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Morphological variability of *Porosia bigibbosa* (Arcellinida: Hyalospheniidae) from East Herzegovina

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Summary

The genus *Porosia* includes two species characterized by presence of two large invaginated pores. These pores are connected by internal tube as in members of the genus *Certesella*. In this paper, morphological variability of *P. bigibbosa* (Penard, 1890) Jung, 1942 based on 175 specimens from East Herzegovina is presented. Shell length ranges from 136 to 178 µm (previously noted 128-177 µm), shell width – from 86 to 118 µm (previously noted 83-123 µm), aperture width – from 40 to 51 µm (previously noted 34-49 µm), aperture-pore distance – from 45 to 62 µm (previously noted 52-70 µm), pore-pore distance – from 48 to 80 µm (previously not measured), and area of the optical section – from 9063 to 15706 µm^2 (previously not measured). The minimal variability (4.5%) was observed for shell length, while maximal variability (9.01%) was recorded for the area of the optical section. Results of size frequency distribution analysis of shell length and shell width indicate that this population possesses continuous polymorphism.

Key words: biometry, morphometry, protists, taxonomy, testate amoebae

Introduction

Penard (1890) described *Nebela bigibbosa* based on moss-dwelling population from the environs of Wiesbaden, Germany. This species is characterized by the presence of two distinct lateral depressions with two large invaginated pores situated on each side of the shell, which are connected by internal tube. For this reason, Jung (1942) proposed the genus *Porosia* for only one species – *N. bigibbosa*. Bobrov and Kosakyan (2015) found a new species in the Japanese mountains which was clearly sharing the morphological peculiarities of the genus *Porosia*. They named this species *P. paracarinata*, emphasizing a distinct morphological character – the lateral keel that can be comparable to the one in *Nebela carinata*. The genus *Porosia* is closely related to the genus *Certesella* but lacks the punctuated neck.

Although *Porosia bigibbosa* was described at the end of the 19th century, its ecology is not yet fully clarified. Some authors found this species in *Sphagnum* mosses as single specimens and described it as rare (Penard, 1903; Wailes and Penard, 1911; Cash et al., 1919), while many others (Bonnet, 1966, 1967, 1977, 1980, 1981, 1987; Todorov, 1993, 1998, 2001; Todorov and Golemansky, 1995; Török, 1995) found this species in humus and litter of the forests. Bonnet (1990) noted that this species has a reputation of a *Sphagnum*-dwelling, but it rarely occurs in some soil habitats like soil mosses, litter, neosols. He

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also concluded that habitats where this species occurs are characterized by high content of organic matter, constantly high moisture, absence of active calcium, and acid environment (pH 3.6–6.8). Török(1995) found *P. bigibbosa* among mixed beech-larch-spruce litter in the Börzsöny Mountains (Hungary). She confirmed Jung's thesis (Jung, 1942) that this species is stenotherm, preferring cold biotopes, and its geographical distribution is concentrated in the Northern Holarctic. She also noted that in Hungary specimens of this species had been only found in the regions above 600 m a.s.l., characterized by a fairly cold and wet climate. She suggested that *P. bigibbosa* requires a rather constant environmental conditions guaranteed by the slowly decomposing pine needles. Todorov (2001) noted that *P. bigibbosa* is frequent and characteristic species for the litter of beech forests in Bulgaria. Todorov (2002) based on data synthesis concluded that this species is not a typical inhabitant of *Sphagnum* mosses but is closely related to the litter of deciduous forests (mainly beech forests) and can be used as an indicator species for these biotopes.

To the best of my knowledge, only four moss-dwelling metapopulations of *P. bigibbosa* were noted: in Germany (Penard, 1890), Great Britain (Brown, 1911), Indonesia (Hoogenraad and de Groot, 1940) and Bulgaria (Todorov, 1998). Recently, I have found specimens of this species in the epigenous moss samples from the Alagovac Lake region, East Herzegovina. The aim of the present study was to describe morphological variability of *P. bigibbosa* based on a moss-dwelling population from East Herzegovina.

Material and methods

The material for the present study was extracted from epigenous mosses collected in the mixed forest in the Alagovac Lake region (43°17'05.9"N, 18°07'58.7"E, ca. 850 m a.s.l.), municipality Nevesinje, East Herzegovina on 24 July 2016. Morphological characters and morphometric variables were studied using a light microscope Zeiss Axio Imager A1. Images were captured using an AxioCam MRc5 (Zeiss) digital color camera. Measurements were conducted in the program AxioVision 4.9.1. The following measurements were taken for the 175 shells: shell length, shell width, aperture width, aperture-pore distance, pore-pore distance (Fig. 1) and area of the optical section (area enclosed by the outline of the shell). The following descriptive statistics were calculated: extreme values (minimum and maximum), median, arithmetic mean and standard error of the arithmetic mean, standard deviation, coefficient of variation (in percentage), skewness and kurtosis. Statistical analysis was conducted using the programs PAST 2.17c and STATISTICA 13.0.

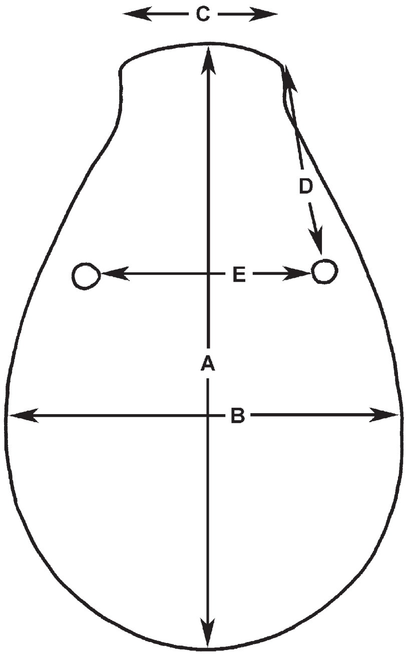

Fig. 1. Shell outline of *Porosia bigibbosa* and position of the measured axes. A – Shell length, B – shell width, C – aperture width, D – aperture-pore distance, E – pore-pore distance.

Results

Description of the species

The shell is colourless, pyriform, laterally compressed, with rounded posterior part, narrow eliptical in lateral view. The shell is composed of oval, circular or elongated euglyphid plates of different sizes; on the posterior part, small sand particles are present, particularly on wide specimens. In broad lateral view, at approximately one third of shell length starting from aperture, two distinct lateral depressions with two large invaginated pores are situated on each side, which are connected by internal tube. The aperture is terminal, oval, slightly

Table 1. Morphometric characterization of *Porosia bigibbosa* from East Herzegovina based on 175 specimens (all measurements in μm, except for area of optical section in μm²)

Characters	Min	Max	M	x	SE	SD	CV	Sk	Ku
Shell length	136	178	159	158.98	0.54	7.15	4.50	– 0.421	0.905
Shell width	86	118	104	104.02	0.44	5.82	5.60	– 0.127	0.008
Aperture width	40	51	45	44.65	0.19	2.46	5.52	0.119	– 0.610
Aperture-pore distance	45	62	55	55.36	0.27	3.54	6.40	– 0.497	0.223
Pore-pore distance	48	80	63	63.13	0.40	5.25	8.32	– 0.073	0.188
Area of the optical section	9063	15706	12590	12559	85.52	1131	9.01	– 0.200	0.302
Shell width/shell length	0.57	0.76	0.65	0.65	0.00	0.03	5.07	0.366	0.195
Aperture width/shell length	0.25	0.33	0.28	0.28	0.00	0.02	6.23	0.504	– 0.320
Aperture width/shell width	0.38	0.52	0.43	0.43	0.00	0.02	5.53	0.869	1.637
Aperture width/aperture-pore distance	0.68	1.02	0.80	0.81	0.01	0.07	8.35	0.460	– 0.020
Aperture width/pore-pore distance	0.59	0.86	0.71	0.71	0.00	0.05	7.42	0.518	0.116
Aperture-pore distance/shell length	0.29	0.41	0.35	0.35	0.00	0.02	5.71	– 0.233	0.810
Aperture-pore distance/shell width	0.41	0.67	0.54	0.53	0.00	0.04	7.84	– 0.184	0.768
Pore-pore distance/shell length	0.31	0.52	0.40	0.40	0.00	0.03	8.13	0.309	0.734
Pore-pore distance/shell width	0.49	0.69	0.61	0.61	0.00	0.03	5.76	– 0.106	0.272

Abbreviations: Min and Max – minimum and maximum values, M – median, x – arithmetic mean, SE – standard error of the arithmetic mean, SD – standard deviation, CV – coefficient of variation in %, Sk – skewness, Ku – kurtosis.

or strongly curved, surrounded by a thick organic lip. Two types of shells were observed in the studied population: typical (Figs 2A–F) and elongated (Figs 2H–I). However, intermediate forms between those shell types were also observed (Fig. 2G).

Morphometry

Morphometric characters of 175 specimens of *P. bigibbosa* from East Herzegovina were measured and the results are given in Table 1. The most frequent shell length (161 μm) was registered in 15 specimens (Fig. 3A); the most frequent shell width (106 μm) was registered in 16 specimens (Fig. 3B), and the most frequent aperture width (44 μm) was registered in 44 specimens (Fig. 3C). Coefficients of variation were relatively low for all measured characters. Namely, coefficients of variation ranged from 4.5% to 9.01%. For basic characters, minimal variability was observed for shell length (4.5%), while the maximal variation coefficient was observed for area of the optical section (9.01%). For ratio characters, minimal variability was observed for shell width/shell length ratio (5.07%), while the maximal variation coefficient was observed for aperture width/aperture-pore distance ratio (8.35%).

Analysis of the size frequency distribution of shell length and shell width indicates that this population possesses continuous polymorphism. Shell length ranged from 136 to 178 μm. However, 62.29% of all measured specimens had a shell length of 154–164 μm, whereas only 19.43% were smaller than 154 μm and only 18.29% were larger than 164 μm. The frequency analysis of the shell width shows similar distribution pattern. Namely, all measured specimens had shell width between 86 and 118 μm. In this case, 58.86% of all specimens had shell width of 99–107 μm, whereas only 15.43% were narrower than 99 μm and only 25.71% were wider than 107

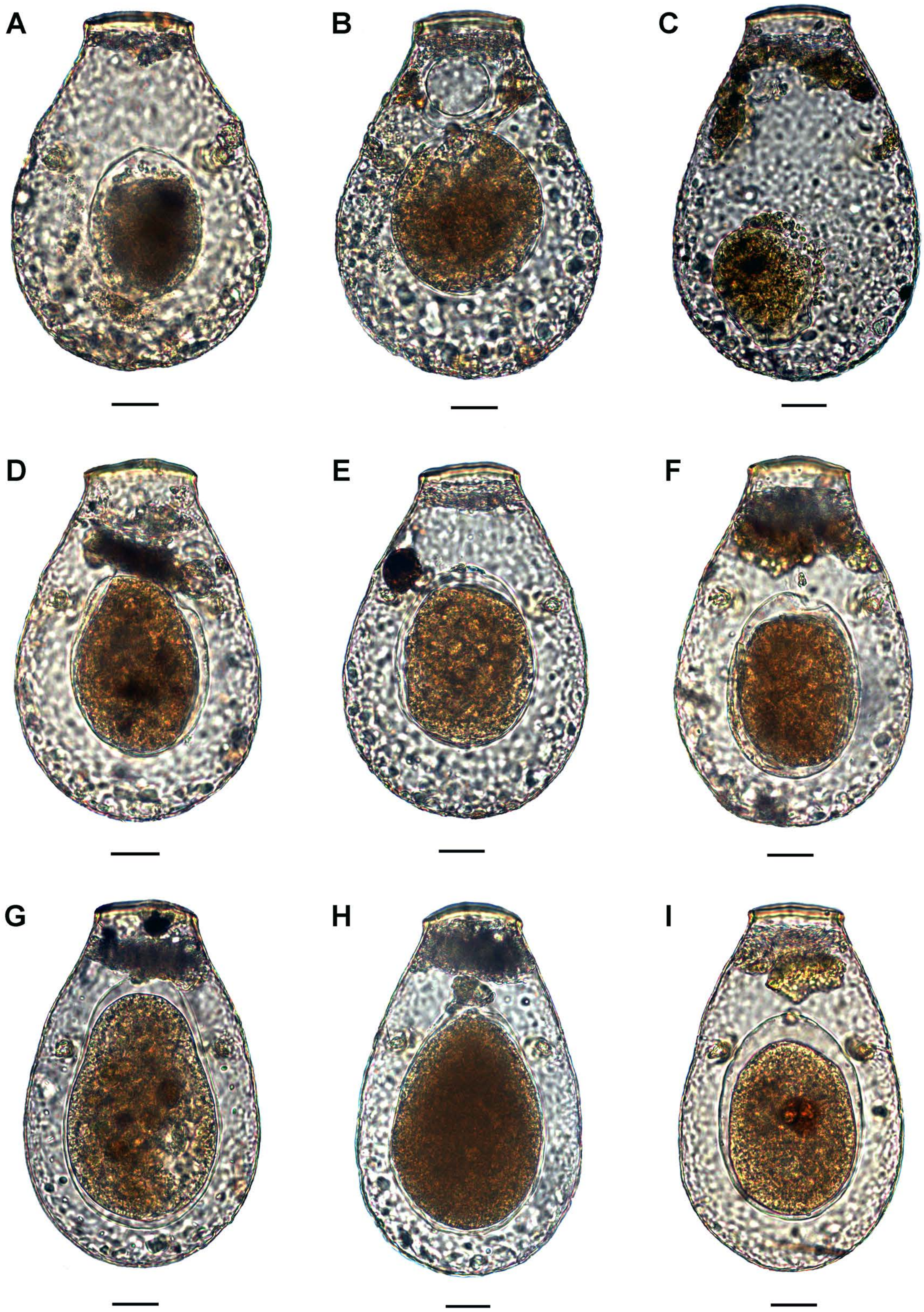

Fig. 2. Light micrographs of *Porosia bigibbosa* from East Herzegovina – broad lateral view of different specimens showing general shell shape and outline. A–F – Typical specimens, G – intermediate specimen, H, I – elongated specimens. Scale bars: 20 μm.

μm. Figures 3D–F show bag plots analyses of the correlation between shell length, shell width and aperture width.

The negative values of skewness for eight characters suggest an asymmetrical distribution with a long tail toward the lower values. However, the asymmetry of these characters was low or moderately low, with the skewness values between –0.073 and –0.497. The other seven characters were described by positive values of skewness (between 0.119 and 0.869), i.e. these characters had an asymmetrical distribution with a long tail toward higher values. Three characters (aperture width, aperture width/shell length ratio, and aperture width/aperture-pore distance ratio) displayed negative kurtosis values, meaning that they were characterized by flatter distribution than a standard Gaussian distribution. This information indicates that the average size group has a higher dispersion. Since the negative value obtained for aperture width/aperture-pore distance ratio is not clearly different from zero (–0.020), the resulting deviation from the normal Gaussian distribution was minimal. However, negative values for aperture width and aperture width/shell length ratio were clearly different from zero (–0.610 and –0.320, respectively), indicating that the average size group has a lower dispersion. Other variables were found to have positive kurtosis values, indicating a distribution that is sharper than the standard Gaussian distribution.

Discussion

Penard (1890) noted shell length of 140–160 μm and shell width 100–110 μm for *P. bigibbosa* population collected in Germany. Wailes and Penard (1911) based on samples from Clare Island (Ireland) noted the following measurements: shell length 135–170 μm, shell width 87–110 μm, and aperture width 34–45 μm. Later, many authors retrieve data from this paper (Cash et al., 1919; Deflandre, 1936; Jung, 1942; Mazei and Tsyganov, 2006). However, there slipped a mistake in two papers (Jung, 1942; Mazei and Tsyganov, 2006) because the authors stated that aperture width ranged from 34 to 35 μm. Hoogenraad and de Groot (1940) reported the original data based on material from Indonesia: shell length 130–170 μm and shell width 83–123 μm. Ogden and Hedley (1980) also noted the original measurements for the specimens from Great Britain: shell length 153–171 μm, shell width 95–115 μm, and aperture width 38–41 μm. The shell measurements of the studied population agree well with the data reported earlier in the literature.

Only Todorov (2002) conducted a detailed morphometric study of *P. bigibbosa*: he characterized morphometrically this species based on 100 specimens from six mountains in Bulgaria. He noted the following morphometric ranges: shell length 128–177 μm, shell width 90–115 μm, aperture width 35–49 μm, and shell width/shell length ratio 0.6–0.7 μm; these parameters are similar to measurements of the studied population from East Herzegovina. This similarity is also confirmed by arithmetic means of these characters: shell length 157.5 μm versus 158.98 μm, shell width 101.9 μm versus 104.02 μm, and aperture width 40.4 μm versus 44.65 μm. The aperture-pore distance is the most discriminant character between specimens from Bulgaria and East Herzegovina (52–70 μm versus 45–62 μm). This difference may be caused by different methodology. Namely, Todorov (2002) measured this character in narrow lateral view, while in the presented study this character was measured in broad lateral view. However, if these characters were measured in the same position, the difference would be even greater. The shells are normally placed in the broad lateral view on the microscope slides. Therefore, it is more practical to measure aperture-pore distance in this position. Populations from Bulgaria and East Herzegovina have relatively low variation coefficients of all measured characters (3.1–12.4% versus 4.5–9.01%). Thus, it is possible to conclude that all populations of *P. bigibbosa* studied up to date are comparatively homogeneous.

Todorov (2002) noted presence of two morphological types in populations from Bulgaria – typical and elongated specimens. I also observed these two morphological types in the studied population from East Herzegovina. It is possible that *Porosia bigibbosa* is a species complex, but without detailed morphometric and molecular data this conclusion is speculative.

The second species, *Porosia paracarinata*, differs from *P. bigibbosa* by larger dimensions (202–236 μm versus 128–177 μm) and presence of the lateral keel. Bobrov and Kosakyan (2012) noted that the main morphological variability of *P. paracarinata* is due to size of the lateral keel which can vary from very wide (13.13 μm) to very narrow (3.75 μm). Molecular data are needed to clarify taxonomic importance of these variations. In addition, these authors concluded that the genus *Porosia* is much more diverse than it was initially

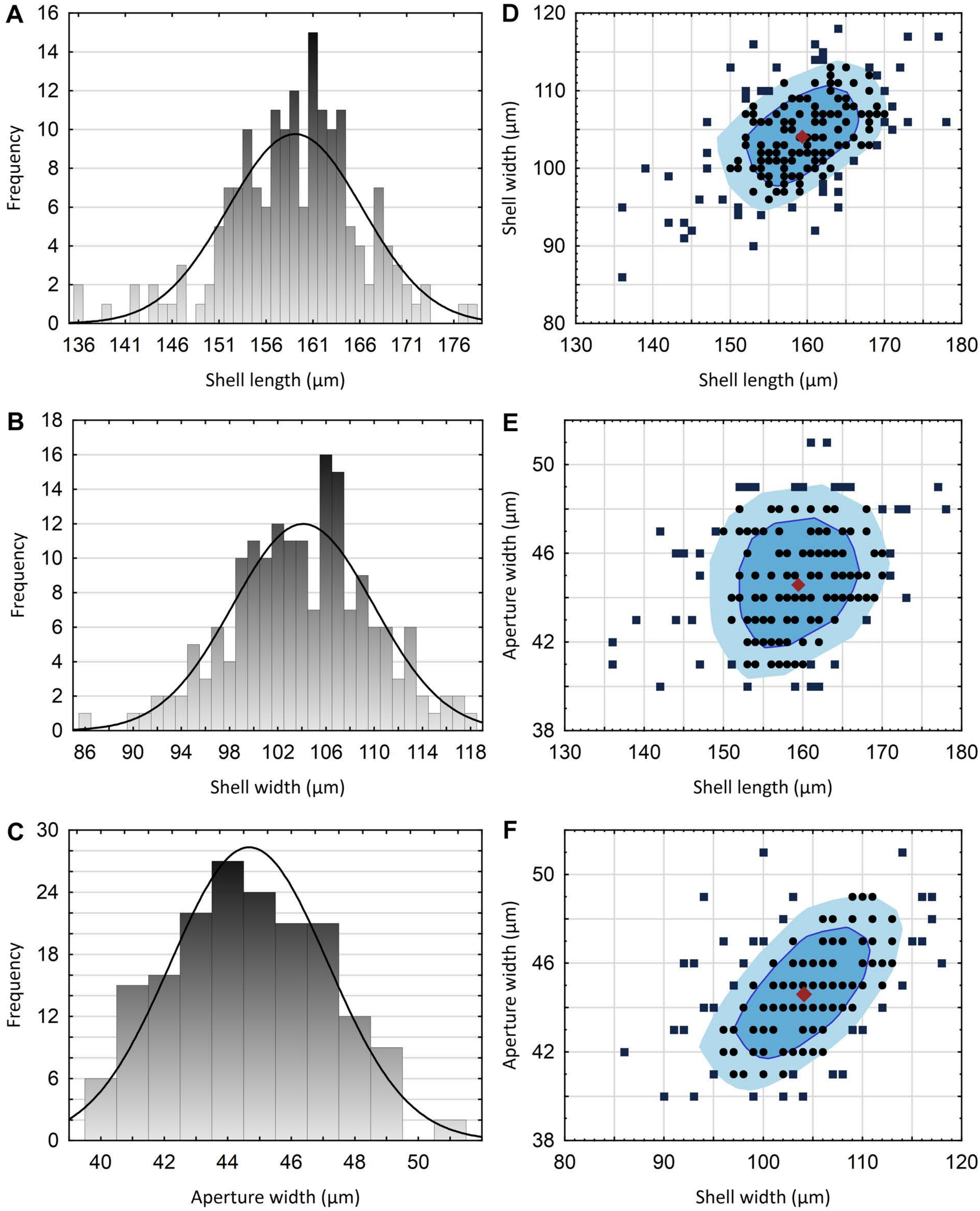

Fig. 3. Morphological variability of *Porosia bigibbosa* population from East Herzegovina based on 175 measured specimens. Histograms show the size frequency distribution of the shell length (A), shell width (B), and aperture width (C); bag plots show correlation between shell length and shell width (D), aperture width and shell length (E), and aperture width and shell width (F). *Legend for bag plots*: depth median ♦, characters on Y axes ●, outliers ■.

expected. Namely, they drew attention to two examples from literature when the findings can have possible affinities to *P. paracarinata*. Chattopadhyay and Das (2003) characterized morphologically and morphometrically *Nebela bohemica* from India. These authors noted short description but have not observed the lateral pores: "Test pyriform in broad view, compressed, fundus rounded and flanks convergent downwards to aperture, oblong in lateral view; aperture oval, straight, with well-defined lip but without any notch". Based on ten specimens, they noted the following measurements: shell length 100.16–104.86 μm, shell width 71.99–73.56 μm, and aperture width 17.22–18.78 μm. Based on Figs 154–155 in Chattopadhyay and Das (2003), Bobrov and Kosakyan (2015) concluded that this species is a member of the genus *Porosia* related to *P. paracarinata*. Namely, this species possess two large lateral pores and a keel. Bobrov and Kosakyan (2015) observed that specimen named as *P. bigibbosa* in Fig. 1g in Lara et al. (2008, p. 167) is much smaller than the typical species and has a distinct lateral keel. The keel of this specimen is starting near the lateral pores but not above lateral pores as in *P. paracarinata*. In addition, the smaller shell length (110 μm) of this specimen indicates that this is most likely another species within the genus *Porosia*.

Conclusions

Data presented in this paper confirm that *Porosia bigibbosa* is a morphometrically uniform species. Different populations of this species are morphologically and morphometrically very similar. This fact supports the assumption that shell length is a species-specific characteristic. Further investigations using additional populations will provide more definitive results due to taxonomic significance of shell shape. Since *P. bigibbosa* is closely related to *P. paracarinata*, it is highly probable that shell length is also an important taxonomic character in *P. paracarinata* complex (species with lateral keel). Consequently, it is possible that specimens from India and Switzerland with lateral keel and small dimensions could well be the members of a new, so far undescribed species from the *P. paracarinata* complex. Further studies based on molecular and morphometric data are needed to clarify the diversity within the genus *Porosia*.

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