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Remarks on the morphology of *Pseudanabaena galeata* BÖCHER

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With 17 figures and 2 tables in the text

Abstract: *Pseudanabaena galeata* was isolated from a shallow, hypertrophic lake, and its cytomorphology, fine structure and morphological variability were studied in both, culture and lake samples. According to this species diagnosis, the trichomes have polar and apical aerotopes with rounded ends. However, in lake samples, trichomes with these characteristics and also with tapered ends were found. These apices are formed after the trichome breakage. The taxonomic position of these forms is discussed in the frame of the new criteria adopted for the classification of Oscillatoriales.

Key words: Cyanophyta, *Pseudanabaena galeata,* culture, lake samples, morphological variation, fine structure, taxonomic discussion.

Introduction

The genus *Pseudanabaena,* established in 1915 by LAUTERBORN, contained originally only two species *(P. catenata* and *P. constricta).* After the characterization proposed by ANAGNOSTIDIS (1961), it became a large group containing more than 33 species and forms described (CHANG et al. 1985). According to the recent taxonomic revision for Oscillatoriales (ANAGNOSTIDIS & KOMÁREK 1988) the genus *Pseudanabaena* has been divided into two genera and 5 subgenera, following the trend to define small taxonomic groups (ANAGNOSTIDIS & KOMÁREK 1985). In this classification, *Pseudanabaena galeata* BOCHER has been included in the subgenus *Ilyonema* having as main diacritical features, polar and apical aerotopes and rounded apices.

The difficult taxonomy of the Oscillatoriales has lead to frequent confusion, especially between *Limnothrix* and *Pseudanabaena* taxa (MEFFERT 1987). This

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aspect is emphasized in the study of morphological forms in nature, due to the fact that several changing factors are affecting algal characteristics, for example, dimensions. Research on the potential variation limits under different sets of conditions seems necessary for recognition of the species from these genera, including studies under monoalgal cultures (Meffert & Krambeck 1977, Meffert & Oberhauser 1982).

Although *Pseudanabaena galeata* has been cited in several taxonomic lists in relation to different habitats (cf. Hojda 1976, Anagnostidis et al. 1988, Chang 1988) and some ultrastructural features have been studied by Bourrelly & Couté (1975) and Guglielmi & Cohen-Bazire (1984), its ecology and morphology in natural populations remain largely overlooked. In this study morphological variability of *P. galeata* from natural populations and in culture, in relation to the recently defined criteria for Oscillatoriales is discussed.

Material and methods

Cultures

Non-axenic batch cultures of *P. galeata* were isolated in October of 1989 from the Albufera of Valencia following standard methods specified in Stein (1979). They were cultivated in 50 and 100 ml Erlenmeyer flasks, in a modified mineral solution of Zehnder & Gorham (1960) with 25 % of macroelements, 2 mmol $NaHCO_3$, FeEDTA complex and microelements. The standard growth of the cultures was carried out under continuous illumination (PAR between 5–15 $\mu E \cdot m^{-2} \cdot s^{-1}$) supplied by fluorescent tubes (Grolux 18W), under a constant temperature of 25 °C and with daily shaking.

Cultures adapted to continuous illumination at 0.5, 2, 15, 25, 60 and 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ and a constant temperature of 25 °C were monitored for morphological changes at the different levels of light.

Micromorphometric measurements

For morphological studies cultures were submitted to standard growth conditions, natural light rhythms (March, 12L:12D) and room temperature (20–26 °C). Samples taken from the Albufera lake during 1980–88 were also examined. Cell and trichome dimensions for a minimum number of 100 individuals were measured at each of the specified conditions under an inverted microscope. From these measurements the average value was calculated, and the standard deviation was used to determine the 95 % confidence limits of the mean (Snedecor & Cohran 1967).

Pigments

Chlorophyll *a* and carotenoid pigments were determined by means of a Water 600 E HPLC. Pigments were extracted in methanol, with ultrasonic treatment and overnight in the dark at 4 °C (MARKER & JINKS 1982). Phycobiliproteins were extracted similarly, using distilled water as solvent, and detection peaks were monitored spectrophotometrically.

Ultrastructure

For the electron microscopy technique, cultures were fixed in a mixture of glutaraldehyde (2.5 %)/paraformaldehyde (2 %) in 0.1 M cacodylate buffer (pH = 7.2) for two hours, washed several times in buffer and postfixed in 1 % osmium tetroxide in the same buffer. The organisms were dehydrated by a graded acetone series and embedded in Spurr's resin (AG. Fluka). Ultrathin sections were poststained as described by REYNOLDS (1963). THIÉRY's staining (THIÉRY 1967) for polysaccharides was performed on some of the grids. Observations were carried out on a Philips 200 transmission electron microscope.

Results and discussion

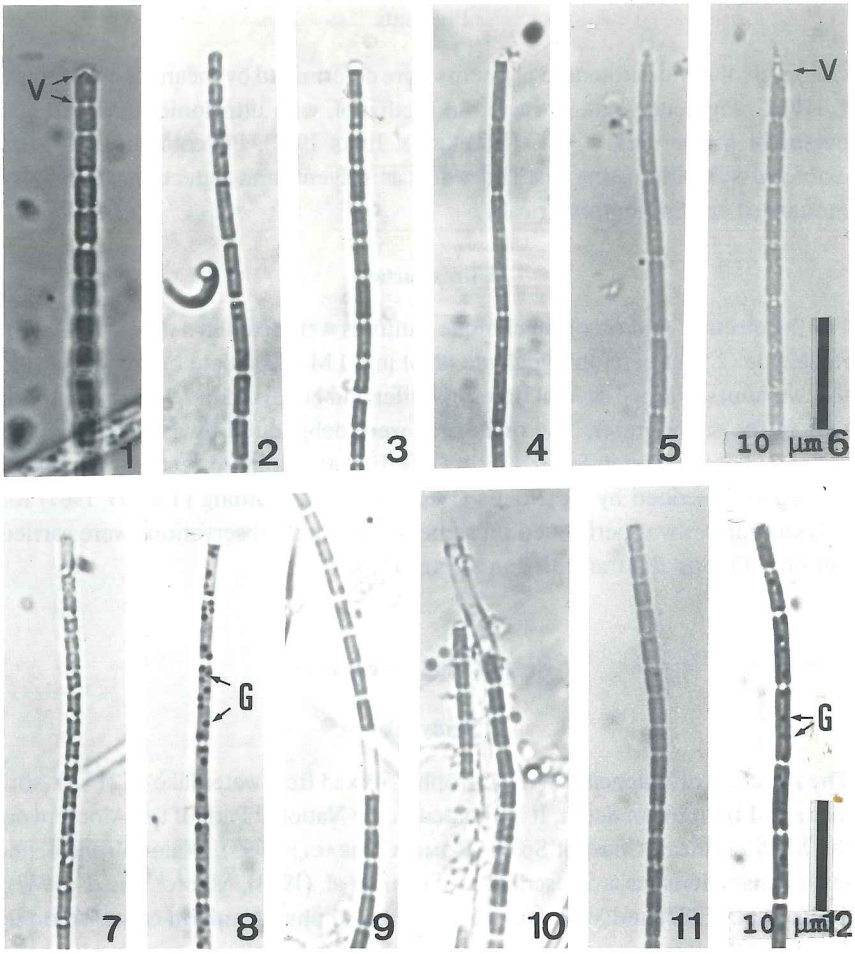
Study lake

The Albufera of Valencia is a hypertrophic, mixed freshwater lake of 21 km^2 surface and 1 m of mean depth. It is situated in the National Park of the Albufera on the Mediterranean Coast of Spain (ROMO & MIRACLE 1993). Water physico-chemistry characteristics are described in SERRA et al. (1984), MIRACLE et al. (1987), SORIA et al. (1987) and VICENTE et al. (1990); and phytoplankton composition in ROMO (1991). It is surrounded by land marsh dedicated almost completely to the cultivation of rice.

Morphology and micromorphometry

According to BOCHER (1949) and ANAGNOSTIDIS & KOMÁREK (1988) the trichomes isolated in culture from the lake of the Albufera and those studied from natural samples of this lake, would correspond to *P. galeata*. The cytomorphological features which confirmed this diagnosis were:

Cylindrical and straight trichomes (Figs 1–12), actively moving by gliding-movement. Trichome length in lake samples and in culture ranged between 30 and 180 μm. The mean number of cell per trichome was quite constant, between 17 and 22 cell per trichome, in both culture and lake samples (Tables 1 and 2). Sheaths absent or occasionally present under prolonged settling of the samples or culture in solid media (Figs 9–10).



Figs 1-12. Morphology of *Pseudanabaena galeata* BÖCHER under light microscopy. (Figs 1-2 and 4-10: Lake samples. Figs 3 and 11-12: culture samples.) (V) polar gas-vacuoles.
1 - Morphotype Ps1. Trichomes with short and wide cells.
2-6 - Morphotype Ps2. Trichomes with longer and thinner cells than morphotype 1.
5 and 6 - Morphotype Ps2 with tapered ends formed after trichome breakage. Note aerotope remaining in the trichome apice (arrow).
7 - Formation of gas-vacuoles after cell division. In the recently divided cell (upper trichome) constrictions are not very prominent.
8 - Polyphosphate granules (G) in a trichome from lake samples.
9-10 - Presence of sheath in trichomes from lake samples under long settling.
11 - Trichome from cultures growing under logarithmic phase, without presence of polyphosphate granules.
12 - Presence of polyphosphate granules from cultures in mid-stationary growth phase. (For further details see text).

Table 1. Micromorphometric data of *Pseudanabaena galeata* BÖCHER.

From culture	trichome length [µm]	diam. [µm]	cel : d	cell numbers	trichome volume [µm³]	cell volume [µm³]
(Logarithmic growth phase)						
Mean	86.57	4.40	1.59	2.76	20	173.28	8.82
Max	89.69	4.70	1.67	2.97	21	195.43	10.14
Min	80.81	3.86	1.53	2.52	19	148.57	7.10
Std	4.08	0.38	0.06	0.18	1	19.21	1.27
n = 300							
(Stationary growth phase)							
Mean	70.04	4.30	1.56	2.76	17	133.83	8.22
Max	83.87	5.81	1.58	3.70	22	159.78	11.25
Min	52.54	3.38	1.54	2.15	11	97.86	6.54
Std	8.48	0.60	0.01	0.38	3	16.94	1.17
n = 1200							
Natural light cycle (March, 12L : 12D)							
(Logarithmic growth phase)							
Mean	95.01	5.14	1.52	3.38	18	172.40	9.33
Max	178	8.91	1.6	5.57	20	357.89	17.91
Min	28	3.24	1.4	2.31	9	43.10	4.99
Std	41.77	1.19	0.06	-	-	-	-
n = 100							
(Stationary growth phase)							
Mean	89.12	5.03	1.5	3.35	18	157.49	8.89
Max	168	8.10	1.60	5.06	21	337.78	16.29
Min	32	3.24	1.40	2.31	10	49.88	4.99
Std	35.82	1.36	0.05	-	-	-	-
n = 100							
Light intensity ($\mu E \cdot m^{-2} \cdot s^{-1}$)							
100	80.79	6	1.88	3.19	13	224.27	16.66
60	105.05	7.75	1.63	4.75	14	219.21	16.17
25	87.01	6.67	1.6	4.17	13	174.94	13.41
2	76.27	3.34	1.52	2.20	23	138.40	6.06
0.5	78.24	3.64	1.52	2.39	21	141.97	6.61
n = 500							

Table 2. Micromorphometry of *Pseudanabaena galeata* morphotypes from the Albufera lake.

	trichome [µm]	length [µm]	diam. [µm]	cel : d	cell numbers	trichome volume [µm³]	cell volume [µm³]
Morphotype Ps.1							
Mean	82.48	3.73	1.63	2.29	22	174.1	7.69
Range	53–126	2.4–5.3	1.4–2.3	1–2.3	14–34	86–409	4.8–10.6
Std	17	0.61	0.11	-	-	50.91	1.5
n = 1500							
Morphotype Ps.2							
Mean	79.39	4.71	1.31	3.60	17	108.3	6.24
Range	31–126	2.0–7.0	0.8–1.7	2.5–8	7–27	16–284	2.7–9.2
Std	18.8	0.90	0.13	-	-	40.9	1.7
n = 4800							
Mean	81	4.22	1.47	2.87	19	135.8	7.0
Lake	31–126	2.0–7.0	0.8–2.3	1.0–8.0	19	16–409	2.7–10.6
Culture	30–178	3.2–8.9	1.4–1.7	2.2–5.6	18	43–358	5 –17.9
BÖCHER 1949	-	2.0–7.0	1.4–2.2	1.4–5.0	-	-	-

Cells longer than broad, with constrictions between cells and presence of terminal and polar aerotopes (Figs 1–12). Cell dimensions in culture were 3–6(–9)×1.5–1.7 µm. Cells were 2–6 times longer than broad (Table 1). Size differences between cultures in logarithmic and stationary growth phase and in those under a natural light/dark rhythm were not significant ($X^2 = 0.13$, $p < 0.01$).

Other features observed in culture provided useful information for the taxonomic diagnosis of this species. All the cells seemed to have the capacity for division, and the new cells grow approximately to the original size before the next division. Reproduction by successive intercellular trichome fragmentation in motile hormogonia.

Ultrastructure and pigments

The ultrastructure of this algae was consistent with that detailed in BOURRELLY & COUTÉ (1975) and in GUGLIELMI & COHEN-BAZIRE (1984) (Fig. 13). The cell wall was 40 nm in width but the interlaminar space and the outer layer (L_3 and L_4) were well defined (Fig. 14) contrary to these authors. Pores in side walls were situated near the cross walls.

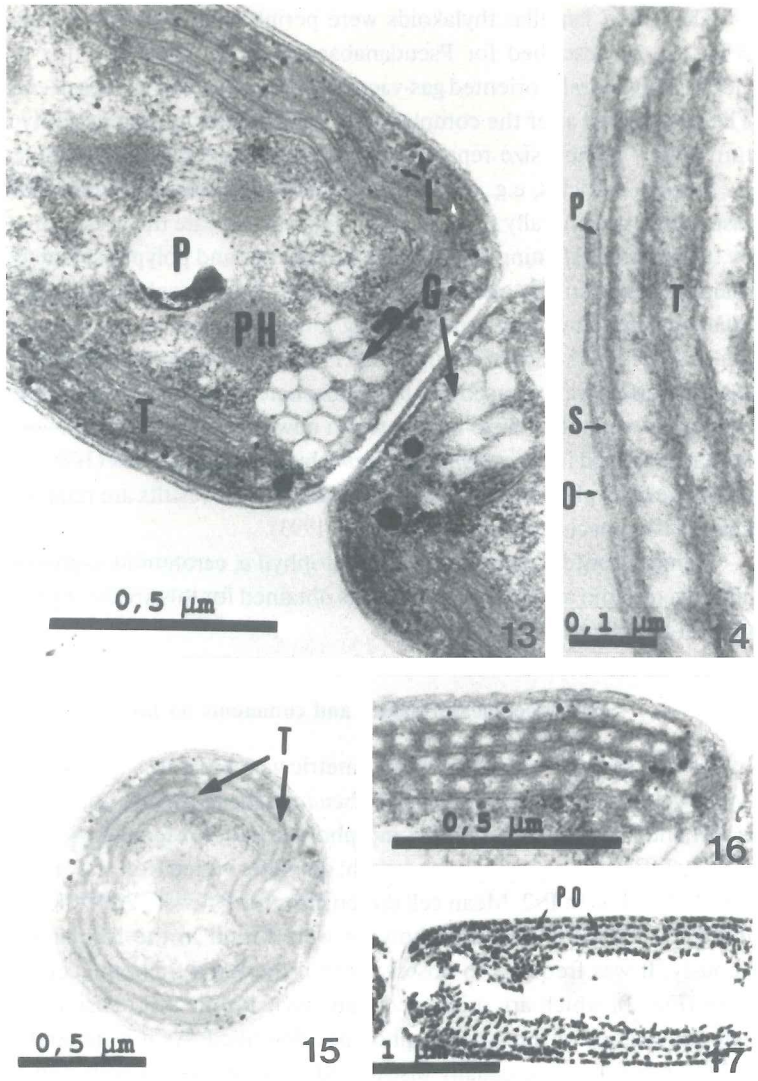


Fig. 13. Longitudinal ultrathin section of *Pseudanabaena galeata,* showing a general view of the peripheral arrangement of thylakoids (**T**) and cell inclusions: (**L**) lipids, (**P**) polyphosphate bodies and (**PH**) polyhedral bodies or carboxisomes, (**G**) gas-vacuoles.

Fig. 14. Cell wall layers: (**PL**) peptidoglycan layer, (**S**) interlaminar space and (**O**) outer layer.

Fig. 15. Longitudinal and transverse section showing number and arrangement of thylakoids.

Fig. 16. Transversal section showing thylakoids and the interthylakoidal location of phycobilisomes.

Fig. 17. THIERY's staining showing polysaccharide reserves (dark dots) situated in the thylakoidal zone.

The 3 to 7 lamellar thylakoids were peripherally and concentrally arranged (Fig. 15), as described for Pseudanabaenaceae by ANAGNOSTIDIS & KOMÁREK (1988). Polyhedrally oriented gas-vacuoles were observed at the cell ends (Fig. 13). They originated after the completion of cell division at the proximity of the septum (Fig. 7). Their size represented between 1 and 6% of the cell length.

Storage particles, e.g., inclusions of lipids and polysaccharide granules were distributed peripherally (Figs 13, 16), being corraborate the presence of this latter by the THIÈRY's staining (Fig. 17). Carboxisomes and polyphosphate bodies were mainly located in the central zone (Fig. 13). Polyphosphate granules were gradually generated during growth, being more abundant in the stationary growth phase (after 10–15 days of culture) than in the starting growing phases (Figs 11 and 12). This effect was reversed and polyphosphate granules were not observed in the trichomes when they were inoculated in new medium. This behaviour is similar to that described in *Plectonema boryanum* by BAXTER & JENSEN (1986), as the result of an overplus phenomenon. In the Albufera these results are related to the ecology of this species (ROMO & MIRACLE, 1993).

Pigment content constituted by chlorophyll *a,* carotenoid, c-phycocyanin and allophycocyanin agreed with the results obtained for this species by GUGLIELMI & COHEN-BAZIRE (1984).

Morphotypes in nature and comments on taxonomy

In the lake samples a greater morphometric variability was found. Two morphotypes were distinguished within the *Pseudanabaena galeata* populations, named as Ps1 and Ps2 (Table 2). The Ps1 morphotype had shorter and wider cells (Fig. 1) than Ps2 (Figs 2–6). The relation length: diameter varied from 2.29 in the morphotype Ps1 to 3.60 in Ps2. Mean cell dimensions for Ps1 were 3.73×1.63 μm and for Ps2 4.7×1.31 μm. Both morphotypes were found in the lake almost simultaneously. It was frequent to observe within the same trichome cells of different sizes (Fig. 2), which are originated from asynchronic cell division and alike the cell dimensions of the two morphotypes described. As it is generally accepted, polymorph forms are usually distributed in water bodies with non-homogeneous and variable conditions. The characteristics of the Albufera lake (hypertrophy, shallowness and water fluctuations) could promote this morphological varibility.

The general dimensions found in culture and lake samples agreed with those specified in the original description of the species (BOCHER 1949) (Table 2).

Occasionally morphotype Ps2 showed slightly tapered apices, which seemed to originate after the trichome breakage (Figs 5 and 6). The apical cells show at its tip gas-vacuole remanent (Fig. 6). Within the same population trichomes with rounded and tapered apices were found. In these latter trichomes the polar position of the aerotopes, as well as, the general morphological features remained

like that described in culture and in the Ps1 form. In this way, drawings from natural population samples shown in WHITTON & PEAT (1969; Fig. 4: e–f and Fig. 8) and in ROJO (1990; Plate X and XIII) are alike to the morphotypes shown in our results.

The morphology of Ps2 with conical ends resembles that described in *Oscillatoria obliqueacuminata* SKUJA (1956), although this species has somewhat larger dimensions (5–10–14 × 1.6–2 μm). This species has been assigned provisionally to the genus *Limnothrix* (MEFFERT 1987). However, the presence of distinct cell constrictions and small polar gas vacuoles (1–10 % of the cell length) approximated to the genus *Pseudanabaena* and makes difficult its definitive taxonomic position within of *Limnothrix* (MEFFERT 1987, 1988).

In this context, the new systematic approach for Oscillatoriales (ANAGNOSTIDIS & KOMÁREK 1988) has confused the classification of constricted and vacuolated trichomes with tapered ends. Within *Pseudanabaena* the sole group characterized with gas-vesicules is the subgenus *Ilyonema,* which is described with only rounded ends. On the other hand, the inclusion of these tapered species within *Limnothrix* (MEFFERT 1988) seems to require further taxonomic consideration. Although they have diacritical features common to both genera, *Pseudanabaena* and *Limnothrix* (ANAGNOSTIDIS & KOMÁREK 1988), the differences listed by MEFFERT (1988) prevent the assignation to this latter genus. It is possible that apical cell morphology should be regarded within the subgenus *Ilyonema* as a morphological criterion with wider variability.

Gas vacuoles and buoyancy

Cultures of *P. galeata* submitted to high light intensities (25–100 $\mu E \cdot m^{-2} \cdot s^{-1}$) show a change in the trichomes morphology. In these cultures, cells were elongated (Table 1), constrictions at the cell-wall were more pronounced, and intracellular aerotopes were reduced, producing a reduction in buoyancy of the cultures. Under low light intensities (2–0.5 $\mu E \cdot m^{-2} \cdot s^{-1}$) trichomes consisted of cells that were smaller in length and diameter (Table 1), maintaining otherwise the standard morphological features described in the standard cultures. Similarly, CHANG (1988) found higher buoyancy in trichomes of *Pseudanabaena galeata* composed of small cells, which showed an enlargement of intracellular vacuoles under conditions of nutrient deficiency.

The morphological changes reported here with respect to illumination seem related to a relative buoyancy control of the position of the trichomes in the water column, as described in the related vacuolated taxon *Oscillatoria redekei* (MEFFERT & OBERHÄUSER 1982). At the same time, this character enhances the opinion regarded over this study of consider a wider morphological variability for *P. galeata* to that originally assigned to this species.

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