RESEARCH ARTICLE

Karyotypic differences and evolutionary tendencies of some species from the subgenus *Obliquodesmus* Mlad. of genus *Scenedesmus* Meyen (Chlorophyta, Chlorococcales)

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Abstract

Karyotype structures of *Scenedesmus acuminatus* (Lagerch.) Chod. and *Scenedesmus pectinatus* Meyen are compared. The karyotype of *S. acuminatus* ($n = 5$) is described for the first time. It reveals four large metacentric and one large submetacentric chromosomes (4M + 1SM). The established karyotype differences have been helpful in clarifying the taxonomic position of these two species. The cytological analyses of other related clonal cultures suggest an evolutionary transition from *S. pectinatus* towards *S. regularis* through *S. pectinatus* f. *regularis*, which correlates with the morphological data about their variability. These results are discussed from the cytogenetic, morphological and evolutionary point of view. On the basis of the karyotypic analysis, it was confirmed that from a taxonomic point of view *S. pectinatus*, *S. acuminatus* and *S. regularis* are separate biological species.

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Introduction

The genus *Scenedesmus* Meyen (Chlorophyta, Chlorococcales) comprises approximately 200 species widely spread in different freshwater basins. The variability of the morphological characteristics used in the taxonomy of *Scenedesmus* species makes it difficult to clearly determine the taxonomic status of some of these species (Chodat and Malinescu 1893; Komárek and Fott 1983; Krienitz 1987; Trainor 1992, 1993; Lürling and Van 1997; Mladenov and Belkinova 1997).

In particular, the taxonomic relationship between *Scenedesmus acuminatus* (Lagerch.) Chod. and *Scenedesmus pectinatus* Meyen has been called to question as early as the beginning of the 20th century. Quite often, these two species are considered in the literature as the same taxon, named by different authors with different names. In some cases, coenobial forms typical of *S. pectinatus* have been discussed as *S. acuminatus* (Uherkovich 1966; Ooshima 1981; Komárek and Fott 1983; Hindák 1990). In other cases, the synonym *S. falcatus* has been used instead of the taxonomically correct name of *S. pectinatus* (Mladenov and Furnadzieva 1995; Hoek *et al*. 1997). Hegewald (1979) highlighted some basic morphological differences between these two species, and issues regarding synonymy were clarified by Hegewald and Silva (1988). Mladenov and Furnadzieva (1995, 1997) studied the variability of *S. acuminatus* and *S. pectinatus* under different rearing conditions and succeeded in differentiating the stable morphological characteristics. Subsequently, studies of the individual development of *S. acuminatus* and *S. pectinatus* revealed ontogenetic differences between these two taxa (Mladenov and Furnadzieva 1999). Molecular and phylogenetic differences between these species also have been reported (An *et al*. 1999), but there have not yet been any studies of possible karyotypic differences between these two species.

Recently, karyological studies of several *Scenedesmus* species have proven helpful in clarifying taxonomic relationships within the genus (Dzhambazov *et al*. 2001, 2002a,b, 2003). Several basic chromosome numbers were pro-

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posed for *Scenedesmus*: $n = 4$ (*S. regularis*, *S. bernardii*, *S. obliquus*, *S. incrassatulus*), $n = 5$ (*S. pectinatus*, *S. obtusiusculus*), and $n = 6$ (*S. nigaardii*, *S. antennatus*), of which so far the most common is $n = 4$.

The aim of the present study, thus, was to compare the karyotypes of *S. acuminatus* and *S. pectinatus* to shed further light on whether *S. acuminatus* and *S. pectinatus* are two different species, or different morphs of the same species. In addition to addressing this broad question, the present study also discusses the form *S. pectinatus* f. *regularis* (Mladenov and Stojanov 1999) and the species *S. regularis*, whose taxonomic identity was called to question (Mladenov 1996; Mladenov and Belkinova 1997).

Materials and methods

Clonal cultures

The clonal cultures used for the karyological study are the following: clone 211, isolated from strain 79/344-Krienitz (Germany) and kept in PACC under No. 5344 named *S. acuminatus* (Lagerch.) Chod; clone 212, isolated from a fish-breeding water body near Plovdiv City and kept in PACC under No. 8913 named *S. acuminatus* (Lagerch.) Chod; clone 223, isolated from a fish-breeding water basin near Trud Village (Plovdiv region) and kept in PACC under No. 8913 named *S. pectinatus* Meyen; clone 12, isolated from strain 8614 and kept in PACC under No. 8614/12 named *S. pectinatus* f. *regularis* Mlad.

The clonal cultures used for the comparative studies are as follows: clone 1, isolated from strain 1998-2 Hegewald (Hungary) and kept in PACC under No. Hg. 98/2/1 named *S. regularis* Svir.; clone 2, isolated from strain 1998-3 Hegewald (Hungary) and kept in PACC under No. Hg. 98/3/2 named *S. pectinatus* Meyen.

Rearing of cultures

The clonal cultures were obtained by the capillary pipette method (Stein 1973) with modifications as described by Mladenov and Furnadzieva (1995). The cultivation and synchronization of the algal cultures was carried out using the apparatus described by Dilov *et al*. (1972). The synchronization was performed by altering light:dark periods of 16:8 hours. The temperature was 33°C and 22°C during the light and dark periods respectively. The intensity of light during the light period was 224 μmol photon $s^{-1}m^{-2}$ (12,000 lux). A BBM nutrient medium was used (Archibald and Bold 1970). The density of the cultures was controlled at the beginning of the light period by diluting with nutrient medium to a concentration of 2.5×10^5 cells/ml. The suspensions were aerated with 100 litres of air per hour per litre of suspension, adding 1% CO_2 during the light cycle.

Karyotype analysis

The karyotype analysis was performed on metaphase plates prepared from synchronized clonal cultures *in vitro*. At the 10th hour of the light period the cultures were treated with Colcemid for 2 h (end concentration 0.20 μg/ml). The metaphase plates were prepared according to the method of Moorhead *et al*. (1960) adapted for plant cells from *in vitro* culture. Briefly, cells were centrifuged at 400 *g* for 5 min, treated with 75 mM KCl solution for 15 min, and hydrolysed in 5 N HCl for 5 min. After fixation and rinsing in methanol:acetic acid (3:1), the cell suspension was pipetted onto microscope slides and allowed to dry under controlled conditions for optimized spreading using the air-dried technique. Chromosomes were stained with 10% Giemsa solution in phosphate buffer (pH 7.2) for 10 min. For the karyotype analysis, metaphase plates of single cells or metaphase plates of coenobial cells, in which the chromosomes were clearly differentiated and within the cells, were chosen. For establishment of morphometric characteristics, 100 metaphase plates each of *S. pectinatus* and *S. acuminatus* were analysed. The nomenclature used for describing karyotype composition followed Levan *et al*. (1964) and contains data about the absolute length of each chromosome (M_L), its relative length (L_r = length of the chromosome/length of all chromosomes), arm ratio (M_r = length of the longer arm/length of the shorter arm) and centromeric index (I_C = length of the shorter arm/absolute length of the chromosome).

Results

Karyotype of Scenedesmus pectinatus Meyen

The karyotype of *S. pectinatus* (clone 223) was identical to the karyotype of *S. pectinatus* (clone 2) described in our previous research work (Dzhambazov *et al*. 2001). The karyotypes of both clonal cultures contain five chromosomes

Table 1. Mean values (± s.e.) of morphological indices* for chromosomes of the karyotype of *S. pectinatus* (clone 223).

Group	Subgroup	Chromosome	Absolute length M_L (μm)	Relative length L_r (%)	Centromeric index I_c (%)	Arm ratio M_r (r)
I	A	1	3.76 ± 0.05	25.63 ± 0.90	48.97 ± 0.96	1.04 ± 0.08
I	A	2	3.69 ± 0.02	25.15 ± 0.43	47.91 ± 0.90	1.08 ± 0.08
I	A	3	3.46 ± 0.03	23.58 ± 0.66	44.44 ± 0.72	1.25 ± 0.02
II	B	4	1.92 ± 0.02	13.08 ± 0.60	44.00 ± 0.66	1.27 ± 0.10
II	C	5	1.84 ± 0.04	12.54 ± 0.78	33.33 ± 0.82	2.00 ± 0.16

* For all values in the table $t > 3$ and $P < 0.01$. $\sum_{L\ \mathrm{abs.}\ n} = 14.69 \pm 0.06\,\mu m$.

(n = 5) with the same morphological features. The reported difference in the absolute length of chromosomes is most likely due to the different pretreatment procedures employed (colchicine and Colcemid). The influence of these substances causes shortening and thickening of the chromosomes, which affects their absolute length. The average values of the morphological indices for the chromosomes from the karyotype of *S. pectinatus* (clone 223) are presented in table 1.

The distribution of chromosomes into groups and subgroups (figure 1) is as follows: two groups (I and II) and three subgroups (A, B and C). The first three chromosomes (1, 2 and 3) belong to the first group (I) and subgroup A. They are large and metacentric (figure 1). The second group comprises the other two chromosomes. The fourth chromosome, which belongs to B subgroup, is small and metacentric; the fifth chromosome is small and submetacentric and belongs to subgroup C. No secondary constrictions in the karyotype or additional B-chromosomes were observed in this clonal culture. The total average (± s.e.) metaphase length of the haploid set of *S. pectinatus* (clone 223) is 14.69 ± 0.06 μm, and the general chromosomal formula is identical to the one established for *S. pectinatus* (clone 2) during our previous investigation, i.e. 3(3M) + 2(1m + 1sm) (Dzhambazov *et al*. 2001).

Unlike *S. pectinatus* (clones 223 and 2), which is characterized by a stable and invariant karyotype, *S. pectinatus* f. *regularis* (clone 12) shows karyotypic heterogeneity and variability in the number of chromosomes (figure 2 and table 2). In 73 of the 100 metaphase plates studied for this clone the karyotype consists of five chromosomes (n = 5), and in 27 plates it consists of four chromosomes (n = 4). At n = 5, the karyotype of clonal culture 12 consists of five chromosomes, whose morphological and morphometrical characteristics are similar to those of *S. pectinatus* (clones 223 and 2) (figures 1 and 2a, and tables 1 and 2). The general chromosomal formula of *S. pectinatus* f. *regularis* (clone 12, n = 5) is also 3(3M) + 2(1m + 1sm), and the total average (± s.e.) metaphase length of the haploid set is 13.98 ± 0.04 μm. The cells of *S. pectinatus* f. *regularis* (clone 12) that are characterized by a karyotype n = 4 have general chromosomal formula 2(1SM+1M)+2(1m+1sm). The chromosomes of the karyotype of these cells (figure 2b and table 2) are divided into two groups (I and II) and four subgroups (A, B, C and D). The first and second chromosomes belong to the first group (I), and the third and fourth chromosomes to the second group (II). The first chromosome is large and submetacentric and belongs to subgroup A. The second chromosome is large and metacentric and is assigned to subgroup B. At the present stage, the second chromosome is classified as metacentric, but in fact it is on the boundary

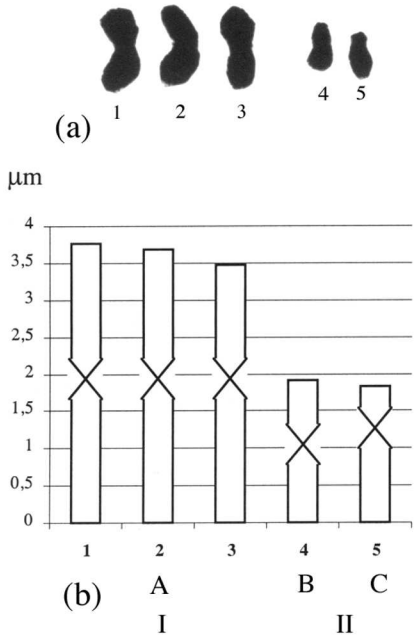

Figure 1. Karyogram (a) and idiogram (b) of *S. pectinatus* (clone 223).

Table 2. Mean values (± s.e.) of morphological indices* for chromosomes of the karyotype of *S. pectinatus* (clone 12).

Group	Subgroup	Chromosome	Absolute length M_L (μm)	Relative length L_r (%)	Centromeric index I_c (%)	Arm ratio M_r (r)
Karyotype with n = 5 (73%)						
I	A	1	3.84 ± 0.03	27.48 ± 0.40	48.00 ± 0.46	1.08 ± 0.04
I	A	2	3.38 ± 0.02	24.19 ± 0.42	45.45 ± 0.68	1.20 ± 0.08
I	A	3	3.07 ± 0.01	21.97 ± 0.28	47.50 ± 0.20	1.10 ± 0.02
II	B	4	1.92 ± 0.04	13.74 ± 0.20	44.00 ± 0.26	1.27 ± 0.08
II	C	5	1.76 ± 0.02	12.59 ± 0.66	34.78 ± 0.62	1.87 ± 0.08
Karyotype with n = 4 (27%)						
I	A	1	4.15 ± 0.02	37.79 ± 0.24	35.18 ± 0.82	1.84 ± 0.28
I	B	2	3.07 ± 0.02	27.95 ± 0.40	38.75 ± 0.30	1.58 ± 0.09
II	C	3	1.92 ± 0.02	17.48 ± 0.60	44.00 ± 0.64	1.27 ± 0.04
II	D	4	1.84 ± 0.04	16.75 ± 0.48	37.50 ± 0.72	1.70 ± 0.12

*For all values in the table $t > 3$ and $P < 0.01$. $\sum_{L\ \mathrm{abs.}\ n}$ = 13.98 ± 0.04 μm (n = 5), $\sum_{L\ \mathrm{abs.}\ n}$ = 10.98 ± 0.06 μm (n = 4).

between the groups of metacentric and submetacentric chromosomes. This chromosome will, most likely at a later stage of karyotype evolution of *S. pectinatus* f. *regularis* (clone 12), be classified as submetacentric. The third chromosome is small and metacentric and belongs to subgroup C, and the fourth one is small and submetacentric and is assigned to subgroup D. The total average (± s.e.) metaphase length of the haploid set at $n = 4$ is 10.98 ± 0.06 μm. The karyotypic heterogeneity and variability in the number of chromosomes of *S. pectinatus* f. *regularis* (clone 12) suggest this clonal culture may be a transitional evolutionary form between *S. pectinatus* Meyen and *S. regularis* Svir.

Karyotype of Scenedesmus acuminatus (Lagerch.) Chod.

The karyotype of *S. acuminatus*, described here for the first time, is characterized by a haploid set of $n = 5$. The average statistical values, received from the morphometric analysis of separate chromosomes of clones 211 and 212, are listed in table 3. While the karyotypes of *S. regularis* and *S. pectinatus* are asymmetrical, the karyotype of *S. acuminatus* is symmetrical and its chromosomes are of similar size and belong to one and the same group (I). Based on their morphology, they are divided into two subgroups (A and B) (figure 3 and table 3). Subgroup A includes the first, second, third and fourth chromosomes, which are large and metacentric. The fifth chromosome, which is large and submetacentric, belongs to subgroup B. No secondary constrictions and additional B-chromosomes were observed in the karyotype of *S. acuminatus*. The general chromosomal formula of the two clonal cultures of *S. acuminatus* (211 and 212) is 5(4M + 1SM). The total average (± s.e.) metaphase length of the haploid set of *S. acuminatus* (clone 211) is 17.54 ± 0.02 μm, and of *S. acuminatus* (clone 212) 18.46 ± 0.04 μm.

Discussion

The comparative karyotype analysis of *S. pectinatus* and *S. acuminatus* showed that these two species differ in the following basic characteristics: karyotype symmetry, morphology of chromosomes, morphometrical indices of chromosomes, general chromosomal formula, and total average metaphase length of the haploid set. These data, thus, agree with the phylogenetic study of An *et al*. (1999).

The karyotype of *S. pectinatus* f. *regularis* (clone 12), which is characterized by variability in the number of chromosomes and karyotype heterogeneity, is of great interest. This clone seems to be a transitional form between *S. pectinatus* and *S. regularis*. It is quite difficult to identify the evolutionary pathway, but from a karyotypical and morphological point of view *S. regularis* could have evolved from *S. pectinatus* through

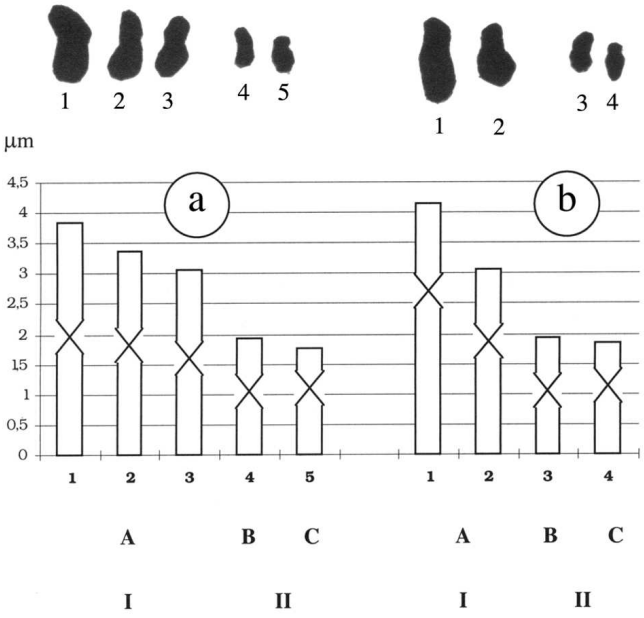

Figure 2. Karyogram and idiogram of *S. pectinatus* (clone 12): (a) with $n = 5$ and (b) with $n = 4$.

Table 3. Mean values (± s.e.) of morphological indices* for chromosomes of the karyotype of *S. acuminatus* (clones 211 and 212).

Group	Subgroup	Chromosome	Absolute length M_L (μm)	Relative length L_r (%)	Centromeric index I_c (%)	Arm ratio M_r (r)
Clone 211						
I	A	1	3.76 ± 0.03	21.46 ± 0.32	40.81 ± 0.62	1.45 ± 0.02
I	A	2	3.46 ± 0.03	19.74 ± 0.44	46.66 ± 0.66	1.14 ± 0.02
I	A	3	3.46 ± 0.03	19.74 ± 0.30	48.88 ± 0.28	1.04 ± 0.04
I	A	4	3.38 ± 0.04	19.29 ± 0.36	45.45 ± 0.12	1.20 ± 0.08
I	B	5	3.46 ± 0.02	19.74 ± 0.58	35.55 ± 0.24	1.81 ± 0.06
Clone 212						
I	A	1	4.23 ± 0.01	22.93 ± 0.60	48.18 ± 0.44	1.09 ± 0.08
I	A	2	3.76 ± 0.02	20.39 ± 0.40	48.97 ± 0.10	1.04 ± 0.08
I	A	3	3.53 ± 0.02	19.14 ± 0.40	45.65 ± 0.82	1.19 ± 0.12
I	A	4	3.46 ± 0.04	18.76 ± 0.32	46.66 ± 0.26	1.14 ± 0.04
I	B	5	3.46 ± 0.03	18.76 ± 0.46	35.55 ± 0.32	1.81 ± 0.08

*For all values in the table $t > 3$ and $P < 0.01$. $\sum_{L\,\mathrm{abs.}\,n} = 17.54 \pm 0.02\,\mu m$ (clone 211), $\sum_{L\,\mathrm{abs.}\,n} = 18.46 \pm 0.04\,\mu m$ (clone 211).

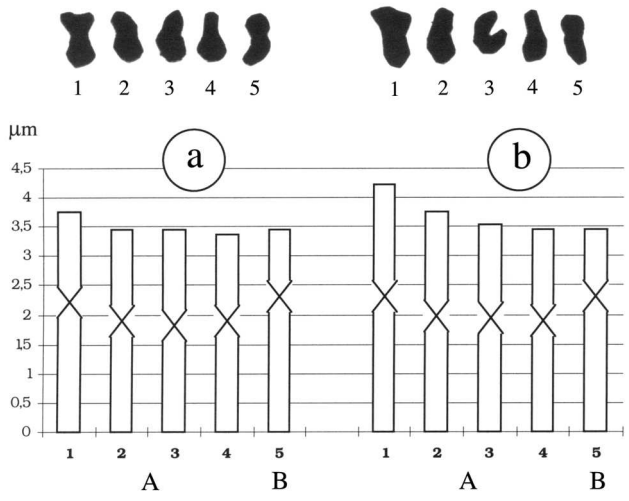

Figure 3. Karyogram and idiogram of *S. acuminatus*: (a) clone 211 and (b) clone 212.

S. pectinatus f. *regularis* as an evolutionary step species. Structural modifications in the chromosomes were observed in 27% of the cells of this clonal culture. These changes are most likely connected with fragmentation of the third chromosome of the basic karyotype ($n = 5$). Subsequently, fusion between the separate fragments and the first and second chromosomes takes place, which leads to formation of translocation chromosomes. This translocation is more strongly manifested in the first chromosome, which turns into submetacentric. The second chromosome associates with a smaller fragment, and despite the fact that at the present stage it remains metacentric it stands on the boundary between the metacentric and submetacentric chromosomes. These data reveal a tendency in the karyotype evolution from *S. pectinatus* towards *S. regularis*. Taking into account that throughout the ontogenetic cycle *Scenedesmus* species are represented only by haploid forms, as well as that these clonal cultures were obtained from single cells by the capillary pipette method, a possible scenario can be drawn. The most likely explanation is that a dicentric chromosome was present in the initial stage, and at a later stage the healed, stabilized chromosomal rearrangements were transmitted to all offspring. In support of this statement, we can point to the established additional B-chromosome (3%) in the karyotype of *S. regularis* (Dzhambazov *et al*. 2001), which most probably is the centromere residual fragment received as a result of the defragmentation of the third chromosome in the karyotype of *S. pectinatus*. New insights into the mechanism of chromosome healing can be obtained by analysing the fate of the third chromosome of several generations of *S. pectinatus* f. *regularis* using fluorescence *in situ* hybridization (FISH).

We can interpret the established morphological variability of *S. pectinatus* (Mladenov and Belkinova 1997), as well as the description of the form *S. pectinatus* f. *regularis* (Mladenov and Stojanov 1999) in light of the results on karyotypic variation from the present study. These results support the view that the tendency of clonal culture 12 *S. pectinatus* f. *regularis* to form coenobia that are identical to *S. regularis* in an environment with increased salt content is genetically determined. Such a morphological transition was not seen in the other clonal culture of *S. pectinatus* (223), whose number of chromosomes is uniform ($n = 5$) and is different from the number for *S. regularis* ($n = 4$).

S. pectinatus is most likely evolutionarily the older species. This plankton species, living in water basins with decreased total salt content, forms coenobia with cells whose ends are hyaline, drawn out, and pointed to the outside of the coenobium centre (figure 4a). These changes considerably increase the relative surface of the coenobium and facilitate its stay in the surface water layers. In populations that exist and develop in water basins with increased concentration of total salt for a long time, the cell ends gradually become shorter and set straight (figure 4b), and afterwards they start winding towards the centre of the coenobium (figure 4c). By these changes the coenobium can preserve its surface existence. This may, thus, be the way in which a morphological transition from one species (*S. pectinatus*) towards another (*S. regularis*) takes place (figure 4c, *S. pectinatus* f. *regularis*). Hegewald *et al*. (2001) established even further modifications in the coenobia of *S. regularis* (figure 5a), expressed in greater shortening and setting straight of the ends of peripheral cells (figure 5b). In cases with even higher salt concentrations, the cell ends even wind to the inside and this

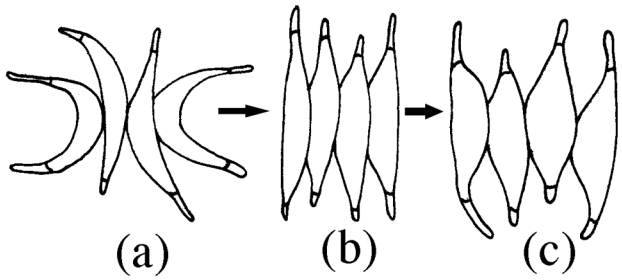

Figure 4. Morphological transition from *S. pectinatus* towards *S. regularis*: (a) coenobium typical for *S. pectinatus* formed in a BBM normal nutrient medium; (b) coenobium of *S. pectinatus* formed in a BBM nutrient medium with increased salt content (BBM + 44 mM NaCl); (c) coenobium of *S. pectinatus* f. *regularis* formed in a BBM nutrient medium with high salt content (BBM + 51 mM NaCl).

makes *S. regularis* morphologically closer to *S. incrassatulus* (figure 5c). Nevertheless, our karyological data show that from a taxonomic point of view *S. pectinatus*, *S. acuminatus* and *S. regularis* are separate biological species. The occurrence of the changes described above in morphology, and the kind of karyotypic variability seen in *S. pectinatus* f. *regularis*, suggest that even clonal cultures of this genus show the ability for rapid evolutionary change in the face of alteration of salt concentration. The causes for such rapid change are, however, not clear at this time.

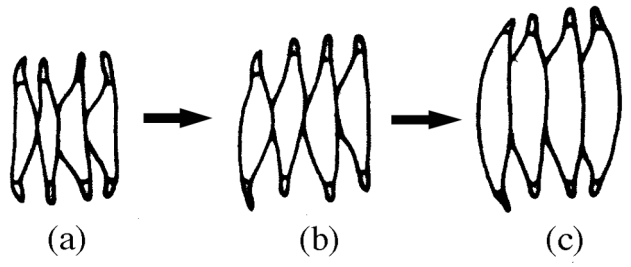

Figure 5. Morphological transition from *S. regularis* towards *S. incrassatulus*: (a) coenobium typical for *S. regularis* formed in a normal BBM nutrient medium; (b) coenobium of *S. regularis* formed in a BBM nutrient medium with increased salt content (BBM + 44 mM NaCl); (c) coenobium of *S. regularis* formed in a BBM nutrient medium with high salt content (BBM + 51 mM NaCl) and resembling *S. incrassatulus* in its morphology.

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