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Morphological variability of two *Quadrulella* species (Arcellinida: Hyalospheniidae) from the Vlasina Lake region of Serbia

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Summary. Here we present morphological and morphometrical data from sphagnicolous population of *Quadrulella longicollis* (Taranek) and both qualitative and quantitative characters of *Q. symmetrica* (Wallich) from the Vlasina Lake region (Serbia). Both broad and narrow types of *Q. longicollis* shells were observed, but intermediate forms were not noted. Subsequent data analysis supports the species status of *Q. longicollis* recently proposed in a molecular phylogenetic study. Based on 603 specimens, eight types of *Q. symmetrica* shells were observed: barrel-shaped shells, short broad shells without a pronounced neck, elongated pyriform shells, elongated square shells, short square shells, slightly curved shells, short broad shells with a pronounced neck and narrow shells. Intermediate forms between these types were also noted. Coefficients of variation were moderately low for all measured characters in the studied population. The minimal variability (4.5%) was observed for shell length, while maximal variability (9.35%) was recorded for the area of optical section. Analysis of the size frequency distribution suggests that *Q. symmetrica* is a size-monomorphic species.

Keywords: Balkan Peninsula, biometry, morphology, morphometry, protists, testate amoebae.

INTRODUCTION

Testate amoebae (Arcellinida and Euglyphida) are cosmopolitan and can be found in marine and freshwater ecosystems, as well as terrestrial habitats. All representatives are free-living and move by pseudopodia. The smallest species are approximately 7 µm in length, while the largest can reach 600 µm. All species are heterotrophic and feed on bacteria, fungi and protists. For this reason, testate amoebae are important components of aquatic and terrestrial ecosystems. Namely, they are important for energy flow and nutrient cycles in ecosystems. For this reason, several authors have suggested the use of these organisms as bioindicators to monitor a wide range of environmental changes. In addition, this group of amoeboid protists is particularly interesting for paleoecological, zoogeographical and evolutionary studies. However, the existence of many undescribed or inadequately described species of these organisms is problematic for multidisciplinary studies; while intra-specific variability presents additional problems.

Taxonomy of testate amoebae at the familial, generic

and species level is based on the shape and size of their shells. Namely, these organisms are treated in taxonomy as asexual because sexual reproduction is known only for a small number of representatives. For this reason, it is not possible to apply the biological species concept for the vast majority of testate amoebae species (Bobrov and Mazei 2004). By the end of the 20th century, the majority of taxonomists began to adopt a more populational concept for understanding species, increasing the importance of morphometry in taxonomical studies. Also, many authors have reported that because testate amoebae are indeed highly variable, insufficient knowledge of morphometry could be problematic during species identification. In light of this, many studies have been devoted exclusively to the morphometric analysis of testate amoebae (e.g. Wanner 1999; Blanco 2001; Bobrov and Mazei 2004; Nicholls 2009; Davidova 2012a, 2012b). In addition, more frequent use of electron microscopy to study morphological characters has even led to the publication of papers focused on the redescription of one species (e.g. Török 2001; Todorov 2002; Todorov and Golemansky 2003, 2007, 2009;

Yang et al. 2004; Yang and Shen 2005; Meisterfeld and Badewitz 2006; Tsyganov and Mazei 2006, 2006/2007; Zapata and Fernández 2008; Todorov et al. 2010; Nicholls 2015).

Taxonomically, the genus *Quadrulella* is a very complicated group composed of a dozen species. The shell is colourless, transparent and pyriform, or with a distinct neck. Some taxa possess shells with a lateral ridge. Members of the genus *Quadrulella* inhabit *Sphagnum* and other terrestrial mosses as well as humus rich soil (Meisterfeld 2002). The present study reports the morphological variability of sphagnicolous testate amoebae *Quadrulella longicollis* (Taranek, 1882) and *Q. symmetrica* (Wallich, 1863) Cockerell, 1911 collected in the Vlasina Lake region of Serbia.

MATERIAL AND METHODS

Material for the present study was extracted from wet Sphagnum mosses collected from an area surrounding Vlasina Lake, south-east Serbia on 28. May 2013. This lake is situated in the central part of a plateau at an altitude of 1213 m. It is the highest and largest artificial lake in Serbia. Vlasina Lake was created between 1947 and 1951 after the construction of a dam on the Vlasina River where it flows from Vlasinsko blato peat bog. For this reason, Vlasina Lake has many specific characteristics (e.g. floating islands). The climate in the area is typically continental. The lake serves to moderate the local temperature and increases the average humidity and precipitation. Morphological characters and morphometric variables were studied using a light microscope Zeiss Axio Imager A1 under 630× magnification. Images were captured using an AxioCam MRc5 (Zeiss) digital color camera. Measurements were conducted in the program AxioVision 4.9.1. Statistical analysis were conducted in the program PAST.

RESULTS

Quadrulella longicollis

Two shell types, broad and narrow, were observed in the studied population as defined below. Broad shell type: Shells are elongated ovoid (Fig. 1A-B), colourless, transparent and compressed laterally, especially in the apertural region. Shells are composed of siliceous, quadrangular plates, which 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. Six specimens were identified, but only three were in ideal position for full measurement. Based on these specimens, the following dimensions were noted: shell length 111-131 µm, shell width 51-57 µm and aperture width 27-28 µm. Narrow shell type: Shells are cylindrical (Fig. 1C-D), colourless, transparent and likely compressed laterally in the apertural region. Shell structures are composed of siliceous, quadrangular plates. Plates 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, surrounded by a thin organic lip. Only two specimens were found, with the following dimensions: shell length 118-123 μ m, shell width 50-51 μ m and aperture width 27-28 μ m.

Quadrulella symmetrica

Shells are ovoid or pyriform, colourless, transparent and compressed laterally especially in the apertural region. Shell structure is more or less symmetrical, although asymmetrical shells were also present. Shells are composed of siliceous, quadrangular plates, which are usually regularly arranged in transverse and longitudinal series (rows), with smaller plates close to the aperture. The aperture is terminal, oval, convex in broad lateral view and often concave in narrow lateral view, surrounded by a thin organic lip. Eight types of shells were observed in the studied heterogeneous population: barrel-shaped shells (Fig. 2A-C), short broad shells without pronounced necks (Fig. 2F-L), elongate pyriform shells (Fig. 3A-D), elongated square shells (Fig. 3F), short square shells (Fig. 3G-H), slightly curved shells (Fig. 3I-L), short broad shells with pronounced neck (Fig. 4A) and narrow shells (Fig. 4C-L). However, intermediate forms between these shell types were also observed (Fig. 2D-E, 3E, 4B).

Morphometric characters of 603 specimens from the Vlasina Lake region were measured and the results are given in Table 1. The most frequent shell length (82 and 83 μ m) was registered in more than 60 specimens (Fig. 5); the most frequent shell width (45 µm) was registered in more than 65 specimens (Fig. 6), and the most frequent aperture width (23 μm) was registered in more than 150 specimens (Fig. 7). Coefficients of variation were moderately low for all measured characters. Namely, coefficients of variation ranged from 4.5% to 9.35%. For basic characters, minimal variability was observed for shell length (4.5%), while the maximal variation coefficient was observed for area of optical section (9.35%). For ratio characters, minimal variability was observed for aperture width/shell width ratio (6.0%), while the maximal variation coefficient was observed for shell width/shell length ratio (6.79%). Analysis of the size frequency distribution of the measured specimens indicates that Quadrulella symmetrica is a size-monomorphic species. For example, shell lengths range from 71 to 93 µm. However, 71.14% of all the specimens had a shell length of 79-86 µm, whereas only 11.77% were smaller than 79 µm and only 17.08% were larger than 86 µm. Histogram analysis revealed nearly the same regularity with respect to shell width distribution. Namely, all measured specimens had a shell width between 39 and 58 μm. In this case, 82.26% of all specimens had a shell width of 43-51 μm , whereas only 17.74% were narrower than 43 μm and wider than 51 µm. Figure 8 shows scatter plot analysis



Fig. 1. Light micrographs of *Quadrulella longicollis* – broad lateral view of different specimens showing general shell shape and outline. **A–B**, Broad shell type; **C–D**, narrow shell type. Scale bar: 20 μm.

of the correlation between shell length and shell width, while Fig. 9 shows scatter plot analysis of the correlation between shell length and area of optical section.

The negative value of skewness for aperture width/shell width ratio suggests an asymmetrical distribution with a long tail toward lower values. However, the asymmetry of this character was low, with a skewness value of -0.098. Moderate positive skewness (0.341) was observed only for shell width/shell length ratio. All other variables were characterized by low positive skewness (between 0.115 and 0.248). High positive values of skewness were not observed for any characters. Three characters (shell length, shell width and area of optical section) displayed negative kurtosis values, meaning they were characterized by flatter distribution than a standard Gaussian distribution. This information indicates that the average size group has a higher dispersion. Because the negative value obtained for area of optical section is not clearly different from zero (-0.238), the resulting deviation from normal Gaussian distribution was minimal. However, negative values for shell length and shell width were clearly different from zero (-0.322 and -0.376, respectively), indicating that the average size group has a lower dispersion. Other variables were found to have positive kurtosis values, indicating a distribution which is sharper than a standard Gaussian distribution. Low positive values (between 0.050 and 0.148) were observed for aperture width, aperture width/ shell length ratio and aperture width/shell width ratio. Further analysis revealed that the frequency distribution of four variables show approximately zero kurtosis, indicating a standard distribution in the studied population. A moderate positive value (0.393) was observed for shell width/shell length ratio. Variables with high positive or high negative

kurtosis values were not observed for any characters. In all, this indicates that the sample population is relatively stable, suggesting that natural selection pressure is not high.

DISCUSSION

Taranek (1882, cited in Penard 1902) distinguished two varieties of Q. symmetrica: Q. symmetrica var. genuina (with simple pyriform shells) and Q. symmetrica var. longicollis (with elongated shells). It is possible that Q. symmetrica var. genuina is a synonym of Q. symmetrica because the description of this variety corresponds to specimens of Q. symmetrica with short broad shells without a pronounced neck. However, without detailed molecular and morphometric data this conclusion is speculative. According to Ogden (1984), three out of four specimens of Q. symmetrica studied were similar to Q. symmetrica var. longicollis: shells were more elongate than the typical form of Q. symmetrica. Ogden 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. However, more recently Kosakyan et al. (2012) used molecular data to conclude that Q. symmetrica var. longicollis is a separate species, and made a new combination of this taxon: Quadrulella longicollis. The present study strongly supports this taxonomic change. Namely, our results demonstrate that no intermediate forms exist between Q. symmetrica and Q. longicollis. Broad specimens are typical, but narrow specimens also occur.

Wailes and Penard (1911) recognised two forms of *Q*. *symmetrica*: (a) with an aperture devoid of lips and to which the sides descend in nearly straight lines and (b) with an ap-



Fig. 2. Light micrographs of *Quadrulella symmetrica* – broad lateral view of different specimens showing general shell shape and outline. **A–C**, Typical barrel shaped shells; **D–E**, intermediate shells between barrel shaped shells and short broad shells without pronounced neck; **F–L**, typical short broad shells without pronounced neck. Scale bar: 20 µm.



Fig. 3. Light micrographs of *Quadrulella symmetrica* – broad lateral view of different specimens showing general shell shape and outline. **A–D**, Typical elongate pyriform shells; **E**, intermediate shell between elongate pyriform shells and elongated square shells; **F**, typical elongated square shell; **G–H**, short square shells; **I–L**, slightly curved shells. Scale bar: 20 µm.



Fig. 4. Light micrographs of *Quadrulella symmetrica* – broad lateral view of different specimens showing general shell shape and outline. **A**, Typical short broad shell with pronounced neck; **B**, intermediate shell between short broad shells with pronounced neck and short broad shells without pronounced neck; **C**-L, typical narrow shells. Scale bar: 20 μm.

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Characters	Min	Max	М	x	SE	SD	CV	Sk	Ku
Shell length	71	93	83	82.87	0.15	3.73	4.50	0.115	-0.322
Shell width	39	58	48	47.72	0.14	3.46	7.25	0.230	-0.376
Aperture width	20	29	24	23.61	0.06	1.49	6.31	0.125	0.096
Area of optical section	2301	4164	3064	3076.58	11.71	287.66	9.35	0.168	-0.238
Shell width/shell length	0.47	0.72	0.57	0.58	0.00	0.04	6.79	0.341	0.393
Aperture width/shell length	0.24	0.35	0.28	0.29	0.00	0.02	6.64	0.248	0.148
Aperture width/shell width	0.40	0.58	0.50	0.50	0.00	0.03	6.00	-0.098	0.050

Table 1. Morphometric characterization of *Quadrulella symmetrica* from *Sphagnum* near Vlasina Lake (Serbia) based on 603 specimens (all measurements in μ m, except for area of optical section in μ m²).

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



Fig. 5. Histogram shows the size frequency distribution of the shell length of 603 specimens of *Quadrulella symmetrica* from a population near Vlasina Lake, Serbia.





Fig. 6. Histogram shows the size frequency distribution of the shell width of 603 specimens of *Quadrulella symmetrica* from a population near Vlasina Lake, Serbia.



Fig. 7. Histogram shows the size frequency distribution of the aperture width of 603 specimens of *Quadrulella symmetrica* from a population near Vlasina Lake, Serbia.

Fig. 8. Scatter plot shows the correlation between shell length and shell width of 603 specimens of *Quadrulella symmetrica* from a population near Vlasina Lake, Serbia. Ellipse represents 95% confidence interval; polygon represent convex hull.



Fig. 9. Scatter plot shows the correlation between shell length and area of optical section of 603 specimens of *Quadrulella symmetrica* from a population near Vlasina Lake, Serbia.

erture furnished with a lip and sides descending in concave lines – a form which, except for the structure of the shell, is hardly distinguishable from a large *Nebela militaris*. The second form mostly corresponds to shells of specimens shown in Fig. 1 in Lahr et al. (2013, p. 328–329). In the population from the Vlasina Lake region, similar shells were not observed. However, elongate pyriform shells from the present study population are very similar to shells described by the above mentioned authors, but are less elongated. Alekperov and Snegovaya (2000) noted shell lengths of 55-80 µm, shell widths 30-40 µm and aperture widths 12-15 µm for a population collected from Apsheron Peninsula, Azerbaijan. Based on Fig. 2j in Alekperov and Snegovaya (2000, p. 142–143), typical specimens in this population are likely characterized by short narrow shells without a pronounced neck.

Penard (1891) described a third variety in a text concerning *Q. symmetrica*:

"I have found this beautiful species abundantly in most of my gatherings, but mostly represented by very small-sized individuals (length 0.100-0.150 mm), which presented this other peculiarity, that the square plates composing the shell, instead of being disposed, as in the typical form, in a high degree of symmetry, showed great disorder in their arrangement, and very often overlapped each other. The sides of the shell, instead of looking like a tolerably continuous curve, appeared like a series of broken short lines. These two varieties, if they must be considered as such (in my opinion, they are more than varieties), were very sharply distinct, and I have not seen any transitional forms."

However, Penard gave a name to this variety in 1902 – Q. symmetrica var. irregularis. This taxon is characterized by a long shell (approximately 100-150 µm based on the original description), with a shell length that is very similar to Q. lon-

gicollis. Deflandre (1936) observed intermediate specimens between these two taxa. Cash et al. (1919) noted: "The typical *Q. symmetrica* varies considerably in form, the outline in broad view ranging from a somewhat wide pyriform shape with convex sides to an elongated form with slightly concave sides; the var. *irregularis* resembles the latter in shape, but the sides are nearly or quite straight and the size is considerably larger." Also, these authors observed that the shell of this variety does not have any neck constriction and noted following measurements: shell length 130-160 µm, shell width 55-60 µm and aperture width 23-30 µm. Based on this, it is possible that *Q. symmetrica* and *Q. symmetrica* var. *irregularis* are synonyms, but investigations of these testate amoebae using additional specimens will provide more definitive results.

At the beginning of the 20th century a new variety was described: Q. symmetrica var. curvata Wailes, 1912. This taxon is characterized by an asymmetrical (slightly curved) shell. In shape, Microquadrula musciphila Golemansky, 1968 is very similar to this variety. The main difference is neck length, with M. musciphila possessing a very short neck. Also, this species is very small: shell length 22-25 µm, shell width 16-19 µm and aperture diameter 5.0-6.5 µm (Golemansky 1968). Edmondson and Kingman (1913) observed only one specimen of Q. symmetrica var. curvata in Lake Hakone, Japan. Specimens with asymmetrical shells were detected in the present study population, representing an intermediate form between this variety and Q. symmetrica. Namely, Q. symmetrica var. curvata have more curved shells and narrower aperture than specimens observed in the present study population. Also, an interesting characteristic of all specimens with slightly curved shells is the presence of a wide aperture and very similar general shell shape. Oliverio et al. (2014) observed two types of specimens: (a) the neck is straight and aperture slightly curved and (b) the neck is slightly curved and the aperture also appears more curved. However, these authors did not find intermediate specimens. To date, a small number of specimens are known with slightly or mostly curved shells and further studies are needed to clarify their taxonomic status.

Phenotypic variability of living organisms can be produced by genetic differences, environmental influences and stochastic events during development (Vogt et al. 2008; Fusco and Minelli 2010). Oliverio et al. (2014) proposed an alternative hypothesis for phenotypic differences between *Hyalosphenia elegans* and *H. papilio*. Namely, these authors assume that the above mentioned species actually represent two different mating types of the same evolutionary lineage. For this reason, it is not impossible that some shell types are pseudocryptic species within the *Q. symmetrica* species complex, as a result of different mating types. All of these possibilities make investigations of the *Q. symmetrica* species complex extremely complicated.

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