that individuals with small (negative values) or large measurements (positive value) predominate in populations. Apertural diameter has negative values of kurtosis, which means higher dispersion of average size group in populations. On the contrary, values of other characters are restricted to a narrow range within overall size variability in maximal number of individuals.

Correlation analysis of characters. Characteristics reflecting correlation between morphological parameters provide additional descriptors of morphological variability of the shell. Absence of a correlation represents a wider phenotypic range of adaptations than a simple proportional reduction or increasing of the shell dimensions. Table 5 shows values of the Spearman correlation coefficient between the morphological characters of *Arcella intermedia*. There is a significant positive and quite strong correlation between shell diameter and shell height. Other shell measurements do not show significant or strong correlations.

Discussion

A great variability of morphological features and poor descriptions of many species (often basing on a single specimen, without any data on morphometry and ecology) cause considerable difficulties in taxonomy of testate amoebae (Bobrov et al., 1995; Bobrov and Mazei, 2004). Recently a practical solution to this

Table 1. Measurements (in μm) of *Arcella intermedia* (Deflandre, 1928) comb. nov. according to different authors

References	Shell diameter	Shell height	Aperture diameter	Length of preapertural cavity
Ogden and Hedley, 1986 (n=7)	55 – 63	23 – 35	11 – 14	
Deflandre, 1928	45 – 56	36 – 42	13 – 18	
Vikol, 1992	47.6±5.9	32.5±6.7		
Bartoš, 1954	35 – 75	26 – 49	31	3 – 9
Present study (n=91)	58 – 76	34 – 54	16 – 23	6 – 14

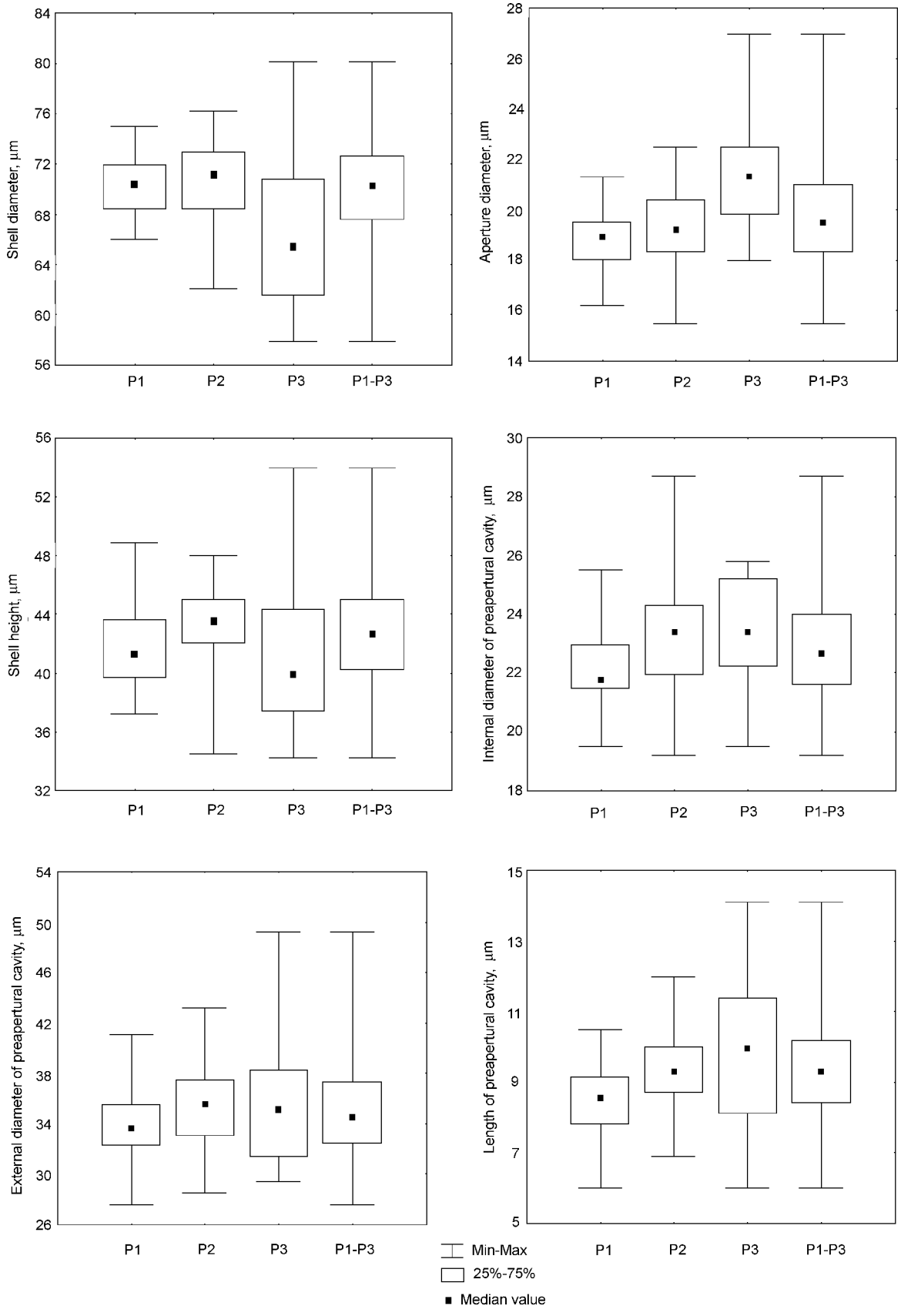

Fig. 3. Morphometry of *Arcella hemisphaerica* from different populations.

Table 2. Morphometric characterization of three populations of *Arcella intermedia*. 1-6 – morphometric characters (see Fig. 2. for character designation).

Character	X	M	SD	SE	CV	Min	Max	N
(1)								
P1	70.7	71.1	3	0.42	4.2	62.1	76.2	50
P2	70.1	70.4	2.4	0.56	3.5	66	75	20
P3	66.4	65.4	5.9	1.35	8.9	57.9	80.1	21
P1-P3	69.6	70.2	4.2	0.44	6	57.9	80.1	91
(2)								
P1	43.2	43.5	2.7	0.39	6.3	34.5	48	50
P2	41.9	41.3	3.1	0.7	7.3	37.2	48.9	20
P3	41.4	39.9	5	1.16	12.1	34.2	54	21
P1-P3	42.5	42.6	3.6	0.37	8.3	34.2	54	91
(3)								
P1	19.3	19.2	1.5	0.21	7.6	15.5	22.5	50
P2	18.7	18.9	1.2	0.29	6.6	16.2	21.3	20
P3	21.4	21.3	2	0.5	9.3	18	27	19
P1-P3	19.6	19.5	1.8	0.19	9.3	15.5	27	89
(4)								
P1	23.1	23.4	1.7	0.26	7.5	19.2	28.7	47
P2	22.1	21.8	1.3	0.31	6.1	19.5	25.5	20
P3	23.3	23.4	2	0.78	8.5	19.5	25.8	9
P1-P3	22.9	22.7	1.7	0.2	7.6	19.2	28.7	76
(5)								
P1	35.2	35.6	3.3	0.49	9.5	28.5	43.2	48
P2	34	33.6	2.8	0.65	8.4	27.6	41.1	20
P3	35.9	35.1	5.4	1.68	15.2	29.4	49.2	12
P1-P3	35	34.5	3.7	0.42	10.5	27.6	49.2	80
(6)								
P1	9.4	9.3	1.1	0.15	11.5	6.9	12	50
P2	8.6	8.6	1.2	0.28	14.2	6	10.5	20
P3	10	9.95	2.1	0.48	21.1	6	14.1	18
P1-P3	9.3	9.3	1.5	0.16	15.6	6	14.1	88
proportion (2)/(1)								
P1	0.60	0.59	0.05	0.610	7.8	0.53	0.69	20
P2	0.61	0.62	0.03	0.005	5.3	0.52	0.68	50
P3	0.62	0.61	0.06	0.013	9.8	0.47	0.76	21
P1-P3	0.61	0.61	0.04	0.005	7.1	0.47	0.76	91
proportion (3)/(1)								
P1	0.45	0.46	0.05	0.011	11.2	0.34	0.55	20
P2	0.45	0.44	0.04	0.006	9.5	0.38	0.58	50
P3	0.52	0.52	0.06	0.014	11.8	0.42	0.66	19
P1-P3	0.46	0.45	0.06	0.006	12.3	0.34	0.66	89

problem has been proposed (Foissner and Korganova, 2000). These authors suggested to lump taxa, which are morphologically and/or morphometrically difficult to distinguish, into "complexes" and to consider closely related forms as separate species if they belong to different taxocenes (i.e., live in different biotops), have a distinct morphometric hiatus and at least a qualitative morphological distinction. At the same time, frequency distribution of characters should have a normal distribution and variation coefficients of characters should not exceed 30%. However, to apply this solution accumulation of sufficient morphometric and ecological data is necessary.

Eight hemispherical *Arcella* forms similar in shell shape can be distinguished: *A. gibbosa, A. gibbosa laevis, A. gibbosa tuberosa, A. hemisphaerica, A. hemisphaerica undulata, A. hemisphaerica tuberculata, A. hemisphaerica intermedia, A. hemisphaerica intermedia undulata*. They have a high shell (shell height/shell diameter ratio is 0.6-0.7) and a round aperture with a collar (Fig. 5). *A. vulgaris penardi* and *A. rotundata alta* are similar in shell shape but have no apertural collar. Other externally similar species have lower (*A. bathystoma, A. papyracea, A. pygamea, A. rotundata, A. vulgaris*) or higher (*A. gibbosa mitriformis, A. mitrata, A. jeanneli*) shell.

The results of present and earlier research (Tsyganov and Mazei, 2006) allow us to make a conclusion about taxonomic status of *Arcella gibbosa* and *A. intermedia* (formerly *Arcella hemisphaerica intermedia undulata*). There is a distinct hiatus in distribution of absolute

Table 3. Statistical significance (Kolmogorov-Smirnov criterion) of shell measurements between different populations of *Arcella intermedia*. P1-P3 – populations (see Materials and Methods).

Sell diameter (upper line) and shell height (lower line)			Aperture diameter (upper line) and length of preapertural cavity		
	P1	P2		P1	P2
P2	NS *		P2	NS **	
P3	** **	** NS	P3	** NS	*** NS

*** P < 0.001; ** 0.01 > P > 0.001; * 0.05 > P > 0.01; + 0.1 > P > 0.05; NS – not significant.

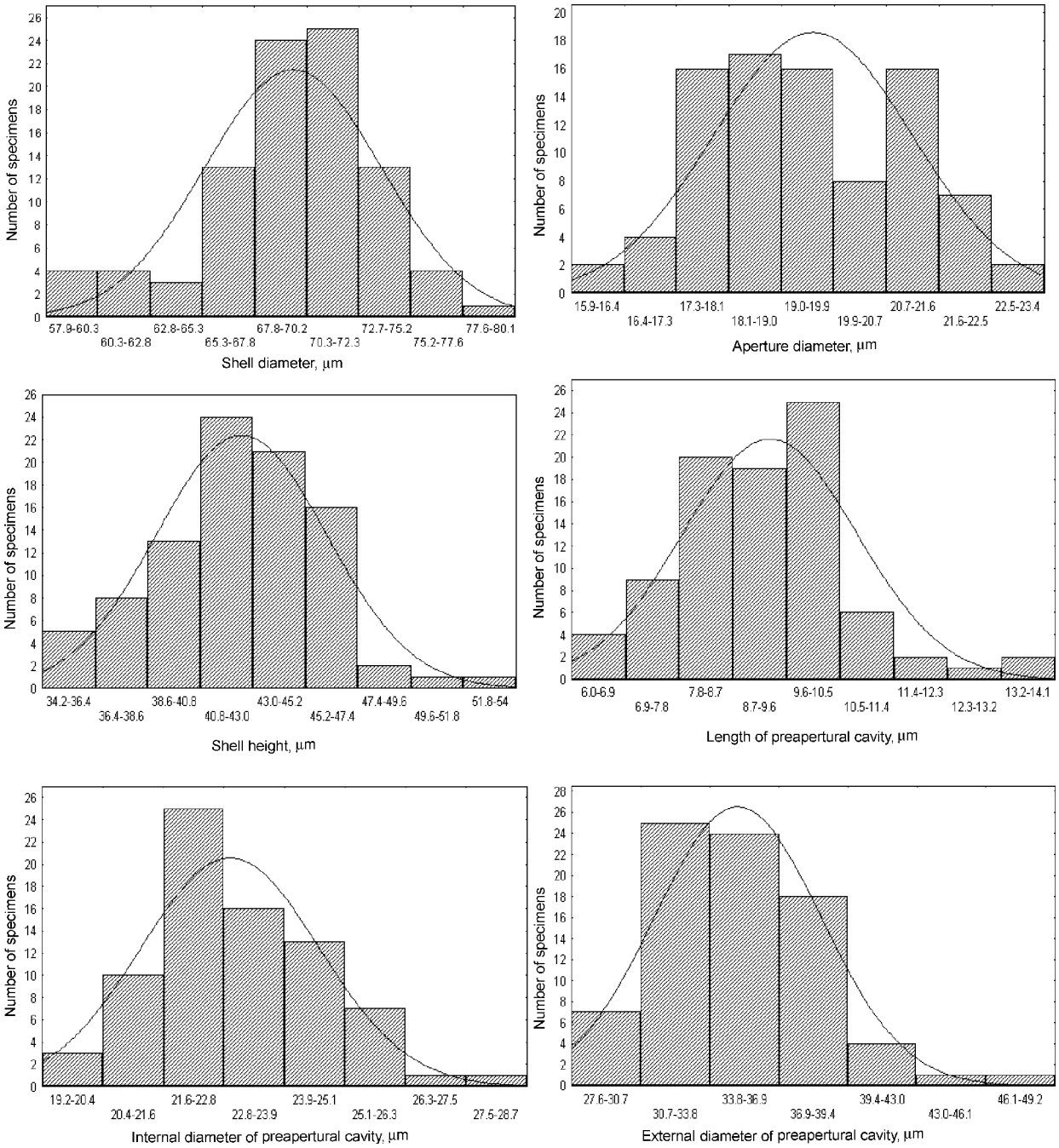

Fig. 4. Size frequency distribution of characters.

values of some quite stable characters, such as shell diameter, shell height, aperture diameter (Fig. 6). Besides, both species occur in the same biotope, which is an evidence of the fact that they occupy different ecological niches in the same community and probably confirm Hutchinson rule. Hutchinson (1959) showed an appreciable constancy of divergence degree of morphological characters related to the oral parts size ratio in several coexisting species of insects, birds and mammals from the same genus. This ratio varies from 1.1 up to 1.4, and its average value 1.28 has been accepted as the lower limit of similarity between competing species. Subsequently many authors have

Table 4. Skewness and kurtosis of characters frequency distribution.
1-6 – shell characters.

Characters	1	2	3	4	5	6
Skewness	-0.61	0.00	0.15	0.41	0.83	0.44
Kurtosis	0.46	0.37	-0.45	0.55	1.70	0.94

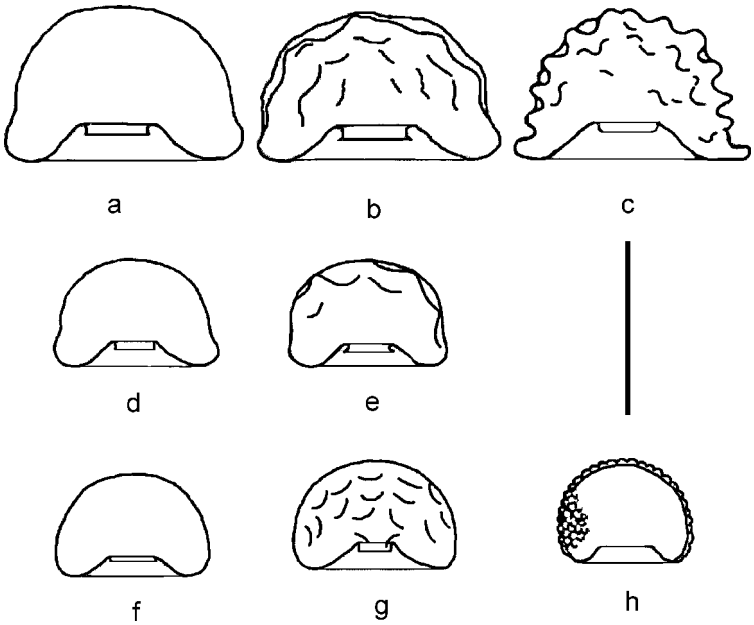

Fig. 5. Shell shape of hemispheric *Arcella* species. **a** - *A. gibbosa laevis*, **b** - *A. gibbosa*, **c** - *A. gibbosa tuberosa*, **d** - *A. hemisphaerica intermedia*, **e** - *A. hemisphaerica intermedia undulata*, **f** - *A. hemisphaerica*, **g** - *A. hemisphaerica undulata*, **h** - A. *hemisphaerica tuberculata* (**a, c-g** - according Deflandre, 1928; **b** - according Decloitre, 1976; **h** - according Štèpánek, 1963). Scale bar: 50 μm.

accepted the size ratio 1.3 to be a biological constant or "rule" applied to groups of sympatric species, especially if it concerns the body size (Pianka, 1969; MacNab, 1971; Uetz, 1977; Pearson and Mury, 1979; Krysik, 1979). In the case of *Arcella gibbosa* and *A. intermedia*, the size ratio for shell diameter is 1.4, that for shell height is 1.5 and that for aperture diameter is 1.2.

Table 5. Spearman correlation coefficients between shell measurements of *Arcella intermedia* in all populations (1-6 – shell characters).

	P1-P6				
	1	2	3	4	5
2	0.54 ***				
3	0.14 NS	0.49 NS			
4	0.31 **	0.36 **	0.49 ***		
5	0.19 NS	0.13 NS	0.24 *	0.34 **	
6	0.05 NS	0.15 NS	0.11 NS	0.18 NS	0.38 ***

*** P < 0.001; ** 0.01 > P > 0.001; * 0.05 > P > 0.01; NS – not significant.

On the other hand, the presence of keel, which is a distinct qualitative character, does not allow considering the form in question as a subspecies of *A. hemisphaerica*. Thus, within the above group of eight forms, we suggest to distinguish three species: *A. gibbosa, A. intermedia, A. hemisphaerica*. The distinction is based on two characters, the first one being presence or absence of keel. Using it, one can separate *A. gibbosa* and *A. intermedia* from A. *hemisphaerica*. The second character is the shell size, which make it possible to distinguish *A. gibbosa* from *A. intermedia*. Within each species, it is possible to pick out forms differing in presence and number of depressions on the dorsal surface. The following diagnoses of the forms under discussion can be given.

***Arcella gibbosa* Penard, 1890**. The shell is circular in apertural view, domed or hemispherical with an enlargement at the basis in lateral view (the distinct basal border, or keel). The apertural surface and the basal border are smooth, but the aboral hemisphere has a series of regular depressions. The aperture is circular, with a distinct collar. Shell diameter 80-110 μm, shell height 50-75 μm, aperture diameter 17-30 μm, length of preapertural cavity 5-15 μ. Shell height/shell diameter ratio 0.5-0.7, aperture diameter/shell diameter 0.20-0.35.

Two subspecies can be distinguished. *Arcella gibbosa laevis* Deflandre, 1928 differs from the type by absence of depressions on aboral hemisphere. *Arcella gibbosa tuberosa* Chardez differs from the type by tuberculate aboral hemisphere.

***Arcella intermedia* (Deflandre, 1928) comb. nov.** (Syn.: *Arcella hemisphaerica intermedia undulata* Deflandre, 1928). The shell is circular in apertural view, domed or hemispherical with an enlargement at the basis in lateral view (the distinct basal border, or keel). Aboral hemisphere has regular depressions. The aperture is circular with or without a distinct collar. Shell diameter 65-75 μm, shell height 35-50 μm, aperture diameter 16-23 μm, length of preapertural cavity 6-12 μm. Shell height/shell diameter ratio 0.5-0.7, aperture diameter/shell diameter 0.35-0.55.

A subspecies, *Arcella intermedia laevis* (Deflandre, 1928) comb. nov. (Syn.: *Arcella hemisphaerica intermedia* Deflandre, 1928),

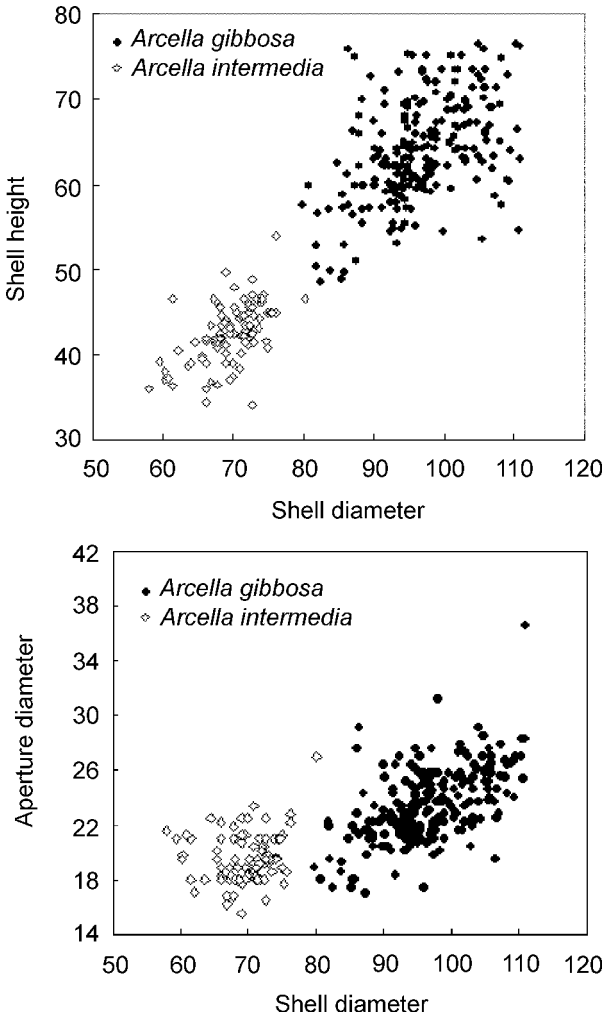

Fig. 6. Morphometric comparison of *Arcella gibbosa* and *Arcella intermedia*.

can be distinguished. It differs from the type by the absence of depression on aboral hemisphere.

***Arcella hemisphaerica* Perty, 1852**. The shell is circular in apertural view, hemisphe-rical or somewhat domed in lateral view. Aboral hemisphere turns into ventral surface at an acute angle. The aperture is circular, with a small collar. Shell diameter 38-68 μm, shell height 23-37 μm, aperture diameter 10-20 μm, length of preapertural cavity 6-9 μm. Shell height/shell diameter ratio 0.48-0.75.

Two subspecies can be distinguished. *Arcella hemisphaerica undulata* Deflandre, 1928 differs from the type by the presence of depressions on aboral hemisphere. *Arcella hemisphaerica tuberculata* Stepanek, 1963 differs from the type by tuberculate aboral hemisphere.

Taxonomic status of other subspecies of *A. hemisphaerica* and *A. gibbosa* (*A. hemisphaerica angulata, A. hemisphaerica depressa, A. hemisphaerica playfairiana, A. hemisphaerica undulata-curvata, A. gibbosa mitriformis)* remains unclear.

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References

Bobrov A.A., Yazvenko S.B. and Warner B.G. 1995. Taxonomic and ecological implications of shell morphology of 3 testaceans (Protozoa, Rhizopoda) in Russia and Canada. Arch. Protistenk. 145, 119-126.

Bobrov A.A. and Mazei Yu.A. 2004. Morphological variability of testate amoebae (Rhizopoda: Testacealobosea and Testaceafilosea) in natural populations. Acta Protozool. 43, 133-146.

Bartoš E. 1954. Korenonožce radu Testacea. Vyd. Slov. Akad. Vied, Bratislava.

Chardez D. 1989. Les Arcelles, Thécamoebiens discrets des mareset des etangs. Naturalistes belges. 70, 17-19.

Deflandre G. 1928. Le genre *Arcella* Ehrenberg. Morphologie-Biologie. Essai phylogé né tique et systematique. Arch. Protistenkd. 64, 152-287.

Decloitre L. 1976. Le genre *Arcella* Ehrenberg. Arch. Protistenk. 118, 291-309.

Foissner W. and Korganova G.A. 1995. Redescription of 3 testate amebas (Protozoa, Rhizopoda) from a caucasian soil - *Centropyxis plagiostoma* Bonnet and Thomas, *Cyclopyxis kahli* (Deflandre) and *Cyclopyxis intermedia* Kufferath. Arch. Protistenk. 146, 13-28.

Foissner W. and Korganova G.A. 2000. The *Centropyxis aerophila* complex (Protozoa: Testacea). Acta Protozool. 39, 257-273.

Hutchinson G.E. 1959. Homage to Santa Rosalia or Why are there so many kinds of animals? Am. Nat. 93, 145-159.

Krysik A.I. 1979. Resource allocation, coexistence, and the niche structure of a streambank salamander community. Ecol. Monogr. 49, 173-194.

McNab B.K., 1971. The structure of tropical bat faunas. Ecology. 52, 352-356.

Penard E. 1890. Etudes sur les Rhizopodes d'eau douce. Mem. Soc. Phys. Hist. Nat. Géneve. 31, 2, 1-230.

Pianka E.R., 1969. Sympatry of desert lizards (Ctenotus) in Western Australia. Ecology. 50, 1012-1030.

Pearson D.L. and Mury E.J. 1979. Character divergence, and convergence among tiger beetles (Coleoptera; Cicindelidae). Ecology. 60, 557-266.

Schönborn W., Foissner W., Meisterfeld R. 1983. Licht- und rasterelectronenmikroskopische Unter-

suchungen zür Schalenmorphologie und Rassenbildung bodenbewohnender Testaceen (Protozoa: Phizopoda) sowie Vorschläge zur biometrischen Characterisierung von Testaceen-Schalen. Protistologica. 19, 553-566.

StatSoft Inc. 1999. STATISTICA for Windows - Computer program manual (http://www.statsoft.com).

Štèpánek M. 1963. Die Rhizopoden aus Katanga (Kongo-Africa). Ann. Mus. Roy. Afr. Centr. Sci. Zool. 117, 9-91.

Tsyganov A.N. and Mazei Yu.A. 2006. Morphology, biometry and ecology of *Arcella gibbosa* Penard 1890 (Rhizopoda, Testacealobosea). Protistology. 4. 279-294.

Uetz G.W. Coexistence in a guild of wandering spiders. J. Anim. Ecol. 46, 531-542.

Address for correspondence: Yuri Mazei. Department of Zoology and Ecology, Penza VG Belinsky State Pedagogical University, Penza, 440026 Russia. E-mail: yurimazei@mail.ru

Editorial responsibility: Andrew Goodkov