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Taxonomy of *Quadrulella longicollis* and *Q. symmetrica* (Arcellinida: Hyalospheniidae) from the central part of the Balkan Peninsula

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Summary

The genus *Quadrulella* comprises most species of hyalosphenid testate amoebae with quadrangular shell plates. The taxonomic status of almost all species is questionable because they are poorly studied. *Quadrulella symmetrica* is the type species with cosmopolitan distribution. In this paper, morphological variability of *Q. symmetrica* based on two populations from the central part of the Balkan Peninsula is presented. Intermediate forms between *Q. symmetrica* s.s. and *Q. madibai* were registered; therefore, taxonomic status of these two recently delimited taxa is discussed. In one studied population, a specimen covered by mixed quadrangular and circular shell plates was observed. Recently, taxonomic status of *Q. longicollis* has been defined as questionable. Here are present morphological and morphometric data of *Q. longicollis* based on 130 examined specimens from Šargan Mountain (Serbia). The present study strongly supports the opinion that this taxon is a separate species within the genus *Quadrulella*.

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

Introduction

Hyalosphenids are one of the well-studied groups of the testate amoebae. This group comprises comparatively large species and most of them live in mosses, well-studied habitats with regard to distribution, taxonomy and ecology of testate amoebae. Therefore, hyalosphenids are considered as important bioindicators and are commonly used in environmental monitoring and paleoecology. The existence of many undescribed or inadequately described morphospecies and especially pseudocryptic species is problematic for multidisciplinary studies. Consequently, a number of relevant taxonomic studies have been undertaken recently (Török, 2001; Todorov, 2002, 2010; Lara et al., 2008; Todorov et al., 2010; Heger et al., 2011; Kosakyan et al., 2012, 2013, 2016; Bobrov and Kosakyan, 2015; Luketa, 2015, 2016, 2017a, 2017b; Nicholls, 2015; Qin et al., 2016; Singer et al., 2015; Pérez-Juárez et al., 2017).

Taxonomy of hyalosphenid testate amoebae at the generic and species level is based on shape, composition and size of their shells. Within the family Hyalospheniidae four types of the shells are present: (1) shells composed only of an organic matrix (e.g. members of the genus *Hyalosphenia*), (2) shells composed of the organic matrix and

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unmodified shell plates of small testate amoebae or other similar materials such as diatom frustules (e.g. members of *Nebela collaris* complex and *Longinebela tubulosa*), (3) shells composed of the organic matrix and modified oval and/or circular siliceous plates (e.g. *Gibbocarina galeata, Longinebela golemanskyi* and *L. speciosa*), and (4) shells composed of the organic matrix and self-secreted square siliceous plates (members of the genera *Quadrulella* and probably *Mrabella*). The most surprising result of the study conducted by Kosakyan et al. (2016) is that square scaled hyalospheniids are not a monophyletic group. Namely, they have obtained sequences from *Quadrulella subcarinata*, a very rare tropical species from Africa, and concluded that this species is not closely related to other *Quadrulella* species. For this reason, these authors established a new genus for this species – *Mrabella*. A very rare species, *Quadrulella plicata*, is also included into the genus *Mrabella* because it has similar shape and keel, but it is smaller than *M. subcarinata*.

Only a small number of papers (Chardez, 1967; Vucetich, 1983; Lopretto and Vucetich, 1997; Luketa, 2015; Pérez-Juárez et al., 2017) were exclusively devoted to the genus *Quadrulella*. According to the recently published taxonomic concept of the genus *Quadrullela* (Kosakyan et al., 2016), this genus includes eleven valid species with pyriform or elongated-pyriform shells composed of self-secreted siliceous quadrangular plates. These testate amoebae inhabit peatlands, fens, wet mosses and humus rich soils. More recently, Pérez-Juárez et al. (2017) described *Q. texcalense* from a Mexican desert. Kosakyan et al. (2016) treated four species (*Q. constricta, Q. lageniformis, Q. tubulata* and *Q. vas*) as incertae sedis and *Q. longicollis* as a questionable species.

The present study reports the morphological and morphometric data for *Q. longicollis* and *Q. symmetrica* based on specimens from the central part of the Balkan Peninsula.

Material and methods

The material for the present study was collected from two localities in the central part of the Balkan Peninsula: (1) *Sphagnum* mosses collected in the Alagovac Lake region (43°17'44.8"N, 18°07'31.9"E), East Herzegovina on 18 April 2014, 19 August 2014, 11 May 2016, and 24 July 2016; (2) epigenous mosses collected on Šargan Mountain (43°49'40.10"N, 19°31'41.40"E), Serbia on 17 August 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 shell parameters were measured: shell length, shell width, aperture width, and area of the optical section. The following descriptive statistics were calculated: extreme values (minimum and maximum), median, arithmetic mean, standard error of the arithmetic mean, standard deviation, coefficient of variation (in percentage), skewness, and kurtosis. Statistical analysis was conducted using the program Statistica 13.2.

Results

Quadrulella longicollis

Description. The shell is elongated ovoid, colorless, transparent and compressed laterally, especially in the apertural region. The shell is composed of siliceous, quadrangular plates that are regularly arranged in transverse and longitudinal series (rows), with smaller plates close to the aperture. The aperture is terminal, oval, and convex in broad lateral view and concave in narrow lateral view, surrounded by a thin organic lip.

Population from Šargan Mountain. Figure 1 shows light micrographs of some specimens from this population, while Figure 2 shows frequency scatter plot analysis of the correlation between shell width and shell length. Morphometric characters of 130 specimens from Šargan Mountain were measured and the results are presented in Table 1. Coefficients of variation were low for all measured characters, ranging from 4.53% to 9.18%. For basic characters, the minimal variability was observed for shell length (4.53%), while the maximal variation coefficient was observed for area of the optical section (9.18%). For ratio characters, the minimal variability was observed for shell width/shell length ratio (5.68%), while the maximal coefficient was observed for aperture width/shell width ratio (7.33%).

The most frequent shell length (122 μm) was registered in 14 specimens (Fig. 3A); the most frequent shell width (54 μm) was registered in 18 specimens (Fig. 3B), and the most frequent aperture width (25 μm) was registered in 37 specimens (Fig. 3C). Analysis of the size frequency distribution of shell length indicates that this population possesses

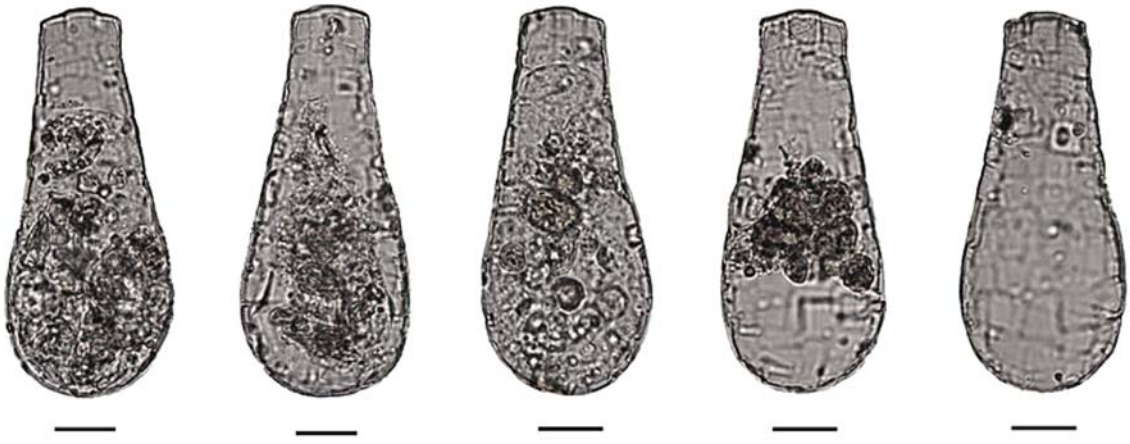

Fig. 1. Light micrographs of *Quadrulella longicollis*: broad lateral view of different specimens from Šargan Mountain, Serbia. Scale bars: 20 μm.

continuous polymorphism. All measured specimens had shell length between 103 and 137 μm. In this case, 69.23% of all specimens had shell length between 115 and 125 μm, whereas only 6.92% were smaller than 115 μm and 23.85% were larger than 125 μm. Analysis of the size frequency distribution of shell width and aperture width indicates that this population is size-monomorphic. Shell width ranged from 45 to 64 μm. However, 56.92% of all measured specimens had shell width of 52–57 μm, whereas 30.00% were narrower than 52 μm and only 13.08% were wider than 57 μm. The frequency analysis of aperture width shows the similar distribution pattern. Namely, all measured specimens had aperture width between 21 and 29 μm. In this case, 71.54% of all specimens had aperture width of 24–26 μm, whereas only 14.61% had aperture narrower than 24 μm and only 13.85% had aperture wider than 26 μm. Figures 3D–F show bag plot analyses of the correlation between shell length, shell width and aperture width.

The negative skewness value (–0.006) for shell length suggests an asymmetrical distribution with a long tail toward lower values. Since the negative value is not clearly different from zero, the asymmetry of shell length distribution was minimal. Moderate positive skewness value (0.298) was registered only for shell width/shell length ratio, while other variables were characterized by low positive values (0.119–0.249). Only shell width displayed negative kurtosis value (–0.113), meaning that this variable was characterized by flatter distribution than a standard Gaussian distribution. Other variables were found to have positive kurtosis values (0.084–0.563), indicating a distribution that

Table 1. Morphometric characterization of *Quadrulella longicollis* from Šargan Mountain (Serbia) based on 130 specimens (measurements in μm).

Characters	Min	Max	M	x	SE	SD	CV	Sk	Ku
shell length	103	137	122	121.94	0.48	5.53	4.53	-0.006	0.460
shell width	45	64	53.5	53.4	0.33	3.73	6.99	0.173	-0.113
aperture width	21	29	25	24.89	0.126	1.44	5.77	0.176	0.084
area of the optical section	3533	6052	4805.5	4825.5	38.83	442.78	9.18	0.205	0.348
shell width/shell length	0.37	0.50	0.44	0.44	0.00	0.02	5.68	0.298	0.176
aperture width/shell length	0.18	0.24	0.21	0.20	0.00	0.01	5.75	0.249	0.563
aperture width/shell width	0.39	0.57	0.47	0.47	0.00	0.03	7.33	0.119	0.309

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.

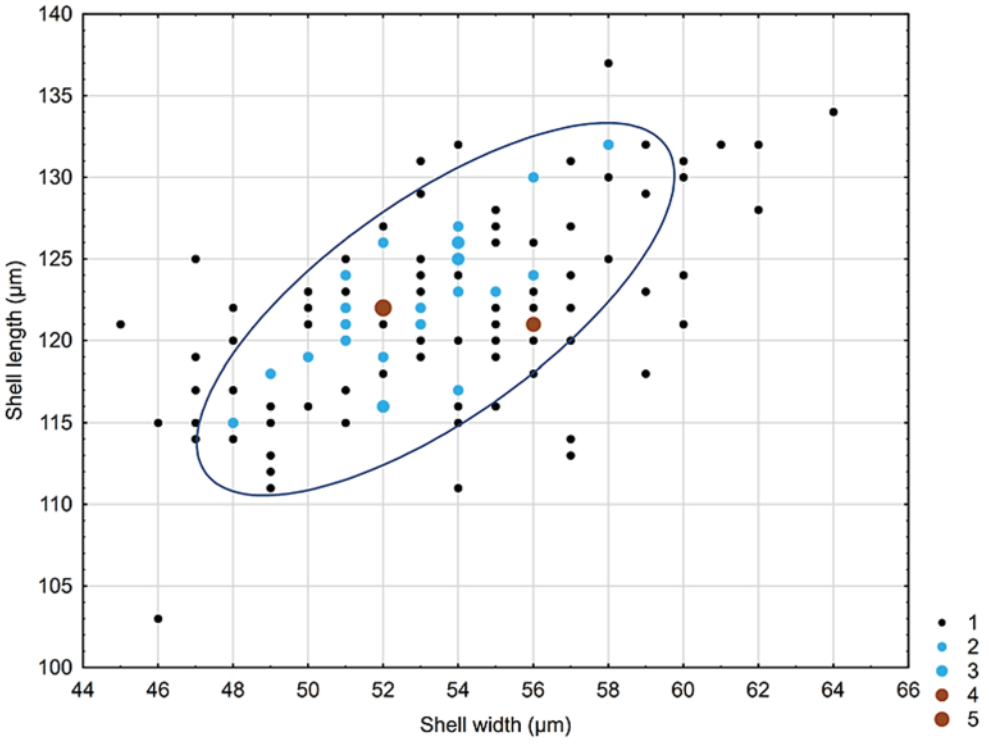

Fig. 2. Frequency scatter plot shows the correlation between shell width and shell length of 130 specimens of *Quadrulella longicollis* from Šargan Mountain, Serbia. Ellipse represents 67% confidence interval.

is sharper than the standard Gaussian distribution. Low positive values (0.084–0.176) were observed for aperture width and shell width/shell length ratio, while moderate positive values (0.309–0.460) were registered for shell length, area of the optical section, and aperture width/shell width ratio. High positive value (0.563) was observed for aperture width/shell length ratio.

Quadrulella symmetrica

Description. The shell is ovoid or pyriform, colorless, transparent and compressed laterally, especially in the apertural region. Shell structure is more or less symmetrical, although asymmetrical shells were also present. The shell is composed of siliceous, quadrangular plates, which are usually regularly arranged in transverse and longitudinal series (rows), with smaller plates close to the aperture. One specimen in population from Šargan Mountain has shell covered by mixed quadrangular and circular plates (Fig. 4). The aperture is terminal, oval, convex in broad lateral view and often concave in narrow lateral view, surrounded by a thin organic lip.

Population from Šargan Mountain. Figure 5 shows light micrographs of some specimens from this population, while Figure 6 shows frequency scatter plot analysis of the correlation between shell width and shell length. Morphometric characters of 432 specimens from Šargan Mountain were measured and the results are given in Table 2. Coefficient of variation was moderate for area of the optical section (11.45%), while the other measured variables were characterized by low variability (from 4.98% to 8.53%). For basic characters, the minimal variability was observed for shell length (4.98%), while the maximal variation coefficient was observed for area of the optical section (11.45%). For ratio characters, the minimal variability was observed for aperture width/shell length ratio (5.82%), while the maximal variation coefficient was observed for aperture width/shell width ratio (6.91%).

The most frequent shell length (76 and 77 µm) was registered in 49 specimens (Fig. 7A); the most frequent shell width (40 and 41 µm) was registered in 55 specimens (Fig. 7B), and the most frequent aperture width (21 µm) was registered in 132 specimens (Fig. 7C). Analysis of the size frequency distribution of shell length, shell width

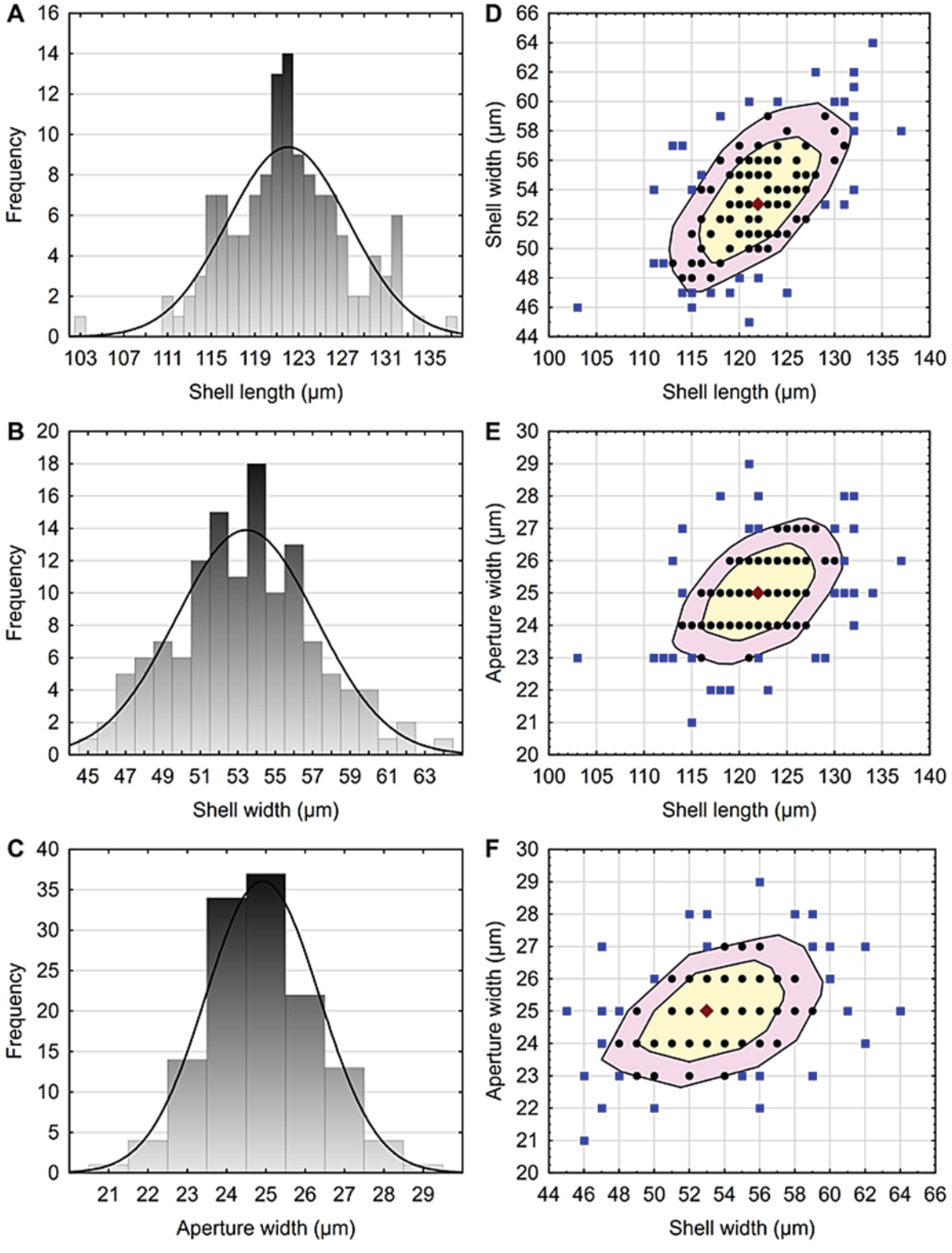

Fig. 3. Morphological variability of *Quadrulella longicollis* based on 130 specimens from Šargan Mountain, Serbia. Histograms show the size frequency distribution of the shell length (A), shell width (B), and aperture width (C); bag plots show the correlation between shell length and shell width (D), aperture width and shell length (E), and aperture width and shell width (F). *Legend for bag plots*: depth median ♦, characters on Y axes ●, outliers ■.

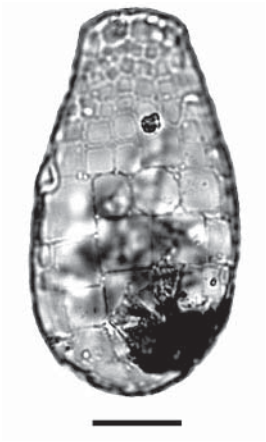

Fig. 4. Specimen of *Quadrulella symmetrica* with mixed quadrangular and circular shell plates observed in a moss-dwelling population from Šargan Mountain, Serbia. Scale bar: 20 μm.

and aperture width indicates that this population is size-monomorphic. Shell length ranged from 64 to 89 μm. However, 72.92% of all measured specimens had shell length of 73–80 μm, whereas only 11.80% were smaller than 73 μm and only 15.28% were larger than 80 μm. The frequency analyses of shell width and aperture width show similar distribution pattern. Namely, all measured specimens had shell width between 33 and 55 μm. In this case, 54.63% of all specimens had shell width of 41–47 μm, whereas 37.96% were narrower than 41 μm and only 7.41% were wider than 47 μm. All measured specimens had aperture width between 17 and 25 μm. However, 75.93% of all specimens had aperture width of 20–22 μm, whereas only 17.36% had aperture narrower than 20 μm and only 6.71% had aperture wider than 22 μm. Figures 7D–F show bag plot analyses of the correlation between shell length, shell width and aperture width.

The negative values of skewness for shell length (–0.156) and aperture width/shell width ratio (–0.162) suggest an asymmetrical distribution with a long tail toward lower values. The asymmetry of aperture width (0.020) and aperture width/shell length ratio (0.090) was low, while high positive skewness values (0.535–0.612) were observed for shell width, area of the optical section and shell width/shell length ratio. The negative values of skewness were not observed, meaning that all variables were characterized by a distribution that is sharper than the standard Gaussian distribution. Low positive values were observed for aperture width (0.081), aperture width/shell length ratio (0.023), and aperture width/shell width ratio (0.131). High positive values (0.501–1.087) were observed for all other variables.

Population from the Alagovac Lake region. Figure 8 shows light micrographs of some specimens from this population, while Figure 9 shows frequency scatter plot analysis of the correlation between shell width and shell length. Morphometric characters of 462 specimens from the Alagovac Lake region were measured and the results are given in Table 3. Coefficient of variation was moderate for area of the optical section (12.08%), while the other measured variables were characterized by low variability (from 4.96% to 9.67%). For basic characters, the minimal variability was observed for shell length (4.96%), while the maximal variation coefficient was observed for area of the optical section (12.08%). For ratio characters, the minimal variability was observed for

Table 2. Morphometric characterization of *Quadrulella symmetrica* from Šargan Mountain (Serbia) based on 432 specimens (measurements in μm).

Characters	Min	Max	M	x	SE	SD	CV	Sk	Ku
shell length	64	89	77	76.92	0.18	3.83	4.98	-0.156	0.501
shell width	33	55	41	41.80	0.17	3.56	8.53	0.569	0.664
aperture width	17	25	21	20.69	0.06	1.27	6.13	0.020	0.081
area of the optical section	1735	3737	2474.5	2486.6	13.69	284.62	11.45	0.535	1.087
shell width/shell length	0.46	0.69	0.54	0.54	0.00	0.04	6.73	0.612	0.550
aperture width/shell length	0.22	0.32	0.27	0.27	0.00	0.02	5.82	0.090	0.023
aperture width/shell width	0.39	0.61	0.50	0.50	0.00	0.03	6.91	-0.162	0.131

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.

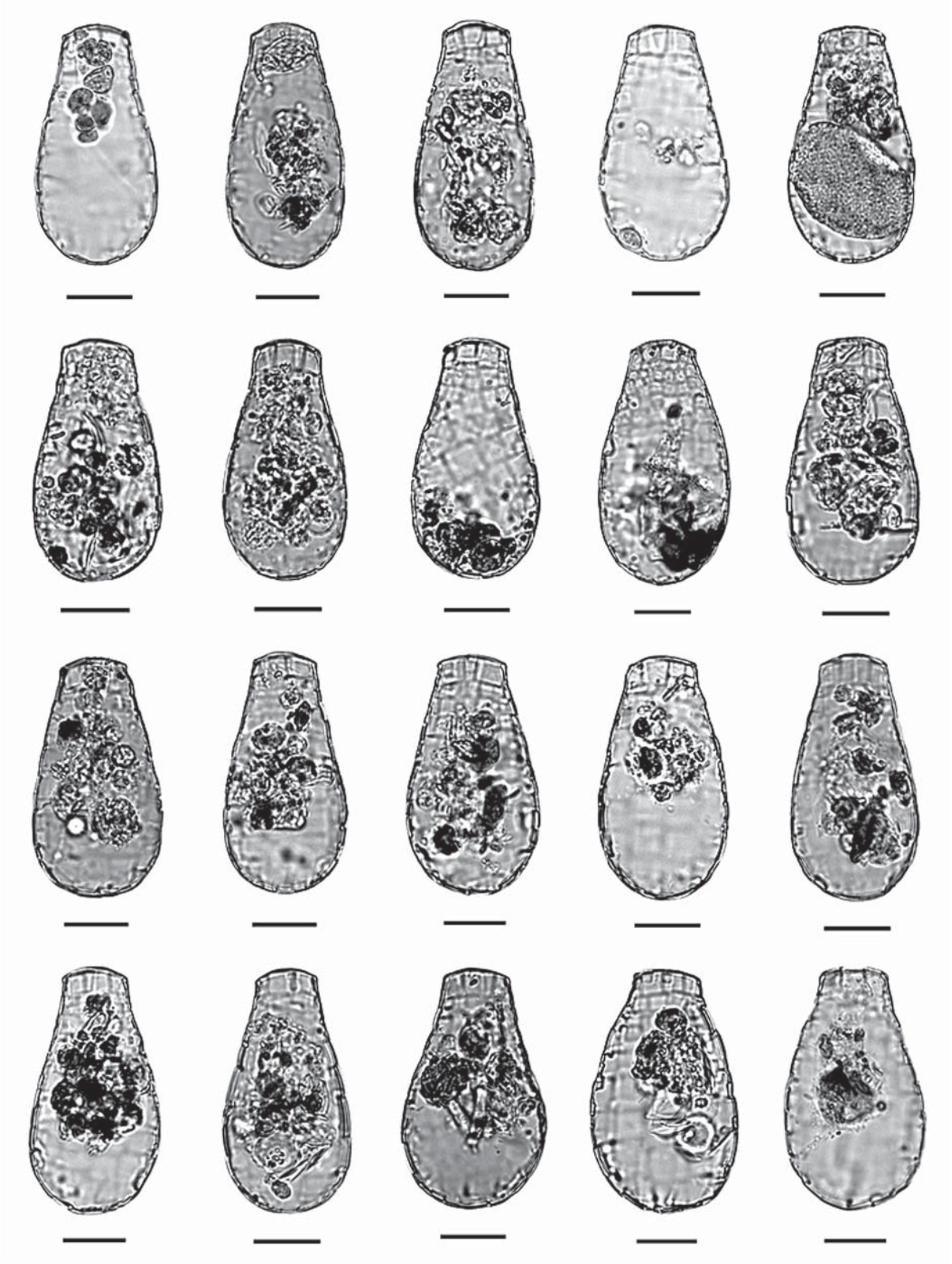

Fig. 5. Light micrographs of *Quadrulella symmetrica*: broad lateral view of different specimens from Šargan Mountain, Serbia. Scale bars: 20 μm.

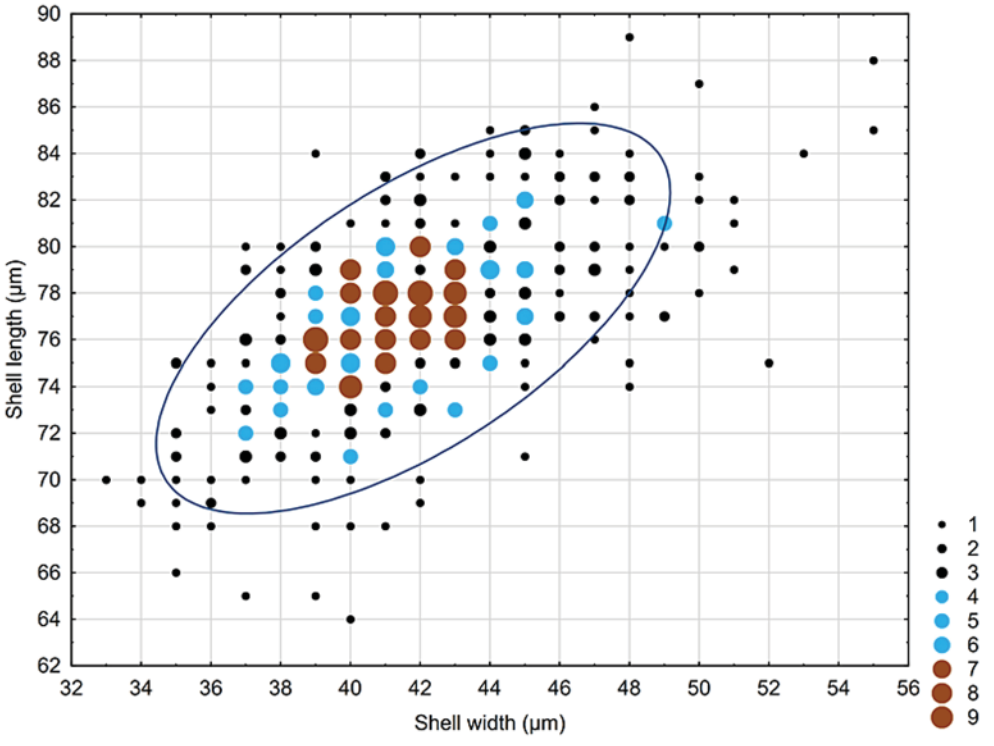

Fig. 6. Frequency scatter plot shows the correlation between shell width and shell length of 432 specimens of *Quadrulella symmetrica* from Šargan Mountain, Serbia. Ellipse represents 67% confidence interval.

aperture width/shell width ratio (6.50%), while the maximal variation coefficient was observed for shell width/shell length ratio (8.51%).

The most frequent shell length (81 µm) was registered in 56 specimens (Fig. 10A); the most frequent shell width (44 µm) was registered in 48 specimens (Fig. 10B), and the most frequent aperture width (22 µm) was registered in 117 specimens (Fig. 10C). Analysis of the size frequency distribution of shell length and aperture width indicates that this population is size-monomorphic. Shell length ranged from 69 to 93 µm. However, 71.21% of all measured specimens had shell length of 77–85 µm, whereas only 7.58% were smaller than 77 µm and only 21.21% were larger than 85 µm. The frequency analysis of aperture width shows similar distribution pattern. Namely, all measured specimens had aperture width between 18 and

Table 3. Morphometric characterization of *Quadrulella symmetrica* from the Alagovac Lake region (East Herzegovina) based on 462 specimens (measurements in µm).

Characters	Min	Max	M	x	SE	SD	CV	Sk	Ku
shell length	69	93	82	82.33	0.19	4.08	4.96	-0.008	0.179
shell width	38	58	45	46.04	0.21	4.45	9.67	0.389	-0.676
aperture width	18	26	22	21.91	0.07	1.55	7.05	0.230	-0.210
area of the optical section	2171	4097	2897.5	2929.81	16.47	353.95	12.08	0.484	0.007
shell width/shell length	0.44	0.68	0.55	0.56	0.00	0.05	8.51	0.233	-0.696
aperture width/shell length	0.20	0.33	0.27	0.27	0.00	0.02	6.86	0.164	0.641
aperture width/shell width	0.39	0.59	0.48	0.48	0.00	0.03	6.50	0.048	-0.012

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.

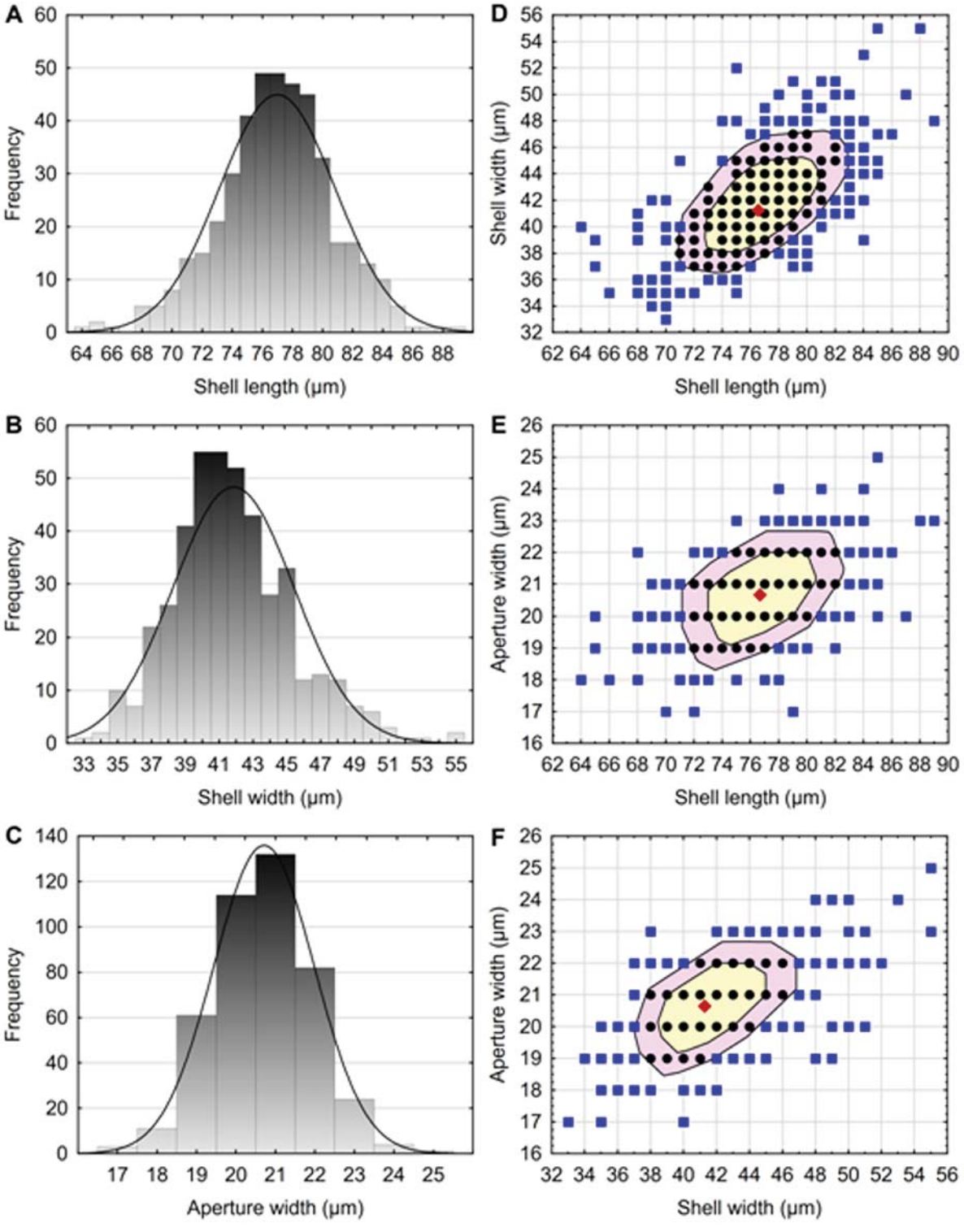

Fig. 7. Morphological variability of *Quadrulella symmetrica* based on 432 specimens from Šargan Mountain, Serbia. Histograms show the size frequency distribution of the shell length (A), shell width (B), and aperture width (C); bag plots show the correlation between shell length and shell width (D), aperture width and shell length (E), and aperture width and shell width (F). *Legend for bag plots*: depth median ♦, characters on Y axes ●, outliers ■.

Fig. 8. Light micrographs of *Quadrulella symmetrica:* broad lateral view of different specimens from the Alagovac Lake region, East Herzegovina. Scale bars: 20 μm.

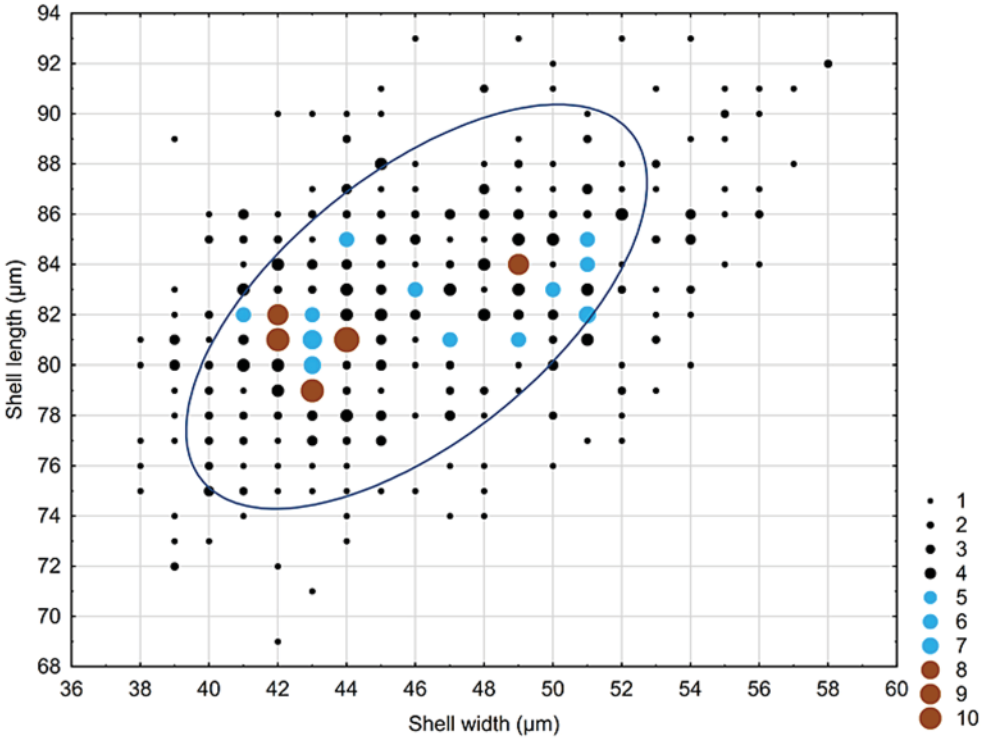

Fig. 9. Frequency scatter plot shows the correlation between shell width and shell length of 462 specimens of *Quadrulella symmetrica* from the Alagovac Lake region, East Herzegovina. Ellipse represents 67% confidence interval.

26 μm. In this case, 67.53% of all specimens had aperture width of 21–23 μm, whereas only 16.88% had aperture narrower than 21 μm and only 15.59% had aperture wider than 23 μm. Analysis of the size frequency distribution of shell width indicates that this population is size-monomorphic. All measured specimens had shell width between 38 and 58 μm. In this case, 43.07% of all specimens had shell width of 45–51 μm, whereas 45.02% were narrower than 45 μm and only 11.91% were wider than 63 μm. Figures 10D–F show bag plot analyses of the correlation between shell length, shell width and aperture width.

The negative value of skewness (–0.008) for shell length suggests an asymmetrical distribution with a long tail toward lower values. Moderate positive skewness values were observed for shell width (0.389) and area of the optical section (0.484). All other variables were characterized by low positive skewness values (0.048–0.233). Four characters (shell width, aperture width, shell width/shell length ratio, and aperture width/shell width ratio) displayed negative kurtosis values, meaning that they were characterized by flatter distribution than a standard Gaussian distribution. Because the negative values obtained for aperture width (–0.210) and aperture width/shell width ratio (–0.012) were not clearly different from zero, the resulting deviation from normal Gaussian distribution was minimal. However, negative values for shell width (–0.676) and shell width/shell length ratio (–0.696) were clearly different from zero, indicating that the average size group has a lower dispersion. Other variables were found to have positive kurtosis values (0.007–0.641), indicating a distribution that is sharper than the standard Gaussian distribution.

Discussion

Taranek (1882) described *Quadrulella symmetrica* var. *longicollis*. This taxon is characterized by more elongate shell than the typical form of *Q. symmetrica* and, based on the original description, a shell length range from 80 to 150 μm. Deflandre (1936) noted that specimens of this taxon are usually longer than 100 μm. Ogden (1984) speculated that these could represent extra large specimens, as often seen in species of the genus *Euglypha*, and thus did not differentiate them from typical *Q. symmetrica*. Cerdá (1986) examined specimens from Bolivia

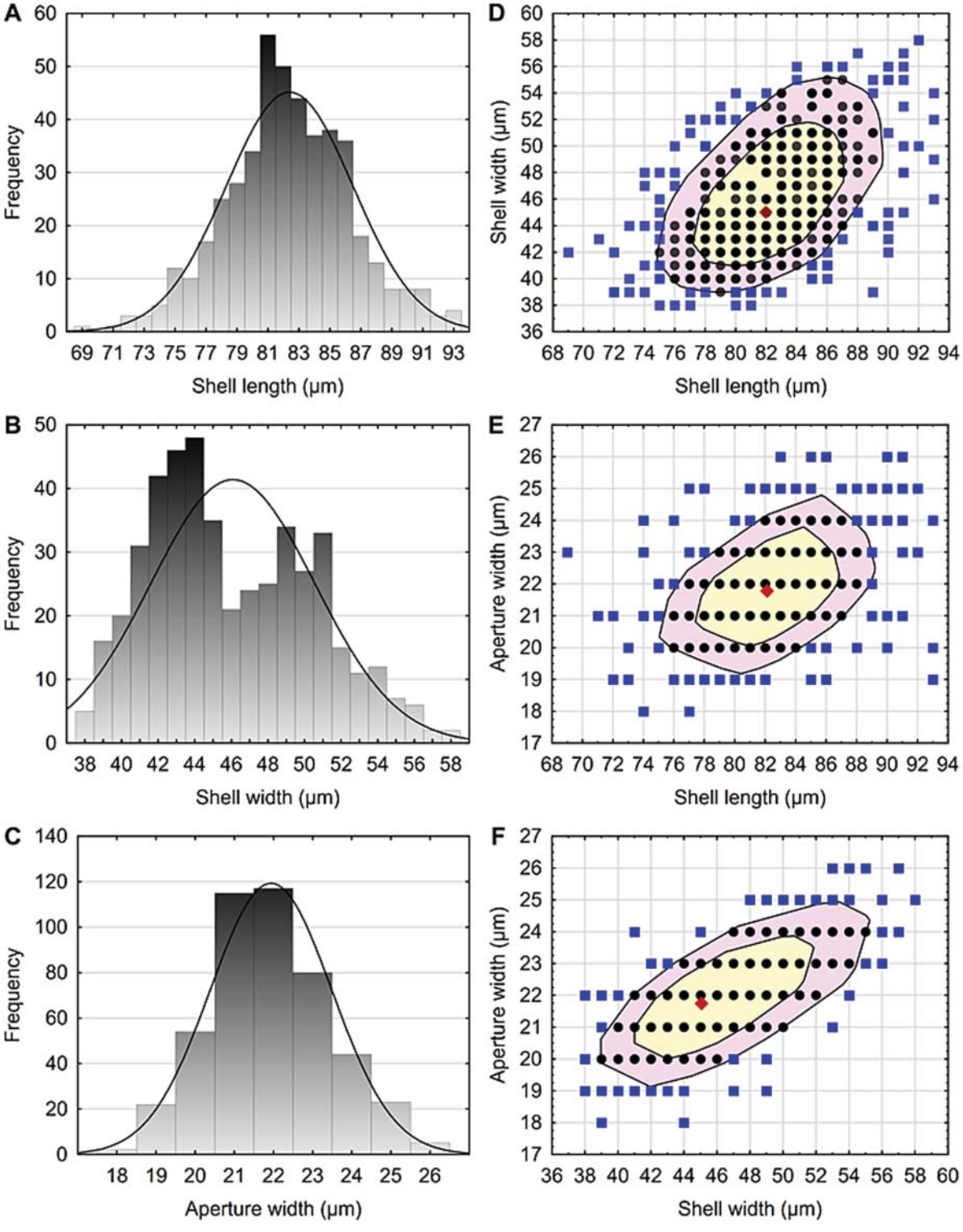

Fig. 10. Morphological variability of *of Quadrulella symmetrica* based on 462 specimens from the Alagovac Lake region, East Herzegovina. Histograms show the size frequency distribution of the shell length (A), shell width (B), and aperture width (C); bag plots show the correlation between shell length 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 ■.

and noted the following measurements: shell length 130–150 μm, shell width 60–70 μm and aperture width 20–30 μm. Kosakyan et al. (2012) based on molecular data concluded that *Q. symmetrica* var. *longicollis* is a separate species and made a new combination of this taxon: *Quadrulella longicollis*. Luketa (2015) based on eight specimens of *Q. longicollis* collected in *Sphagnum* mosses from the Vlasina Lake region (Serbia) noted the following measurements: shell length 111–131 μm, shell width 50–57 μm and aperture width 27–28 μm. Kosakyan et al. (2016) concluded that population from the Vlasina Lake region may represent the true *Q. longicollis*. Luketa (2015) based on shell shape observed two shell types: broad and narrow. In the population from Šargan Mountain analysed in the present study, only broad shell types were observed.

Kosakyan et al. (2016) described new *Sphagnum*-dwelling species from Switzerland with variable neck length: *Quadrulella variabilis*. Based on seven sequenced specimens, they noted the following measurements: shell length 66–69 μm, shell width 35–40.5 μm and aperture width 17–18.5 μm. Also, they concluded that the sequenced specimen of *Q. longicollis* from the study published in Kosakyan et al. (2012) might be a member of *Q. variabilis*. Namely, this specimen has shell length 96 μm and it is longer than typical specimens of *Q. variabilis*, but they have similar size of aperture and shell plates. Gauthier-Lièvre (1957) observed *Nebela* (*Quadrulella*) *symmetrica* var. *longicollis* from the Republic of Congo (Middle Africa) and noted the following measurements: shell length 80–95 μm, shell width 40–46 μm and aperture width 20–23 μm. Green (1979) observed two specimens of this taxon in the open water of the Lake Sonfon (Sierra Leone, West Africa) and noted the following measurements: shell length 90 μm, maximum shell width 45 μm, and aperture diameter 19 μm. The specimen from Kosakyan et al. (2012) morphometrically is very similar to specimens from the above mentioned African populations.

Kosakyan et al. (2016) considered that the typical *Q. longicollis* is longer than 100 μm and thus does not overlap with the sequenced *Q. variabilis*. It is possible that the specimen from Kosakyan et al. (2012) represent an extremely short specimen of *Q. longicollis*. Namely, the shell size range proposed by Taranek (1882) included the specimen described in Kosakyan et al (2012). Another possibility is that the *Q. longicollis* complex includes four species: *Q. variabilis* (shell length 66–69 μm), an undescribed species (shell length 80–96 μm), *Q. longicollis* s.s. (shell length 110–130 μm), and a very long undescribed species (shell length 130–150 μm). Detailed morphological, morphometric, ecological and molecular studies using additional populations are needed to clarify true taxonomic status of all taxa from *Q. longicollis* complex.

To the best of my knowledge, only two examples of *Quadrulella* members were noted with shells covered by mixed quadrangular and circular plates. Gauthier-Lièvre (1957) observed specimens of *Q. tropica* from Africa with shells covered by mixed quadrangular and circular plates. Later, Chung et al. (1992) noted some specimens of *Q. symmetrica* from South Korea with shell surface covered by quadrangular and circular shell plates. In addition, in the present study a specimen of *Q. symmetrica* from Šargan Mountain with similar shell structure was observed. These findings put forward new questions on the evolution of square plates. The specimens of *Q. symmetrica* with mixed shell plates may be classical mutants, mutants with atavistic nature, or a beginning of new phyletic lineage. In the first assumption, round plates are abnormal self-secreted plates. The second assumption indicates that the ancestor of the genus *Quadrulella* possessed the shell completely covered by round self-secreted plates. The third assumption suggests that specimens with mixed shell coverage present new species if round plates are the products of neutral or positive mutations. Further molecular and morphological studies based on large number of populations will resolve the taxonomic status of square scaled hyalosphenids.

Kosakyan et al. (2012) revealed an unexpected morphological and genetic variability of *Q. symmetrica*, but they did not propose new taxonomic changes. However, Kosakyan et al. (2016) based on detailed study of this morphospecies concluded that *Q. symmetrica* is not a single species. Namely, they demonstrated that this taxon hosts at least three different genetic species that are well supported by the morphological characteristics: *Q. symmetrica* s.s., *Q. variabilis* and *Q. madibai*. *Quadrulella variabilis* is morphologically very similar to *Q. symmetrica* s.s., from which it differs by the dimensions of the shell and the size of scale plates (shell length 66–69 μm, maximum plate size 7–9 μm in *Q. variabilis* versus shell length 72–85 μm, maximum plate size 10–12 μm in *Q. symmetrica* s.s.). An interesting case is *Q. madibai*, which is morphologically similar to *Q. symmetrica* s.s. because of its large shell plates

Table 4. Comparative morphometric data of *Quadrulella symmetrica* based on three populations from the Balkan Peninsula.

Characters	Location	N	Min	Max	x	References
shell length	Vlasina Lake region	603	71	93	82.87	Luketa, 2015
	Šargan Mountain	432	64	89	76.92	This study
	Alagovac Lake region	462	69	93	82.33	This study
shell width	Vlasina Lake region	603	39	58	47.72	Luketa, 2015
	Šargan Mountain	432	33	55	41.80	This study
	Alagovac Lake region	462	38	58	46.04	This study
aperture width	Vlasina Lake region	603	20	29	23.61	Luketa, 2015
	Šargan Mountain	432	17	25	20.69	This study
	Alagovac Lake region	462	18	26	21.91	This study
area of the optical section	Vlasina Lake region	603	2301	4164	3076.58	Luketa, 2015
	Šargan Mountain	432	1735	3737	2486.60	This study
	Alagovac Lake region	462	2171	4097	2929.81	This study
shell length/shell width	Vlasina Lake region	603	0.47	0.72	0.58	Luketa, 2015
	Šargan Mountain	432	0.46	0.69	0.54	This study
	Alagovac Lake region	462	0.44	0.68	0.56	This study
aperture width/shell length	Vlasina Lake region	603	0.24	0.35	0.29	Luketa, 2015
	Šargan Mountain	432	0.22	0.32	0.27	This study
	Alagovac Lake region	462	0.20	0.33	0.27	This study
aperture width/shell width	Vlasina Lake region	603	0.40	0.58	0.50	Luketa, 2015
	Šargan Mountain	432	0.39	0.61	0.50	This study
	Alagovac Lake region	462	0.39	0.59	0.48	This study

Abbreviations: N – number of measured specimens, Min – minimum value, Max – maximum value, x – arithmetic mean

(maximum plate size 9–11 μm in *Q. madibai* versus 10–12 μm in *Q. symmetrica* s.s.). However, *Q. symmetrica* s.s. can be discriminated from *Q. madibai* based on its less slender and elongated shell (shell length/shell width ratio is 1.7–1.9 in *Q. symmetrica* s.s. versus 2.0–2.3 in *Q. madibai*). In addition, the general outline of the shell in *Q. madibai* is globally more tubular and does not present a distinct neck. Based on the detailed morphological and morphometric data presented in this study, it is not possible to distinguish *Q. symmetrica* s.s. and *Q. madibai*. Probably, *Q. madibai* represents only one extreme line of clones. Further molecular and morphological studies based on large number of populations will resolve the taxonomic status of these two taxa. Comparative morphometric data of three *Q. symmetrica* populations from the Balkan Peninsula are presented in Table 4.

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