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Geitlerinema amphibium (Ag. ex Gom.) Anagnostidis (Cyanophyceae): morphology, ultrastructure and ecology

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With 24 figures and 4 tables in the text

Abstract: Morphometric, ultrastructural and ecophysiological studies on *Geitlerinema amphibium* were performed following the recent criteria adopted for Oscillatoriales. Results showed the presence of junctional pores involved in the trichome breakage and polygonal thylakoid arrangement, which are not included in the description of the genus. The species morphometry and polymorphism in cultures and field samples, show an approximation of this taxon to the diacritical features of *Geitlerinema ionicum,* suggesting that both taxa corresponded to the same species. Data on its ecology were supplied from a four-year monitored study. The characteristics of the ecology and culture seem to confirm this species as tychoplanktonic, being associated in this study with the phytoplankton of a shallow hypertrophic lake. Low light intensities and high temperatures were found to be related to optimal growth conditions for this species, both in culture and in the field.

Key words: Cyanophyte, *Geitlerinema,* cultivation, morphology, ultrastructure, ecology, Spain, lake.

Introduction

The taxonomy of Oscillatoriales has been recently submitted to revision (Anagnostidis & Komárek 1988) taking into account new criteria that combine both the traditional taxonomy of the group, mainly based upon morphological and ecological features, with the information supplied by the biochemistry, physiology and ultrastructure obtained from unialgal cultures. However, as these authors pointed out, some further studies on specific taxa of this order are required to confirm the new classification.

Geitlerinema correspond to a genus described recently by Anagnostidis (1989) which contained especies originally included in *Oscillatoria* and later

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reclassified by Anagnostidis & Komárek (1988) within *Phormidium,* subgenus *Geitlerinema.* The new genus includes fine Oscillatoriales of 1–4 μm wide, cells mainly longer than wide, trichomes intensely motile, often with ends gradually attenuated, bent or coiled, and usually containing large cyanophycin granules or localized carotenoid bodies.

In the Albufera of Valencia (Spain) the filamentous Cyanophyta constitute the most important group, quantitatively and qualitatively (Romo 1991). Within them it was isolated a taxon the diacritical features of which correspond to *Geitlerinema amphibium* (Ag. ex Gom.) Anagnostidis. Although this alga has been cited in several phytoplanktonic lists (e. g. Chung 1976, Campos & Sena 1989) and has been studied at a morphological and morphometric level by Anagnostidis (1961), Kondrateva (1968), Economou-Amilli & Anagnostidis (1981), Anagnostidis et al. (1981) and Anagnostidis (1989), its ultrastructure and ecology remain largely unknown. In this study the taxonomy and ultrastructure of this alga is discussed in the frame of the recent Oscillatoriales classification, complemented with physiological and ecological studies.

Material and methods

Cultures

Non-axenic batch cultures of *Geitlerinema amphibium* were isolated from the Albufera of Valencia. They were cultivated in 50 and 100 ml Erlenmeyer flasks, in a modified mineral solution of Zehnder (Zehnder & Gorham 1960) with 25 and 50 % 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 18 W), under a constant temperature of 25 °C, and with daily shaking. The pH during growth ranged between 8.4 and 9.8. It was tested that these high pH did not limite growth during the logarithmic phase.

Micromorphometric measurements

G. amphibium morphometry in cultures submitted to standard growth conditions, natural light rhythms and room temperature, as well as wild samples studied in the lake during 1985–88, were determined. Cell and trichome length and width 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 calculate the 95 % confidence limits of the mean.

Culture experiments

To calculate *G. amphibium* saturation light intensities, growth in 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 spectophotometrically at 750 nm (Stein 1979). Similarly, cultures were grown at 10 and 25 °C in tempered chambers under saturation light intensity (20 $\mu E \cdot m^{-2} \cdot s^{-1}$). Growth rates were calculated from the logarithmic curve by means of a linear regression, and the standard deviation of this regression was used to calculate the 95 % confidence limits of the growth rate (Snedecor & Cochran 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 determined spectrophotometrically.

Lake studies

Water samples from the Albufera lake were taken approximately monthly from the upper 30 cm of the water column, at three stations from 1985 to 1988. They were fixed in Lugol's iodine solution and counted with an inverted microscope (Utermöhl 1958). The biomass of the phytoplankton was calculated by multiplying the number of individuals by the volume of the algae estimated by approximation to the nearest simple geometric solid (Rott 1981). Since seasonal differences among the three sites were found to be unsignificant, only the average value of the samples is discussed in the article. Additional, physico-chemistry data (dissolved algal nutrients, Secchi depth, pH and temperature) were obtained from parallel projects on the same lake (courtesy of J. M. Soria).

Statistical tests were made with the SPSS Programs package. A multivariant regression analysis between population biomass of the algae in the lake and environmental parameters (temperature, mean illumination under the water column, nitrate, amonium, DIN, DIP) were tested, the former being considered as the dependent variable.

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 (THIERY 1967) for polysaccharides was performed on some of the grids. Observations were carried out on a Philips 200 transmission electron microscope. X-ray energy dispersive analysis supplied by a Hitachi MT 80 MET was used to ascertain the composition of the intracellular granules and polyphosphate bodies (SICKO-GOAD et al. 1975).

Results and discussion

Study zone

The Albufera of Valencia is a hypereutrophic, mixed freshwater lake of 23 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. 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 cultivation of rice, which are usually flooded with lake water. This use of the lake primarily determines its seasonal water level and retention time (ROMO & MIRACLE, 1993).

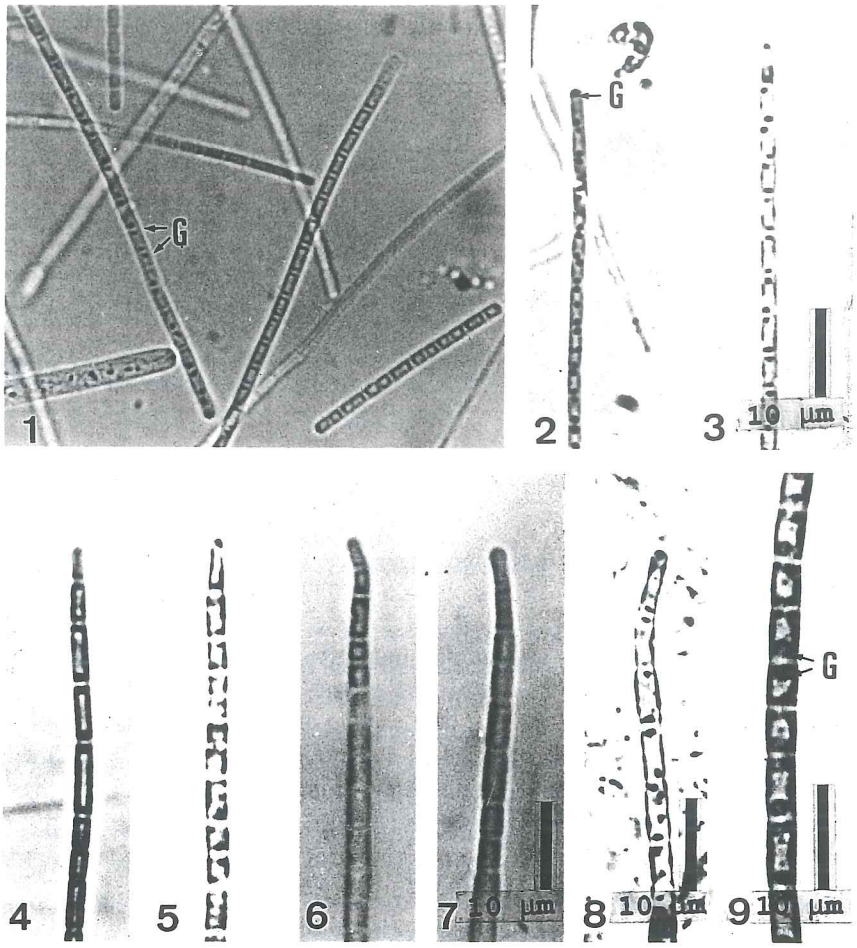
The taxonomy of *Geitlerinema amphibium*

Morphology

Cylindrical trichomes, straight or flexuous, isolated or forming fascicle-like aggregates. Easily deposits in culture (tychoplanktic). Motile, moving by trembling and waving. Sheath absent.

Cells cylindrical, longer than broad without constrictions at the cross walls. Usually containing, depending of growth state, cyanophycin granules or localized carotenoids bodies. Aerotopes absent.

Apical cell of the trichomes were rounded, in both lake and culture samples (Figs 1–3) but in culture bent and attenuated trichomes with sometimes enlarged cells at the ends were also observed (Figs 4–8). This morphology of the apices with the presence of a terminal granule observed in both, culture and field samples (Figs 2–3) resemble the diacritical features described for *Geitlerinema ionicum* (SKUJA) ANAGN. (1989) (ANAGNOSTIDIS et al. 1981, ANAGNOSTIDIS 1989). Moreover, cell dimensions are similar between the two species, although *G. ionicum* present a slighly smaller trichome width (Table 1). These results suggest that both species probably correspond to the same taxon. This supposition is also sup-



Figs 1–9. *Geitlerinema amphibium* (AG. ex GOM.) ANAGN.: morphology under light microscopy.
1–2 – Trichome view from lake samples, with visible apical cell rounded and granules at the cross wall.
3–9 – Trichomes view from laboratory cultures.
3 – Apical cell rounded from culture samples.
4–8 – Apical cell with bent, attenuated or enlarged ends.
9 – Carotenoid and cyanophycin granules enlargement at the cross wall, in cultures under terminal growth phase.

ported by the simultaneous presence of both taxa in half of the scarce *G. ionicum* citations (SKUJA 1937, ANAGNOSTIDIS et al. 1981).

Culture colour is greenish or blue-green.

The photosynthetic pigments identified in culture were: chlorophyll *a*, carotenoid, *c*-phycocyanin and allophycocianin.

Table 1. *Geitlerinema amphibium* and *Geitlerinema ionicum* morphometric data taken from bibliography.

G. amphibium	cell [μm]	diameter [μm]
ANAGNOSTIDIS (1961)	2.5–8.0	1.2–3.0
KONDRATJEVA (1968)	4.0–9.0	2.0–4.0
ANAGNOSTIDIS et al. (1981)	2.3–5.0	1.8–2.6
ECONOMOU-AMILLI & ANAGNOSTIDIS (1981)	2.5–8.4	1.4–2.8
ANAGNOSTIDIS (1989)	2.5–9.0	1.4–3.0
This study: (Lake)	2.2–5.0	1.2–1.6
(Culture)	2.4–8.1	1.5–1.8
Range	2.2–9.0	1.2–4.0
G. ionicum		
SKUJA (1937)	2.0–5.5	1.0–1.3
ANAGNOSTIDIS (1989)	3.0–4.0	1.0–1.5

Micromorphometry

G. amphibium dimensions in both, lake sample and cultures were alike. The trichome length was variable, between 50–400 μm. In culture, mean cell dimensions were (2–) 3–4 (8) × 1.5–1.8 μm. Samples from the lake agreed with these dimensions, although cell width was slightly lower, ranging between 1.2 and 1.6 μm (Table 2). Cell size differences between cultures in logarithmic and stationary growth phases and those with nutrient dilution (Z/4) were not significant ($X^2 = 0.32$, $p < 0.01$). However, cultures submitted to natural light/dark rhythm (approximately 12 L:12 D) showed longer cell length with an average value of 4.64 μm in the logarithmic phase, being within of the range found for the wild samples (Table 2). The general dimensions found in this study agree with that described in the literature (Table 1).

Ultrastructure

The cylindrical trichomes of *G. amphibium* showed a dense peripherical thylakoid-containing cytoplasm and a central area which contains a variable number of granules (Figs 10–12).

The cell wall structure is similar to that for Cyanophyta (DREWS & WECKESSER 1982). It consists of an electrontransparent periplasmatic space just outside the cell membrane, followed outside by a 10 nm in width electron dense peptidoglycan layer. External to this, there is an interlaminar space of 16 to 18 nm and a unit

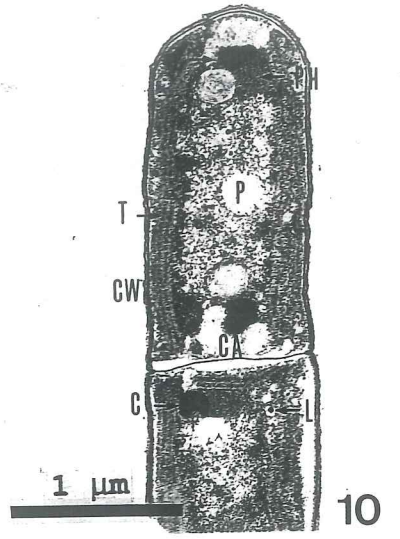
Table 2. Micromorphometric data of *Geitlerinema amphibium*.

From culture

	trichome [µm]	cell [µm]	diam. [µm]	cel:d	cell numbers	trichome volume [µm³]	cell volume [µm³]
(Logarithmic growth phase)							
Mean	150.07	3.32	1.64	2.03	45	313.82	6.99
Max	161.52	3.39	1.69	2.06	50	316.69	7.60
Min	138.62	3.25	1.58	2.01	41	310.95	6.37
Std	11.45	0.07	0.05	0.03	4	2.87	0.62
n = 200							
(Stationary growth phase)							
Mean	222.64	3.06	1.55	1.97	74	419.82	5.79
Max	288.4	3.4	1.58	2.15	104	530.24	6.67
Min	175.5	2.76	1.51	1.80	56	333.07	5.07
Std	45.32	0.24	0.03	0.13	19	83.91	0.66
n = 400							
Medium (Z/4). Stationary growth phase.							
Mean	205.09	3.58	1.59	2.26	57	404.75	7.06
Max	212.76	3.71	1.59	2.35	62	422.45	7.27
Min	197.41	3.45	1.58	2.17	53	387.06	6.85
Std	7.67	0.13	0.00	0.09	4	17.70	0.21
n = 200							
Natural light cycle (12L:12D)							
(Logarithmic growth phase)							
Mean	229.26	4.64	1.57	2.26	49	443.83	8.98
Max	375	8.1	1.8	4.50	46	954.26	20.61
Min	47	3.24	1.5	2.16	15	83.06	5.73
Std	72.46	0.85	0.06	-	-	-	-
n = 100							
(Stationary growth phase)							
Mean	174.75	3.75	1.57	2.26	47	338.30	7.26
Max	372	6.05	1.7	3.56	61	844.37	13.73
Min	54	2.43	1.5	1.62	22	95.43	4.29
Std	85.3	0.82	0.06	-	-	-	-
n = 100							

From Albufera lake

	trichome [µm]	cell [µm]	diam. [µm]	cel:d	cell numbers	trichome volume [µm³]	cell volume [µm³]
Mean	112	3.17	1.59	2.00	35	216.74	6.29
Range	41–220	2.2–5.0	1.2–1.6	1.6–3.6	13–69	79–442	4.4–9.9
Std	39.6	0.60	0.06	-	-	81	0.42
n = 1000							



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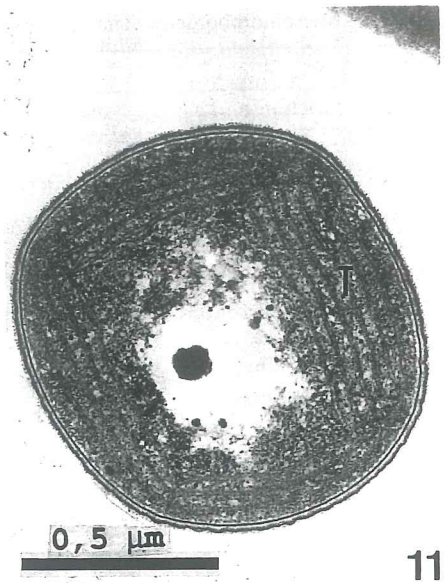

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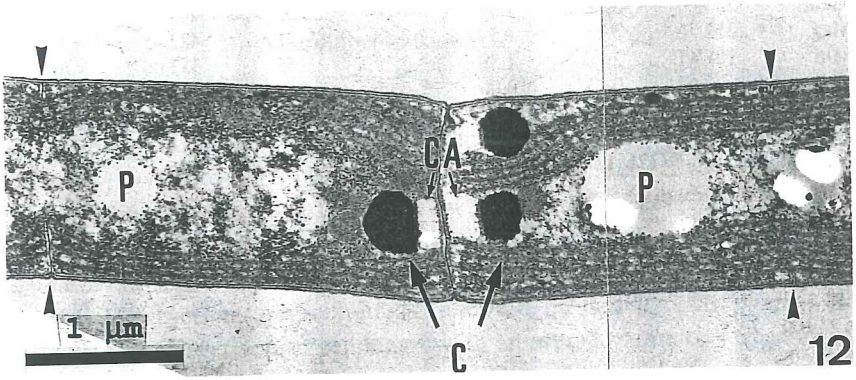

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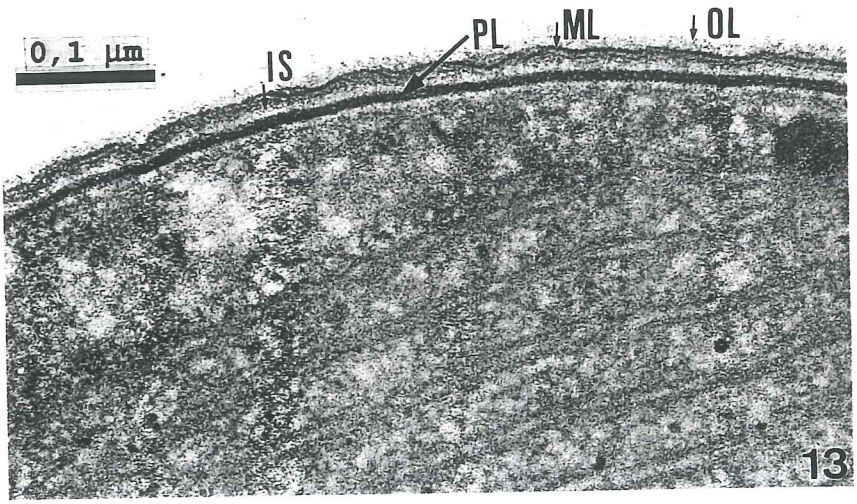

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membrane layer (Fig. 13). It was also observed an outer envelope of between 6 to 12 nm, alike to the "couche surnuméraire" described by GUGLIELMI & COHEN-BAZIRE (1982 a) in some *Oscillatoria* species. At the septum plasmodesms were observed.

Beside the cross-wall and distributed in a regular parallel row, junctional pores can be observed (Figs 14–15) passing through the peptidoglycan layer (Fig. 16). It should noted that the presence of these junctional pores has not been described in any of the Pseudanabaenaceae taxa. However, the presence and function of these pores are poorly documented (LAMONT 1969, GUGLIELMI & COHEN-BAZIRE 1982 b, 1984) and at the moment its taxonomic weight as criterion still not yet ascertained (ANAGNOSTIDIS, K., pers. comm.).

Inclusions of lipids observed as dense granules are distributed mainly at the thylakoidal zone (Fig. 10).

The thylakoids number from 3 to 5, are polygonal and peripherally arranged (Figs 11 and 17). This disposition differs from the concentric arrangement described by ANAGNOSTIDIS (1989) for *Geitlerinema,* although both distributions are characteristic of Pseudanabaenaceae (ANAGNOSTIDIS & KOMÁREK 1988). In this context, a further extension of this feature is suggested for the genus *Geitlerinema,* including in its description a polygonal and concentric arrangement of the thylakoids.

All the cells of the trichomes have capacity for division, and the cells grow into the original size before the next division (Fig. 12). This begins with an ingrowth of the peptidoglycan layer, forming an invagination and pushing the peripheral thylakoids toward the centre (Fig. 18). Sometimes, asymetrical cell division is produced with only an invagination in one of the cell sides (Fig. 19) in a way similar to that described in *Arthronema africanum* (KOMÁREK & LUKAVSKÝ 1988). After the division some thylakoids could be closed at the septum level, remaining parallel to it.

Polyphosphate bodies can be observed as cell inclusions in the nucleoplasmatic zone (Fig. 20). By means of X-ray energy dispersive analysis they were found to contain calcium, sulphur and phosphorus, which agrees with the results obtained in *Plectonema boryanum* by SICKO-GOAD & JENSEN (1974).

The cyanophycin granules are located, usually one in number, at each side of the septum (Figs 10, 12 and 20), and due to its prominent size they could also be

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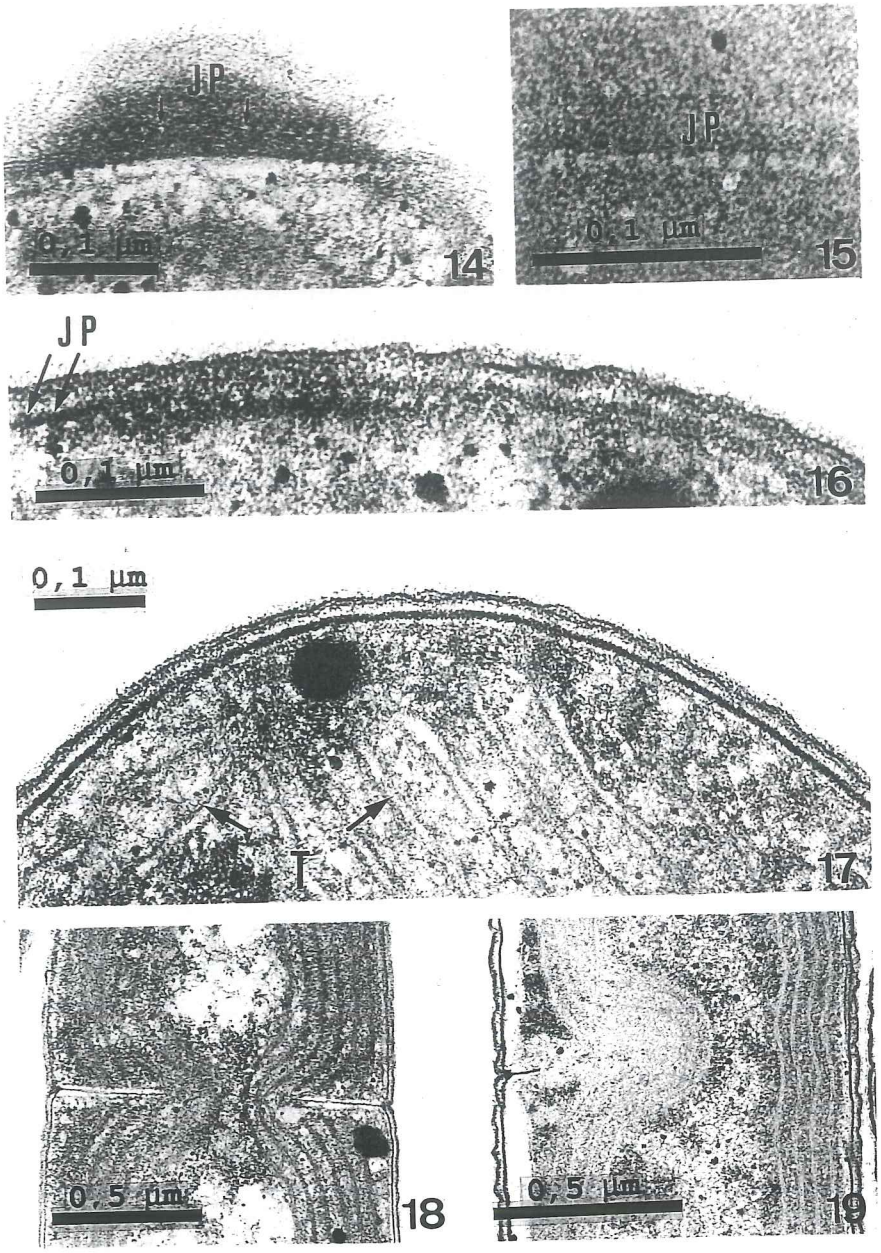
Figs 10–13. Longitudinal ultrathin section of *Geitlerinema amphibium,* showing a general view of the peripheral arrangement of thylakoids (**T**) and cell inclusions: (**CW**) cell wall, (**C**) cyanophycin granules, (**CA**) carotenoids, (**L**) lipids, (**P**) polyphospate bodies and (**PH**) polyhedral bodies or carboxisomes.

11 – Transverse section showing number and thylakoids arrangement.

12 – Carotenoid and cyanophycin distribution within the cell. Arrowheads: ingrowth of the peptidoglycan layer and thylakoidal invagination during cell division.

13 – Structure of the cell wall. Note the outside layer (**OL**) external to the outer membrane (**ML**). (**IS**) interlaminar space, (**PL**)peptidoglycan layer.



Figs 14–19.
Figs 14 and 15. Magnified ultrathin section showing the presence of junctional pores, distributed in a parallel row.
Fig. 16. Junctional pores passing through the peptidoglycan layer (arrows).
Fig. 17. Polygonal thylakoids arrangement.
Fig. 18. Symetrical cell division.
Fig. 19. Asymetrical cell division.

seen under optical microscopy (Figs 1–3). Their distribution and number were quite constant, in both cultures and wild samples. However, their size and formation were variable as a consequence of altering growth conditions, with total absence under strong nutrient limitation (Figs 6 and 7).

Near the cross-wall situated at each side of the septum, there are 1 or 2 carotenoid granules (Figs 12 and 20). Their size seems to increase in culture during the terminal growth phase, being its vision under light microscopy overlapped with the cyanophycin granules (Fig. 9).

The polyhedral bodies limited by a monolayer membrane are mainly distributed in the nuclear area (Figs 10 and 20).

The THIERY's staining showed polysaccharide granules distributed beneath the thylakoids and forming aggregates near the septum (Figs 21 and 22). A higher intracellular polysaccharides content was observed in the stationary growth phase than in logarithmic. This staining also showed that the outer envelope is rich in polysaccharides.

Trichomes reproduce by division into motile hormogonia, without necridic cells. The breakage takes place perpendicularly to the long axis of the trichome through the junctional pores (Fig. 23), in a similar way to that described by LAMONT (1969) in the genus *Oscillatoria.* In *G. amphibium* the trichome breakage is helped by an accumulation of carotenoids near the septum and an inceasing of the cell volume (Fig. 23). Intercellular trichome breakage which characterized the Pseudanabaenaceae family was not observed. However, other genus of this family, *Limnothrix,* also divises by transcellular cell breakage (MEFFERT 1988).

In a preliminary analysis of ARNs, *G. amphibium* appears filogenetically close to *Limnothrix redekei* and *Pseudanabaena galeata* (HÖFLE, pers. comm.), which together with the features presented above seems to ratify its present taxonomic position within *Geitlerinema* and Pseudanabaenaceae.

Growth parameters

G. amphibium showed a plateau of optimum growth rate between 15 and 25 $\mu E \cdot m^2 \cdot s^{-1}$ (Table 3), which is lower than that found in well studied planktonic species such as *Planktothrix agardhii* (FOY et al. 1976, VAN LIERE 1979) and *Limnothrix redekei* (MEFFERT 1971, FOY et al. 1976), which present saturation light intensities above 25 $\mu E \cdot m^{-2} \cdot s^{-1}$. The $Q_{10} = 2.08$ calculated for this alga (Table 3), was similar to EPPLEY's determinations (1972).

Population dynamic and ecology

The annual population growth of *G. amphibium* in the Albufera of Valencia took place from June to November, with nearly constant interannual population maxima in September (Fig. 24). These peaks ranged between 2.7 and 6.4 $mg \cdot l^{-1}$

Table 3. *Geitlerinema amphibium* growth rate under different light and temperature conditions. r = specific growth rate; K = doubling time (r/Ln2).

Light intensity			Temperature		
$\mu E/m^2s$	r [hours]	K [day]	°C	r [hours]	K [day]
0.5	0.003 ± (0.001)	0.111	25	0.027 -	1.054
2	0.017 ± (0.001)	0.607	10	0.013 ± (0.001)	0.474
15	0.028 ± (0.003)	0.988			
25	0.029 ± (0.002)	1.018			
60	0.018 ± (0.004)	0.614			
100	- -	-			

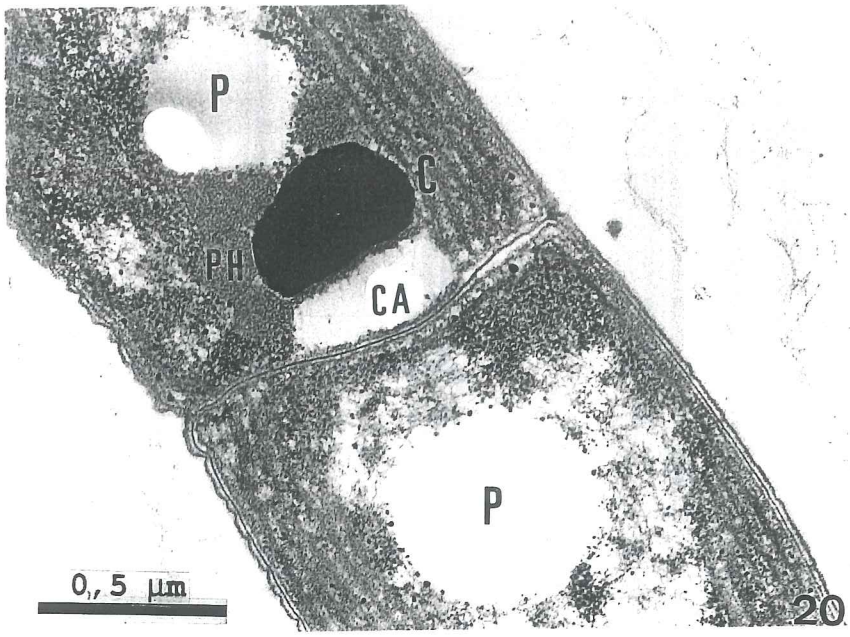
Table 4. Physico-chemical values during maximum population growth of *Geitlerinema amphibium* in the Albufera of Valencia during 1985–88.

	Mean values	Range
Temperature [°C]	25.90	25–28
pH	8.75	8.3–9.5
Mean illumination [%]	18	15–21
Chloride [meq/l]	13.00	8.14–17.7
DIN [μM]	56.70	1.07–162
DIP [μM]	0.13	0.10–0.16
DIN:DIP	436	208–1300

and between 2.3 and 4×10^4 ind. m^{-1} representing approximately 7% and 16% of the total phytoplanktonic biomass and abundance of the lake, respectively.

The physico-chemical values found in the lake during the maximum population peaks are shown in Table 4. In accordance to these values, the biomass variation of this species under the whole period studied showed a statistically significant ($p < 0.05$) relationship with water temperature ($r = 0.36$), soluble nitrate ($r = 0.65$), both with positive regression coefficients, and mean water column illumination ($r = 0.62$) with negative coeficient, when they were entered in a multivariant

Figs 20–23. Enlarged longitudinal section showing location of cell inclusions. Polyphosphate bodies (**P**) and carboxisomes (**PH**) situated at the nucleoplasmatic zone, carotenoid (**CA**) and cyanophycin granules (**C**) beside the cell wall.
Figs 21 and 22. THIERY's staining showing polysaccharide reserves (dark dots) situated and forming aggregates near the septum or in an interthylakoidal position.
Fig. 23. Trichome breakage at the cross wall level and carotenoid granules size increased during the breakage.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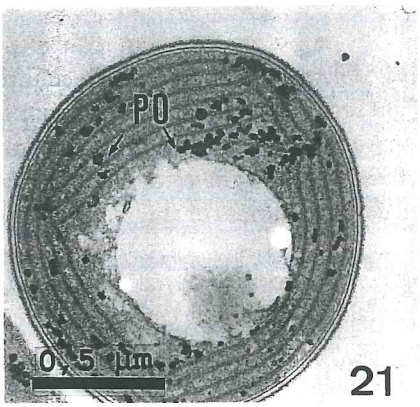
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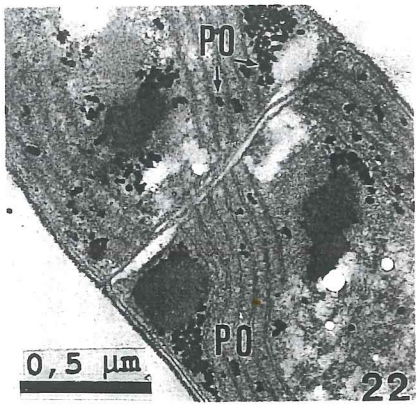
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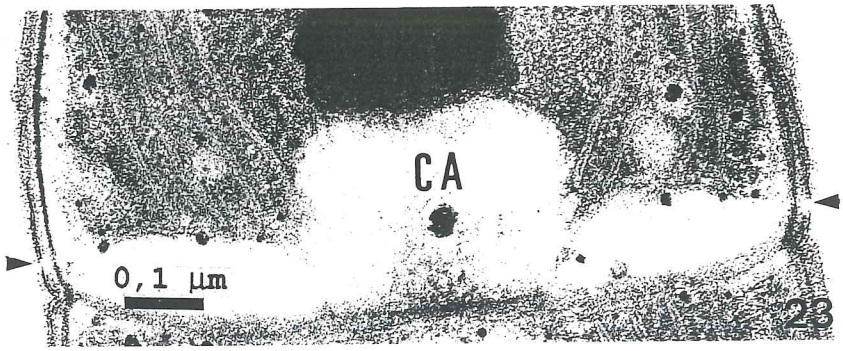
CA
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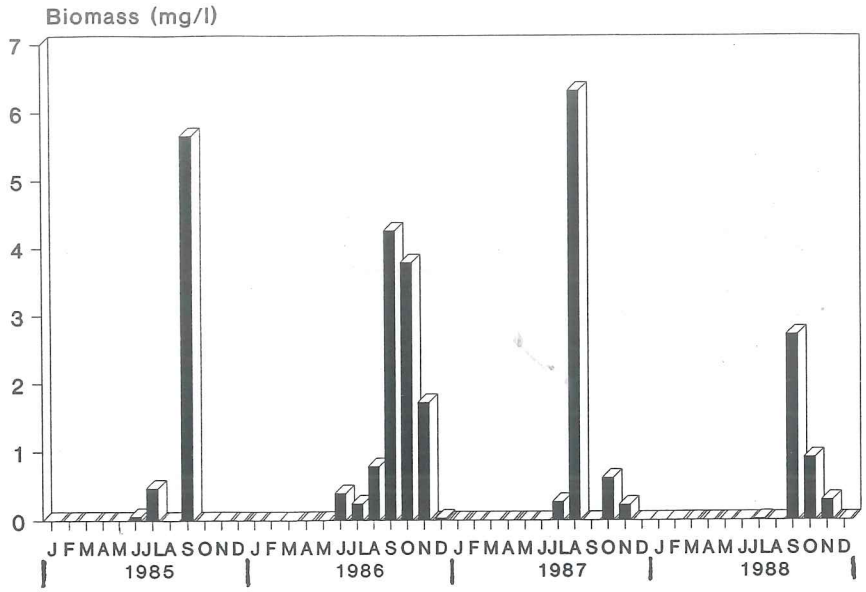

Fig. 24. Population dynamics of *Geitlerinema amphibium* in the Albufera of Valencia, from 1985 to 1988.

regression analysis. These results are consistent with those obtained in culture, where this species showed low levels of light saturation and optimal response to increasing temperatures, which suggest that its optimal growth conditions are related to these conditions. The results obtained can not be compared with the bibliography, due to has not been reported data for nutrient or ambiental growth requeriments of this algae.

The growth of this species in the Albufera seems to be favoured by the conditions produced in the lake as a consequence of the annual paddy harvesting, which takes place in September. At this time, the rice fields are drained and water flows from them into the lake, carrying suspended material and causing a decrease in water transparency and an increase in nutrient load, especially nitrate (SORIA et al. 1987), which is used as fertilizer. Moreover, turbulence produced during this period mantained this species in suspension within of the shallow water column, which is described as tychoplanktonic (ANAGNOSTIDIS 1989).

On the other hand, although the hydrologic cycle of the Albufera lead to a close interaction between the lake and the rice-field environs, and despite the fate that *G. amphibium* has been also described in these latter habitats (ANAGNOSTIDIS et al. 1981, PETROVSKA 1971, LIU & LI 1989), we found evidences of its growth in the lake due to it was practically absent from the main drainage ditches flowing into the lake, during the seasonal period examined in 1985. However, the possibility of its presence within the whole marsh system is not discarded.

KONDRATEVA, N. (1968): Viznachnik Prisnovodnikh Vodorostej Ukrayinskoyi RSR I. Sinjozeleni Vodorosti - Cyanophyta, 2. Hormogoniophyceae. - 523 pp., Akad. Nauk. Ukrayinskoyi RSR, "Naukova Dumka" Kiev.

LAMONT, H. C. (1969): Sacrificial cell death and trichome breakage in an Oscillatoriacen blue-green alga. The role of murein. - Arch. Mikrobiol. **69:** 237-259.

LIU, Y. & LI, S. (S. H. LEY) (1989): Species composition and vertical distribution of blue-green algae in rice soils, Hubei, China. - Nova Hedwigia **48:** 55-67.

MARKER, A. F. H. & JINKS, S. (1982): The spectrophotometric analysis of chlorophyll *a* and pheopigments in acetone, ethanol and methanol. - Arch. Hydrobiol./Beih. Ergebn. Limnol. **16:** 3-17.

MEFFERT, M. E. (1971): Cultivation and growth of two planktonic *Oscillatoria* species. - Mitt. Internat. Verein. Limnol. **19:** 189-205.

– (1988): *Limnothrix* MEFFERT nov. gen. The unsheathed planktic cyanophycean filaments with polar and central gas vacuoles. - Arch. Hydrobiol./Suppl. 80, Algological Studies **50-53:** 269-276.

MIRACLE, M. R.; SORIA, J. M.; VICENTE, E. & ROMO, S. (1987): Relaciones entre la luz, los pigmentos fotosintéticos y el fitoplancton en la Albufera de Valencia, laguna litoral hipertrófica. - Limnética **3:** 25-34.

PETROVSKA, L. (1971): Algenota flora na orizovite polinja vo Kochansko. (Die Algenflora auf den Reisfeldern in der Umgebung von Kotschani). - Skopje Univ. Prir. mat. Fak. God. Zb. Biol. Annu. Biol. **23:** 163-177.

REYNOLDS, E. S. (1963): The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. - J. Cell Biol. **17:** 208-212.

ROMO, S. (1991): Estudio del fitoplancton de la Albufera de Valencia, una laguna hipertrófica y somera, entre 1980 y 1988. - 303 pp., PhD Thesis. University of Valencia (Spain).

ROMO, S. & MIRACLE, M. R. (1993): Long-term periodicity of *Planktothrix agardhii, Pseudanabaena galeata* and *Geitlerinema* sp. in a shallow hypertrophic lagoon, the Albufera of Valencia (Spain). - Arch. Hydrobiol. **126:** 469-486.

ROTT. E. (1981): Some results from phytoplankton counting intercalibrations. - Schweiz. Z. Hydrol. 43 (1): **xxx.**

SICKO-GOAD, L. M.; CRANG, R. E. & JENSEN, T. E. (1975): Phosphate metabolism in blue-green ale. IV. - In situ analysis of polyphosphate bodies by X-ray energy dispersive analysis. - Cytobiology **11:** 430-437.

SICKO-GOAD, L. M. & JENSEN, T. E. (1974): X-ray energy dispersive analysis of polyphosphate bodies in *Plectonema boryanum.* - In: C. J. ARCENEAUX (Ed.): 32nd Ann. Proc. Electron Microscopy Soc. Amer. St. Louis, Missouri, p. 168-169.

SKUJA, H. (1937): Süßwasseralgen aus Griechenland und Kleinasien. - Nova Hedwigia **77:** 15-70.

SERRA, M.; MIRACLE, M. R. & VICENTE, E. (1984): Interacciones entre los principales parámetros limnológicos de la Albufera de Valencia. - Limnetica **1:** 9-19.

SNEDECOR, G. W. & COCHRAN, W. G. (1967): Statistical Methods, 6th edition. - Iowa State University Press, Ames, Iowa.

SORIA, J. M.; MIRACLE, M. R. & VICENTE, E. (1987): Aporte de nutrientes y eutrofización de la Albufera de Valencia. - Limnetica **3** (2): 227-242.

STEIN, J. R. (1979): Handbook of Phycological Methods. Culture Methods and Growth Measurements. New York. - 448 pp., Cambridge University Press.

THIERY, J. (1967): Mise en évidence des polysaccharides sur coupes fines en microscopie électronique. - J. Microscopie **6:** 987-1018.

UTERMÖHL, H. (1958): Zur Vervollkommnung der quantitativen Phytoplankton Methodik - Mitt. Internat. Verein. Limnol. **9:** 1-38.

VAN LIERE, L. (1979): On *Oscillatoria agardhii* GOMONT, experimental ecology and physiology of a nuisance bloom-forming cyanobacterium. PhD thesis. 100 pp.

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References

Anagnostidis, K. (1961): Untersuchungen über die Cyanophyceen einiger Thermen in Griechenland. - Inst. Syst. Bot. & Pflanzengeogr., Univ. Tessaloniki **7:** 1-322.

– (1989): *Geitlerinema,* a new genus of oscillatorialean cyanophytes. - Pl. Syst. Evol. **164:** 33-46.

Anagnostidis, K.; Economou-Amilli, A. & Makris, K. (1988): On the morphotypes of *Phormidium boryanum* (Bory ex Gom.) Anagn. & Kom. and *Phormidium janthiphorum* (Fior.-Mazz. ex Gom.) Elenk. A taxonomic consideration. - Arch. Hydrobiol./Suppl. 80, Algological Studies **50-53:** 71-91.

Anagnostidis, K.; Economou-Amilli, A. & Tsangridis, A. (1981): Taxonomic and floristic studies on algae from rice-fields of Kalochorion-Thessaloniki, Greece. - Nova Hedwigia **34:** 1-67.

Anagnostidis, K. & Komárek, J. (1988): Modern approach to the classification system of cyanophytes. 3 Oscillatoriales. - Arch. Hydrobiol./Suppl. 80, Algological Studies **50-53:** 327-472.

Campos, I. F. P. & Senna, P. A. C. (1989): Nostocophyceae cyanophyceae of the Bonita lake federal district Brazil. - Rev. Bras. Biol. **49**(1): 83-96.

Chung, J. (1976): A study on the cyanophyceae from Chun-ra-book-do area. - Korean J. Bot. **19**(1): 19-30.

Drews, G. & Weckesser, J. (1982): Function, structure and composition of cell walls and external layers. - In: Carr, N. G. & Whitton, B. A. (eds.): The biology of Cyanobacteria. Bot. Monogr. **19:** 333-357. - Blackwell Sci. Publ., Oxford-London-Edinburgh-Boston-Melbourne.

Economou-Amilli, A. & Anagnostidis, K. (1981): Periphyton algae and bacteria from springs of Levadia Boeotia, Greece. - Hydrobiologia **80:** 67-89.

Eppley, R. W. (1972): Temperature and phytoplankton growth in the sea. - Fishery Bull. **70:** 1063-1085.

Foy, R. H.; Gibson, C. E. & Smith, R. V. (1976): The influence of daylength, light intensity and temperature on the growth rates of planktonic blue-green algae. - Br. phycol. J. **11:** 151-163.

Guglielmi, G. & Cohen-Bazire, G. (1982 a): Étude comparée de la structure et de la distribution des filaments extracellulaires ou fimbriae chez quelques cyanobactéries. - Protistologica **18:** 167-177.

– (1982 b): Structure et distribution des pores et des perforations de l' enveloppe de peptidoglycane chez quelques cyanobactéries. - Protistologica **18:** 151-165.

– (1984): Étude taxonomique d'un genre de cyanobactérie oscillatoriacée: la genre *Pseudanabaena* Lauterborn. I. Étude ultrastructurale. - Protistologica **20:** 377-391.

Komárek, J. & Lukavský, J. (1988): *Arthronema,* a new cyanophyte genus from Afro-Asian deserts. - Arch. Hydrobiol./Suppl. 80, Algological Studies **50-53:** 249-267.

VICENTE, E.; MIRACLE, M. R. & SORIA, J. M. (1990): Global model for nutrient flux and biomass production in the Albufera of Valencia, Spain. - Rapp. Comm. Int. Medit. 32 (1): xx-xx.

ZEHNDER, A. & GORHAM, P. R. (1960): Factors influencing the growth of *Microcystis aeruginosa* KÜTZ. emend. ELENK. - Can. J. Microbiol. 6: 645-660.

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