Morphological variability of *Tetrastrum triangulare* and *T. komarekii* (*Chlorophyta*, *Chlorococcales*) in clonal cultures

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Dedicated to Assoc. Prof. Dr Stefan Draganov in honour of his 75th anniversary

Abstract. Variability of some diagnostic features, such as pyrenoids, syncoenobia, and type and size of coenobia of two species of the genus *Tetrastrum, T. triangulare* and *T. komarekii*, were studied in extensive and intensive cultures. Clone cultures with a specific initial coenobium for both species were used. Variability in clone cultures was traced out at three different temperatures. The influence of nutritious solutions with different concentrations was investigated by intensive cultivation. The presence of a pyrenoid is the main diacritical feature for the two species, as it was observed in all *T. triangulare* cells, but not in *T. komarekii* cells. Development of syncoenobia can be used as an additional diacritical feature. Regarding the coenobia size in our cultures, *T. komarekii* has shown by about 1/3 smaller size than *T. triangulare*.

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

Introduction

Tetrastrum komarekii is described by Hindák (1977) and its synonyms are *Crucigenia quadrata* Morr. sensu auct. post. p.p. and *T. triangulare* (Chodat) Komárek sensu auct. post. p.p. Square or rhomboid coenobia with four crosswise arranged cells are typical for the species. This species is closest to *T. triangulare* and the only difference between them is the lack of pyrenoid in *T. komarekii*.

Tetrastrum triangulare (Chodat) Komárek (basionym *Staurogenia triangulare* Chodat) is characterized by square coenobia, forming together syncoenobia of four coenobia each, and by the presence of a pyrenoid easily observed in the cell.

According to Hindák (1980), the presence or absence of a pyrenoid is one of the permanent genetically fixed features. That is why, the species *T. komarekii* can be clearly distinguished from the other species by the lack of a pyrenoid. Komárek & Fott (1983) and John & al. (2002) believe that it is necessary to study further the connections between these two species.

The aim of this study is to investigate the diacritical features in the cultures of these two species and in particular the presence or absence of a pyrenoid and the formation of syncoenobia. Another aim is to analyze the coenobia sizes and types developed by these two species and, finally, to trace out variability of the above-mentioned features after extensive and intensive cultivation of clone cultures in different nutritious media and at different temperatures.

Material and methods

On the basis of literary data on genus *Tetrastrum* (diagnostic and iconographic) and of our own observations we have typified five different coenobia in the present species, according to the patterns of cell arrangement (Fig. 1).

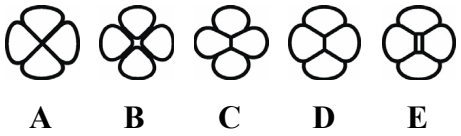

Fig. 1. Basic types of coenobia in genus *Tetrastrum*: **A** – a square coenobium, the top parts of the four cells are in very close contact; **B** – a square coenobium, with opening in the interior; **C** – a rhomboid coenobium of different lengths along the two symmetric axes, with crosswise arranged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

From the species of *T. triangulare*, five clones with initial coenobium corresponding to each of the coenobia types established by us were isolated from strain no. 8713, maintained in the algal collection of the Paisiy Hilendarski University of Plovdiv (PACC). The strain comes from the phytoplankton of Mandra Reservoir, Bourgas district, East Bulgaria. The five clones were cultivated in the nutritious media BBM, G_1 and Z, with different composition and concentration gradient.

The results obtained on the variability of the five clones were identical. Therefore, only two of them will be commented on here: clone no. 8713/9 with initial coenobium of type B, and no. 8713/22 with initial coenobium of type D (Fig. 2).

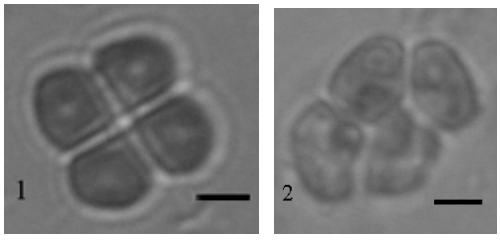

Fig. 2. Initial coenobium of *T. triangulare*, strain no. 8713: **1**-clone no. 8713/9; **2**-clone no. 8713/22. Scale bar 10 μm.

The strain no. 510 of *T. komarekii*, originally defined as Řeháková 1960/4, was received from the algal collection of CCALA at Třeboň, the Czech Republic. Two clones were isolated from the strain: no. 5084/1 with initial coenobium of type B, and no. 5084/8 with initial coenobium of type E (Fig. 3).

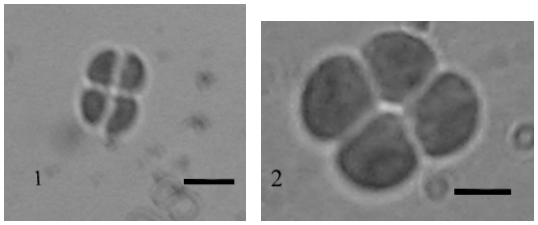

Fig. 3. Initial coenobium of *T. komarekii* strain no. 5084: **1**-clone no. 5084/1, **2**-clone no. 5084/8. Scale bar 10 μm.

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 an Ackerman test-glass, under light conditions of 15/9 light/dark and 12 klux. During that light period, the suspensions were blown with 100 l air/h/100 ml 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), in three concentrations – 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).

Variability of the clone cultures under intensive cultivation was studied under three different temperatures: minimum (16 °C), optimum (24 °C) and maximum (36 °C).

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 the coenobium size.

Results

Presence and absence of pyrenoids

The presence or absence of pyrenoids as a main diacritical feature was traced out in two clones of *T. triangulare* (8713/9, 8713/22). We have observed pyrenoids

in the coenobia cells of both studied clones in all experimental setups (extensive cultivation and intensive cultivation in different nutritious media and temperatures). The pyrenoids were clearly visible under the light microscope.

We analyzed two clones of *T. komarekii* (5084/1, 5084/8) but no pyrenoid was observed. This supports Hindák's (1980) statement that the presence or absence of a pyrenoid belongs to the stationary, genetically fixed features. In some phases of the ontogenesis, or owing to the nutritious conditions in the environment, the pyrenoid may be indistinct in some specimens, but its presence or absence can be determined in nature, as well as in cultures. That is why, due to the absence of a pyrenoid, *T. komarekii* can be clearly distinguished from all other species of the genus. This means that the main diacritical feature between the two species is clearly indicated and studied in the strains Hindák 1975/155 and 1975/174 (Hindák 1977), which was confirmed by us also for the clone cultures.

Syncoenobium

In his diagnosis of *T. komarekii*, Hindák (1977) mentioned syncoenobia of 16, or even of more cells, whereas Komárek & Fott (1983) noted that in *T. triangulare* 16-celled daughter syncoenobia seldom remained connected for a longer time.

In our clone cultures, syncoenobia were observed only in the *T. triangulare* clones. During the process of intensive cultivation, growth is faster and daughter coenobia depart relatively faster. In normal and dissolved media a greater number of syncoenobia were observed. Formation of a greater number of syncoenobia depended also on temperature. Temperature increase caused enhancement the number of syncoenobia. This might be explained with ecological adjustment to the density of the environment.

Our study supports the opinion of Komárek & Fott (1983) who maintained that the presence of syncoenobia can be regarded as an additional diacritical feature between the two species.

Types of coenobia in the clone cultures

***Tetrastrum triangulare* (clones no. 8713/9 & 8713/22)**

Microscopic analysis of the different percentage of coenobium types has shown that in extensive cultivation of clone no. 8713/9 type D prevailed (35 %), followed by the initial type B (25 %), and then by types E (16 %), C (14 %), and A (10 %). Under the same conditions for clone no. 8713/22, again type D (42 %) showed the highest percentage, which is the initial type for this clone (Fig. 4)

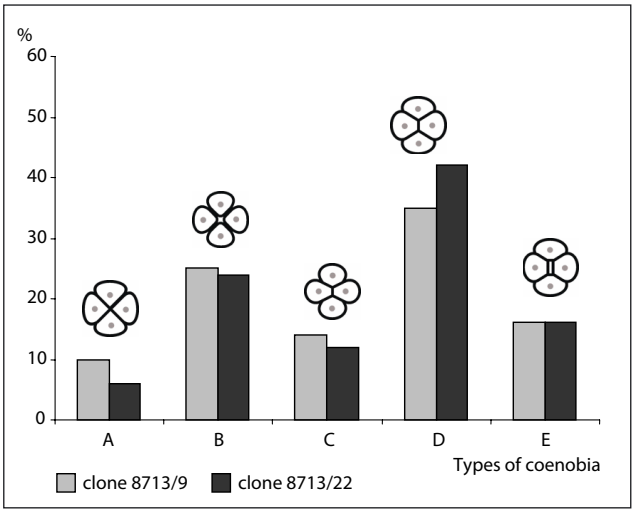

Fig. 4. Prevailing types of coenobia in *T. triangulare*, strain no. 8713 (in %) during extensive cultivation – clones no. 8713/9 & 8713/22**.**

In our study, during intensive cultivation in the nine nutritious media variants we observed similar results for both clones. For no. 8713/9 in BBM nutritious media, type D registered the highest percentage. It was followed by coenobium B and by lower values of the types C, E, and A (Fig. 5).

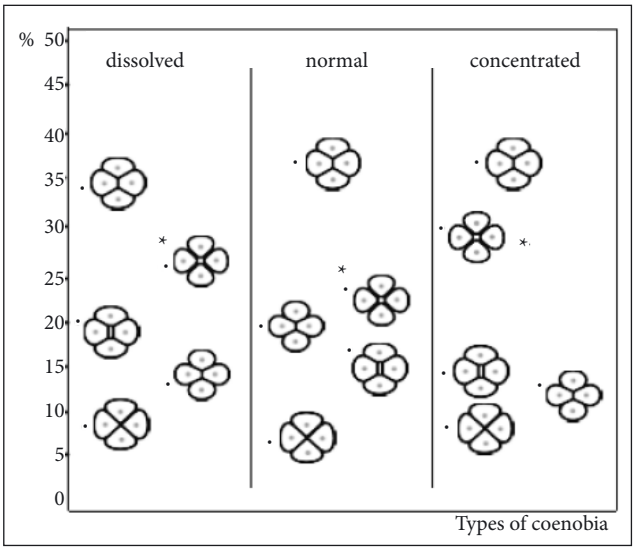

Fig. 5. Prevailing types of coenobia in *T. triangulare*: strain no. 8713 (in %) during intensive cultivation in nutritious medium BBM with different concentration – clone no. 8713/9. * – Initial coenobium.

***Tetrastrum komarekii* (clones no. 5084/1 & 5084/8)**

We cultivated clone no. 5084/1 under extensive conditions, where the prevailing coenobia type was E (35 %), followed by the initial type B (28 %) and type D (20 %). Lesser percentage was registered by the coenobia types C (10 %) and A (7 %). Under the same conditions for clone no. 5084/8, coenobia of the initial type E (38 %) occurred most frequently, followed by types B (20 %) and D (20 %), while types C (14 %) and A (8 %) registered a much lesser percentage (Fig. 6).

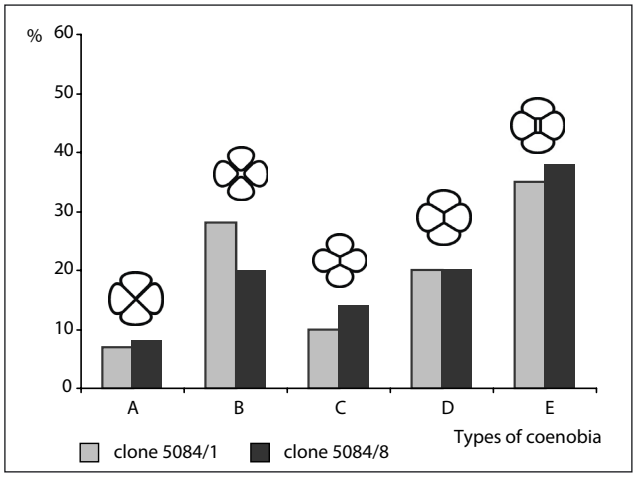

Fig. 6. Prevailing types of coenobia in *T. komarekii* strain no. 5084 (in %) during extensive cultivation in clones no. 5084/1 & 5084/8.

The results obtained during intensive cultivation of both clones were not unidirectional, as they were for *T. triangulare*. For clone no. 5084/1 and nutritious medium G_1, coenobia types E and B showed similar percentage and were followed by types A, D and C (Fig. 7).

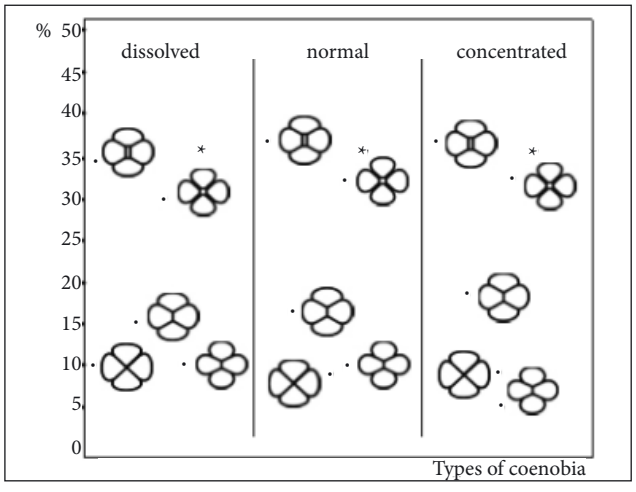

Fig. 7. Prevailing types of coenobia in *T. komarekii* strain no. 5084 (in %) during intensive cultivation in nutritive medium G_1, with different concentration in clone no. 5084/1. * – Initial coenobium.

A close pattern was observed for BBM nutritive medium. The only exception was observed in the dissolved nutritious medium Z, where the prevailing coenobia type was the initial one (irrespective of the small percentage difference) – type B (35 %), followed by type E (32 %).

Clone no. 8713/8 in all three concentrated nutritious media did not develop any coenobia. On the contrary, the percentage of coenobia greatly increased in the dissolved and normal nutritious media. The prevailing coenobia type was the initial one – type E, followed by types B and D and, with lesser values, by C and A.

Coenobia sizes

Owing to the fact that there are different coenobia types (rhomboid and square), the length and width of the coenobia were taken into account for the rhomboid ones, whereas for the square ones only one of the sizes was examined.

***Tetrastrum triangulare* (clones no. 8713/9 & 8713/22)**

Under extensive conditions, clone culture no. 8713/9 had on the average a length of 6.8 μm and a width of 6.2 μm. Coenobia sizes varied from 5.4 μm to 9.0 μm in length and 4.5–7.5 μm in width (Table 1). Under optimum temperature, coenobia were the largest (7.6 μm), contrary to the minimum and maximum temperatures, when the values were lower (7.2 μm and 7.1 μm). The average width was the greatest under the optimum growth temperature (7.0 μm) and under maximum temperature reached 6.5 μm.

Table 1. Variability of coenobia length and width under different cultivation conditions in *T. triangulare* strain no. 8713, clone no. 8713/9; x – average; σ – standard deviation; CV – coefficient of variation.

Clone	Conditions of cultivation	Length (μm)			Width (μm)		
		min-max	x±σ	CV [%]	min-max	x±σ	CV [%]
8713/9	Extensive	5.4–9.0	6.8±0.9	13.2	4.5–7.5	6.2±0.8	12.9
	T min	3.2–7.8	7.2±1.0	13.8	3.6–6.8	6.6±0.9	13.6
	T opt	4.0–8.8	7.6±1.0	13.1	3.2–7.6	7.0±1.0	14.0
	T max	4.0–8.0	7.1±1.0	14.1	3.2–6.8	6.5±0.9	13.8

During intensive cultivation in different nutritious media, the length varied from 7.9 μm to 8.6 μm. The longest were the coenobia grown in the dissolved variant of BBM medium: 8.6 μm (Fig. 8). The average length decreased in the dissolved variant of nutritious media G_1 (7.9 μm).

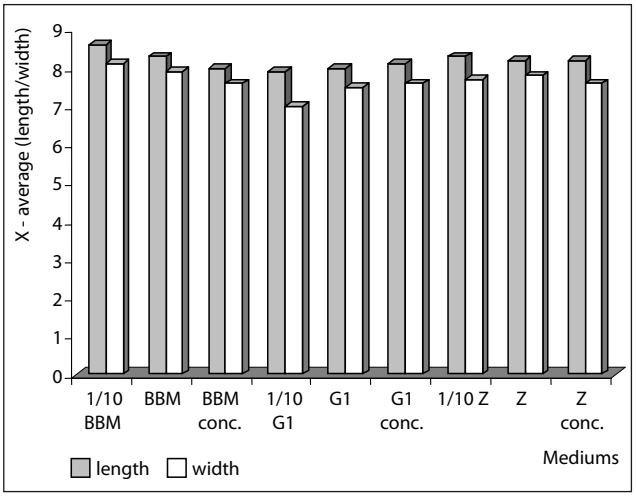

Fig. 8. Average coenobia sizes (μm) in *T. triangulare*, strain no. 8713 for intensive cultures in different nutritious media, clone no. 8713/9.

The average width of the intensive cultures in different nutritious media also varied extensively: from 7.0 μm to 8.2 μm. The highest value of this feature was reached in BBM nutritious media: 8.1 μm. Lower values were measured in G_1 nutritious medium: 7.0 μm. Similar results were registered for clone no. 8713/22.

***Tetrastrum komarekii* (clones no. 5084/1 & 5084/8)**

Under extensive conditions for the clone culture no. 5084/8, the average length of the coenobia was 6.9 μm and the average width was 6.0 μm. Coenobia sizes varied between 4.2 μm and 9.0 μm in length and between 3.5 μm and 7.5 μm in width (Table 2). Under temperature influence the average length varied between 6.3 μm and 7.0 μm (Table 2). Under optimum temperature the coenobia were the largest: 7.0 μm. The average width varied from 5.3 μm to 6.3 μm under the same conditions and reached the maximum size of 6.3 μm under optimum temperature.

Table 2. Variability of coenobia length and width under different cultivation conditions in *T. komarekii* strain no. 5084, clone no. 5084/8; x – average; σ – standard deviation; CV – coefficient of variation.

Clone	Conditions of cultivation	Length (μm)			Width (μm)		
		min-max	x±σ	CV [%]	min-max	x±σ	CV [%]
5084/8	Extensive	4.2–9.0	6.9±0.9	13.0	3.5–7.5	6.0±0.8	13.3
	T min	4.0–8.6	6.3±0.9	14.2	3.2–7.5	5.8±0.8	13.7
	T opt	4.0–9.0	7.0±1.0	14.0	3.2–8.6	6.3±0.9	14.2
	T max	4.5–8.0	6.4±0.9	14.0	3.5–7.4	5.3±0.7	13.2

In relation to the type of nutritious media, again the largest were the coenobia grown in G_1 medium, with average length of 7.2 μm and average width of 6.4 μm (Fig. 9). In Z nutritious medium, the average length and width were 6.4 μm and 5.3 μm, respectively.

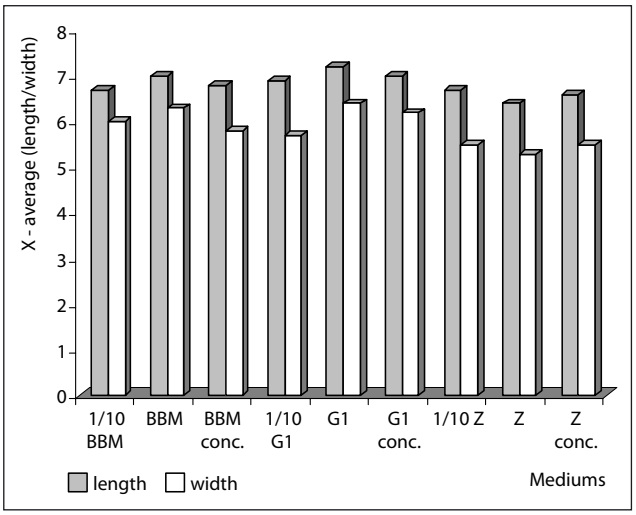

Fig. 9. Average coenobia sizes (μm) in *T. komarekii* strain no. 5084 for intensive cultures in different nutritious media, clone no. 5084/8.

Similar results were obtained for clone no. 5084/1.

Most authors who had studied natural materials from both species reported larger coenobia sizes in *T. triangulare* (Table 3).

Table 3. Coenobia size (μm) of *T. komarekii* and *T. triangulare* according to different authors and the present study.

Authors	*T. komarekii*	*T. triangulare*
Hindák (1977) (natural sample)	5.0–12.0 × 5.0–11.0 μm	5.0–17.5 μm
John & al. (2002) (natural sample)	5.0–15.0 μm	7.0–15.0 μm
Komárek & Fott (1983) (natural sample)	5.0–12.0 × 5.0–11.0 μm	5.0–17.5 μm
Echenique & al. (2004) (natural sample)	10.0–13.0 μm	10.0–13.0 μm
Present study (natural sample)	6.5–12.0 μm	7.5–12.0 μm
Present study (clone cultures)	5.3–7.0 μm	6.2–9.0 μm

Discussion

Our study of cultures showed that the presence of a pyrenoid is the main diacritical feature for the two species, as it was observed in all *T. triangulare* cells but not in *T. komarekii* cells. The development of syncoenobia can be used as an additional diacritical feature. Regarding the coenobia sizes in our cultures, *T. komarekii* has shown by 1/3 smaller sizes than *T. triangulare*. This corresponds to the data reported by other

authors for natural materials of these species. The sizes varied between 5.3–7.0 μm for *T. komarekii* cultures and between 6.2-9.0 μm for *T. triangulare,* within the limit under 3.0 μm.

Mention deserves the fact that *T. triangulare* clones grew better and developed strongly in all studied media variants, while *T. komarekii* were relatively grew slower and developed to a lesser extent.

Furthermore, it was established that *T. triangulare* was better developed and formed larger coenobia in different BBM nutritious media, whereas for *T. komarekii* similar results were observed in G_1 nutritive medium.

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