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THE VARIABILITY OF EXOSKELETON ELEMENTS IN *POLYPLACOCYSTIS AMBIGUA* (PROTISTA, CENTROHELIDA)

L. P. Gaponova, I. V. Dovgal

Schmalhausen Institute of Zoology, NAS of Ukraine,
Bogdan Chmielnicky str., 15, Kyiv, 01601 Ukraine
E-mail: lgaponova@gmail.com

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The Variability of Exoskeleton Elements in *Polyplacocystis ambigua* (Protista, Centrohelida). Gaponova L. P., Dovgal I. V. — Article dealt with the investigation of variability of the scales of periplast in *Polyplacocystis ambigua* (Penard, 1904). New quantative character of periplast scale (wide of rim) was introduced. Renewed diagnosis of *P. ambigua* where the quantative characteristics of perilpast scales are taken into account is given.

Key words: variation, periplast scales, *Polyplacocystis*, Centrohelida, Protista.

Изменчивость структурных элементов перипласта *Polyplacocystis ambigua* (Protista, Centrohelida). Гапонова Л. П., Довгаль И. В. — Приведены результаты исследования изменчивости чешуек перипласта цетрохелидного солнечника *Polyplacocystis ambigua* (Penard, 1904). Предложен новый количественный признак — ширина каймы чешуек. Приведено переописание вида, составленное с учетом характера изменчивости структурных элементов перипласта.

Ключевые слова: изменчивость, чешуйки перипласта, *Polyplacocystis*, Centrohelida, Protista.

Introduction

Polyplacocystis ambigua (Penard, 1904) is a protist widespread in fresh waters of different regions of the world (Микрюков, 2002). Similarly other centrohelid heliozoans of the genus *Polyplacocystis* Mikrjukov, 1996, *P. ambigua* is characterized by exoskeleton (periplast) comprising only tangential plate-scales, which are formed by only one plate (Mikrjukov, 1996).

The scales of *P. ambigua* are formed by a thin, flat mesh of siliceous material, with the edges curved over to form a rim (Patterson and Dürrschmidt, 1988). In this species the periplast is composed of three scales types: fusiform, naviculoid and oval scales.

Since the original description (Penard, 1904) and further studies (Rainer, 1968) scales types of *P. ambigua* were characterized by both qualitative (shapes of scales) and quantitative characters (length and breadth of scales) (tabl. 1). Despite based on many characters, these descriptions were incomplete, because light microscope could not provide sufficient magnification for the measurements of all necessary characters. For example, Penard (1904) did not indicate the breadth of fusiform scales. In part, diagnosis of Rainer (1968) contained information about length of oval scales only (tabl. 1).

The application of scanning electron microscopy allows study of the fine structure of *P. ambigua* scales and obtaining additional data for species identification (Takahashi, 1959; Bardele, 1981). As a result the three main types of periplast scales were marked out in *P. ambigua* by Nicholls and Dürrschmidt (1985) (tabl. 1).

Variation of scale shape of *P. ambigua* was considered as continuous series of changes of scales shapes which distinguished by only two clusters of scales (long/thin and short/broad scales) (Dürrschmidt and Patterson (1986–1987). In some following publications the quantitative characters of *P. ambigua* scales were indicated as a highly variable and either excluded from species descriptions (Siemensma and Roijackers, 1988) or only quantitative diapasons of scale ranges were indicated (Patterson and Dürrschmidt, 1988).

Then Siemensma (1991) and Mikrjukov (Микрюков, 2002) have provided morphological descriptions of *P. ambigua* based on both qualitative and quantitative character of periplast scales. Three scale types based on the scales shapes and their length and breadth ratios were distinguished by Mikrjukov (Микрюков, 2002) in the diagnosis of *P. ambigua*. These three types of scales constitute discrete scales groups according to their quantitative characters (tabl. 1).

Table 1. Periplast scale dimensions in *P. ambigua* (μm) according to literary data
Таблица 1. Размерные характеристики чешуек перипласта *P. ambigua* (мкм) по литературным данным

Authors	Fusiform scales		Naviculoid scales		Oval scales	
	Length	Breadth	Length	Breadth	Length	Breadth
Penard, 1904	20	–	12	3	5–6	3–3.5
Rainer, 1968	10–20	–	8–13	–	5–6	3–4
Nicholls and Dürrschmidt, 1985	10–15	0.4–0.7	6–10	1.5–2.5	4.5–6	3–4
Patterson and Dürrschmidt, 1988			1.5 x 4 to 2.5 x 8			
Siemensma, 1991	14	0.4–0.7	9	–	5–6	–
Микрюков, 2002	10–15	0.4–0.7	6–10	1.5–2.5	4.5–6	3–4

Therefore, both qualitative and quantitative characters were applied for the descriptions of *P. ambigua* by one group of authors (Penard, 1904, Rainer, 1968, Nicholls and Dürrschmidt, 1985, Siemensma, 1991, Микрюков, 2002). However, from another points only qualitative characters were considered as valuable for the diagnosis of *P. ambigua* (Patterson and Dürrschmidt, 1988; Siemensma and Roijackers, 1988). Thereby, at present opinions differ on interpretation of periplast scales as morphological characters of *P. ambigua*. Furthermore, the investigations on limits and type of variation of periplast scales in *P. ambigua* did not carry out.

The goal of our study was to recognize and evaluate the variability of quantitative characteristics of periplast scales in *P. ambigua*. Obtained data allow us to propose the own version of diagnosis of *P. ambigua* which might be used for the correct identification of the species.

Material and methods

The water samples containing the specimens of *P. ambigua* were collected by plankton net (diameter of mesh is 70 мm) in a pond near the village Khotov at the vicinity of Kyiv (Ukraine) on August 31, 2006.

Living specimens of *P. ambigua* and some other protists were maintained in Petri dishes with water at the room temperature (20–25°C) in the laboratory during three months. Over this period of time the liquid remained after the swelling and dissolving of rice seeds in the boiled water was used for feeding of maintained living culture of *P. ambigua*. Boiled precipitated water was also added into the Petri dishes with specimens of *P. ambigua*.

Specimens were examined from September to November, 2007 using light microscope (Carl Zeiss-50) under the magnification 160. The living cells of centrohelids were isolated, air-dried without preliminary fixation and examined under the scanning electron microscope JSM–35C at the magnifications 1200–18000.

Five periplasts of *P. ambigua* were studied. 935 scales belonging to three main types of scales (fusiform, naviculoid and oval) were used for the measurements.

The abbreviations of measurements used for the study are as the following: L — length of scales, B — breadth of scales, WR — width of rims. The width of scales rims was measured five times in connection with its different width in a various parts of the scale.

The original measurements of the siliceous scales were taken on the basis of the electron scanning micrographs using the computer program ScopePhoto 2.0.

The differences of morphological characters of periplast scales were estimated using discriminant analysis (Халафян, 2007). Twenty six undamaged and well-visible periplast scales of *P. ambigua* were used for the measurements of each from seven quantitative characters.

Following algorithms were developed for the identification of scale types: using length and breadth of scales and five measurements of width of scale rims (model I), using length of scales and five measurements of width of scale rims (model II), using breadth of scales and five measurements of width of scale rims (model III), using five measurements of width of scale rims (model IV), using length and breadth of scales and one measurement of width of scale rims (model V) and using length and breadth of scales (model VI).

The values of Wilks' Lambda statistics with corresponding Fisher statistics (F) and significance level (p) were determined for each of above listed models. Morphological characters of the *P. ambigua* periplast scales were accepted in accordance with proposed by Patterson and Dürrschmidt (1988) (fig. 1).

The scanning electron micrographs were taken in the Laboratory of Electron Microscopy of Kholodny Institute of Botany of National Academy of Sciences of Ukraine (Kyiv).

Results and discussion

The periplast scale dimensions are given in the table 2. Under these data the dimensional features of different types of scales overlap.

However, they can be identified using multivariate analysis with best results if set of 7 quantitative characters (model I) was analyzed (tabl. 3).

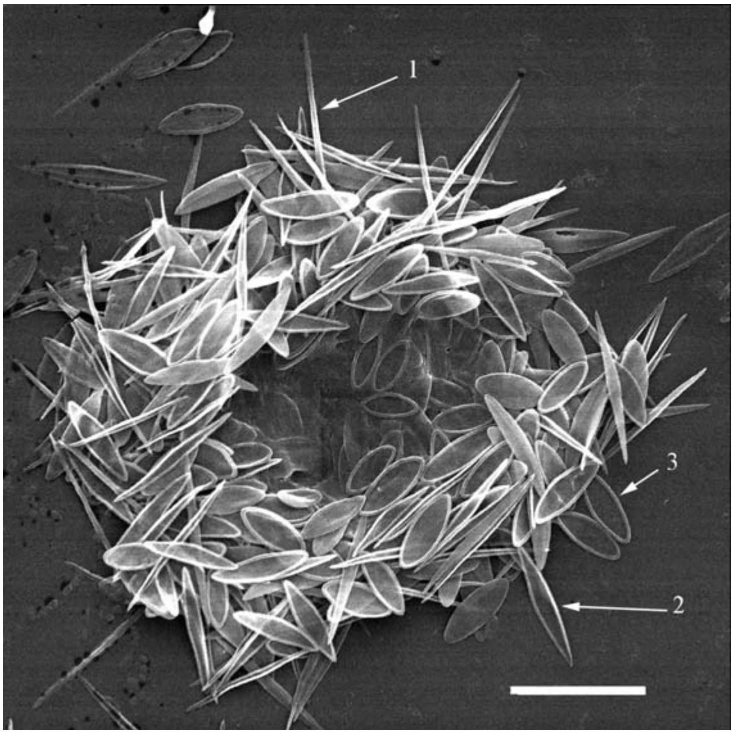

Fig. 1. Periplast of *Polyplacocystis ambigua*: *1* — fusiform scales; *2* — naviculoid scales; *3* — oval scales. Scale bar 10 μm.

Рис. 1. Перипласт *Polyplacocystis ambigua*: *1* — веретенообразная чешуйка; *2* — навикулоидная чешуйка; *3* — овальная чешуйка. Масштабная линейка 10 мкм.

Table 2. Dimensions of *P. ambigua* periplast scales (μm, own data)

Таблица 2. Размерные характеристики чешуек перипласта *P. ambigua* (мкм, собственные данные)

Variable	Type of scales	Number of scales	Minimum	Maximum	Mean	Standard Error
Length	fusiform scales	30	7.68	14.16	11.57	0.252
	naviculoid scales	33	5.47	11.94	8.26	0.288
	oval scales	131	3.67	9.22	5.50	0.089
Breadth	fusiform scales	30	0.38	1.11	0.72	0.039
	naviculoid scales	33	0.95	2.09	1.60	0.052
	oval scales	131	1.28	2.62	1.98	0.024
Wide of rim	fusiform scales	167	0.09	0.26	0.17	0.002
	naviculoid scales	119	0.11	0.33	0.21	0.003
	oval scales	455	0.14	0.38	0.25	0.002

Table 3. Effectiveness of classifications of *P. ambigua* periplast scales types using different sets of quantitative characters (models I–VI)

Table 3. Эффективность классификаций типов чешуек перипласта *P. ambigua* по разным наборам количественных признаков (модели I–VI)

Model	Wilks' Lambda	F	Degrees of freedom	p, %	% of correct identifycation
I	0.06	7.19	14; 34	< 0.0001	100
II	0.08	7.47	12; 36	< 0.0001	92.3
III	0.22	4.27	10; 38	< 0.0005	92.3
IV	0.10	6.19	12; 36	< 0.0001	84.6
V	0.13	12.30	6; 42	< 0.0001	84.6
VI	0.14	18.08	4; 44	< 0.0001	80.8

The distribution of scales in the space of two canonical variables is shown on the figure 2. Three scales types can be distinguished based on the value of the first canonical variable. It was shown in our study that fusiform and oval scales can not be surely differentiated by the value of second canonical variable. The first canonical variable can be interpreted as dimension and shape of scales. The second canonical variable construed as wide of rims.

Usage of 7 quantitative characters (model I) allow to identify the scale types for 100% scales of selected sample. For our observations the usage of character sets including five measurements of scale rim (models II, III and IV) doesn't make worse the quality of identification. Finally, models V and VI don't discriminate the naviculoid scales from other types. Thus the model I considered the most valuable for the description of quantitative dissimilarity between different types of scales.

As was stated above in the diagnoses of *P. ambigua* (Penard, 1904, Rainer, 1968, Nicholls, Dürrschmidt, 1985, Patterson, Dьrrschmidt, 1988, Siemensma, 1991, Микрюков, 2002) only length, breadth of periplast scales and ratio between these characters were in use (tabl. 1).

However, our current study shows that the application of these characters is insufficient for discrimination of scales types whereas the presence of three types of the scales is important characteristic of the species. However, using of a new quantitative character (width of scale rim with five consequent measurements) was efficient. The application of the character in combination with traditional dimensions, allows surely discriminate three types of scales in *P. ambigua* by dimensional characteristics.

The obtained dimensions of periplas scales of *P. ambigua* were used in redescription of the species below.

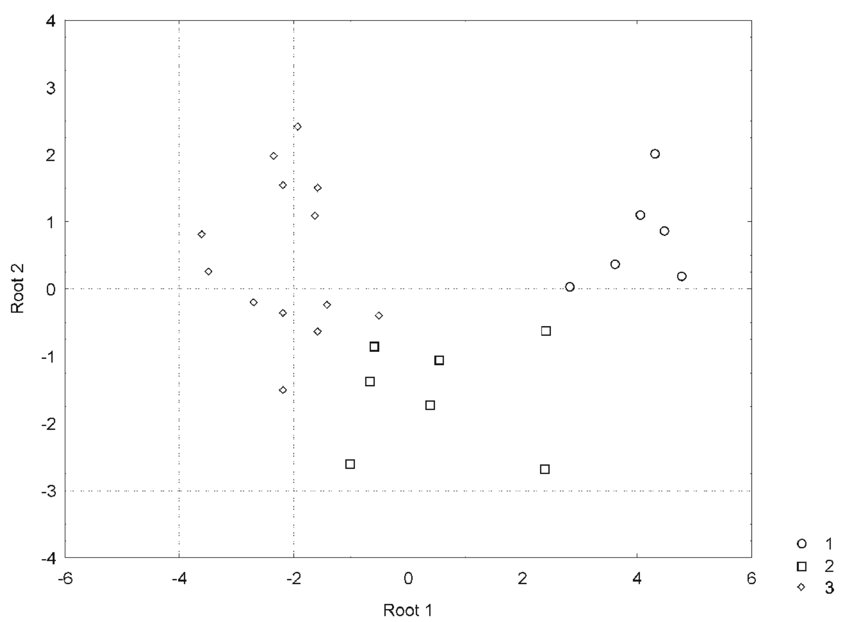

Fig. 2. Scatterplot of canonical scores computed for three scale types of *Polyplacocystis ambigua* using model I: *1* — fusiform scales; *2* — naviculoid scales; *3* — oval scales.

Рис. 2. Распределение трех типов чешуек перипласта *Polyplacocystis ambigua* в пространстве двух первых канонических переменных (модель I): *1* — веретенообразные чешуйки; *2* — навикулоидные чешуйки; *3* — овальные чешуйки.

Polyplacocystis ambigua (Penard, 1904) charact. emend

Diagnosis. Centrohelid heliozoan with periplast (20–50 μm in diameter) formed from scales with reticular external texture of distal surface. All scales surrounded by marginal rim which can be smooth or delicately ribbed. Scales are of three main shapes:

1) long, narrow fusiform scales with sharp-pointed apices (L: 7,68–14,16 μm (after Penard, 1904 L: 20; after Rainer, 1968 L: 10–20; after Nicholls and Dürrschmidt, 1985 L: 10–15; after Siemensma, 1991 L: 14; after Микрюков, 2002 L: 10–15); B: 0,38–1,11 μm (after Nicholls and Dürrschmidt, 1985, Siemensma, 1991 and Микрюков, 2002 B: 0,4–0,7); WR: 0,09–0,26 μm);

2) narrowly ellipsoidal or naviculoid scales (L: 5,47–11,94 μm (after Penard, 1904 L: 12; after Rainer, 1968 L: 8–13; after Nicholls and Dьrrschmidt, 1985 L: 6–10; after Siemensma, 1991 L: 9; after Микрюков, 2002 L: 6–10); B: 0,95–2,09 μm (after Penard, 1904 B: 3; after Nicholls and Dürrschmidt, 1985 B: 1,5–2,5; after Микрюков, 2002 B: 1,5–2,5); WR: 0,11–0,33 μm);

3) broadly elliptical or oval scales with rounded poles (L: 3,67–9,22 μm (after Penard, 1904 L: 5–6; after Rainer, 1968 L: 5–6; after Nicholls and Dürrschmidt, 1985 L: 4,5–6; after Siemensma, 1991 L: 5–6; after Микрюков, 2002 L: 4,5–6); B: 1,28–2,62 μm (after Penard, 1904 B: 3–3,5; after Rainer, 1968 B: 3–4; after Nicholls and Dürrschmidt, 1985 B: 3–4; after Микрюков, 2002 B: 3–4); WR: 0,14–0,38 μm).

Distribution. Switzerland (Penard, 1904) (type locality), Argentina, Canada, Chile, Germany, Japan, New Zealand, Sri Lanka, The Netherland (Микрюков, 2002), Ukraine (Polissya).

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Микрюков К. А. Центрохелидные солнечники (Centroheliozoa). — М. : КМК, 2002. — 136 с.

Халафян А. А. STATISTICA 6. Статистический анализ данных. 3-е изд. — М. : Бином-Пресс, 2007. — 512 с.

Bardele C. Symbiosis in cell evolution // Life and its environment on the early earth / Eds L. Margulis, W. H. Freeman. — San Francisco, 1981. — 419 p.

Dürrschmidt M., Patterson D. J. On the organization of the Heliozoa Raphidiophris ambigua and R. pallida Schulze // Ann. Sci. Nat. Zool. — 1986–1987. — **13**, Sér. 8. — P. 135–155.

Mikrjukov K. A. Revision of genera and species composition of lower Centroheliozoa. II. Family Raphidiophryidae n. fam. // Arch. Protistenkd. — 1996. — **147**, N 2. — P. 205–212.

Nicholls K. H., Dürrschmidt M. Scale structure and taxonomy of some species of Raphidocystis, Raphidiophrys, and Pompholyxophrys (Heliozoea) including descriptions of six new taxa // Can. J. Zool. — 1985. — **63**, N 8 — P. 1944–1961.

Patterson D. J., Dürrschmidt M. The formation of siliceous scales by Raphidiophrys ambigua (Protista, Centroheliozoa) // J. Cell Sci. — 1988. — **91**. — P. 33–39.

Penard E. Les Heliozoaires d'eau douce. — Geneve : Kündig, 1904. — 341 p.

Rainer H. Urtiere, Protozoa, Wurzelfussler, Rhizipoda, Sonnentiechen, Heliozoa // Die Tierwelt Deutschland. Bd. 56 / Ed. F. Dahl. — Jena : Gustav Fisher Verlag, 1968. — 176 S.

Siemensma F. J. Klasse Heliozoa Haeckel, 1866 // Nackte Rhizipoda und Heliozoea. 2 / Eds F. C. Page, F. J. Siemensma. — Stuttgart : Gustav Fisher Verlag, 1991. — S. 171–297.

Siemensma F. J., Roijackers R. M. M. A study of new and little-known acanthocystid heliozoeans, and a proposed division of the genus Acanthocystis (Actinopoda, Heliozoea) // Arch. Protistenkd. — 1988. — **135**, N 1–4. — P. 197–212.

Takahashi E. Studies on the genera Mallomonas, Synura and other plankton in freshwater by electron microscope // I. Bull. Yamagata Univ. (Agr. Sci.). — 1959. — **3**. — P. 117–151.