Morphological variability in clonal cultures of *Tetrastrum staurogeniaeforme (Chlorophyta, Chlorococcales)*

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Abstract. The variability of taxonomic features, such as number and length of the spines and shape of the coenobium, of *Tetrastrum staurogeniaeforme* was studied. Clone cultures, with a specific initial coenobium were used. The influence of nutritive solutions in different concentrations on intensive cultivating was investigated.

Key words: clone cultures, morphology, taxonomy, *Tetrastrum*, variability

Introduction:

Using the original picture of Schröder, Lemmerman (1915) diagnosed the species *Tetrastrum staurogeniaeforme* (Schröd.) Lemm. as possessing a four-cell coenobium, rhomboid or square, mostly with 5 very short spines.

In the first monograph on genus *Tetrastrum* (Ahlstrom & Tiffany 1934) attention was paid on the strong variability of species. Hindák (1980, 1984), who investigated natural material and laboratory cultures of *T. staurogeniaeforme*, emphasized the strong variability of species in number and length of spines on the cells. His maintained that, as identification of the species was relatively wide-ranging, some established taxa in genus *Tetrastrum* were identical with *T. taurogeniaeforme* species. Komárek & Fott (1983) also doubted that the length and structure of spines were suitable for use as taxonomic features. They contested the existence of the following varieties and forms described earlier by different authors, namely: *T. s.* var. *longispinum* G.M. Smith, *T. s.* f. *crassispinosus* Hortob. & Németh, *T. s.* f. *crassispinum* Hortob., *T. s.* f. *obtusum* Hortob, and *T. s.* f. *exaltatum* Hortob.

The present article is an extension of our study on the variability of the species *T. staurogeniaeforme*, based on natural samples (Velichkova & Kiryakov 2005). The purpose of the study was to analyze the variability of taxonomic features, namely, the number of spines on the cells and their length under intensive cultivation in different nutritious solutions with varying gradient of concentration. We also investigated the shape of the coenobium (the type of the coenobium) and the extent (in per cent) of its overlapping in the clone culture.

Materials and methods

On the basis of literature data on genus *Tetrastrum* (diagnosis and iconographies) and on our own observations we have typified five different coenobia of the present species, according to the way of cell arrangement (Fig. 1).

From *T. staurogeniaeforme* strain no. 8828 in the algae collection of the Paisiy Hilendarski University of Plovdiv (PACC) we isolated five clones with initial coenobium corresponding to each of the coenobia types established by us (Fig. 2). The strain comes from the phytoplankton of the Mandra Reservoir (Bourgas

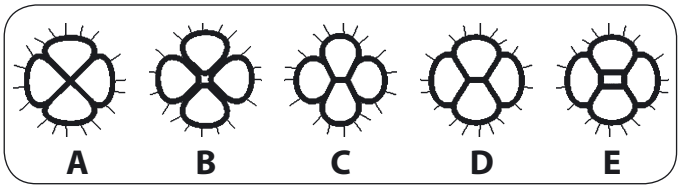

Fig. 1. Basic types of coenobia in genus *Tetrastrum*:
A – a square coenobium, the top parts of the four cells are in very close contact, the spines are shorter than the cells; **B** – a square coenobium, with opening in the interior, the spines are shorter than the cells; **C** – a rhomboid coenobium, of different length along the two symmetric axes, with crosswise laying cells – as the top parts of two cells have small contact area, the spines are shorter than the cells; **D** – a rhomboid coenobium, of different length along the two symmetric axes, with four cells distributed crosswise – as the two cells are in contact with most of their inner parts, the spines are shorter than the cells; **E** – a rhomboid coenobium, of different length along the two symmetric axes, with four cells distributed crosswise, the interior of the coenobium is with a rectangular opening, the spines are shorter than the cells.

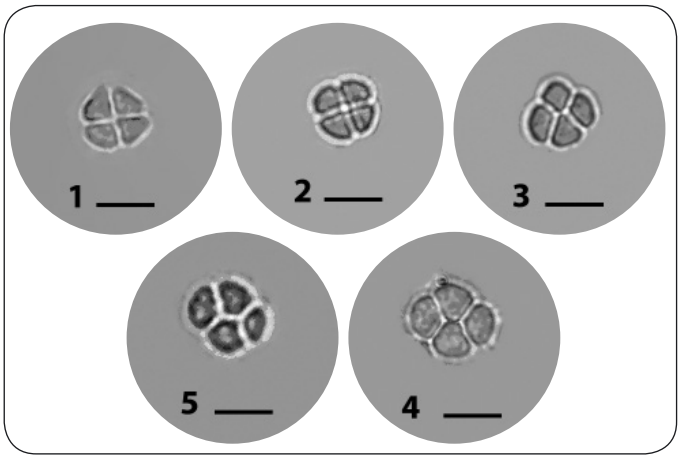

Fig. 2. Initial coenobium of *T. staurogeniaeforme* strain no. 8828:
1, clone no. 8828/3; **2**, clone no. 8828/1; **3**, clone no. 8828/5; **4**, clone no. 8828/4; **5**, clone no. 8828/15.
Scale bar 10 μm.

district). The five clones were cultivated in nutritious media with different composition and concentration gradient (BBM, G_1 and Z).

The obtained results on the variability of the five clones were identical. Therefore, we comment here only on two of them: clone no. 8828/1 with initial coenobium of type **B**, and clone no. 8828/4 with initial coenobium of type **D**.

The cloning was accomplished by the method of capillary pipette (Stein 1973), modified by Mladenov & Furnadžieva (1995). The initial clone was cultivated preliminarily in luministat, with continuous light conditions of 7 klux, 24±1 °C temperature and continuous aeration. These clone cultures (algae suspensions) served as initial material for intensive cultivation in nutritious solutions with a different gradient of concentration.

The intensive cultivation was conducted in a cultivating installation according to Dilov & al. (1972). It was completed in Ackerman test-glass in light conditions 15/9 light/dark and 12 klux. During this light period, the suspensions were blown with 100 l air/h/100ml mixture, enriched with 1 % CO_2. Three nutritious media were used: BBM (Archibald & Bold 1970), G_1 – Göttingen Basal Medium 1 (Schlösser 1982) and Z – Zehnder Medium (Staub 1961) – dissolved, natural and concentrated. We have increased concentration by adding 3 $g.l^{-1}$ NaCl to the nutritious media and have decreased it by 10× dissolving (without changing the concentration of microelements).

The microscopic investigation was accomplished by studying 500 coenobia for every variant of the experiment so as to read the coenobium type, and 50 coenobia were microscopically analysed for number and length of the spines.

Results

Number and length of spines on the cells

The number and length of the spines are used as taxonomic features for differentiation of species and infraspecific taxa in the genus. Therefore, we were interested to learn more about their variability not only in the nature, but also in the clone cultures.

In regard to the number of spines we have found that in the normal nutritious media (BBM, G_1, Z) the number of spines on the cells exceeds 5-6, while in the concentrated media the number of spines on the cells is under 3-6 (Table 1).

Table 1. Number of spines on the cells of the coenobium in clones no. 8828/1 & 8828/4

Media / Clone	1/10BBM	BBM	BBM+3g.l^{-1}	1/10G1	G_1	G1+3g.l^{-1}	1/10Z	Z	Z+3g.l^{-1}
8828/1	4–6	5–6	3–5	4–6	5–6	3–6	4–6	5–6	3–5
8828/4	4–5	4–6	3–5	4–6	4–6	3–6	4–6	4–6	3–6

The length of the spines has shown low variability within the range of 1.5–2.5 μm. Shorter spines were seen in dissolved nutritious media, whereas long spines were found in concentrated ones, especially in the Z concentrated medium (Table 2).

Table 2. Length of spines on the cells of the coenobium in clones no. 8828/1 & 8828/4; x – average, σ – standard deviation, CV – coefficient of variation

Clone \ Media	Length (μm) – clone 8828/1			Length(μm) – clone 8828/4		
	min–max	x±σ	CV [%]	min–max	x±σ	CV [%]
1/10BBM	1.5–2.0	1.6±0.5	31.2	1.5–2.0	1.9±0.6	31.5
BBM	1.5–2.5	2.0±0.6	30.0	1.5–2.5	2.0±0.6	30.0
BBM+3g.l^{-1}	1.5–2.5	2.2±0.7	31.3	2.0–2.5	2.2±0.7	31.3
1/10G_1	1.0–2.5	1.7±0.5	29.4	1.5–2.0	1.8±0.6	31.1
G_1	1.5–2.5	2.0±0.6	30.0	1.5–2.0	2.0±0.6	30.0
G1+3g.l^{-1}	1.5–2.5	2.3±0.7	30.4	1.5–2.5	2.3±0.7	30.4
1/10Z	1.0–2.5	1.8±0.6	31.1	1.5–2.5	1.7±0.5	29.4
Z	2.0–2.5	2.0±0.6	30.0	1.5–2.5	1.9±0.6	31.5
Z+3g.l^{-1}	2.0–2.5	2.4±0.7	29.1	2.0–2.5	2.4±0.7	29.1

In the monograph on *Tetrastrum* (Ahlstrom & Tiffany 1934) based on data by different authors the number of spines for *T. staurogeniaeforme* was recorded as 1-8 at the vacant end of the cell, with their length ranging between 3–10 μm. Hortobágyi (1968), after studying species from natural samples, counted five spines on each coenobium cell, 1 to 4 μm long.

In his investigations of *T. staurogeniaeforme* Hindák (1984) found great variability in the number and length of the spines in natural samples, namely: 1–20 in number and 5–29 μm long (Plate104, Figs 1-5). The clone presented by the author (Hindak 1984) (Plate 104, Figs 6-11) for study, Hindak 1978/30, isolated from the Stávek Fish Farm, had shown a different number of spines 3-6, which varied in length from 1–2 μm, according to the presented scale. He observed a rare coenobium without spines.

Our results on the number and length of the spines in the different clones during intensive cultivation corresponded with those obtained by Hindák (1984).

Types of coenobia in clone cultures

In our study of intensive cultivation of clone no. 8828/1 in three variants of the nutritious media we have observed that the rhomboid type **D** claims the highest percentage share of coenobia, followed by the initial square coenobium type **B**. Lower in percentage share were the coenobia of type **C** and the seldom occurring types **A** and **E** (Fig. 3).

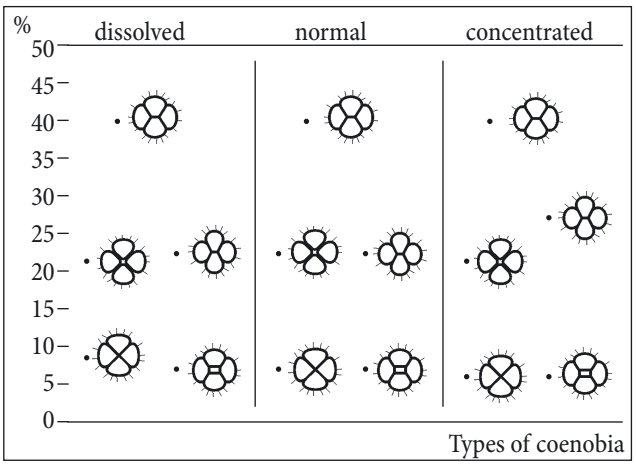

Fig. 3. Prevailing types of coenobia (in %) during intensive cultivation in nutritive medium BBM with different concentration (clone no. 8828/1).

During intensive cultivation of clone no. 8828/4 in nutritious media with gradient of concentration the initial type **D** appeared as the dominant coenobium type. In the three nutritious media BBM, G_1 and Z and their variants, after the initial coenobium, we have found the types **B** and **C** in close distribution. Types **A** and **E** occurred seldom (Fig. 4).

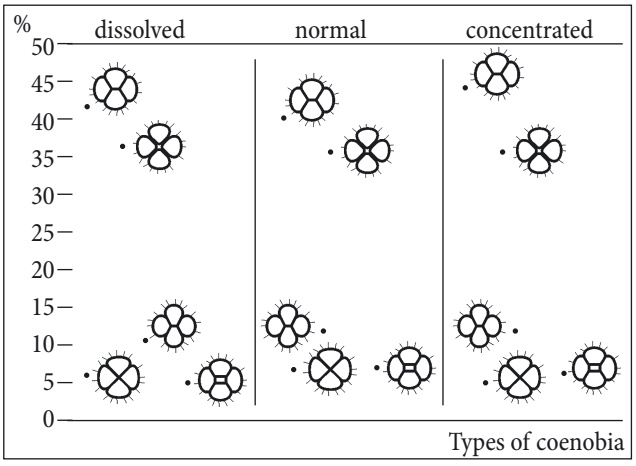

Fig. 4. Prevailing types of coenobia (in %) during intensive cultivation in nutritive medium Z with different concentration (clone no. 8828/4).

Discussion

In our study of natural samples (Velichkova & Kiryakov 2005) the number of spines on the cells in *T. staurogeniaeforme* varied between 3-7, but mostly they were five. The size of the spines was in the range of 1–3 μm. A single specimen was found where the spines were longer than the cell itself (8–20 μm).

Hindák (1984), who studied natural samples of *T. staurogeniaeforme* with a focus on the variability of the number and length of spines, had found respectively 2-3 spines in number, 15–29 μm long.

According to Komárek & Fott (1983), *T. staurogeniaeforme* showed strong variability in the length of spines, which could vary from 3–23 μm in different populations. Similarly, the number of spines on each cell can be 3-(5)-7.

The study of Hegewald & Schnepf (1976) of a Hegewald 1975/55 strain of *T. staurogeniaeforme* isolated from a reservoir in South India and then cultured in nutritious media retgistered low variability in the form of coenobium and the length of spines. In their illustrations they showed a rhomboid coenobium with short spines on the cells and a frequently encountered coenobium without spines (Abb. 1a-d). They were the first to publish the structure of the spines by electron microscopy.

As it was underlined earlier, Hindák (1984) in the cultured strain Hindák 1978/30 isolated from the Stávek Fish Farm also saw a coenobium with short spines under the cells and a rare case of coenobium without spines (Plate 104, Figs 6-11).

In our study of the five *T. staurogeniaeforme* clones the spines were always shorter than the cells and we seldom observed a spineless coenobium. The length of the spines remained constant in the three nutritious media with various gradient of concentration.

Most of the authors who had studied *T. staurogeniaeforme* presented in their iconographies a rhomboid coenobium, often of type **D**. The five types of coenobium established by us correspond to the diagnosis of species and the figures given by Komárek & Fott (1983). In our study we have established that type **D** was dominant in all nutritious media, regardless of the initial coenobium.

The results of our study regarding the variability of the number and length of spines in clone cultures of *T. staurogeniaeforme* cultivated in different nutritious media, as well as the literature data on the variability of clones of that particular species (Hegewald & Schnepf 1976; Hindák 1984) showed low variability of these taxonomic features. This gives us ground to maintain that the taxonomic status of the variety based of the length of spines must be acknowledged as *T. s.* var. *longispinum*.

Further analysis must be performed on the taxa described by the thickness and sharpness of spines: *T. s.* f. *crassispinosus*, *T. s.* f. *crassispinum*, *T. s.* f. *obtusum*. Furthermore, the electron microscopic structure of the spines from Hegewald & Schnepf (1976) should be taken in account.

References

Ahlstrom, E. & Tiffany, L. 1934. The algal genus *Tetrastrum*. – Amer. J. Bot., **21**: 499-507.

Archibald, P. & Bold, H. 1970. Phycological studies, XI. The genus *Chlorococcum* Meneghini. – Univ. Texas Publ. **7015**.

Dilov, Ch.; Božkova, M. & Staev, S. 1972. Praktische Anlage zur Kultivierung von Mikroalgen unter Laboratoriumsbedingungen. – Dokl. Selskoct. Akad. "George Dimitrov", **4**(2): 169-175 (in Russian).

Hegewald, E. & Schnepf, E. 1976. Zellwandultrastrukturen in der Grünalgengattung *Tetrastrum* Chod. – Algol. Stud., **17**: 389-393.

Hindák, F. 1980. Studies on the chlorococcal algae (*Chlorophyceae*). II. – Biol. Práce Slov. Akad. Vied, **26**(6): 1-196.

Hindák, F. 1984. Studies on the chlorococcal algae (*Chlorophyceae*). III. – Biol. Práce Slov. Akad. Vied, **30**(1): 279-285.

Hortobágyi, T. 1962. Algen aus den Fischteichen von Buzák V. – Nova Hedwigia, **4**(1-2): 21-53.

Hortobágyi, T. 1967. *Tetrastrum*-Arten aus den Fischteichen von Buzsák, Ungarn. – Rev. Roumaine Biol., Sér. Bot., **12**(1): 41-46.

Hortobágyi, T. 1968. Die Algen Vietnams. III. *Euglenophyta*, *Chlorophyta* I. – Acta Bot. Acad. Sci. Hung., **8**: 41-58.

Hortobágyi, T. & Németh, J. 1963. Neue Algen aus den Fischteichen von Gödöllö. – Acta Bot. Acad. Sci. Hung., **9**(3-4): 307-321.

Komárek, J. & Fott, B. 1983. *Chlorophyceae* (Grünalgen) Ordnung: *Chlorococcales*. – In: **Huber-Pestalozzi, G.** (ed.), Das Phytoplankton des Sübwassers. Systematik und Biologie. **7**(1). Stuttgart.

Lemmerman, E., Brunnthaler, J., Pascher, A. 1915. Die Süsswasser – Flora Deutschlands, Österreichs und der Schweiz. *Chlorophyceae* II. *Tetrasporales*, *Protococcales*, Einzellige Gatungen Unsicherer Stellung. Heft 5. Jena.

Mladenov, R. & Furnadžieva, S. 1995. Morphologische Veränderlichkeit klonaler Kulturen von *Scenedesmus falcatus* Chod. und *Scenedesmus acuminatus* (Lagerh.) Chod. – Algol. Stud., **79**: 93-107.

Schlösser, U. 1982. Sammlung von Algenkulturen. – Ber. Deutsch. Bot. Ges., **95**: 181-276.

Smith, G.M. 1926. The plankton algae of the Okoboji region. – Trans. Amer. Microscop. Soc., **45**(3): 156-233.

Staub, R. 1961. Ernärungsphysiologisch–autökologische Untersuchungen an der planktonische Blaualga *Oscillatoria rubescens* DC. Schweis. – Z. Hydrol. (Aarau), **23**: 82-198.

Stein, J. 1973. Handbook of Phycological Methods. Culture Methods and Growth Measurements. Cambridge Univ. Press, Cambridge.

Velichkova, K., Kiryakov, I. 2005. The morphological variability of *Tetrastrum staurogeniaeforme* (Schröd.) Lemm. in the nature. – In: **Gruev, B., Nikolova, M. & Donev, A.** (eds), Proc. Balkan Sci. Conf. Biol. Part 1, pp. 257-265. Plovdiv (in Bulgarian).