

Types and division of the coenobia of genus *Tetrastrum* (*Chlorophyta*, *Chlorococcales*)

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Abstract: The aim of this study was to investigate shape variability in clone cultures of *Tetrastrum staurogeniaeforme*, *T. triangulare*, *T. komarekii*, and *T. heteracanthum* coenobia. Clone cultures with a species-specific initial coenobium were used. The influence of different nutrient solutions was investigated by intensive cultivation. For each of the investigated species, five coenobia with different shape were observed. In addition to the observed different types of coenobia formation, it was found that the most pronounced type can differ from the initial coenobium. Division of cells and formation of young coenobia were traced out in synchronous cultures. It was found out that the daughter coenobium shape was not always identical to that of the mother coenobium.

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

Introduction

The genus *Tetrastrum* was initially described by Chodat (1895), who based his investigation on the *Staurogenia* species characteristic with presence of spines on cells. Ahlstrom & Tiffany (1934) presented the first monographs on genus *Tetrastrum*, consisting of five species containing two infraspecific taxa. According to Ahlstrom & Tiffany (1934), the species in this genus were not clearly distinct, due to variation in the number of spines, their size and shape within the coenobium. With regard to the coenobia shape, the authors noted the presence of two different shapes: cruciform and axial. The cruciform type was asymmetrical, as it was longer than wide, and the four cells forming the coenobium were not centrally located (*T. staurogeniaeforme*). On the contrary, the axial coenobium shape was more symmetrical and with four centrally located forming cells (*T. heteracanthum*). However, it was

observed that the individuals of *T. staurogeniaeforme* may also have an axial arrangement, and those from *T. heteracanthum* a cruciform one.

Interestingly, subsequent publications (Koršikov 1953; Ergašev 1979; Komárek & Fott 1983; John & al. 2002) have also noted the presence of a third type: square or rhomboid-like, with no cavity in the middle. Furthermore, Kirjakov & Vodeničarov (1986) reported in iconography of the genus that there was a coenobium with a square or rhomboid-shaped cavity formed by the cells. Similar coenobia were also reported by Hortobágyi (1967) and Hindák (1980). It was suggested that the type of coenobium was strongly dependent on the cell division during formation of the daughter coenobia. According to Komárek (1974), arrangement of the daughter cells in the newly formed coenobium is identical to the mother coenobium.

The purpose of our study was to determine the appearance and distribution of the different types of coe-

nobia within the species of genus *Tetrastrum* and to examine the cells division profile of the mother coenobia.

Material and methods

In this study, five species of the genus were used: *T. staurogeniaeforme* (Schröd.) Lemm., *T. triangulare* (Chod.) Kom., *T. komarekii* Hindák, and *T. heteracanthum* (Nordst.) Chod.

On the basis of published literature (diagnosis and iconographies) and on our observations of genus *Tetrastrum*, we have classified these species according to their cell arrangement into five different coenobia types (Fig. 1).

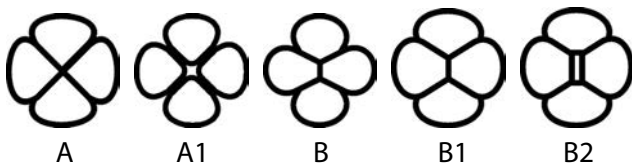


Fig. 1. Types of coenobia of genus *Tetrastrum*: **A** – a square coenobium, the four cells are in very close contact; **A1** – a square coenobium with a cavity formed by the cells; **B** – a rhomboid coenobium of different lengths along the two axes of symmetry consisting of four crosswise distributed cells, as the two cells have a small contact area; **B1** – a rhomboid coenobium of different lengths along the two axes of symmetry consisting of four crosswise distributed cells, where two cells are in contact with most of their inner parts; **B2** – a rhomboid coenobium of different lengths along the two axes of symmetry consisting of four crosswise distributed cells, where the cavity has a rectangular shape.

Five clones were isolated from each *T. staurogeniaeforme* strain No 8828 and *T. triangulare* strain No 8713, with the initial coenobium corresponding to all coenobia types established by us. The strains were from the algae collection of the Paisiy Hilendarski University of Plovdiv (PACC) isolated from a phytoplankton reservoir (Bourgas district).

The *T. komarekii* strain No 510, originally defined by Řeháková 1960/4, was provided 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 A1, and No 5084/8 with initial coenobium of type B2.

The *T. heteracanthum* strain No 1223, originally defined by M. F. Santhos in 1999, was obtained from the algal collection of the University of Coimbra, Portugal. From it, we isolated two clones: No 5083/6 with initial coenobium type A1, and No 5083/8 with initial coenobium type B2.

The cloning was performed according to Stein (1973), by the capillary pipette method modified by Mladenov & Furnadzieva (1995). For a short time, the initial clone was cultivated in a cultivation chamber (Luministat), with constant light of 7 klx at 24°C, and continuous aeration. The clone cultures were then used for intensive cultivation in nutrient media of different composition and concentration gradient.

The intensive cultivation was performed according to Dilov & al. (1972). Clones were cultured in Ackerman glass test tubes at a photoperiod of 15 hours light/ 9 hours dark with 12 klx. At such light regimen, the suspensions were purged with 100 L air/h/100ml mixture enriched with 1% CO₂. For cultivation, three culture media were used: BBM – Bold's Basal Medium (Archibald & Bold 1970), G₁ – Göttingen Basal Medium 1 (Schlösser 1982) and Z – Zehnder Medium (Staub 1961). We have increased the concentration by adding 3 g.l⁻¹ NaCl to the culture media and have decreased it by 10-fold dilution (without changing the concentration of microelements).

Clone synchronization was performed by alternating 15/9 hours photoperiods, with temperature during the light phase of 24°C and during the dark phase of 22°C. The intensity of light during the light phase was 12 000 lx. A BBM culture solution was used.

Density of cultures was controlled at the beginning of the light period by dilution with culture medium to a concentration of 2.5 × 10⁵ cells/ml. The suspensions were aerated with 100 L air/h/100 ml and 1% CO₂ during the light period.

The coenobium types were evaluated microscopically. From each variant, 500 coenobia were examined.

Results

Types of coenobia in the clone cultures

The obtained results on the variability of clones isolated from the corresponding species were identical. Therefore, our comments here are extended to only one clone of each strain and in five culture media (BBM).

T. staurogeniaeforme (clone No 8828/5) (Fig. 2)

We have investigated the influence of three variants of the BBM culture media on the intensive cultivation of clone No 8828/5 (with initial coenobium type B). In this clone, most coenobia had a rhomboid type B1 cell arrangement (42%), followed by the in-

itial square coenobium type A1 (35.6%). The occurrence of coenobia of type B was lower (10.3%), as well as of types A (6%) and B2 (6%) (Table 1). The results about the *T. staurogeniaeforme* clone No 8828/1 and clone No 8828/4 are given in our earlier article (Velichkova & Kiryakov 2006).

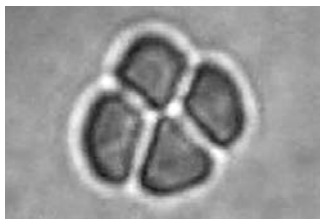


Fig. 2. Initial coenobium of *T. staurogeniaeforme* strain No 8828/5 (scale bar 10µm).

T. triangulare (clone No 8713/22) (Fig. 3)

In the BBM culture media variants, we have observed that the coenobium shape of type B1 was dominating, followed by the coenobium type A1. The types B2, B and A were also observed (Table. 1). Detailed results for *T. triangulare* about the shape and size of coenobia are given in our earlier article (Velichkova & Kiryakov 2007).

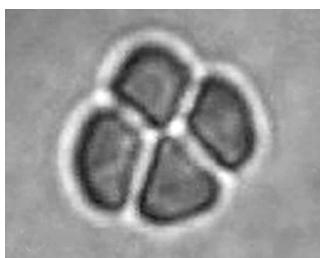


Fig. 3. Initial coenobium of *T. triangulare* strain No 8713/22 (scale bar 10µm).

T. komarekii (clone No 5084/1) (Fig. 4)

In clone No 5084/1 from *T. komarekii*, no great difference was observed between the BBM culture medium variants. The coenobium type B2 occurred most frequently, followed by type A1. The other types (B1, B and A) were also observed, but in lower percentage (Table 1). Detailed results for *T. komarekii* about the shape and size of the coenobia are given in our earlier article (Velichkova & Kiryakov 2007).

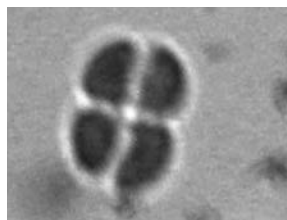


Fig. 4. Initial coenobium of *T. komarekii* strain No 5084/1 (scale bar 10µm).

T. heteracanthum (clone No 5083/8) (Fig. 5)

The majority of newly formed coenobia of clone No 5083/8 in the BBM culture media variants were of the initial (rhomboid) type B2 (55%), followed by the square coenobium type A1 (45%) (Fig. 6). Nevertheless, in our study we have observed, even though in a small percentage, that the other types A, B, B1 were also present in the *Tetrastrum* coenobia.

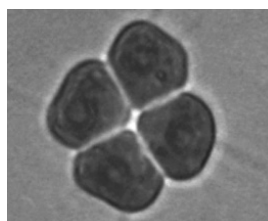


Fig. 5. Initial coenobium of *T. heteracanthum* strain No 5083/8 (scale bar 10µm).

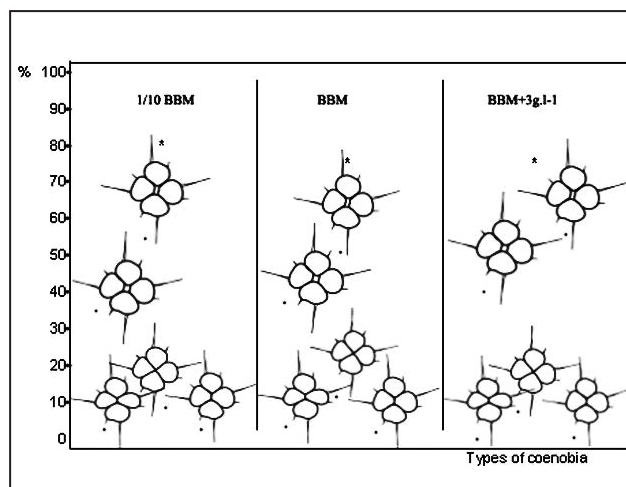


Fig. 6. Prevailing types of *T. heteracanthum* strain No 5083 coenobia (in %) during intensive cultivation of clone 5083/8 in nutrient me

Table 1. Coenobia types (%) of *T. staurogeniaeforme*, *T. triangulare* and *T. komarekii* clones in the BBM culture media variants.

Media	Coenobia types	<i>T. staurogeniaeforme</i> clone 8828/5 (%)					<i>T. triangulare</i> clone 8713/22 (%)					<i>T. komarekii</i> clone 5084/1 (%)				
		A	A1	B	B1	B2	A	A1	B	B1	B2	A	A1	B	B1	B2
1/10 BBM		6	37	10	41	6	6	26	14	34	20	10	23	14	18	35
BBBM		7	35	11	40	7	6	25	17	36	16	8	22	15	19	36
BBM+3g.l ⁻¹		5	35	10	45	5	5	29	14	35	17	9	23	15	17	36

Division of the coenobia

According to Komárek (1974), the cells division is strongly dependent on the form of the mother coenobium – square or rhomboid. Based on this, the cell division can occur in two ways. For both ways, the position of the daughter coenobia inside mother cells is identical to the mother coenobium.

In our study, the clones were able to form all five coenobia types. We have also observed the coenobium division and formation in synchronous culture.

In synchronous cultures, the young coenobia were more pronounced by the third hour of the light period of culturing. Until the ninth hour of the light photoperiod, the young coenobia passed the stage of growing and started to mature. By the fifteenth hour of the light period, a number of cells started forming a division protoplast. By the third hour of the dark photoperiod, the daughter coenobia were released and the process continued till the sixth hour. By the ninth hour of the dark period an increased amount of young coenobia were observed, which entered into the light period (Fig. 7).

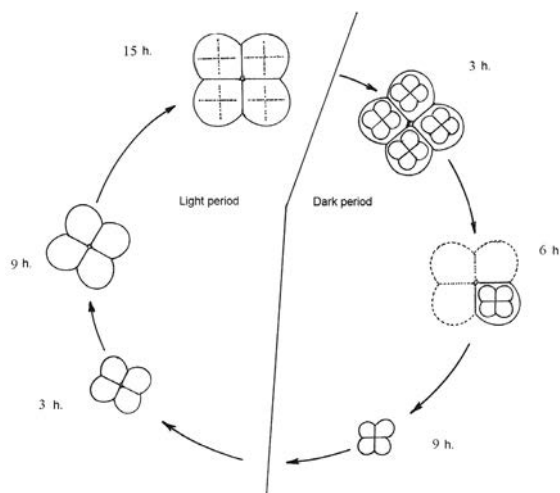


Fig. 7. Cycle of growth (division, formation and release of the coenobia) in the genus *Tetrastrum*.

During the division of the protoplast, the mother cells divide into two or four new cells, as the division planes of the two dividing cells are at a right angle. Only one coenobium was usually formed from one mother cell and released from it by destruction of the cell wall.

Discussion

Komárek & Fott (1983) found out that *T. triangulare* has only the square coenobium type. In contrast, we

have observed not only the above-described square type, but also rhomboid-shaped coenobia. The obtained results from intensive cultivation of the five *T. triangulare* clones give us ground to add the coenobia types (square or rhomboid, with or without a interior cavity) to the diagnosis of species.

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 decreased by 10-fold dilution media, a greater number of syncoenobia were observed (Velichkova & Kiryakov 2007).

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

The major types of coenobia described for *T. heteracanthum* are two: square coenobium with a square cavity, or rhomboid coenobium with a rhomboid cavity (Komárek & Fott 1983; Hindák 1988; Hortobágyi 1968). We have also found out the other three types of coenobia in our cultures, where the cells were closely connected. Therefore we consider it necessary to add these features to the diagnosis of species.

The obtained results have shown that in the investigation of species from genus *Tetrastrum*, five types of coenobia were developed. In addition to the observed different types of coenobia formation, the most pronounced type observed can differ from the initial coenobium. In fact, an interesting observation was that the dominant type was the rhomboid B1 coenobium, followed by the square type A1. Such results were obtained in our earlier studies related to genus *Tetrastrum* (Velichkova & Kiryakov 2005, 2006, 2007).

Contrary to Komárek (1974), the division planes observed by us were not always corresponding to the type of the mother coenobium, thus suggesting that the daughter coenobia were not strongly dependent on the initial, mother type. That is why, in some of the observed divisions, the daughter coenobia corresponded to the initial type and in others, as in the example with the square-shaped mother coenobium we have observed formation of the rhomboid daughter coenobia (Fig. 8).

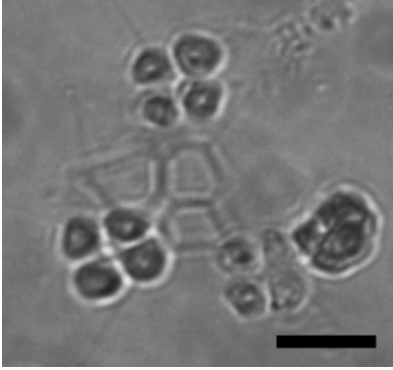


Fig. 8. Division of mother coenobium and formation of rhomboid and square types of daughter coenobia (*T. heteracanthum*, strain No 5083) (scale bar 10µm).

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