

# Morphometrics of three hyalosphenid testate amoebae from the Velež Lake region, East Herzegovina

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## Summary

Taxonomy of hyalosphenid testate amoebae at the species level is mainly based on the shape and size of their shells. In this paper, the morphometric data for *Padaungiella lageniformis* (member of the family Padaungiellidae), *Nebela minor* and *Porosia bigibbosa* (members of the family Hyalospheniidae s.s.) from the Velež Lake region (East Herzegovina) are presented. The analyzed population of *P. lageniformis* contains specimens with shell length between 83 and 112  $\mu\text{m}$ , with the arithmetic mean of 99.07  $\mu\text{m}$ . These data support synonymization of *P. wailesi* (shell length 75–100  $\mu\text{m}$ ) and *P. lageniformis* s.s. (shell length 100–160  $\mu\text{m}$ ). Recently, the wide taxonomic concept of *N. tincta* has been proposed based on molecular and morphological analyses conducted on a small number of specimens. This concept implies synonymization of *N. minor* with *N. tincta*, but morphometric analysis of *N. minor* presented here does not support the wide taxonomic concept of *N. tincta*. In addition, morphometric analysis of *P. bigibbosa* has shown that it is a much more diversified taxon than previously thought. These investigations have shown that further morphometric studies of hyalosphenid testate amoebae are necessary for the clarification of their taxonomic relations.

**Key words:** Arcellinida, Hyalospheniidae, Padaungiellidae, protists, taxonomy, testate amoebae

## Introduction

The group Arcellinida includes free-living lobose amoebae characterized by shell which is built in very different ways. Kosakyan with co-authors (2012) included the genera with a shell entirely composed of rigid proteinaceous sheet or with addition of building units into the family Hyalospheniidae. The shell possesses terminal aperture bordered by a thick organic lip. Kosakyan with co-authors

(2016) included all members of hyalosphenid testate amoebae into the family Hyalospheniidae with 13 genera, while Luketa (2015a) included 3 genera with elongated necks into the family Padaungiellidae.

The family Padaungiellidae includes testate amoebae characterized by rigid, flask-shaped shells covered by predated siliceous oval or quadrangular plates. This family includes three closely related genera characterized by an elongated neck: *Alocodera*, *Apodera* and *Padaungiella*. Only four studies

have been dealing with morphometrics of padaungiellid testate amoebae (Zapata and Fernández, 2008; Todorov et al., 2010; Luketa, 2015a, 2017a). The family Hyalospheniidae s.s. includes testate amoebae characterized by dorso-ventrally compressed shells that are composed of an organic matrix and building units. These building units are often oval or circular, but some species have shells without building units. The shell is often pyriform or oval, rarely vase-shaped. Hyalospheniids are cosmopolitan and can be found in freshwater and marine ecosystems, as well as terrestrial habitats. This family comprises about 100 valid species, but only several species were studied by modern morphometric methods (Török, 2001; Todorov, 2002, 2010; Luketa, 2015b, 2016, 2017b, 2017c; Nicholls, 2015).

The present study reports the morphometric data for *Padaungiella lageniformis* (member of the family Padaungiellidae), *Nebela minor* and *Porosia bigibbosa* (members of the family Hyalospheniidae s.s.) based on specimens collected in the Velež Lake region, East Herzegovina.

## Material and methods

The material for the present study was extracted from epigenous mosses collected in the forest. The samples were taken in the Velež Lake region (43°21'11.8"N, 18°03'24.3"E, ca. 1000 m a.s.l.), municipality Nevesinje, East Herzegovina, on 28 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. Six shell parameters were measured for *Padaungiella lageniformis* (shell length, shell width, aperture width, neck length, neck width, and area of the optical section), three for *Nebela minor* (shell length, shell width, and aperture width), and six for *Porosia bigibbosa* (shell length, shell width, aperture width, aperture-pore distance, pore-pore distance, 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.

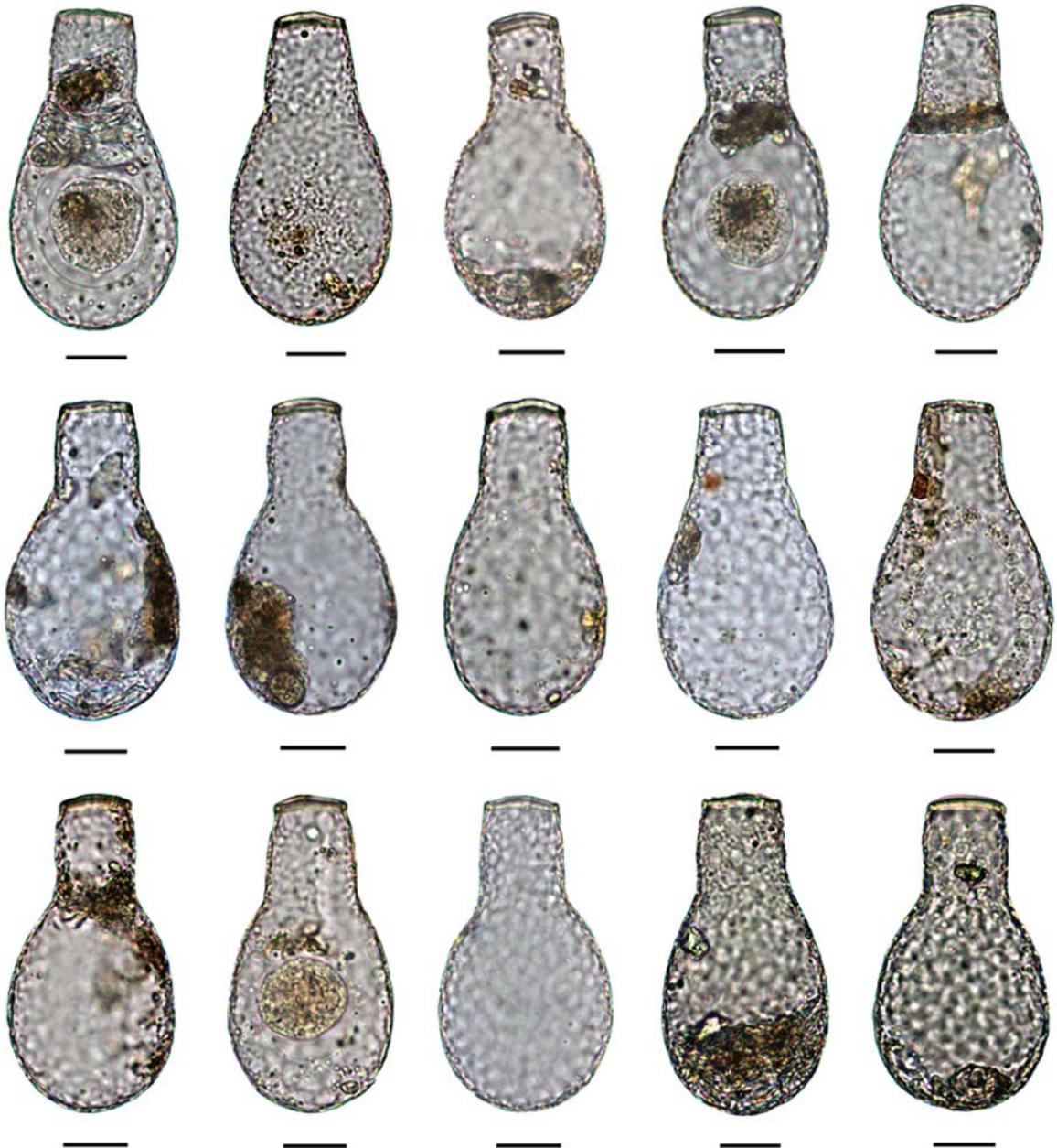
## Results and Discussion

### *PADAUNGIELLA LAGENIFORMIS*

**Description.** The shell is transparent, colorless or slightly yellow, pyriform, always laterally compressed, rounded aborally, prolonged into a tubular neck. The shell is covered by a mixture of predated or collected oval, circular and elongated plates; these plates are usually arranged haphazardly and overlap. The aperture is ellipsoid with regular outline and it is surrounded by a thick organic lip; it is characterized by an uneven and undulating rim. In frontal view the apertural lip is convex, while in lateral view it is concave. Figure 1 shows light micrographs of specimens from the studied population.

**Morphometry.** Morphometric characters of 142 specimens of *P. lageniformis* from the Velež Lake region were measured and the results are given in Table 1. Coefficients of variation were moderate for three measured characters (area of the optical section, neck length/aperture width ratio, and neck width/neck length ratio) and ranged from 10.47 to 10.88%; other measured parameters were characterized by low variability (from 5.34 to 9.73%). For basic characters, the minimal variability was observed for shell length (5.61%), while the maximal variation coefficient was observed for area of the optical section (10.64%). For ratio characters, the minimal variability was observed for shell width/shell length ratio (5.34%), while the maximal variation coefficient was observed for neck width/neck length ratio (10.88%).

The most frequent shell length (100 µm) was registered in 15 specimens (Fig. 2A); the most frequent shell width (53 µm) was registered in 24 specimens (Fig. 2B), and the most frequent aperture width (23 µm) was registered in 40 specimens (Fig. 2C). Analysis of the size frequency distribution of shell length indicates that this population is characterized by continuous polymorphism. All measured specimens had shell length between 83 and 112 µm. In this case, 62.68% of all specimens had shell length between 93 and 102 µm, whereas only 10.56% were smaller than 93 µm and 26.76% were larger than 102 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 62 µm. However, 63.38% of all measured specimens had shell width of 51–56 µm, whereas 23.24% were narrower than 51 µm and only 13.38% were wider than 56 µm.



**Fig. 1.** Light micrographs of *Padaungiella lageniformis*: broad lateral view of different specimens from the Velež Lake region, East Herzegovina. Scale bars: 20  $\mu\text{m}$ .

The frequency analysis of aperture width shows similar distribution pattern. Namely, all measured specimens had aperture width between 19 and 27  $\mu\text{m}$ . In this case, 69.72% of all specimens had aperture width of 22–24  $\mu\text{m}$ , whereas only 12.68% had aperture narrower than 22  $\mu\text{m}$  and only 17.60% had aperture wider than 24  $\mu\text{m}$ . Figures 2D–F show bag plot analyses of the correlation between shell length, shell width and aperture width.

The negative values of skewness for five characters suggest an asymmetrical distribution with a long tail toward lower values. The asymmetry of shell length (–0.172) and neck length/aperture width ratio (–0.029) was low, while moderate negative values of skewness (between –0.279 and –0.426) were observed for aperture width, neck length, and aperture width/shell width ratio. High negative skewness values were not observed for any character. All other variables

**Table 1.** Morphometric characterization of *Padaungiella lageniformis* from the Velež Lake region (East Herzegovina) based on 142 specimens (measurements in  $\mu\text{m}$ , except for area of the optical section in  $\mu\text{m}^2$ ).

Characters	Min	Max	M	x	SE	SD	CV	Sk	Ku
shell length	83	112	99.5	99.07	0.47	5.56	5.61	-0.172	-0.199
shell width	45	62	53	53.15	0.29	3.40	6.39	0.194	0.009
aperture width	19	27	23	23.15	0.13	1.52	6.55	-0.279	0.139
neck length	21	36	29	29.11	0.23	2.73	9.38	-0.296	0.398
neck width	23	31	27	26.51	0.15	1.81	6.82	0.199	-0.008
area of the optical section	2906	4996	3763	3782	33.78	403	10.64	0.106	-0.155
shell width/shell length	0.48	0.62	0.53	0.54	0.00	0.03	5.34	0.511	0.133
aperture width/shell length	0.19	0.29	0.23	0.23	0.00	0.02	6.51	0.146	1.656
neck length/shell length	0.23	0.37	0.29	0.29	0.00	0.02	8.35	0.087	0.200
neck width/shell length	0.23	0.33	0.27	0.27	0.00	0.02	6.65	0.850	0.724
aperture width/shell width	0.32	0.52	0.44	0.44	0.00	0.03	7.17	-0.426	0.886
neck length/shell width	0.41	0.75	0.55	0.55	0.00	0.05	9.73	0.224	0.868
neck width/shell width	0.44	0.59	0.50	0.50	0.00	0.03	5.51	0.524	0.202
neck length/aperture width	0.91	1.60	1.26	1.26	0.01	0.13	10.47	-0.029	-0.054
neck width/aperture width	1.00	1.50	1.13	1.15	0.01	0.08	6.71	1.151	2.882
neck width/neck length	0.72	1.29	0.90	0.92	0.01	0.10	10.88	0.782	1.127

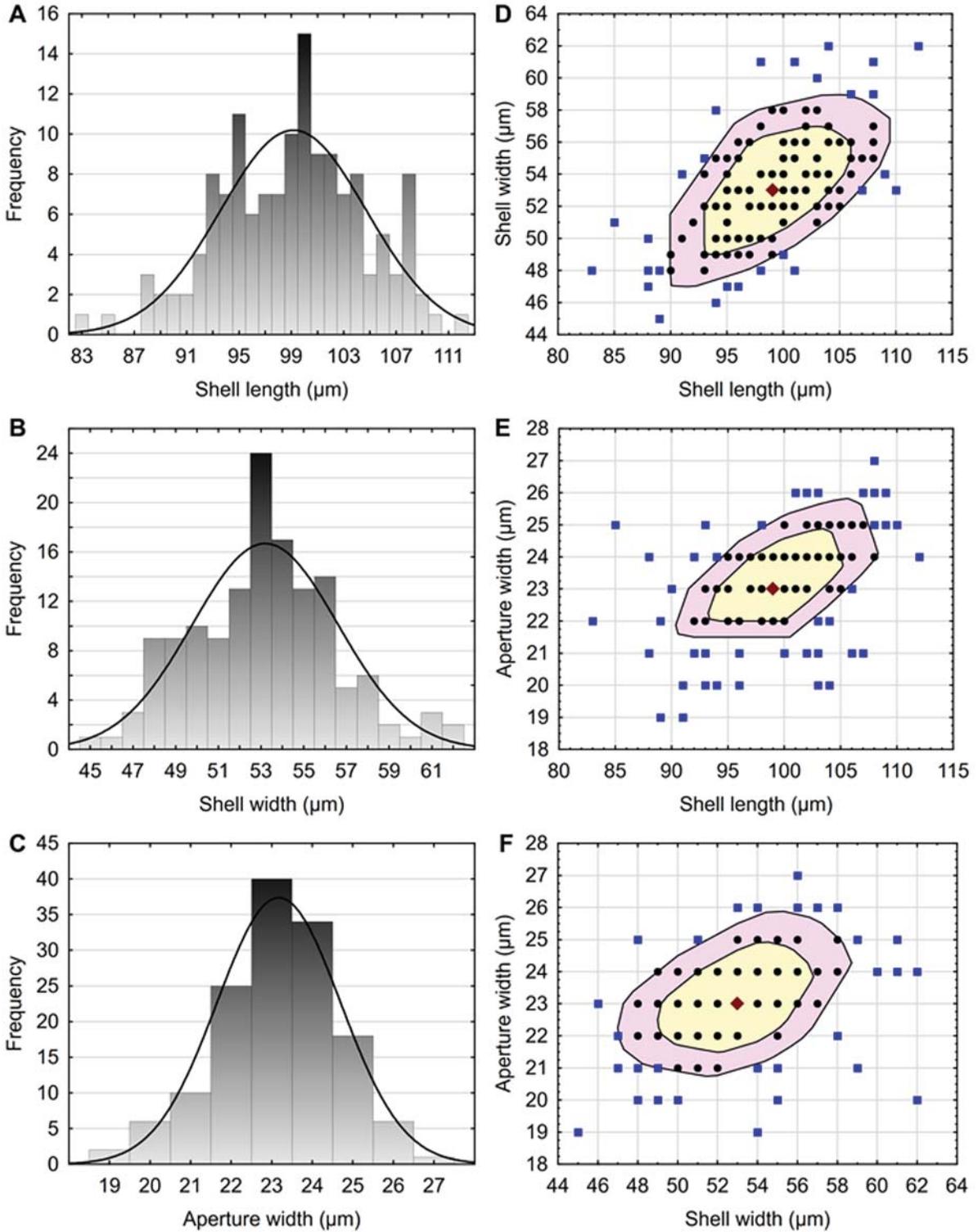
*Abbreviations:* Min and Max – minimum and maximum values, M – median, x – arithmetic mean, SE – standard error of the arithmetic mean, SD – standard deviation, CV – coefficient of variation in %, Sk – skewness, Ku – kurtosis.

were characterized by positive values of skewness (0.087–1.151), indicating a distribution with a long tail toward higher values. The asymmetry of six characters (shell width, neck width, area of the optical section, aperture width/shell length ratio, neck length/shell length ratio, and neck length/shell width ratio) was low, with skewness values ranging between 0.087 and 0.224. Moderate positive skewness values were not observed for any character. High positive skewness values (0.511–1.151) were observed for five ratio characters (shell width/shell length, neck width/shell length, neck width/shell width, neck width/aperture width, and neck width/neck length ratios).

Four characters (shell length, neck width, area of the optical section, and neck length/aperture width ratio) displayed negative kurtosis values, meaning that they were characterized by flatter distribution than a standard Gaussian distribution. Since the negative values obtained for these characters (between –0.008 and –0.199) are not clearly different from zero, the resulting deviation from

the normal Gaussian distribution was minimal. Other variables were found to have positive kurtosis values, indicating a distribution that is sharper than the standard Gaussian distribution. Low positive values (0.009–0.202) were observed for shell width, aperture width, shell width/shell length ratio, neck length/shell length ratio, and neck width/shell width ratio. Moderate positive value (0.398) was observed for neck length. High positive values (0.724–2.882) were observed for six ratio characters (aperture width/shell length, neck width/shell length, aperture width/shell width, neck length/shell width, neck width/aperture width, and neck width/neck length ratios).

**Comments.** Kosakyan et al. (2012) based on molecular data placed five members of the genus *Nebela* with an elongated neck (*N. lageniformis*, *N. nebeloides*, *N. tubulata*, *N. wailesi*, and *N. wetekampi*) into a separate genus named *Padaungiella*. Based on comprehensive morphometric data, Luketa (2017a) concluded that *P. wailesi* is synonym for the type species *P. lageniformis*; thus, the genus *Padaungiella*



**Fig. 2.** Morphological variability of *Padaungiella lageniformis* based on 142 specimens from the Velež 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  $\blacklozenge$ , characters on Y axes  $\bullet$ , outliers  $\blacksquare$ .

included only four valid species. However, further molecular and morphometric studies based on different populations of *P. lageniformis* are needed to clarify their taxonomic relations. Namely, Kosakyan et al. (2012) based on COI sequences concluded that *P. wailesi* is close related to *P. nebeloides*, while *P. lageniformis* is a basal clade. The taxonomic relations between the type species and two rare members of the genus *Padaungiella* (*P. nebeloides* and *P. wetekampi*) are not fully clarified. The main reason is the lack of detailed morphometric data.

Wailes (1912) described *Nebela lageniformis* var. *minor* based on samples from North America and noted that shell length varied from 85 to 100  $\mu\text{m}$ . Deflandre (1936) proposed new taxonomic status and name for this taxon – *Nebela wailesi* – and noted that shell length ranged between 75 and 100  $\mu\text{m}$ . Chattopadhyay and Das (2003) reported also typical forms with shell length between 75 and 85  $\mu\text{m}$  based on ten specimens from India. Recently this species was included into the genus *Padaungiella* (Kosakyan et al., 2012). Shell length is the main character for separating *P. wailesi* (shell length less than 100  $\mu\text{m}$ ) and *P. lageniformis* (shell length more than 100  $\mu\text{m}$ ). However, few authors reported the intermediate populations between these two species. Jung (1942a) noted specimens from Chile with shell length between 91 and 105  $\mu\text{m}$ . Luketa (2015a) reported a population with values of shell length between 91 and 127  $\mu\text{m}$  based on material from the Vlasina Lake region (Serbia). Luketa (2017a) noted similar values of shell length (93–128  $\mu\text{m}$ ) for a moss-dwelling population from the Alagovac Lake region (East Herzegovina). He also reported size-dimorphic population with small (shell length 91–100  $\mu\text{m}$ ) and large (101–139  $\mu\text{m}$ ) classes from *Sphagnum* peatland near Alagovac Lake. An important proof that *P. wailesi* is synonym for *P. lageniformis* is a population from the Velež Lake region presented in this study; shell length in this population ranges between 83 and 112  $\mu\text{m}$ , while arithmetic mean is 99.07  $\mu\text{m}$ . Comparative morphometric data of shell length based on different populations of *P. lageniformis* from the Balkan Peninsula are given in Table 2.

Luketa (2017a) concluded that shell width/shell length ratio is a very important taxonomic character for distinguishing *P. lageniformis* (0.45–0.67) and *P. nebeloides* (0.34–0.46). Data for population from the Velež Lake region support this conclusion because values for this ratio range between 0.48 and 0.62. Comparative morphometric data of shell width/shell length ratio based on different populations of

*P. lageniformis* from the Balkan Peninsula are given in Table 3. Luketa (2017a) did not give taxonomic importance to shell width values for distinguishing these two species. Based on recently published data and analysis reported in this study (Table 4), it is possible to conclude that shell width is a strongly variable character. Nevertheless, arithmetic means of shell width in different *P. lageniformis* populations range from 53.15 and 73.98  $\mu\text{m}$  (Table 4), while these values for *P. nebeloides* populations range between 47.6 and 48.0  $\mu\text{m}$  (Todorov et al., 2010). Therefore, it is possible to conclude that arithmetic mean of shell width is an important taxonomic character for distinguishing these two species.

Jung (1942a) described *Schaudinnia wetekampi* from Chile, while Kosakyan et al. (2012) included this species into the genus *Padaungiella*. This species is very similar to *P. lageniformis* and key difference is length of neck. Many authors had been reporting values for typical populations of *P. lageniformis* between 30 and 50  $\mu\text{m}$  (Hoogenraad and de Groot, 1940; Jung, 1942a; van Oye, 1949; Gauthier-Lièvre, 1953; Luketa, 2017a) and for typical *P. wailesi* from 25 to 34  $\mu\text{m}$  (Jung, 1942a; Chattopadhyay and Das, 2003). Luketa (2017a) reported values of neck length for two intermediate populations from 22 to 42  $\mu\text{m}$ . Population from the Velež Lake region is the most similar to these two intermediate populations because neck length ranges from 21 to 36  $\mu\text{m}$ . Chattopadhyay and Das (2003) reported specimens of *P. lageniformis* with very long neck (53–59  $\mu\text{m}$ ) from India. In large class of size-dimorphic population from the Alagovac Lake region, Luketa (2017a) observed small number of specimens with very long neck (up to 55  $\mu\text{m}$ ).

In summary, *P. lageniformis* is characterized by neck length between 21 and 59  $\mu\text{m}$ . Comparative morphometric data of neck length based on different populations of *P. lageniformis* from the Balkan Peninsula are present in Table 5. For *P. wetekampi* only data from original description are known – neck length range from 63 and 68  $\mu\text{m}$ . For this reason, further molecular and morphometric studies are needed to clarify taxonomic status of this species.

Values for neck length/shell length ratio in different populations of *P. lageniformis* range between 0.21 and 0.40, while values for arithmetic mean of this ratio character range from 0.28 to 0.30 (Table 6). Values for other *Padaungiella* species are not published yet, but this character may be taxonomically important because values for the arithmetic mean are very stable.

**Table 2.** Shell length in different populations of *Padaungiella lageniformis* (all measurements in  $\mu\text{m}$ ).

Country	Location	Microhabitat	N	Min	Max	x	CV	Sk	Ku	References
Serbia	Vlasina Lake region	dry <i>Sphagnum</i> mosses	334	91	127	111.27	5.46	-0.191	0.037	Luketa, 2015a
	Šargan Mountain	epigenous mosses	49	101	153	129.67	7.67	-0.564	1.323	Luketa, 2017a
Bosnia and Herzegovina	Alagovac Lake region	epigenous mosses	403	93	128	109.58	5.83	0.132	0.002	
		wet <i>Sphagnum</i> mosses	15	91	100	96.6	2.82	—	—	
	1024		101	139	120.99	5.49	-0.148	-0.187		
Velež Lake region	epigenous mosses	142	83	112	99.07	5.61	-0.172	-0.199	This study	

*Abbreviations:* N – number of specimens, Min and Max – minimum and maximum values, x – arithmetic mean, CV – coefficient of variation in %, Sk – skewness, Ku – kurtosis.

**Table 3.** Shell width/shell length ratio in different populations of *Padaungiella lageniformis* (all measurements in  $\mu\text{m}$ ).

Country	Location	Microhabitat	N	Min	Max	x	CV	Sk	Ku	References
Serbia	Vlasina Lake region	dry <i>Sphagnum</i> mosses	334	0.46	0.63	0.54	4.37	0.040	0.666	Luketa, 2015a
	Šargan Mountain	epigenous mosses	49	0.50	0.66	0.57	4.88	0.381	0.943	Luketa, 2017a
Bosnia and Herzegovina	Alagovac Lake region	epigenous mosses	403	0.45	0.63	0.54	5.23	0.295	0.002	
		wet <i>Sphagnum</i> mosses	15	0.54	0.63	0.57	5.35	—	—	
	1024		0.45	0.67	0.56	4.83	0.139	0.676		
Velež Lake region	epigenous mosses	142	0.48	0.62	0.54	5.34	0.511	0.133	This study	

*Abbreviations:* N – number of specimens, Min and Max – minimum and maximum values, x – arithmetic mean, CV – coefficient of variation in %, Sk – skewness, Ku – kurtosis.

**Table 4.** Shell width in different populations of *Padaungiella lageniformis* (all measurements in  $\mu\text{m}$ ).

Country	Location	Microhabitat	N	Min	Max	x	CV	Sk	Ku	References
Serbia	Vlasina Lake region	dry <i>Sphagnum</i> mosses	334	51	73	60.33	6.23	0.112	-0.077	Luketa, 2015a
	Šargan Mountain	epigenous mosses	49	56	90	73.98	7.75	-0.482	2.451	Luketa, 2017a
Bosnia and Herzegovina	Alagovac Lake region	epigenous mosses	403	47	73	59.21	7.98	0.537	-0.032	
		wet <i>Sphagnum</i> mosses	15	53	60	55.47	4.08	—	—	
	1024		56	83	67.50	6.59	0.118	-0.002		
Velež Lake region	epigenous mosses	142	45	62	53.15	6.39	0.194	0.009	This study	

*Abbreviations:* N – number of specimens, Min and Max – minimum and maximum values, x – arithmetic mean, CV – coefficient of variation in %, Sk – skewness, Ku – kurtosis.

**Table 5.** Shell neck length in different populations of *Padaungiella lageniformis* (all measurements in  $\mu\text{m}$ ).

Country	Location	Microhabitat	N	Min	Max	x	CV	Sk	Ku	References
Serbia	Vlasina Lake region	dry <i>Sphagnum</i> mosses	334	23	39	31.09	8.66	0.063	0.006	Luketa, 2015a
	Šargan Mountain	epigenous mosses	49	29	48	38.47	9.98	-0.288	0.290	Luketa, 2017a
Bosnia and Herzegovina	Alagovac Lake region	epigenous mosses	403	23	42	32.09	9.54	0.253	-0.122	
		wet <i>Sphagnum</i> mosses	15	22	30	26.60	7.76	—	—	
	1024		23	55	34.12	10.48	0.911	2.685		
	Velež Lake region	epigenous mosses	142	21	36	29.11	9.38	-0.296	0.398	This study

Abbreviations: N – number of specimens, Min and Max – minimum and maximum values, x – arithmetic mean, CV – coefficient of variation in %, Sk – skewness, Ku – kurtosis.

*NEBELA MINOR*

**Description.** The shell is transparent, colorless, narrow to wide pear-shaped, laterally compressed, with small lateral pores (the number of which can vary) that can be difficult to observe. The shell is composed mainly of oval or circular plates. The shell neck is always absent. The aperture is terminal, oval, slightly curved or strongly curved, surrounded by a thin organic lip. Figure 3 shows light micrographs of specimens from the studied population.

**Morphometry.** Morphometric characters of 1352 specimens of *N. minor* from the Velež Lake region were measured and the results are presented in Table 7. Coefficients of variation were low for all measured characters, ranging from 4.72% to 6.43%. For basic characters, the minimal variability was observed for shell length (4.72%), while the maximal variation coefficient was observed for aperture

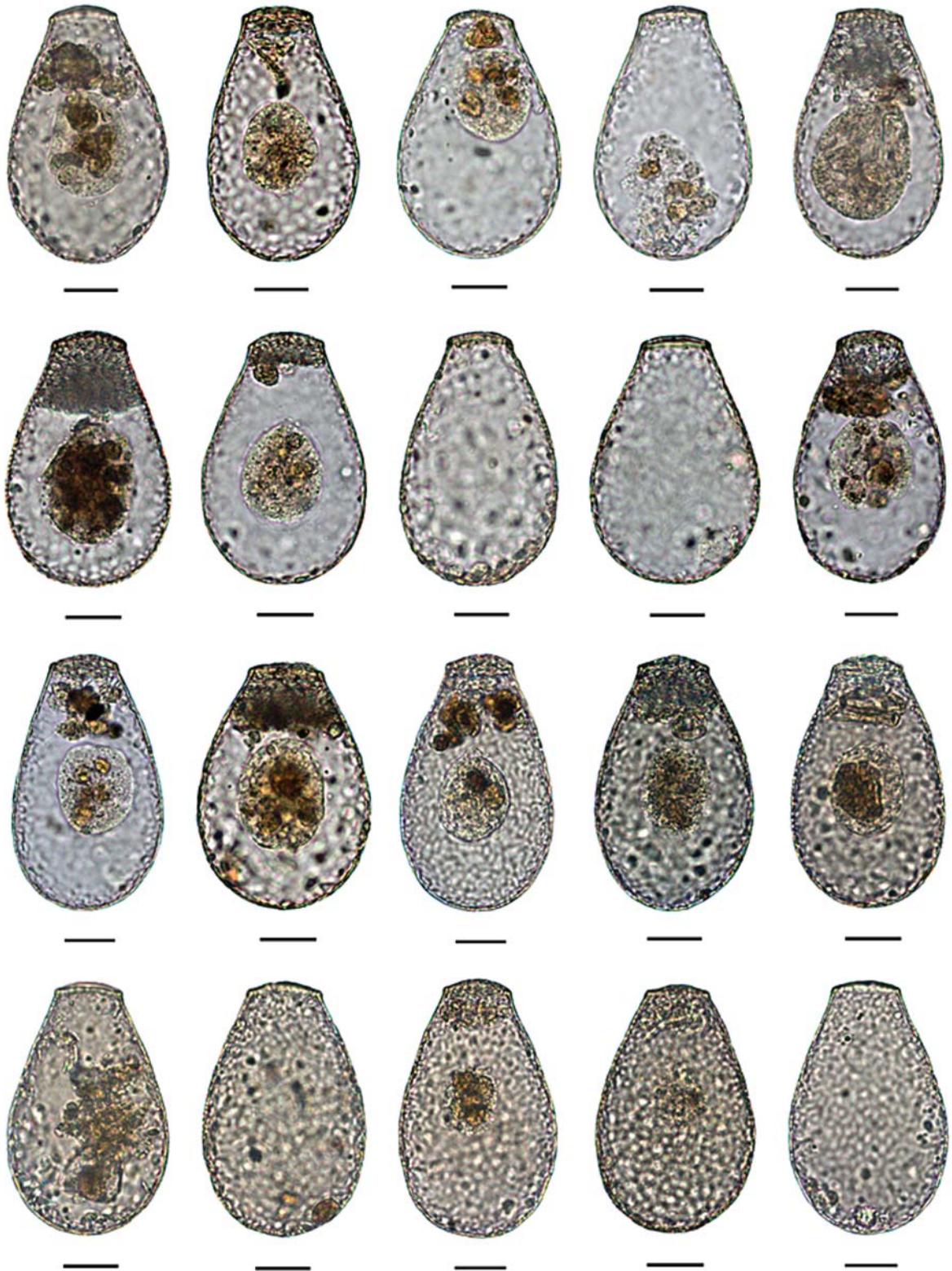
width (5.85%). For ratio characters, the minimal variability was observed for aperture width/shell width ratio (5.59%), while the maximal variation coefficient was observed for aperture width/shell length ratio (6.43%).

The most frequent shell length (95  $\mu\text{m}$ ) was registered in 132 specimens (Fig. 4A); the most frequent shell width (58  $\mu\text{m}$ ) was registered in 181 specimens (Fig. 4B), and the most frequent aperture width (26  $\mu\text{m}$ ) was registered in 377 specimens (Fig. 4C). Analysis of the size frequency distribution of shell length, shell width and aperture width indicates that this population is size-monomorphic. Shell length ranged from 79 to 108  $\mu\text{m}$ . However, 72.26% of all measured specimens had a shell length of 89–98  $\mu\text{m}$ , whereas only 8.43% were smaller than 89  $\mu\text{m}$  and only 19.31% were larger than 98  $\mu\text{m}$ . The frequency analysis of shell width shows slightly different distribution pattern. Namely, all measured

**Table 6.** Shell neck length/shell length ratio in different populations of *Padaungiella lageniformis* (all measurements in  $\mu\text{m}$ ).

Country	Location	Microhabitat	N	Min	Max	x	CV	Sk	Ku	References
Serbia	Vlasina Lake region	dry <i>Sphagnum</i> mosses	334	0.22	0.33	0.28	6.81	-0.100	0.130	Luketa, 2015a
	Šargan Mountain	epigenous mosses	49	0.24	0.34	0.30	6.44	-0.048	1.112	Luketa, 2017a
Bosnia and Herzegovina	Alagovac Lake region	epigenous mosses	403	0.24	0.37	0.29	8.30	0.229	-0.172	
		wet <i>Sphagnum</i> mosses	15	0.23	0.31	0.28	7.46	—	—	
	1024		0.21	0.40	0.28	8.34	0.670	1.707		
	Velež Lake region	epigenous mosses	142	0.23	0.37	0.29	8.35	0.087	0.200	This study

Abbreviations: N – number of specimens, Min and Max – minimum and maximum values, x – arithmetic mean, CV – coefficient of variation in %, Sk – skewness, Ku – kurtosis.



**Fig. 3.** Light micrographs of *Nebela minor*: broad lateral view of different specimens from the Velež Lake region, East Herzegovina. Scale bars: 20  $\mu\text{m}$ .

**Table 7.** Morphometric characterization of *Nebela minor* from the Velež Lake region (East Herzegovina) based on 1352 specimens (measurements in  $\mu\text{m}$ ).

Characters	Min	Max	M	x	SE	SD	CV	Sk	Ku
shell length	79	108	95	94.64	0.12	4.47	4.72	-0.265	0.144
shell width	51	78	60	60.13	0.09	3.43	5.70	0.648	1.088
aperture width	20	32	26	25.83	0.04	1.51	5.85	0.076	0.515
shell length/shell width	1.25	1.88	1.58	1.58	0.00	0.09	5.92	-0.249	0.072
aperture width/shell length	0.21	0.34	0.27	0.27	0.00	0.02	6.43	0.285	0.624
aperture width/shell width	0.34	0.52	0.43	0.43	0.00	0.02	5.59	-0.010	0.199

*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.

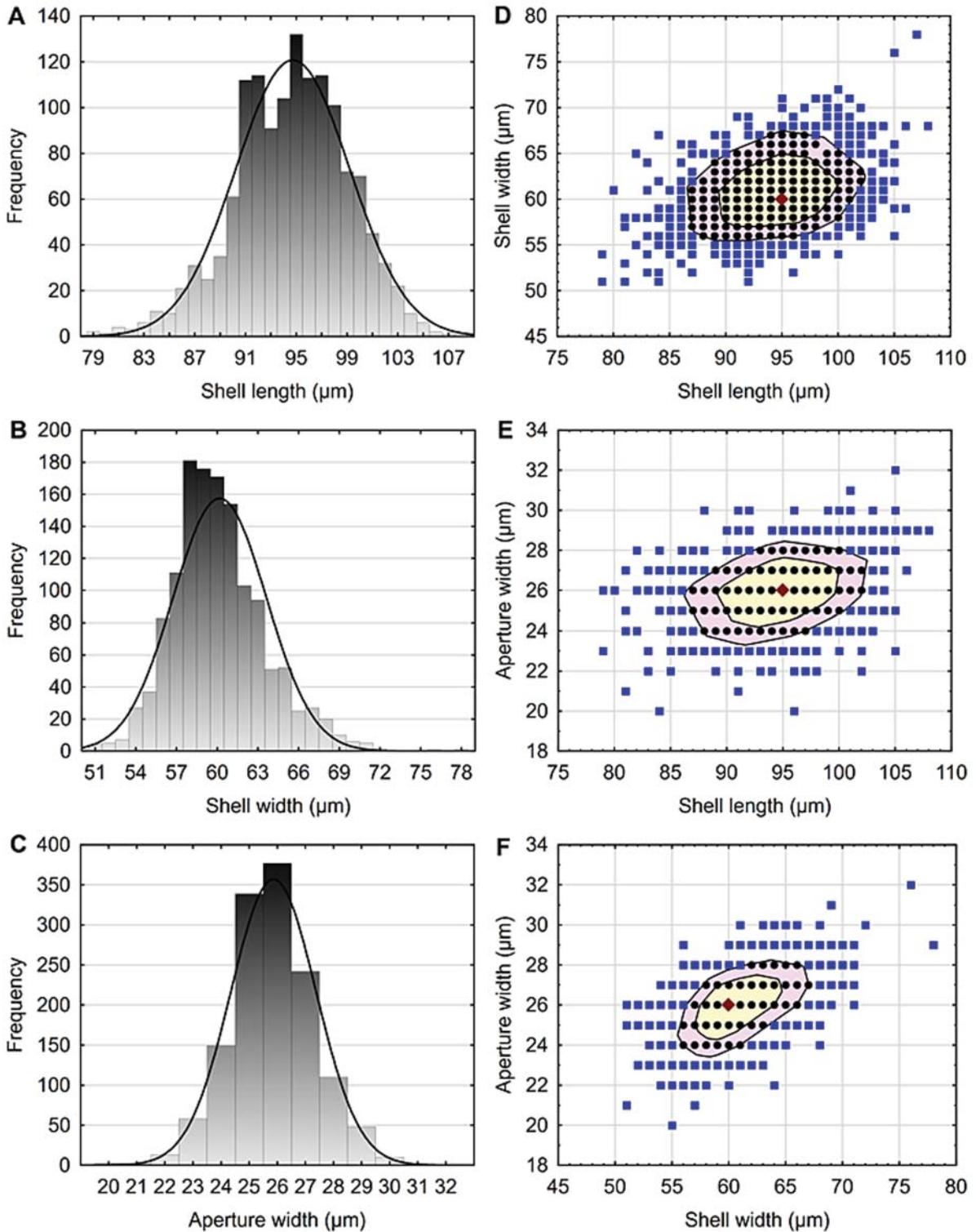
specimens had shell width between 51 and 78  $\mu\text{m}$ . In this case, 52.29% of all specimens had shell width of 60–69  $\mu\text{m}$ , whereas 46.67% were narrower than 60  $\mu\text{m}$  and only 1.04% were wider than 69  $\mu\text{m}$ . All measured specimens had aperture width between 20 and 32  $\mu\text{m}$ . However, 90.01% of all specimens had aperture width of 24–28  $\mu\text{m}$ , whereas only 5.55% had aperture narrower than 24  $\mu\text{m}$  and only 4.44% had aperture wider than 28  $\mu\text{m}$ . Figures 4D–F show bag plot analyses of the correlation between shell length, shell width and aperture width.

The positive values of skewness for three characters suggest an asymmetrical distribution with a long tail toward higher values. The asymmetry of aperture width was low (0.076), while the asymmetry of aperture width/shell length ratio was moderate (0.285). High positive skewness value was observed only for shell width (0.648). All other variables were characterized by negative values of skewness (between -0.010 and -0.265), indicating a distribution with a long tail toward lower values. However, only the asymmetry of shell length was moderate (-0.265), while the asymmetry of shell length/shell width ratio (-0.249) and aperture width/shell width ratio (-0.010) was low. The negative kurtosis values were not observed for any character. Therefore, all measured characters have a distribution that is sharper than the standard Gaussian distribution. Low positive values (0.072–0.199) were observed for shell length, shell length/shell width ratio, and aperture width/shell width ratio. Moderate positive values were not observed for any character, while high positive values (0.515–1.088) were registered for shell width, aperture width, and aperture width/shell length ratio.

**Comments.** Leidy (1879) originally described *Nebela tinctoria* as *Hyalosphenia tinctoria*. He noted that

this species possessed shell composed of pale yellow transparent, structureless, chitinous membrane. Later Awerintzew (1906) noted that many specimens corresponding to the first description of *H. tinctoria* had shells composed of plates. Therefore, he transferred this species to the genus *Nebela*. Jung (1942b) in his important review noted that *N. tinctoria* was characterized by shell length 76–92  $\mu\text{m}$  and shell width 56–64  $\mu\text{m}$ . Similar measurements of the shell were also typical for two morphologically close species: *N. minor* (described by Penard, 1893) and *N. parvula* (described by Cash and Hopkinson, 1909). Kosakyan et al. (2013) concluded that these two taxa are synonyms for *N. tinctoria* because the only one difference is presence or absence of lateral pores. Namely, their molecular analysis suggests that the presence of pores is not a valid taxonomic criterion. However, Corbet (1973) observed that shell length/shell width and aperture width/shell width ratios were important taxonomic characters in this species group. Lüftenegger et al. (1988) used only presence or absence of lateral pores for separation *N. tinctoria* and *N. parvula*. Kosakyan et al. (2013) analyzed only typical specimens of *N. tinctoria*. In addition, they measured small number of specimens. For more definitive conclusions about taxonomic validity of *N. minor* and *N. parvula* extensive studies based on higher number of populations will be necessary. A special challenge is the determination of phenotypic characters for the separation of these taxa.

Penard (1893) described *Nebela minor* based on *Sphagnum*-dwelling population from Rocky Mountains (North America) and noted shell length between 90 and 100  $\mu\text{m}$ . Penard (1902) observed specimens from Switzerland (Europe) with shell length between 55 and 70  $\mu\text{m}$ . Gerjcke (1932) noted the following measurements based on specimens



**Fig. 4.** Morphological variability of *Nebela minor* based on 1352 specimens from the Velež 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  $\blacklozenge$ , characters on Y axes  $\bullet$ , outliers  $\blacksquare$ .

from South Africa: shell length 60–75 µm and shell width 31–39 µm. Gauthier-Lièvre (1953) reported the original morphometric data based on material from northern Africa: shell length 85–95 µm, shell width 50–55 µm, and aperture width 38–42 µm. Gauthier-Lièvre (1957) observed one specimen from Cameroon (Middle Africa) characterized by shell length 110 µm, shell width 60 µm, and aperture width 26 µm. Golemansky (1962) reported basic morphometric data based on specimens extracted from tree mosses in Guinea (West Africa): shell length 88 µm, shell width 48–64 µm, and aperture width 16–20 µm. Cerdá (1986) noted measurements for one specimen from Bolivia (South America): shell length 90 µm, shell width 40 µm, and aperture width 15 µm. In these studies, arithmetic means and ratios of the measured characters were not presented.

Kosakyan et al. (2013) for *N. collaris* s.s. noted the following measurements: shell length 95–115 µm (mainly 109–112 µm), shell width 74–81 µm, aperture width 28–32 µm, and shell length/shell width ratio 1.38–1.47. Luketa (2017c) conducted a detailed morphometric study of a population from Krečko brdo Hill (East Herzegovina) based on 765 specimens. He noted specimens with shell length between 87 and 115 µm. Because the arithmetic mean is 103.48 µm, this population has been identified as *N. collaris* s.s. Some authors (e.g. Corbet, 1973; Kosakyan et al., 2013) delimited *N. minor* and *N. collaris* based on morphometric data of shell length. Corbet (1973) noted that shell length of *N. minor* ranged from 80 to 100 µm, while shell length of *N. collaris* ranged from 107 to 184 µm. Kosakyan et al. (2013) included *N. minor* into the species *N. tinctoria* with shell length between 90 and 95 µm. According to Kosakyan et al. (2013), another taxonomic character for distinguishing *N. tinctoria* and *N. collaris* is shell width: 62–71 µm versus 74–81 µm, correspondingly. Based on shell width, population from Krečko brdo Hill belongs to *N. tinctoria* because this variable ranges from 53 to 73 µm. Morphometric analysis presented in this study shows that population from the Velež Lake region is similar to population from Krečko brdo Hill. However, population from the Velež Lake region is very close to typical populations of *N. minor* previously reported from different parts of the world. For example, shell length ranges from 79 to 108 µm, with arithmetic mean of 94.64 µm. At the same time, shell width ranges from 51 to 78 µm and it is a proof that this character is not valid for delimitation of *N. tinctoria* and *N. collaris* if we accept

that *N. minor* is synonym for *N. tinctoria*. For these reasons, population from Krečko brdo Hill is likely an extreme population of *N. minor*. Comparative morphometric data of two *N. minor* populations from East Herzegovina are presented in Table 8. According to comparison of morphometric data for these two populations, it is possible to conclude that aperture width/shell length and aperture width/shell width ratios are stable characters. When we have data about these ratios for other taxa included into the *N. collaris* complex, it will be possible to determine if these characters are significant for the species identification.

#### *POROSIA BIGIBBOSA*

**Description.** The shell is transparent, colorless or slightly yellow, pyriform, laterally compressed, rounded aborally, narrow elliptical in lateral view. The shell is composed of oval, circular, or elongated euglyphid plates of different sizes; on the posterior part, small sand particles were not present in the studied population. In broad lateral view, at approximately one third of the shell length starting from aperture, two distinct lateral depressions with two large invaginated pores are situated on each side; two internal tubes joined by tops connect these pores. The aperture is terminal, oval, slightly or strongly curved, surrounded by a thick organic lip. In the studied population, it was not possible to distinguish two types of shells. Figure 5 shows light micrographs of specimens from the studied population.

**Morphometry.** Morphometric characters of 253 specimens of *P. bigibbosa* from the Velež Lake region were measured and the results are given in Table 9. Coefficients of variation were low for all measured characters (from 4.27 to 9.15%). For basic characters, the minimal variability was observed for shell length (4.27%), while the maximal variation coefficient was observed for pore-pore distance (9.15%). For ratio characters, the minimal variability was observed for shell width/shell length ratio (5.31%), while the maximal variation coefficient was observed for pore-pore distance/shell length ratio (9.03%).

The most frequent shell length (140, 143 and 144 µm) was registered in 20 specimens (Fig. 6A); the most frequent shell width (88 µm) was registered in 24 specimens (Fig. 6B), and the most frequent aperture width (40 µm) was registered in 44 specimens (Fig. 6C). Analysis of the size frequency distribution of shell length and aperture width

**Table 8.** Comparative morphometric data of *Nebela minor* based on two populations from East Herzegovina (measurements in  $\mu\text{m}$ ): Krečko brdo Hill (based on data from Luketa, 2017c; in this paper population is identified as *N. collaris* s.s.) and Velež Lake region (based on data from this study).

Character	Location	N	Min	Max	x
shell length	Krečko brdo Hill	765	87	115	103.48
	Velež lake region	1352	79	108	94.64
shell width	Krečko brdo Hill	765	53	73	62.40
	Velež lake region	1352	51	78	60.13
aperture width	Krečko brdo Hill	765	21	31	26.45
	Velež lake region	1352	20	32	25.83
shell length/shell width	Krečko brdo Hill	765	1.33	1.91	1.66
	Velež lake region	1352	1.25	1.88	1.58
aperture width/shell length	Krečko brdo Hill	765	0.19	0.31	0.26
	Velež lake region	1352	0.21	0.34	0.27
aperture width/shell width	Krečko brdo Hill	765	0.33	0.50	0.42
	Velež lake region	1352	0.34	0.52	0.43

**Table 9.** Morphometric characterization of *Porosia bigibbosa* from the Velež Lake region (East Herzegovina) based on 253 specimens (measurements in  $\mu\text{m}$ , except for area of the optical section in  $\mu\text{m}^2$ ).

Characters	Min	Max	M	x	SE	SD	CV	Sk	Ku
shell length	122	166	143	143.33	0.38	6.12	4.27	-0.112	0.998
shell width	80	107	91	91.07	0.32	5.08	5.58	0.465	0.196
aperture width	34	48	41	40.87	0.16	2.50	6.12	0.091	0.110
aperture-pore distance	38	58	48	48.26	0.20	3.14	6.50	0.024	0.416
pore-pore distance	39	64	51	51.02	0.29	4.67	9.15	0.278	-0.020
area of the optical section	7935	13173	9831	9913	53.33	848.22	8.56	0.391	0.542
shell width/shell length	0.56	0.75	0.63	0.64	0.00	0.03	5.31	0.592	0.409
aperture width/shell length	0.24	0.35	0.29	0.29	0.00	0.02	6.12	0.374	0.475
aperture width/shell width	0.39	0.53	0.45	0.45	0.00	0.02	5.36	0.125	-0.167
aperture width/aperture-pore distance	0.64	1.12	0.85	0.85	0.00	0.07	8.49	0.291	0.812
aperture width/pore-pore distance	0.61	0.98	0.80	0.81	0.00	0.07	8.26	0.102	-0.107
aperture-pore distance/shell length	0.28	0.40	0.34	0.34	0.00	0.02	5.53	-0.005	0.448
aperture-pore distance/shell width	0.39	0.67	0.53	0.53	0.00	0.04	7.73	0.037	0.413
pore-pore distance/shell length	0.29	0.47	0.35	0.36	0.00	0.03	9.03	0.510	0.004
pore-pore distance/shell width	0.43	0.68	0.56	0.56	0.00	0.04	7.05	0.034	0.384

*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.

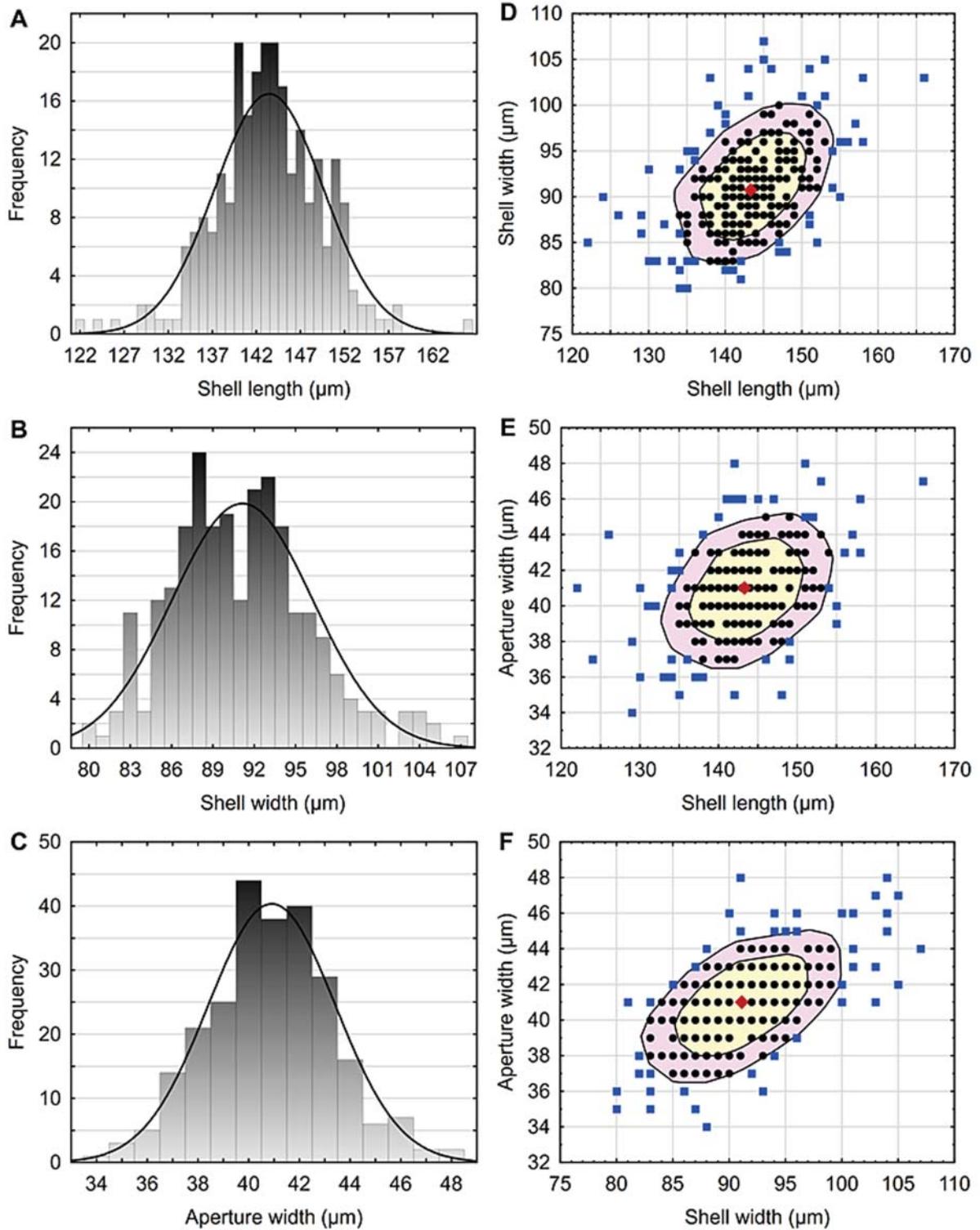


**Fig. 5.** Light micrographs of *Porosia bigibbosa*: broad lateral view of different specimens from the Velež Lake region, East Herzegovina. Scale bars: 20  $\mu\text{m}$

indicates that this population is size-monomorphic. Shell length ranged from 122 to 166  $\mu\text{m}$ . However, 76.68% of all measured specimens had shell length of 138–151  $\mu\text{m}$ , whereas only 15.02% were smaller than 138  $\mu\text{m}$  and only 8.30% were larger than 151  $\mu\text{m}$ . The frequency analysis of aperture width shows similar distribution pattern. Namely, all measured specimens had aperture width ranging between 34 and 48  $\mu\text{m}$ . In this case, 69.57% of all specimens had aperture width of 39–43  $\mu\text{m}$ , whereas only 17.39% had aperture narrower than 39  $\mu\text{m}$  and only 13.04% had aperture wider than 43  $\mu\text{m}$ . Analysis of the size

frequency distribution of shell width indicates that this population possesses continuous polymorphism. All measured specimens had shell width between 80 and 107  $\mu\text{m}$ . In this case, 58.10% of all specimens had shell width between 89 and 98  $\mu\text{m}$ , whereas 34.39% were narrower than 89  $\mu\text{m}$  and only 7.51% were wider than 98  $\mu\text{m}$ . Figures 6D–F show bag plot analyses of the correlation between shell length, shell width and aperture width.

The positive values of skewness for thirteen characters suggest an asymmetrical distribution with a long tail toward higher values. The asymmetry of



**Fig. 6.** Morphological variability of *Porosia bigibbosa* based on 253 specimens from the Velež 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  $\blacklozenge$ , characters on Y axes  $\bullet$ , outliers  $\blacksquare$ .

aperture width, aperture-pore distance, aperture width/shell width ratio, aperture width/pore-pore distance ratio, aperture-pore distance/shell width ratio, and pore-pore distance/shell width ratio was low, with skewness values ranging between 0.024 and 0.125. Moderate positive values of skewness (0.278–0.465) were observed for shell width, pore-pore distance, area of the optical section, aperture width/shell length ratio, and aperture width/aperture-pore distance ratio. High positive skewness values (0.510–0.592) were observed only for shell width/shell length ratio (0.592) and pore-pore distance/shell length ratio. Shell length and aperture-pore distance/shell length ratio were characterized by negative values of skewness (between –0.005 and –0.112), suggesting an asymmetrical distribution with a long tail toward lower values.

Three characters (pore-pore distance, aperture width/shell width ratio, and aperture width/pore-pore distance ratio) displayed negative kurtosis values, meaning that they were characterized by flatter distribution than a standard Gaussian distribution. Since the negative values obtained for these characters (between –0.020 and –0.167) are not clearly different from zero, the resulting deviation from the normal Gaussian distribution was minimal. Other variables were found to have positive kurtosis values, indicating a distribution that is sharper than the standard Gaussian distribution. Low positive values (0.004–0.196) were observed for shell width, aperture width, and pore-pore distance/shell length ratio. Moderate positive values (0.384–0.475) were observed for aperture-pore distance, shell width/shell length ratio, aperture width/shell length ratio, aperture-pore distance/shell length ratio, aperture-pore distance/shell width ratio, and pore-pore distance/shell width ratio. High positive values (0.542–0.998) were observed for shell length, area of the optical section, and aperture width/aperture-pore distance ratio.

**Comments.** Penard (1890) described *Nebela bigibbosa* based on a moss-dwelling population from Germany and noted basic morphometric data. Jung (1942b) proposed the genus *Porosia* for only this species because it is characterized by the presence of two distinct lateral depressions with two large invaginated pores situated on each side of the shell. After the original description of this species, several authors noted basic morphometric data based on the populations from Ireland (Wailles and Penard, 1911), Indonesia (Hoogenraad and de Groot, 1940), and Great Britain (Ogden and

Hedley, 1980). In these studies, arithmetic means and ratios of measured characters were not provided. Comparative morphometric data of *P. bigibbosa* according to different authors are presented in Table 10.

Todorov (2002) first conducted a detailed morphometric study of *P. bigibbosa* based on 100 specimens from six mountains in Bulgaria and showed arithmetic means for all measured characters. Of ratio characters, he calculated only shell width/shell length ratio. Recently, Luketa (2016) conducted a detailed morphometric study of *P. bigibbosa* based on a moss-dwelling population (175 specimens) from the Alagovac Lake region (East Herzegovina). Based on comparison of all published data, Luketa (2016) concluded that *P. bigibbosa* is a morphometrically uniform species. Namely, all populations have very similar values of minimum and maximum for all measured characters. In addition, all values of arithmetic means for all measured characters in Bulgarian populations and population from the Alagovac Lake region are very similar. Morphometric data shown in the present study confirm that *P. bigibbosa* is morphometrically uniform species according to minimum and maximum values. However, in contrast to populations from Bulgaria and a population from the Alagovac Lake region, the population from the Velež Lake region shows very different values for arithmetic means of some basic characters: shell length (157.5  $\mu\text{m}$  versus 158.98  $\mu\text{m}$  versus 143.33  $\mu\text{m}$ ), shell width (101.9  $\mu\text{m}$  versus 104.02  $\mu\text{m}$  versus 91.07  $\mu\text{m}$ ), aperture width (40.4  $\mu\text{m}$  versus 44.65  $\mu\text{m}$  versus 40.87  $\mu\text{m}$ ), aperture-pore distance (59.6  $\mu\text{m}$  versus 55.36  $\mu\text{m}$  versus 48.26  $\mu\text{m}$ ), pore-pore distance (not measured, 63.13  $\mu\text{m}$  versus 51.02  $\mu\text{m}$ ), and area of the optical section (not measured, 12559  $\mu\text{m}^2$  versus 9913  $\mu\text{m}^2$ ).

Todorov (2002) calculated only shell width/shell length ratio and noted values between 0.60 and 0.70. Luketa (2016) also calculated ratio between these two characters based on population from the Alagovac Lake region and noted similar values (0.57–0.76). Population from the Velež Lake region shows very similar values (0.56–0.75) as two previously reported analyses. According to these data, it is possible to conclude that *P. bigibbosa* is a uniform species and that shell width/shell length ratio is a stable taxonomic character. In addition, other ratio characters have similar values in these two populations from East Herzegovina; the most discriminant character is arithmetic

**Table 10.** Comparative morphometric data of *Porosia bigibbosa* according to different authors (measurements in  $\mu\text{m}$ ).

Country	Shell length	Shell width	Aperture width	Shell width/ shell length	References
Germany	140–160	100–110	—	—	Penard, 1890
Ireland	135–170	87–110	34–45	—	Wailes and Penard, 1911
Indonesia	130–170	83–123	—	—	Hoogenraad and de Groot, 1940
Great Britain	153–171	95–115	38–41	—	Ogden and Hedley, 1980
Bulgaria	128–177	90–115	35–49	0.60–0.70	Todorov, 2002
Bosnia and Herzegovina	136–178	86–118	40–51	0.57–0.76	Luketa, 2016
Bosnia and Herzegovina	122–166	80–107	34–48	0.56–0.75	This study

mean of aperture width/pore-pore distance ratio (0.71 versus 0.80). Todorov (2002) noted low variation coefficients for all measured characters (3.1–7.0%), except for pore diameter (12.4%). Two studied populations from East Herzegovina have low variation coefficients for all measured characters (4.50–9.01% versus 4.27–9.15%). Thus, it is possible to conclude that two populations of *P. bigibbosa* from East Herzegovina studied up to date are morphometrically homogeneous. Because pore diameter was not measured in populations from East Herzegovina, it is not possible to conclude about the nature of variation of this character.

## Conclusions

Data presented in this paper support synonymization of *Padaungiella wailesi* (shell length 75–100  $\mu\text{m}$ ) and *P. lageniformis* s.s. (shell length 100–160  $\mu\text{m}$ ). The population described here contains specimens with shell length between 83 and 112  $\mu\text{m}$ , with the arithmetic mean of 99.07  $\mu\text{m}$ . Additionally, morphological and morphometric data presented in this paper do not support the recently proposed wide taxonomic concept of *Nebela tincta*. Namely, this data call into question the synonymization of *N. minor* and *N. tincta*. Further molecular and morphometric studies are needed to clarify taxonomic relations between these two taxa. The presented morphometric analyses of *Porosia bigibbosa* refute the previously published conclusion that this species is a morphometrically uniform taxon. The most discriminant characters between populations are arithmetic means of shell length, shell width, aperture-pore distance, pore-pore distance, area of the optical section,

and aperture width/pore-pore distance ratio. Further morphometric studies based on different populations are needed to clarify interpopulation diversity within *P. bigibbosa* and evaluate taxonomic importance of these variations.

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