

ACTA PROTOZOLOGICA

Morphology and Biometry of *Nebela tenella* Penard, 1893 (Amoebozoa: Arcellinida)

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Summary: Shell ultra-structure and morphometrical variability of *Nebela tenella* were investigated using scanning electron microscopy (SEM). *N. tenella* was isolated from two widely separated populations, one from Switzerland and another from Canada. The shell's structural elements were similar to those of the other nebelids, but *N. tenella* has a characteristic peculiarity – always present depressions on the shell surface, which makes an uneven outline of the shell. Moreover, light microscopy and SEM study showed that the collar of the *N. tenella* represents a turned-over continuation of the neck, which is covered by the same idiosomes as on the shell body. The biometrical analysis showed that the majority of the basic characters of *N. tenella* vary moderately and give continuous series of transition forms. According to the shell depth and the ratio depth/width both populations were significantly different of each other. Variation coefficients showed that the variability of the characters differs in both populations and the Swiss population is more stable than the Canadian one. All new obtained data for *N. tenella* raise the question whether the shell's size, cross section and ultramorphology are reliable enough as characters for the differentiation of *N. tenella* and *N. griseola*, and whether they are two distinct species or should just be considered as ecophenotypic variation within one species?

Key words: Testate amoebae, Amoebozoa, Arcellinida, *Nebela tenella*, biometry, morphology.

INTRODUCTION

The genus *Nebela* is one of the most numerous testatean genera and consist of more than 120 species and around 100 infra-sub-specific taxa. The majority of them inhabits *Sphagnum* mosses, freshwaters and forest's litter, and is distributed worldwide. Most of the species have comparatively large dimensions and are clearly

distinguished from each other. However, some comparatively rare taxa have been described using mainly light microscopy, and data about their ultramorphology and biometry in the literature are incomplete. *N. tenella* is one of these species whose morphometry and biometry is poorly studied. It was recorded the first time by Penard (1893) in *Sphagnum* moss sampled in Jura Mountains (Switzerland). Although, many authors from different countries have later published data about *N. tenella*, still scarcity of data about its ultramorphology and biometry is available in the literature (Schouteden 1906; Cash and Hopkinson 1909; Heinis 1911; Wailes 1912; Steinecke 1913; van Oye 1933; Jung 1936,

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1942a, b; Harnisch 1938; Hoogenraad and de Groot 1952; Bartoš 1954; Ertl 1955; Graaf 1956; Lepši 1960; Stěpének 1963).

The aim of the present study was to investigate the details of the shell ultrastructure of *N. tenella* and to determine the morphological and biometrical characteristics of the *N. tenella* sampled from two distant populations from Switzerland and Canada.

MATERIAL AND METHODS

The material was extracted from *Sphagnum* moss from the following two sites:

- 1) Praz-Rodet bog (46°34'00"N lat., 6°10'25"E long.; 1035 m a.s.l.) – a small peat bog about 5 ha in size situated at the south-western end of the Joux Valley in the Jura Mountains (Switzerland) in May 2008;
- 2) Peggys cove (44°29'42"N lat., 63°53'47"W long.; 3 m a.s.l.) – peat land, oligotrophic peat bog, south-west of Halifax (Canada) in August 2008.

The shell dimensions of 100 specimens of the Swiss population and 45 specimens of the Canadian population were measured with optical microscope "Amplival" (Zeiss-Jena) using 40× objective and 10× oculars lens. The isolated specimens were placed in a drop of glycerol to achieve optimal orientation for the measurement of their shell depth and apertural collar. They were orientated in lateral or apertural view while measurements were carried out.

For scanning electron microscopy (SEM) specimens were isolated by searching through small isolates of material in a petri dish. Specimens were extracted using a glass micropipette, washed several times in distilled water, and then individual shells were positioned with a single-hair brush onto a small drop of Araldite on a previously cleaned standard aluminium stub and air-dried. The shells were coated evenly with gold in a vacuum coating unit. The photomicrographs were obtained using a JEOL JSM-5510, operating at 10 kV.

The biometric description was made according to Schönborn *et al.* (1983). The following basic statistics were calculated: arithmetic mean, median (M), standard deviation (SD), standard error of mean (SE), coefficient of variation in % (CV), extreme values (Min and Max). Frequency distribution analysis was carried out in order to describe variation of characters. Statistical analysis was performed using the computer program STATISTICA, version 6.0 (Stat Soft 1999).

RESULTS

Nebela tenella Penard, 1893; Figs 1–22; Tables 1–3

Shell morphology: The shell is comparatively small, transparent, colourless, compressed laterally. In broad view it is ovoid, tapering towards the aperture

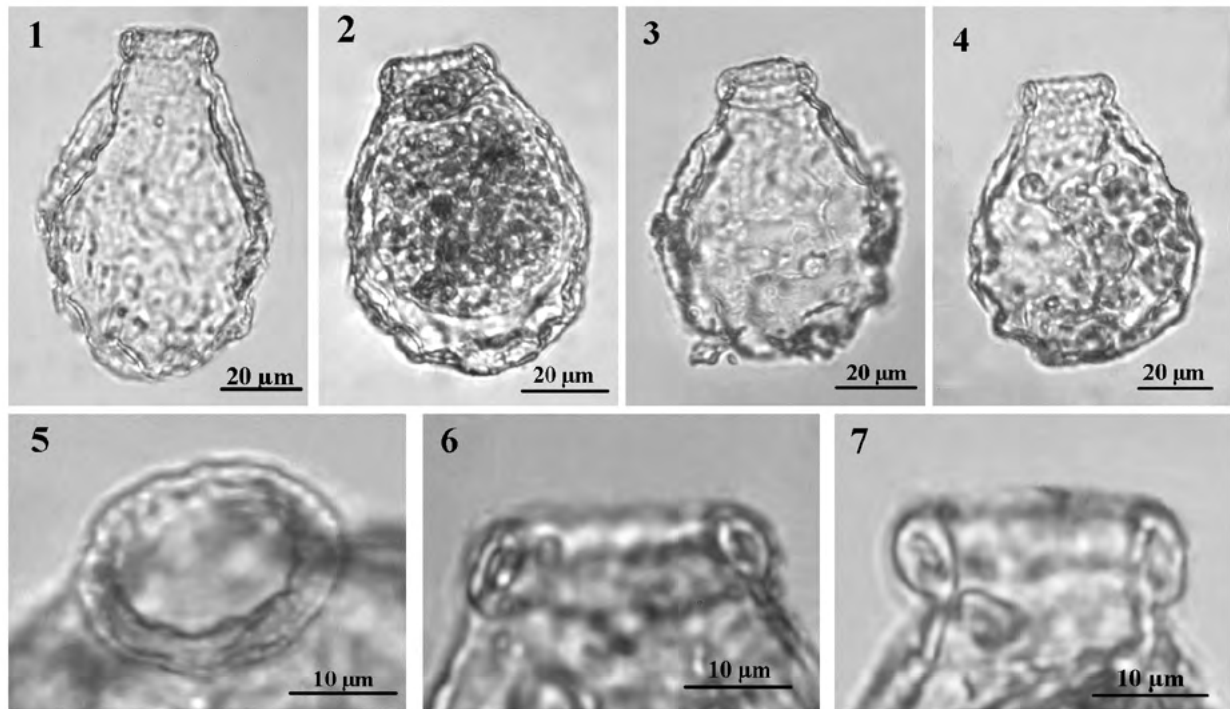
and forming a brief neck, with a pronounced annular collar around the aperture, which represent a turn-over continuation of the neck (Figs 1–7). It has a smooth but uneven outline and is mainly composed of siliceous particles (idiosomes), which are almost invisible in light microscopy (Figs 1–4). The cross section of the shell is elongate-elliptical.

Our SEM investigations of the shell's ultrastructure show that it is composed of many ellipsoidal or oval siliceous particles with different sizes, sometimes mixed with rod-like particles, diatom frustules and some flattened particles of quartz. They are all bound together by organic cement, which is evident and easily seen at junctions (Figs 16–19). The shell surface is characterized by typical depressions, more or less pronounced, but always present, which give an uneven outline of the shell (Figs 8–11). The aperture is oval, with an uneven outline and is surrounded by a characteristic collar. The particles covering the collar have the same form and size as on the shell body (Figs 12–15).

Biometry: Morphometrical characteristics of both populations are given in Table 1. Median values of majority of the basic characters (shell length, width, large and small axis of aperture, length of neck, collar's width and ratio width/length) give a continuous series of transition forms. Individuals from both populations differ significantly in shell depth and ratio depth/width.

Analysis of the variation coefficients shows that variability of characters differs in both populations (Table 1). As a whole the Swiss population is more stable than the Canadian one. Coefficient of variation of all the taxonomical characters measured in the first population is less than 10% and shows that shell parameters are moderately variable. The most stable characters in this population were the large and small axis of aperture, shell length, shell width and width/length ratio (CV range from 3.6 to 5.7%). Length of the neck and the ratio depth/width had maximal variation coefficient (8.5 and 9.2%, respectively). Variability of the shell depth and collar's width were intermediate between the constant and the most variable characters (6.8 and 6.9%, respectively).

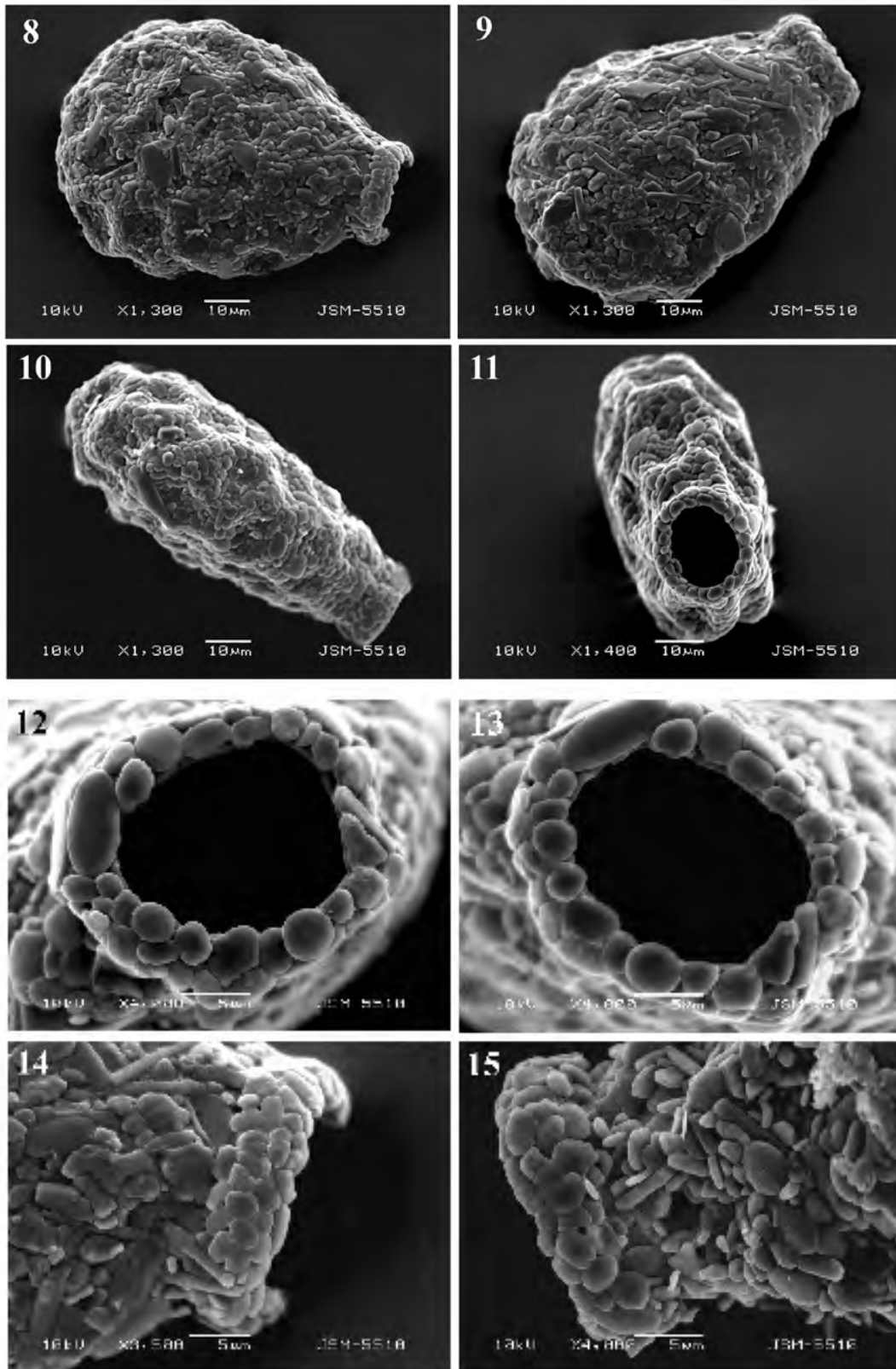
Variability of all characters in the Canadian population was higher than that in the Swiss population. The coefficient of variation in the Canadian population was almost double that of the Swiss population for some characters (shell depth, large axis of aperture and length of neck). Maximal coefficient of variation was marked for ratio depth/width (CV = 11.6%), shell depth (CV



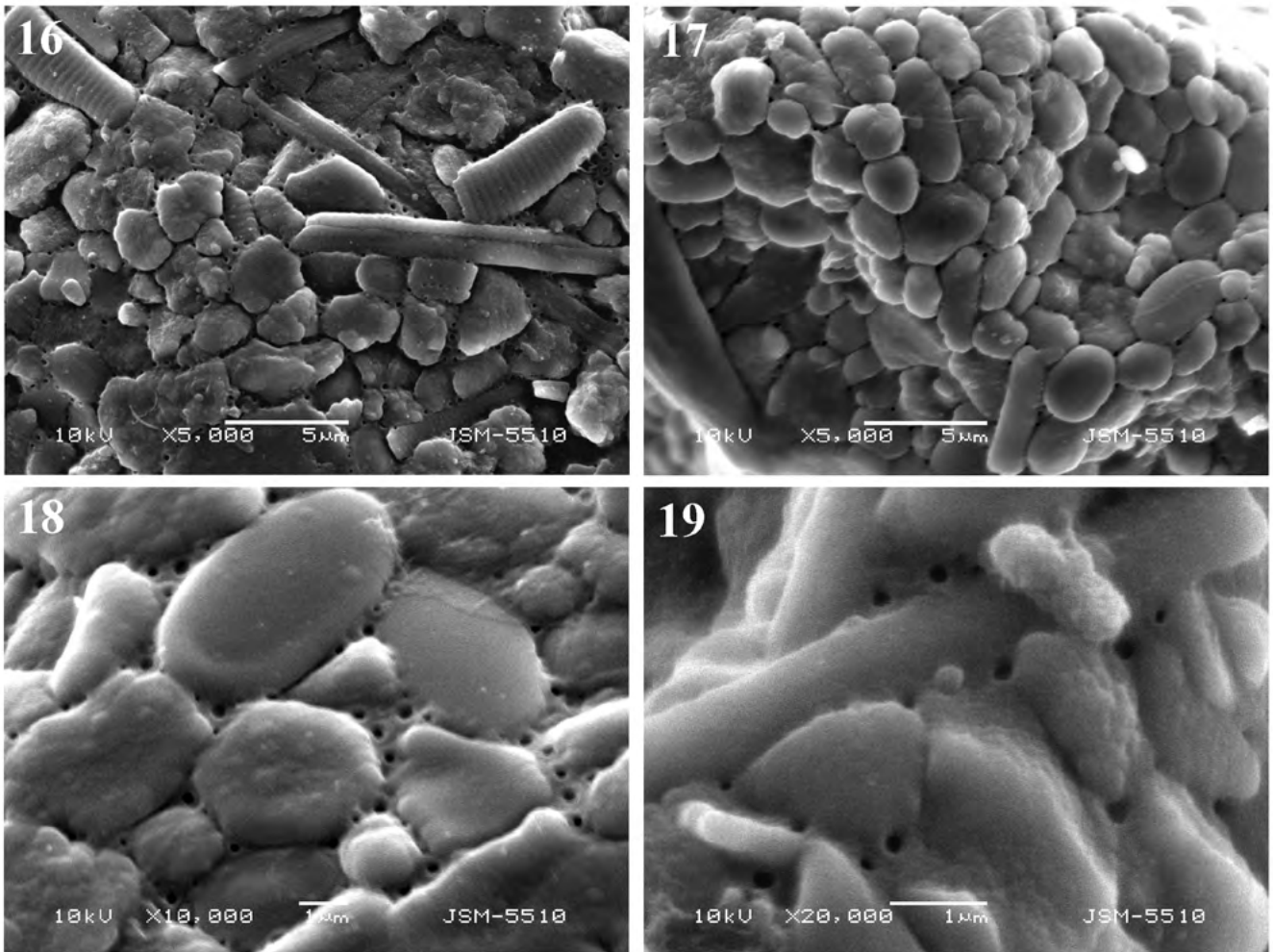
Figs 1–7. LM photographs of *N. tenella*. 1–4 – broad lateral view of four specimens showing different shell's shape; 5 – apertural view showing the collar; 6–7 – lateral view of aperture to illustrate that the collar is a turned-over continuation of the shell's neck.

Table 1. Morphometric characterization of two population of *Nebela tenella*: M – median, SD – standard deviation, SE – standard error of the mean, CV – coefficient of variation in %, Min – minimum, Max – maximum, n – number of individuals examined (measurements in µm).

Character	Population	Mean	M	SD	SE	CV	Min	Max	n
Length	Swiss	79.8	80	4.1	0.41	5.1	73	87	100
	Canadian	79.4	79	4.61	0.69	5.8	70	87	45
Width	Swiss	57.0	58	3.26	0.32	5.7	50	64	100
	Canadian	56.5	57	5.35	0.8	9.5	45	66	45
Depth	Swiss	30.8	31	2.1	0.21	6.8	27	37	100
	Canadian	39.0	39	4.89	0.73	12.5	31	47	45
Large axis of aperture	Swiss	16.7	17	0.6	0.06	3.6	16	18	100
	Canadian	17.0	17	1.34	0.2	7.9	15	20	45
Small axis of aperture	Swiss	14.9	15	0.7	0.07	4.7	14	16	100
	Canadian	15.2	15	1.18	0.18	7.8	13	18	45
Length of neck	Swiss	5.9	6	0.5	0.05	8.5	5	7	100
	Canadian	6.2	6	0.97	0.14	15.6	5	8	45
Collar's width	Swiss	2.9	3	0.2	0.02	6.9	2.5	3	100
	Canadian	3.0	3	0.33	0.05	11.0	2.5	3.5	45
Ratio width/length	Swiss	0.71	0.71	0.04	0.004	5.6	0.61	0.81	100
	Canadian	0.71	0.71	0.05	0.01	7.0	0.62	0.84	45
Ratio depth/width	Swiss	0.54	0.53	0.05	0.005	9.2	0.46	0.65	100
	Canadian	0.69	0.7	0.08	0.001	11.6	0.52	0.84	45



Figs 8–15. SEM photographs of *N. tenella*. **8–9** – broad lateral view of two specimens showing the depressions on the shell surface; **10** – narrow lateral view; **11–12** – apertural view of two specimens; **13** – apertural view to show the apertural collar and the shape of aperture; **14–15** – lateral view of the aperture showing the structure of the collar.



Figs 16–19. SEM photographs of *N. tenella*. 16–17 – detail of shell surface; 18–19 – higher magnification of shell surface showing details of organic cement.

= 12.5%) and length of neck (CV = 15.6%). Only two characteristics (shell length and width/length ratio) were as stable as in the Swiss population (CV = 5.8 and 7.0%, respectively).

Analysis of the size frequency distribution also indicated that the two populations differ in their homogeneity (Figs 20A–C). The data for the Swiss population showed that *N. tenella* is a size-monomorphic species with comparatively well-expressed main-size class of the main shell characters. For example, all measured individuals had a shell length of 73–87 µm. Moreover, 86% of them were within the limits of 74–85 µm, whereas only 4% had shell length less than 74 µm and 10% – more than 85 µm (Fig. 20A). Regarding the shell width and depth, all measured individuals range in close

limits – width, 50–64 µm and depth, 27–37 µm. The presence of comparatively well-expressed main-size class of the shell width (54–60 µm), as well as of the shell depth (30–32 µm), and the lack of the subsidiary peaks (bell-shaped curves) indicate a normal distribution (Figs 20B, C). The average width and depth of *N. tenella* in this population were 57.0 ± 3.26 (n = 100) and 30.8 ± 2.1 (n = 100). These arithmetical means correlate with the main-size classes of these characters, and testify for monomorphism of the species.

The size frequency distribution analysis of the Canadian population differs greatly from that of the Swiss population. This indicates that *N. tenella* is a size-poly-morphic species, characterized by increasing size range (shell length 70–87 µm, shell width 45–66 µm and shell

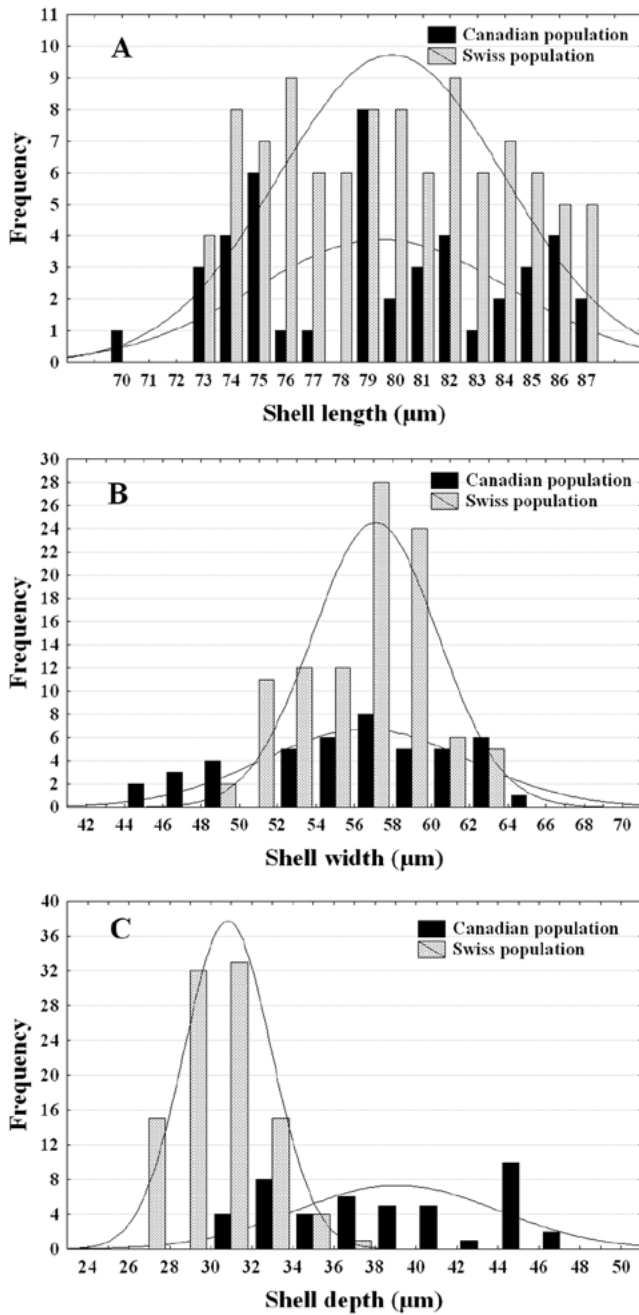


Fig. 20. Histograms showing the size frequency distribution of the shell length (A), width (B) and depth (C) in *N. tenella*.

depth 31–47 μm) and by reduced main size-class in favour of subsidiary classes (the curves are flattened and not well-expressed bell-shaped) (Figs 20A–C). The average length, width and depth of shells amount to 79.4 ± 4.61 ($n = 45$), 56.5 ± 5.35 ($n = 45$) and 39.0 ± 4.89 ($n = 45$), respectively. These arithmetical means do not

Table 2. Skewness and kurtosis of characters' frequency distribution in *Nebela tenella*.

Character	Skewness		Kurtosis	
	Swiss population	Canadian population	Swiss population	Canadian population
Length	0.07	-0.01	-1.13	-1.08
Width	-0.23	-0.51	-0.41	-0.42
Depth	0.47	0.07	-0.07	-1.3
Large axis of aperture	0.02	0.19	-0.69	-1.04
Small axis of aperture	0.17	0.38	-0.93	-0.74
Length of neck	-0.17	0.45	0.57	-0.67
Collar's width	-1.99	-0.10	2.0	-0.66
Ratio width/length	0.17	0.35	0.56	-0.31
Ratio depth/width	0.32	-0.15	-0.57	-0.66

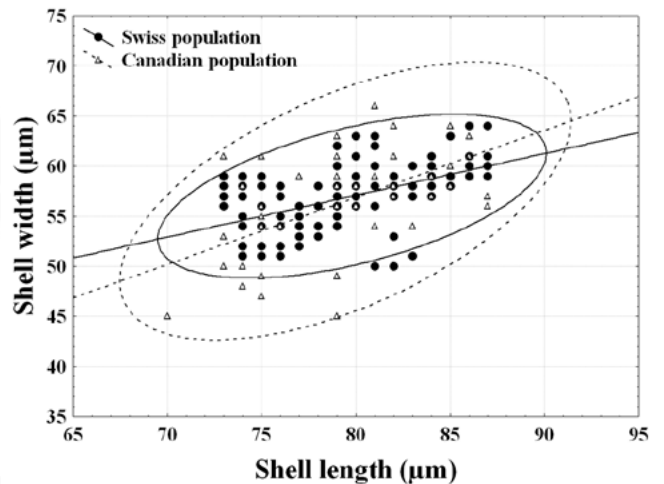


Fig. 21. Scatter plot of shell length versus shell width in two populations of *N. tenella* (with 95% confidence ellipses).

agree with the main size classes of the characters, and testify to the polymorphism of the species.

Frequency distribution of most characters, in both populations, is characterized by approximately a zero level of skewness, which indicates that specimens with average measurements predominate in populations

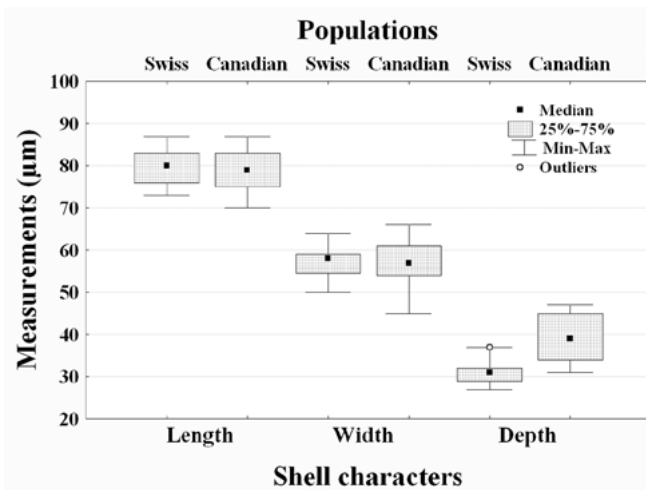


Fig. 22. Box-plots showing the variability of the shell length, width and depth in the Swiss and Canadian populations of *N. tenella*.

(Table 2). At the same time, skewness of frequency distribution of shell width (for Canadian population) and collar's width (for Swiss population) is clearly different from zero and has negative values, showing that individuals with small measurements for these characters predominate in these populations. On the contrary, skewness of frequency distribution of neck's length (for Canadian population) and shell depth (for Swiss population) has positive values, clearly different from zero, showing that individuals with large measurements predominate there. In both populations, most of the characters (shell length, width, and depth, large and small axis of aperture and ratio depth/width) had negative values of kurtosis, indicating higher dispersion of average size group in these populations. On the contrary, a maximal number of individuals in the Swiss population possess values of collar's width, length of neck and ratio width/length restricted to a narrow size limits within overall size variability.

The above mentioned characteristics of both studied populations are also confirmed by the scatter plot of shell length versus shell width in *N. tenella* (Fig. 21) and by the boxplots for the main shell characters in *N. tenella* (Fig. 22), where it is clearly indicated that the individuals of the Canadian population have bigger dispersion than these in the Swiss population.

DISCUSSION

In his monograph about the genus *Nebela* Deflandre (1936) placed *N. tenella* together with two other species (*N. griseola* and *N. cratera*) in sub-generic "Section I." The main peculiarity that distinguishes species of this section is the presence of a more or less pronounced apertural collar, which represents a turned-over continuation of the shell's neck. Later Jung (1942a) divided the genus *Nebela* into 10 separate genera and allocated species of the Deflandre's "Section I," including *N. tenella*, to a newly named genus *Physochila*. The separation of the genus *Nebela* was not widely adopted and most authors have continued to regard *Nebela* as a single genus, a practice we follow in this paper.

The shell ultra-structure of *N. tenella* has been unclear for a long time, because the resolving power of the optical microscopes has not allowed observation of the structural elements. Our SEM investigations provide new information in these aspects and show that the shell's structural elements in *N. tenella* are similar to those of the other nebelids. Furthermore, the shell of *N. tenella* has another peculiarity – typical depressions on the shell surface, more or less pronounced, but always present, which gives an uneven outline of the shell. This unevenness of the shell's outline was previously mentioned by many authors, but has not been explained by the presence of such permanent depressions on the shell surface (Penard 1902; Wailes and Penard 1911; Deflandre 1936; Jung 1942a, b). Surprisingly, our SEM images of *N. tenella* (Figs 8–19) are in good agreement with these ones of *N. griseola*, given by Ogden and Hedley (1980). Unfortunately, the last mentioned data were based on two specimens only, and are, to the best of our knowledge, the only SEM data for the ultra-structure of *N. griseola*. Because of that they don't permit us to make more comprehensive comparison between the ultra-structure of *N. tenella* and *N. griseola*.

Some of the earlier researchers compared *N. tenella* and *N. griseola*, and recognized that differences between the two species are expressed in the following: 1) The shell of *N. tenella* is clearly compressed, whereas *N. griseola* has a much slighter (almost nil) compression; 2) *N. tenella* has a thickened collar around the aperture that is not a turned-over continuation of the neck as in *N. griseola*, but is an agglutination of particles forming a semicircular beading round the aperture; 3) The aperture in *N. tenella* is large-elliptical and in *N. griseola* it is round; 4) *N. tenella* is smaller than *N. griseola* (Pe-

Table 3. Comparative morphometric data for *Nebela griseola* from different authors (measurements in μm).

Authors	Region	Characters			
		Length	Width	Depth	Diameter of aperture
Penard 1911	Australia	70–75	–	–	–
Wailes and Penard 1911	Ireland	80–85	–	–	–
Wailes 1912	New Jersey, USA	80–85	53–60	43–50	16–18
Cash <i>et al.</i> 1919	British Isles	80–87	52–60	45–55	18–23
Deflandre 1936	Compilation	70–85	50–58	–	–
Hoogenraad and de Groot 1940	Sumatra	63–70	47–57	–	–
Hoogenraad and de Groot 1940	Java	70–73	49–54	–	–
Hoogenraad and de Groot 1951	South America, Brazil	83–90	63–70	–	–
Jung 1942b	Compilation	67–97	47–65	–	19–32
Ogden and Hedley 1980	England	82–88	62–69	50–51	20–21

nard 1902; Wailes and Penard 1911; Deflandre 1936; Hoogenraad and de Groot 1940; Jung 1942a, b).

Our biometrical studies of *N. tenella* showed that the majority of its characters vary moderately and give a continuous series of transition forms. Individuals from both studied populations differ significantly in shell depth and in depth/width ratio. This high dispersion of the last two parameters shows that *N. tenella* is quite variable in respect to the shell's depth and that some of the observed specimens (mainly from the Canadian population) have close characteristics to *N. griseola* (depth/width ratio attains to 0.84). Further, our values for the main shell's characters of *N. tenella* are too close to those of *N. griseola* given by many other authors (Table 3) (Penard 1911; Wailes and Penard 1911; Wailes 1912; Cash *et al.* 1919; Deflandre 1936; Hoogenraad and de Groot 1940, 1951; Jung 1942b; Ogden and Hedley 1980). Such similarity and some difficulties in the differentiation of these two species were previously mentioned by different authors (Wailes and Penard 1911; Wailes, 1912; Deflandre 1936; Hoogenraad and de Groot 1940; Jung 1942a, b). Moreover, when Deflandre (1936) discussed the distribution of *N. tenella* he pointed out that there was confusion between *N. tenella* and *N. griseola* in many of the reports for these species.

Besides, both LM and SEM studies showed that the collar in *N. tenella* represents a turned-over continuation

of the neck (like in *N. griseola*). This fact is contrary to the opinion accepted until now that in this respect *N. tenella* and *N. griseola* differ from one another (Penard 1893, 1902; Wailes and Penard 1911; Deflandre 1936).

All the above mentioned facts raise the question whether the shell's size, cross section and ultramorphology are reliable enough as characters for the differentiation of *N. tenella* and *N. griseola*, and whether they are two distinct species or maybe one polymorphic species? The available to this moment data for the shell size and shape of these species clearly show that light microscopically they are distinguishable with difficulty. This could lead to errors in the determination of these species in future, and thus will be make inexact many faunistical, ecological and biogeographical studies, containing data for them. Therefore, we think that it should be doing more complete SEM ultramorphological study of many specimens with typical characteristics of *N. griseola*, which will allow to be made more precise assessment about the similarities and differences in the ultra-structure of *N. tenella* and *N. griseola*. That will answer the question as to whether the two species are distinct, or they should just be considered as ecophenotypic variation within one species. Additional modern molecular and genetic investigations should also be used to resolve this problem and to understand if both morphologically close species are genetically distinct.

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