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Morphological variability of *Gibbocarina galeata* and *G. penardiana* comb. nov. (Arcellinida: Hyalospheniidae) from East Herzegovina

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Summary

The genus *Gibbocarina* has been recently established and includes only two species (*G. galeata* and *G. gracilis*) characterized by presence of a hollow keel. Morphology and morphometry of *Nebela penardiana* from East Herzegovina were investigated based on 47 specimens and a new combination was proposed: *Gibbocarina penardiana*. In addition, morphological variability of a sympatric population of *G. galeata* was analyzed based on 121 specimens. It is possible to distinguish these species based on morphological and morphometric data. Morphometric analysis shows that shell width is one of the best discriminating characters (95–139 μm in *G. galeata* versus 64–77 μm in *G. penardiana*). The minimal variability was observed for shell length (5.17% in *G. galeata* and 4.44% in *G. penardiana*), while the maximal variability was recorded for area of the optical section (11.31% in *G. galeata* and 8.50% in *G. penardiana*).

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

Introduction

The family Hyalospheniidae includes mostly comparatively large species with rigid shell composed of organic matrix and building units. These building units may be self-secreted siliceous plates or recycled shell plates of small euglyphid testate amoebae. Some species have shells without building units. Hyalosphenid testate amoebae are common and diverse in peatlands and mosses, but also can be found in some other terrestrial and freshwater habitats. Golemansky (1970) found one population of *Hyalosphenia cuneata* in a marine ecosystem.

The family Hyalospheniidae includes some well-studied genera of the testate amoebae. Modern taxonomy at the generic and species level within this family is based on shape and dimensions of shell (Kosakyan et al., 2013, 2016). However, the overall morphological variability of hyalospheniids is studied rather poorly, although recently several investigations have been dealing with the morphometric analysis of these testate amoebae (Török, 2001; Todorov, 2002; Heger et al., 2011; Luketa, 2015a, 2016; Nicholls, 2015).

Based on molecular data, Kosakyan with co-authors (2012) redefined the family Hyalospheniidae and included eight genera into this family: *Alocodera, Apodera, Certesella, Hyalosphenia, Nebela, Padaungiella, Porosia* and *Quadrulella*. However, Luketa (2015b) established the family Padaungiellidae for three closely related genera characterized by an elongated neck (*Alocodera,*

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Apodera and *Padaungiella*). The paraphyly of the genus *Nebela* s.l. is well established by molecular studies (Kosakyan et al., 2012, 2016; Lahr et al., 2013; Oliverio et al., 2014). For this reason, Kosakyan with co-authors (2016) divided the genus *Nebel*a s.l. into five monophyletic genera: *Nebela* s.s., *Longinebela, Planocarina, Gibbocarina* and *Cornutheca*.

Jung (1942) also predicted the non-monophyly of the genus *Nebela* s.l. based on morphological characters and proposed some new genera. However, he did not designate a type species for each genus and, therefore, the names for these genera are not valid. One of Jung's genera is *Umbonaria* that includes species with a hollow keel and corresponds well to the recently established genus *Gibbocarina*. Jung (1942) includes four species into the genus *Umbonaria*: *U. gracilis, U. pulcherrima, U. orbicularis* and *U. galeata*.

The genus *Gibbocarina* comprises only two species (*G. galeata* and *G. gracilis*) characterized by elongated-pyriform shell shape, the lateral sides of the shell tapering towards the aperture. Members of this genus differ from the other similar taxa by presence of a hollow keel (Kosakyan et al., 2016). The present study reports the morphological variability of *G. galeata* and *G. penardiana* comb. nov. based on specimens collected in East Herzegovina.

Material and methods

The material for the present study was extracted from *Sphagnum* mosses collected in the peatland located in the Alagovac Lake region (43°17'44.8"N, 18°07'31.9"E, ca. 850 m a.s.l.), municipality Nevesinje, East Herzegovina on 11 May and 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 studied shells: shell length, shell width, aperture width, 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, 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.

Results

Gibbocarina galeata (Penard, 1890)

Description. The shell is transparent, colorless, elongated in broad lateral view, laterally compressed, with the lateral sides gradually tapering toward the aperture; lateral margins distinctly compressed giving the impression of a thick lateral keel; in narrow lateral view the shell is elongated elliptical with a pointed aboral end. A small lateral pore is present on each side (sometimes difficult to observe), almost at the point where the keel begins. Shell surface is covered with siliceous plates (circular and oval) seemingly recycled from predated testate amoebae. The aperture is oval, linear or slightly curved in broad lateral view, usually surrounded by a thin organic lip. Figure 1 shows light micrographs of specimens from the studied population.

Morphometry. Morphometric characters of 121 specimens of *G. galeata* from East Herzegovina were measured and the results are given in Table 1. Coefficient of variation was moderate only for area of the optical section (11.31%), while other measured characters were characterized by low variability (from 5.17% to 7.36%). For basic characters, the minimal variability was observed for shell length (5.17%), while the maximal variation coefficient was observed for area of the optical section (11.31%). For ratio characters, the minimal variability was observed for shell width/shell length ratio (5.63%), while the maximal variation coefficient was observed for aperture width/shell length ratio (7.33%). Figure 2 shows scatter plot analysis of the correlation between shell length and shell width for the studied specimens of *G. galeata* and *G. penardiana*.

The most frequent shell length (184 and 189 μm) was registered in 8 specimens (Fig. 3A); the most frequent shell width (113 μm) was registered in 9 specimens (Fig. 3B), and the most frequent aperture width (38 μm) was registered in 20 specimens (Fig. 3C). Histogram analysis revealed nearly the same regularity with respect to shell length, shell width and aperture width distribution. All measured specimens had shell length ranging between 163 and 213 μm. In this case, 61.98% of all specimens had shell length of 181–197 μm, whereas only 21.49% were smaller than 181 μm and only 16.53%

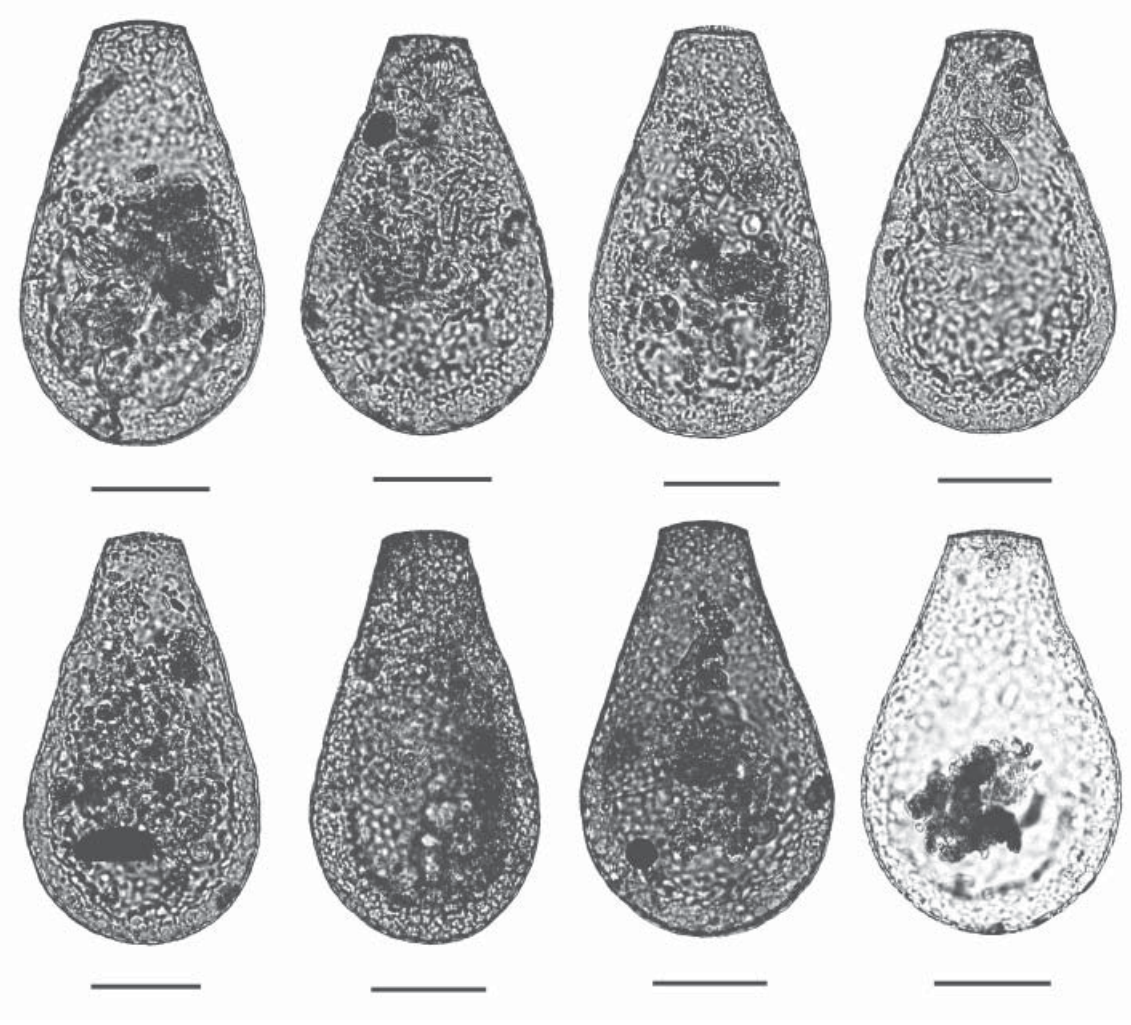

Fig. 1. Light micrographs of *Gibbocarina galeata*: broad lateral view of different specimens from the Alagovac Lake region, East Herzegovina. Scale bars: 50 μm.

were larger than 197 μm. The frequency analysis of the shell width shows similar distribution pattern. Namely, all measured specimens had shell width ranging between 95 and 139 μm. However, 55.37% of all measured specimens had a shell length of 111–125 μm, whereas only 36.36% were narrower than 111 μm and only 8.26% were wider than 126 μm. Figures 3D–F show bag plots analyses of the correlation between shell length, shell width and aperture width.

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 –0.112. Low positive skewness values were observed for aperture width (0.018) and shell width/shell length ratio (0.158), while moderate positive values (between 0.305 and 0.463) were registered for shell length, shell width, area of the optical section and aperture width/shell length ratio. Four characters (shell length, shell width/shell length ratio, aperture 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. Since the negative value obtained for shell length was not clearly different from zero (–0.021), the resulting deviation from the normal Gaussian deviation was minimal. However, negative values for shell width/shell length ratio, aperture width/shell length ratio and aperture width/shell width ratio were clearly different from zero (between –0.332 and –0.628), indicating that the average size group has a lower dispersion. Other

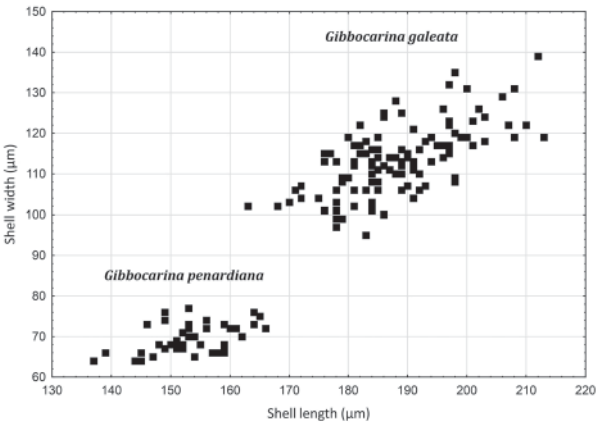

Fig. 2. Scatter plot shows the correlation between shell length and shell width of *Gibbocarina penardiana* comb. nov. (47 specimens) and *G. galeata* (121 specimens) from the Alagovac Lake region, East Herzegovina.

variables were found to have low positive values of kurtosis (0.150–0.237).

GIBBOCARINA PENARDIANA (DECLOITRE, 1936) COMB. NOV.

Description. The shell is transparent, colorless, elongated-pyriform in broad lateral view, slightly compressed, with the lateral sides gradually tapering toward the aperture; lateral margins are not distinctly compressed and do not extend around the aboral region; in narrow lateral view the shell is elongated elliptical with a rounded aboral end. A small lateral pore is present on each side (sometimes difficult to observe) at a distance of one-third of the shell length from the aperture. Shell surface is covered with siliceous plates (circular and oval) seemingly recycled from predated testate amoebae. The aperture is oval, slightly curved in broad lateral view, surrounded by a thin organic lip. Figure 4 shows light micrographs of specimens from the studied population.

Morphometry. Morphometric characters of 47 specimens of *G. penardiana* from East Herzegovina were measured and the results are given in Table 2. Coefficients of variation were low for all measured characters (from 4.44% to 8.50%). For basic characters, the minimal variability was observed for shell length (4.44%), while the maximal variation coefficient was observed for area of the optical section (8.50%). For ratio characters, the minimal variability was observed for shell width/shell length ratio (4.76%), while the maximal coefficient of variation was observed for aperture width/shell width ratio (5.80%).

The most frequent shell length (151 µm) was registered in 6 specimens (Fig. 5A); the most frequent shell width (68 µm) was registered in 8 specimens (Fig. 5B), and the most frequent aperture width (30 µm) was registered in 16 specimens (Fig. 5C). Histogram analysis of shell length indicates that this population is size-monomorphic. All measured specimens had shell length ranging

Table 1. Morphometric characterization of *Gibbocarina galeata* from East Herzegovina based on 121 specimens (measurements in µm, except for area of the optical section in µm²).

Characters	Min	Max	M	x	SE	SD	CV	Sk	Ku
shell length	163	213	187	188.02	0.88	9.72	5.17	0.318	–0.021
shell width	95	139	113	113.46	0.76	8.35	7.36	0.398	0.216
aperture width	32	47	38	38.21	0.24	2.64	6.92	0.018	0.237
area of the optical section	12297	21302	15459	15583	160.16	1762	11.31	0.463	0.150
shell width/shell length	0.52	0.68	0.60	0.60	0.00	0.03	5.63	0.158	–0.332
aperture width/shell length	0.18	0.24	0.20	0.20	0.00	0.01	7.33	0.305	–0.384
aperture width/shell width	0.27	0.38	0.34	0.34	0.00	0.02	7.02	–0.112	–0.628

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.

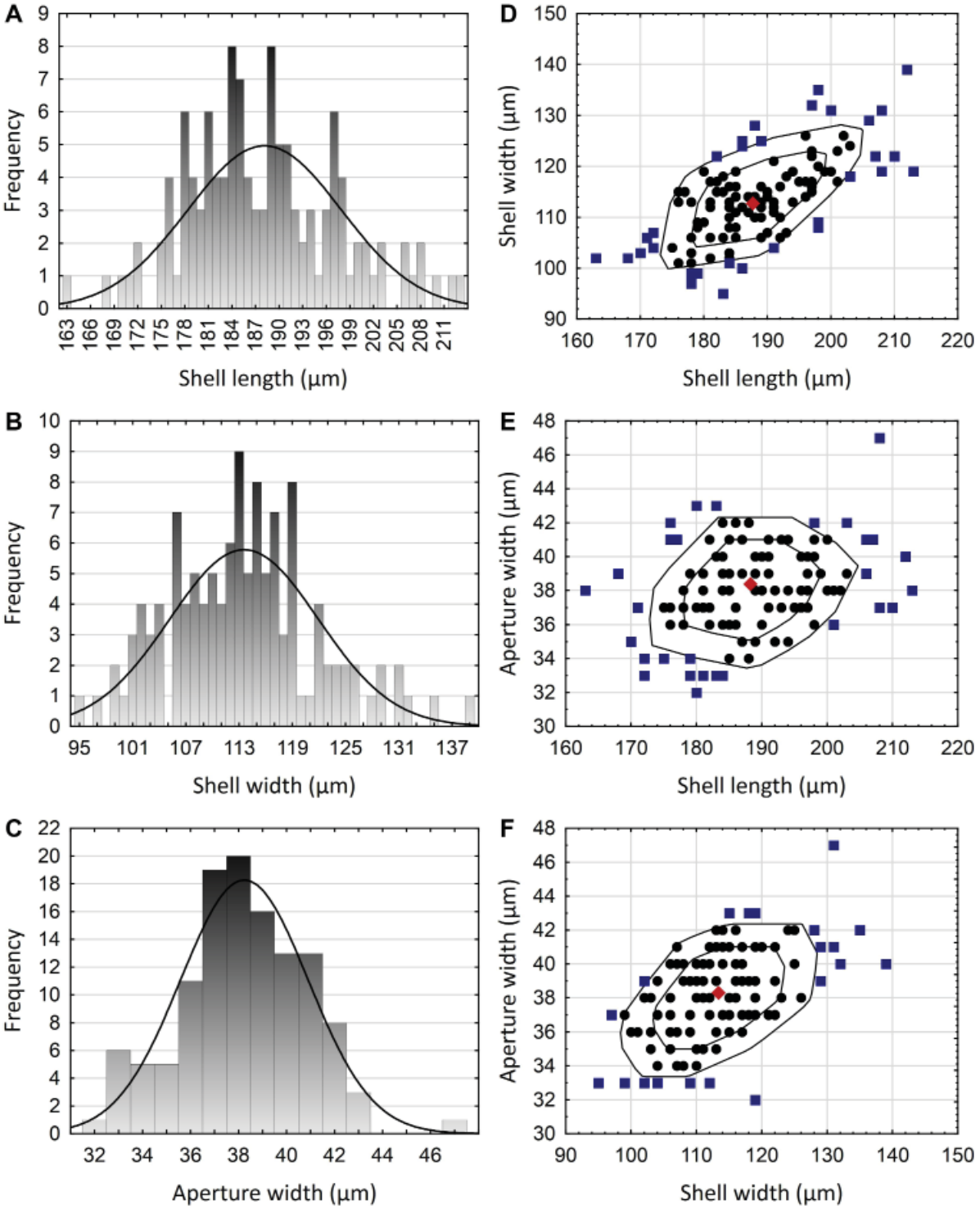

Fig. 3. Morphological variability of *Gibbocarina galeata* based on 121 specimens from the Alagovac Lake region (East Herzegovina). Histograms show the size frequency distribution of the shell length (A), shell width (B), and aperture width (C); bag plots show the correlation between shell length and 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 ■.

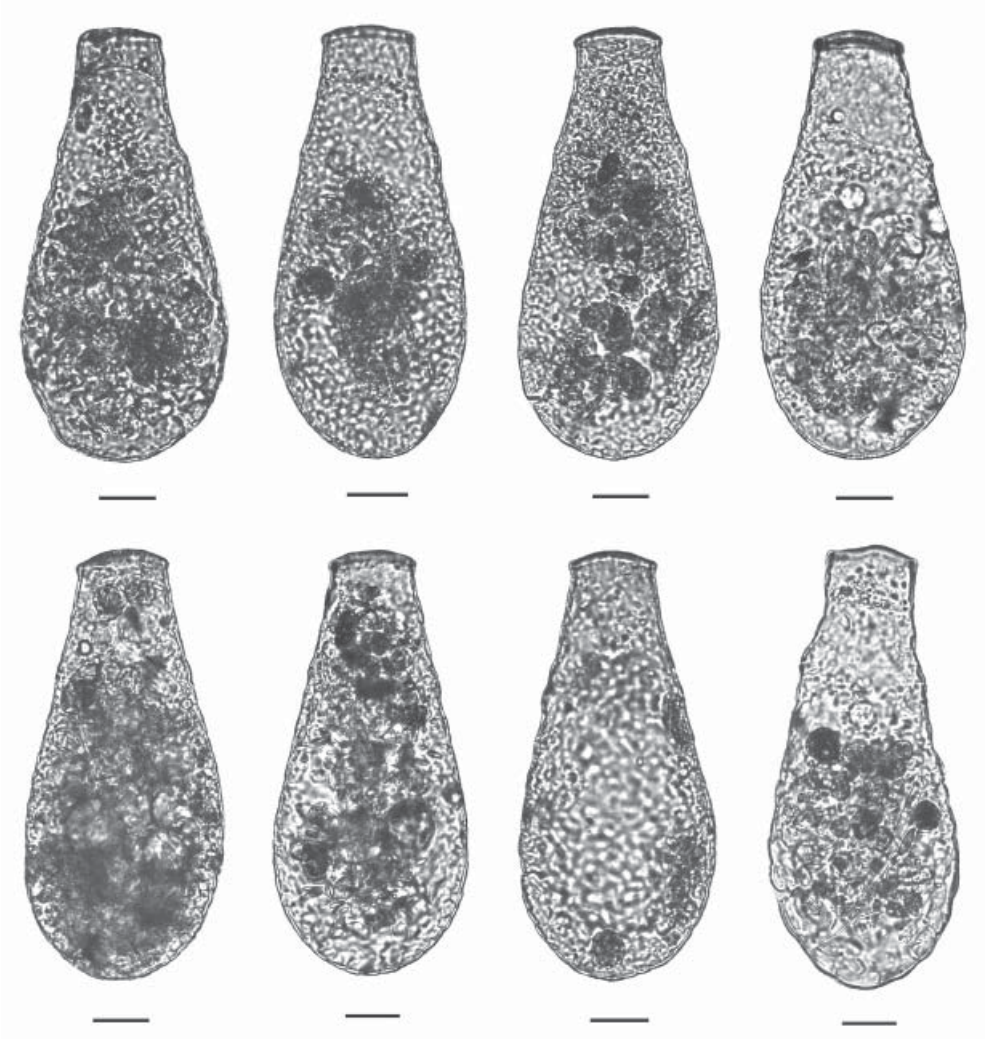

Fig. 4. Light micrographs of *Gibbocarina penardiana* comb. nov.: broad lateral view of different specimens from the Alagovac Lake region, East Herzegovina. Scale bars: 20 μm.

between 137 and 166 μm. In this case, 57.45% of all specimens had shell length of 147–157 μm, whereas only 17.02% were smaller than 147 μm and only 25.53% were larger than 157 μm. Analysis of the size frequency distribution of shell width indicates that this population possesses continuous polymorphism. Namely, all measured specimens had shell width ranging between 64 and 77 μm. In this case, 63.83% of all measured specimens had a shell length of 64–70 μm, while 36.17% were wider than 70 μm. Figures 5D–F show bag plots analyses of the correlation between shell length, shell width and aperture width.

The negative values of skewness for shell length, aperture width/shell length ratio, and aperture width/shell width ratio suggest an asymmetrical distribution with a long tail toward lower values. However, the asymmetry of these characters was low, with the skewness values between –0.113 and –0.132. Area of the optical section displayed low positive skewness value (0.134), while moderate positive value (0.406) was observed for shell width. High positive values were observed for shell width/shell length ratio (0.655) and aperture width (1.215). Three characters displayed negative kurtosis values, meaning that they were characterized by flatter distribution than a standard Gaussian distribution. Because the negative value obtained for shell length was not clearly different from zero (–0.127), the resulting deviation from normal Gaussian distribution was minimal. However, negative values for shell width and aperture width/shell width ratio were clearly different from zero (–0.881 and –0.588, respectively), indicating that the average size group

Table 2. Morphometric characterization of *Gibbocarina penardiana* comb. nov. from East Herzegovina based on 47 specimens (measurements in μm, except for area of the optical section in μm²).

Characters	Min	Max	M	x	SE	SD	CV	Sk	Ku
shell length	137	166	152	152.70	0.99	6.78	4.44	–0.132	–0.127
shell width	64	77	68	69.19	0.53	3.62	5.23	0.406	–0.881
aperture width	28	37	30	30.57	0.25	1.72	5.61	1.215	3.063
area of the optical section	6281	9864	7939	7904	98.00	672	8.50	0.134	0.973
shell width/shell length	0.42	0.51	0.45	0.45	0.00	0.02	4.76	0.655	0.656
aperture width/shell length	0.17	0.23	0.20	0.20	0.00	0.01	5.29	–0.127	1.069
aperture width/shell width	0.38	0.49	0.44	0.44	0.00	0.03	5.80	–0.113	–0.588

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.

has a lower dispersion. Other variables were found to have high positive values of kurtosis (between 0.656 and 3.063), indicating a distribution which is sharper than a standard Gaussian distribution.

Discussion

Kosakyan et al. (2012) conducted a molecular study of the family Hyalospheniidae that shows close phylogenetic relations between *Nebela galeata* and *N. penardiana*. However, Kosakyan et al. (2016) included into the genus *Gibbocarina* only the type species *N. galeata* and *N. gracilis*. Data presented in this paper show that *N. penardiana* is closely related to *G. galeata*. Therefore, I proposed a new taxonomic combination: *Gibbocarina penardiana*. Key morphological difference between these two species is visibility of hollow keel (clearly visible keel in *G. galeata* versus less visible keel in *G. penardiana*). Also, it is possible to distinguish these species based on morphometric data: shell width 95–139 μm in *G. galeata* versus 64–77 μm in *G. penardiana*, area of the optical section 12297–21302 μm² in *G. galeata* versus 6281–9864 μm² in *G. penardiana*, shell width/shell length ratio 0.52–0.68 in *G. galeata* versus 0.42–0.51 in *G. penardiana*, and aperture width/shell width ratio 0.27–0.38 in *G. galeata* versus 0.38–0.49 in *G. penardiana*. Table 3 shows comparative morphometric data of *Gibbocarina* species according to different authors.

Taranek (1881, cited in Penard, 1902) described *Nebela americana* that is very similar in shape to *G. penardiana*, but shell of *N. americana* is not compressed. Before Deflandre's description of *N. penardiana* (Deflandre, 1936), some authors (Cash and Hopkinson, 1909; Wailes, 1912) described populations with compressed shells as *N. americana*. Further observations of *N. americana* by modern methods are needed to clarify taxonomic status of this species. In addition, Wailes (1912) described *N. americana* var. *falcata* with curved shells. Later, four infraspecific taxa of *N. penardiana* were described based on small number of specimens: *N. penardiana* var. *suecica* Grospietsch, 1954, *N. penardiana* var. *minor* Gauthier-Lièvre, 1957, *N. penardiana* f. *elongata* Gauthier-Lièvre, 1957 and *N. penardiana* var. *retorta* Decloitre, 1977. These taxa are very rare and their taxonomic status is questionable.

Penard (1910) described *Nebela gracilis*, a taxon which is very similar in shell shape to *G. galeata*, but it is characterized by smaller dimensions: shell length 98–110 μm and shell width 45–50 μm. Later, few authors reported wider range of shell values (see Table 3). Hoogenraad and de Groot (1927, cited in Hoogenraad and de Groot, 1940) described *N. galeata* f. *minor* from the Netherlands, and later they (Hoogenraad and de Groot, 1940) concluded that this taxon is a part of population complex: original population of *N. galeata* f. *minor*, *N. gracilis*, and population of *N. galeata* f. *minor* from Indonesia. Further molecular and morphometric data are needed for many populations of this complex for clarifying status of these taxa. Wailes (1912) described *N. gracilis* var. *stomata* and noted: "This variety differs from the type in the possession of two

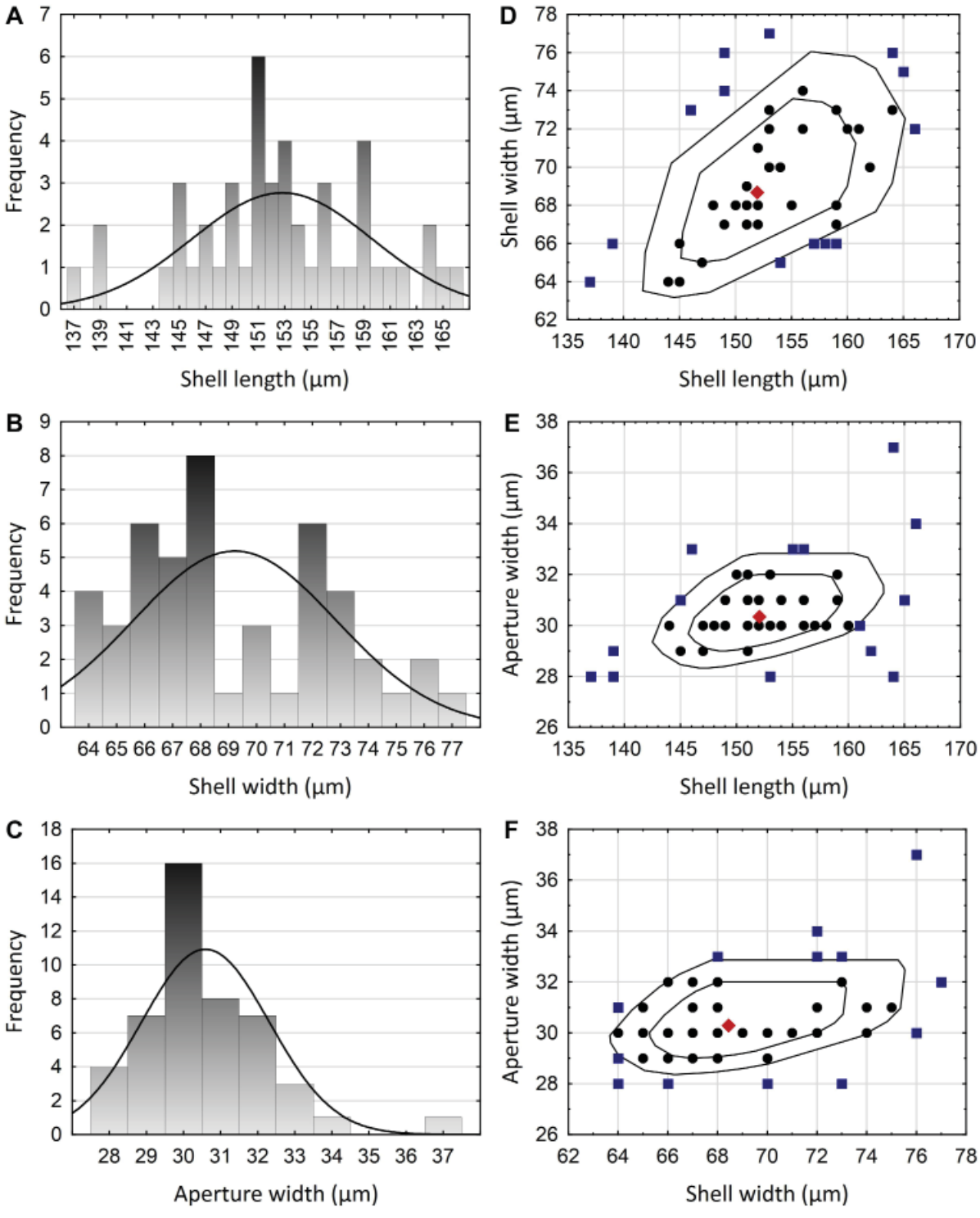

Fig. 5. Morphological variability of *Gibbocarina penardiana* comb. nov. based on 47 specimens from the Alagovac Lake region (East Herzegovina). Histograms show the size frequency distribution of the shell length (A), shell width (B), and aperture width (C); bag plots show the correlation between shell 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 ■.

Table 3. Comparative morphometric data of *Gibbocarina* species according to different authors (all measurements in μm).

Species	Shell length	Shell width	Aperture width	References
G. galeata	180–270	130–190	—	Penard, 1890
	180–200	100	—	Cash and Hopkinson, 1909
	180–200	98–114	31–40	Jung. 1942
	170–185	115–120	32–35	Gauthier-Lièvre, 1957
	200–210	90–100	30–40	Alekperov and Snegovaya, 2000
	180–283	94–190	26–51	Kosakyan et al., 2016
	163–213	95–139	32–47	Present study
G. gracilis	98–110	45–50	—	Penard, 1910
	97–130	42–65	14–23	Wailes, 1912
	90–130	50–60	19–25	Jung, 1942
G. penardiana	160–175	65–75	30–35	Cash and Hopkinson, 1909*
	120–168	60–90	20–23	Wailes, 1912*
	140–175	65–75	30–35	Deflandre, 1936
	140–175	65–75	30–35	Jung, 1942
	160–180	70–85	—	Gauthier-Lièvre, 1953
	180–185	80–85	45	Gauthier-Lièvre, 1953
	120–170	60–82	32–45	Gauthier-Lièvre, 1953
	100–110	60–62	40	Gauthier-Lièvre, 1953
	78–92	41–52	21–31	Gauthier-Lièvre, 1953
	150	68	30	Gauthier-Lièvre, 1957
	136–150	63–73	32–44	Golemansky, 1962
	115–164	61–91	27–36	Laminger, 1972
	115–161	65–80	23–34	Ogden and Hedley, 1980
	99–169	50–86	17–32	Ogden, 1984
	130–164	68–75	25–33	Chung et al., 1992
	170–210	70–90	35–50	Alekperov and Snegovaya, 2000
	175–186	87–92	35–38	Alves et al., 2007
	137–166	64–77	28–37	Present study

* Reported as *Nebela americana* (see Discussion).

evaginated pores, one on either side of the neck. Similar protuberances occur on other Nebelae, i.e. *N. tuberosa* and *N. americana*." It is clear that evaginated pores are not a valid taxonomic character at the species level. Because variety is not an accepted taxonomic category in modern zoology, it is evident that *N. gracilis* var. *stomata* is a synonym to *N. gracilis*.

Nebela galeata var. *orbicularis* is a very rare taxon described by Deflandre (1936). This variety is characterized by wide pyriform shell and the following measurements: shell length 166–208 μm, shell width 140–170 μm, and aperture width 25–35 μm. Jung (1942) proposed species rank for this variety. Further observations are needed to clarify taxonomic status of this morphotype.

Awerinzew (1907) described *N. pulcherrima* as a very characteristic morphospecies with flask-like shell shape. This species possesses hollow keel and its measurements are very similar to those of *G. galeata:* shell length 180–185 μm, shell width 100 μm, and aperture width 40 μm. It is possible that this species is also a member of the genus *Gibbocarina*. However, flask-like shell shape is very specific for members of the family Padaungiellidae. *Nebela pulcherrima* is a poorly known species and further studies using modern methods will provide results that are more definitive.

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