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Morphological and ultrastructural studies of *Nostoc foliaceum* MOUGEOT ex BORN. et FLAH. (Cyanophyceae) growing on soil

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With 12 figures and 1 table

Abstract: *Nostoc foliaceum* MOUGEOT ex BORN. et FLAH. (Nostocales, Nostocaceae) is restricted to North America and Europe, and its description was limited to MOUGEOT's original illustrations (1854). This report extends the geographic distribution to South America and on the basis of material collected from the field and cultured (Buenos Aires Province, Argentina), provides taxonomical characters by which this species can be compared with *Nostoc commune* VAUCHER ex BORN. et FLAH. In addition, an exhaustive morphological and ultrastructural study, particularly of the development and ultrastructure of the akinetes, is provided.

Resumen: La distribución de *Nostoc foliaceum* MOUGEOT ex BORN. et FLAH. (Nostocales, Nostocaceae) está restringida a América del Norte y Europa, este trabajo extendería la misma a América del Sur. Sobre la base de material de campo (Provincia de Buenos Aires, Argentina) y de cultivo se realizó un exhaustivo estudio morfológico y ultraestructural, con especial mención al desarrollo y ultraestructura de las acinetas. Además se analizaron similitudes y diferencias entre *Nostoc foliaceum* y *Nostoc commune* VAUCHER ex BORN. et FLAH. respecto del hábitat, talo y caracteres taxonómicos. Se considera en el futuro realizar un estudio filogenético molecular con el propósito de dilucidar la posible sinonimia entre ambas especies.

Key words: cultures, Cyanoprokaryota, Cyanophyceae, morphology, *Nostoc foliaceum*, soil algae, taxonomy, ultrastructure

Introduction

The genus *Nostoc* VAUCHER ex BORN. et FLAH. is included in the family Nostocaceae, order Nostocales (KOMÁREK & ANAGNOSTIDIS 1989).

Many species of this genus have been reported from different types of aquatic and terrestrial environments. Some of the species most frequently

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observed growing on soil are *Nostoc commune* VAUCHER ex BORN. et FLAH., *N. muscorum* AG. ex BORN. et FLAH., *N. ellipsosporum* (DESM.) RABENH. ex BORN. et FLAH., *N. humifusum* (CARM.) ex BORN. et FLAH., *N. passerianum* BORN. et THUR., *N. foliaceum* MOUGEOT ex BORN. et FLAH. and *N. verrucosum* VAUCHER ex BORN. et FLAH.

According to BORNET & FLAHAULT (1888), *Nostoc foliaceum* was first described by MOUGEOT in 1854. TILDEN (1910) reproduced the original illustrations by MOUGEOT, and since then this species has only been cited in the general monographs by FORTI (1907), GEITLER (1932) and STARMACH (1966), with no new contributions to the original description and illustrations.

Nostoc foliaceum has previously been recorded in North America (TILDEN 1910) and Europe (BORNET & FLAHAULT 1888, FORTI 1907, GEITLER 1932, SKUJA 1964, STARMACH 1966).

The objectives of the present work are to provide a detailed description of *N. foliaceum* using light and electron microscopy, and to compare it with *N. commune.*

Materials and methods

The material was collected at the beginning of December 1999 from garden soil of a sandy loam type, in the locality of San Clemente del Tuyú (36º 22' S, 56º 44' W), Buenos Aires Province, Argentina. The soil sample was taken from the surface layer to a depth of 5 cm, and then transferred to a sterile container.

Part of the sample was air-dried before being deposited in the Cryptogamic Collection (BAc Nº 45691) of the Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Argentina. The remaining part of the sample was rehydrated for further observations, specific identification, isolation and culture. Unialgal cultures were grown on soil-water medium (PRINGSHEIM 1946), and liquid and agarised BBM (ARCHIBALD & BOLD 1970). They were kept under controlled conditions of temperature (25 °C ± 1 °C) and photoperiod (12:12), and at a light intensity of 23 μmol m^{-2} s^{-1}.

Taxonomic studies were based on the works by BORNET & FLAHAULT (1888), FORTI (1907), TILDEN (1910), FRÉMY (1930), GEITLER (1932), DESIKACHARY (1959), STARMACH (1966) and KOMÁREK & ANAGNOSTIDIS (1989).

The light microscopes used for photographs and illustration were a Zeiss Standard 14 with a M35 digital camera and a Wild M20, both equipped with a drawing tub.

For transmission electron microscopy (TEM) examination, the material was fixed in 1 % glutaraldehyde and 2 % osmium tetroxide, after which it was dehydrated in a graded acetone series and embedded in Spurr resin.

Ultrathin sections were made with a diamond knife and stained with uranyl acetate and lead citrate. The sections were studied and photographed with a Jeol 1200 EX2 EM at the Instituto Nacional de Tecnología Agropecuaria, Castelar, Buenos Aires.

Results and discussion

Description of material collected from the field

Young aggregates were spherical, from 1 to 7 cm in diameter. Macroscopic thalli on the soil surface exhibited an intense bluish-green colour, and turned dark brown or black when dry (Fig. 1). Thalli extended widely over the lawn, and showed flattened or elongated, folded or concave shapes, and a firm to spongy consistency (Fig. 2).

Examination with light microscopy indicated that trichomes at the periphery of the aggregates were surrounded by yellow sheaths reaching up to 10 μm in diameter (Fig. 4). Inside the aggregate, trichomes of intense bluish-green colour were immersed in hyaline mucilage; these were long, straight or flexuous, sometimes interwoven with each other or tightly arranged. Vegetative cells were almost spherical to cylindrical in shape (4–5 μm in diameter, and 4–7 μm in length).

Terminal heterocysts were spherical and about the same size as the vegetative cells, while intercalary heterocysts varied from spherical to elongated in shape (4–6 μm in diameter, 4–6 up to 8 μm in length) (Figs 4–5).

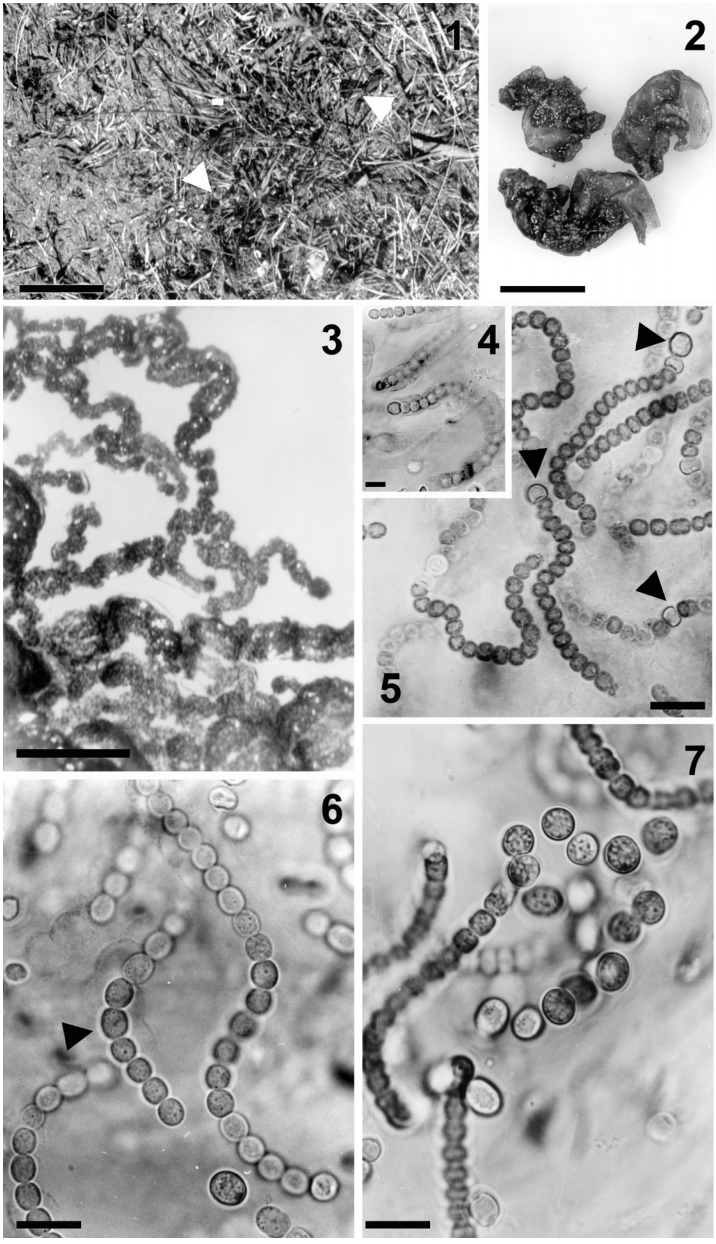
Description of the cultured material

Part of the rehydrated material was cultured in the media indicated above.

On the agarised medium, the aggregates had irregular shapes, and a bright, branched appearance (Fig. 3). Mc Guire (1984) pointed out that this character allows to distinguish between *N. foliaceum* and *N. commune*, which shows an unbranched thallus.

After 30 days, some vegetative cells increased in size, kept on accumulating reserve substances, thickened their walls, and gradually differentiated into akinetes. In the trichome, akinetes were observed in series (2 or more) and located distant from the intercalary heterocysts (Fig. 6). The shape of mature akinetes varied from spherical to oval (6–7 μm in diameter and 7–10 μm in length). The smooth epispore varied from colourless to yellowish (Figs 6–8).

Mature akinetes detached from the trichome germinated into hormogones (Figs 7–8).



Figs 1–7. *Nostoc foliaceum* MOUGEOT ex BORN. et FLAH.: **1** – Aggregates growing on soil (arrows). [Scale bar = 10 cm]. **2** – Hydrated wild macroscopic aggregates. [Scale bar = 5 cm]. **3** – Culture aspect on agarized BBM. [Scale bar = 2 cm]. **4** – Trichomes surrounded by sheath. [Scale bar = 10 µm]. **5** – General morphology of 3 weeks old *N. foliaceum* strain showing terminal and intercalary heterocysts (arrows). [Scale bar = 10 µm]. **6** – Filaments of one month old culture with akinetes in series (arrows). [Scale bar = 15 µm]. **7** – Isolated akinetes of 45 days old cultures.

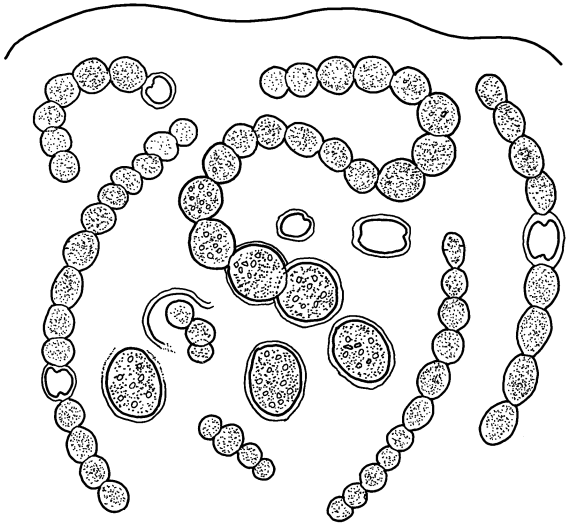


Fig. 8. *Nostoc foliaceum* MOUGEOT ex BORN. et FLAH. Detail of the aggregate with heterocysts, akinetes and akinetes germinating into hormogones. [1.000 x].

Description of the material observed by TEM

Vegetative cells

Ultrastructural studies revealed the presence of a microfibrillar layer external to the cell wall, which corresponded to the sheath (2.5–3 μm in width) (Figs 9–10).

The disposition of the thylakoids in the Nostocales is a uniform and stable character (KOMÁREK & ANAGNOSTIDIS 1989). In *N. foliaceum* also, as in the rest of the Nostocales species so far studied, thylakoids are irregularly arranged filling the whole lumen of the cell (Figs 9–10).

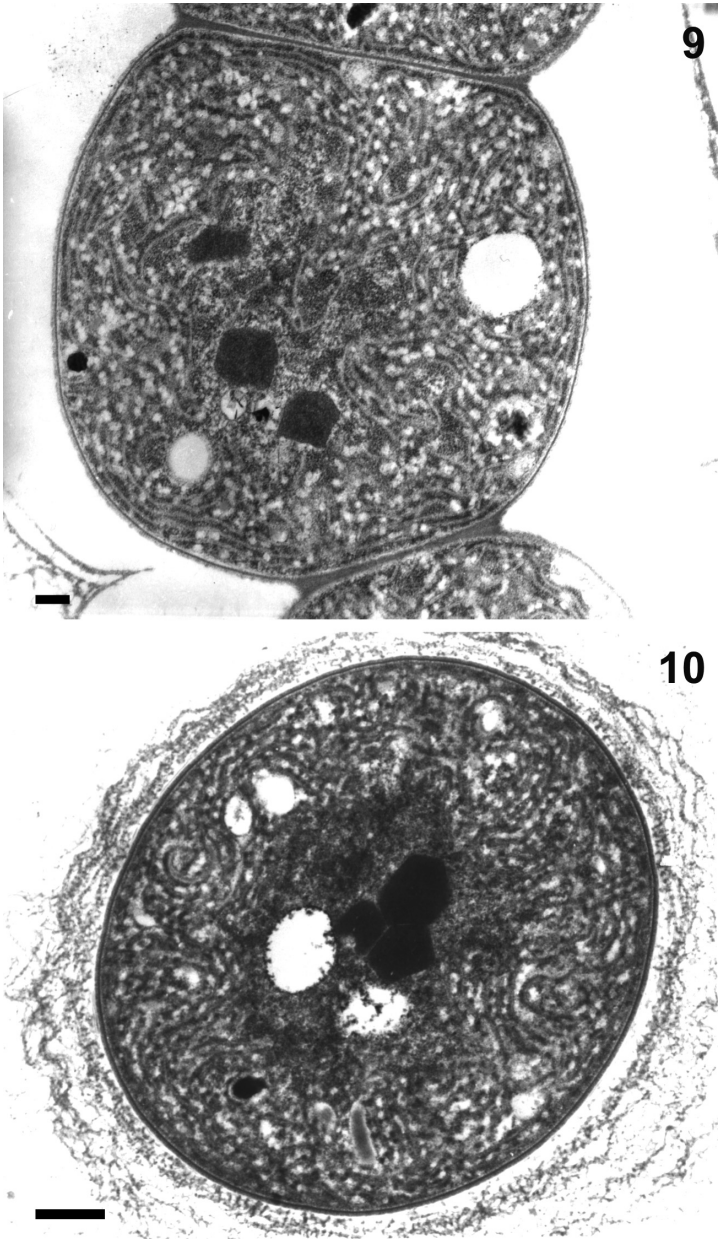
The phycobilisomes were located on thylakoid membranes, and there were abundant α granules between thylakoids (Fig. 9).

The carboxysomes or polyhedral bodies (0.25–0.30 μm in diameter) (Fig. 9), together with approximately round empty spaces (0.2–0.6 μm in diameter) were located towards the centre of the cell (Figs 9–10). The latter were probably caused by the loss of polyphosphate bodies, resulting from the technique used to cut the material (BROWN & BISALPUTRA 1969).

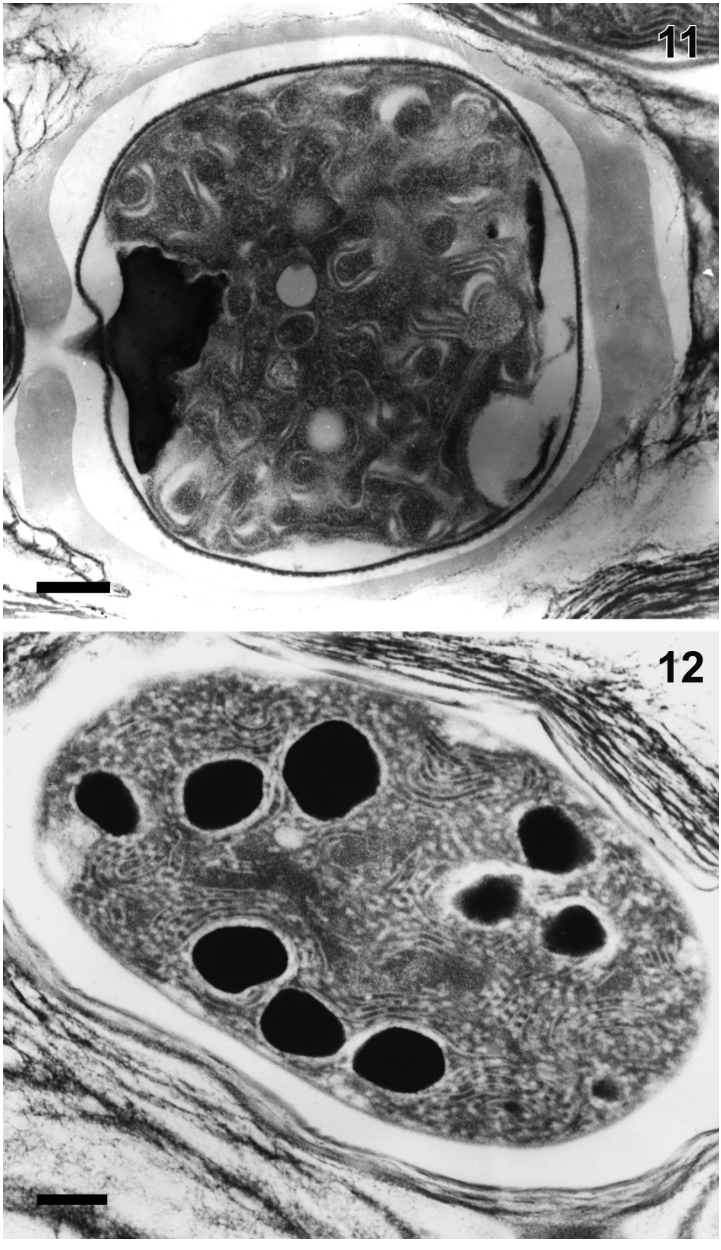
In describing the vegetative cells of *N. foliaceum*, JENSEN (1979, 1985) mentioned the presence of trilamellar bodies close to the transverse wall, but these inclusions were not observed in the material studied herein.

Heterocysts

The heterocysts showed a thickened outer wall and contained unorganized thylakoids (Fig. 11). The abundant deposition of cyanophycin granules in



Figs 9–10. *Nostoc foliaceum* MOUGEOT ex BORN. et FLAH.: **9** – Detail of vegetative cell with thylakoidal lamellae and carboxysomes. [Scale bar = 200 nm]. **10** – Detail of vegetative cell with central carboxysomes. [Scale bar = 500 nm].



Figs 11–12. *Nostoc foliaceum* MOUGEOT ex BORN. et FLAH.: **11** – Terminal heterocyst with persistent thylakoids and the pore filled with electron-dense material. [Scale bar = 500 nm]. **12** – Akinete: wall, thylakoids and lipidic inclusions. [Scale bar = 500 nm]

Table 1. Characteristics of *Nostoc foliaceum* MOUGEOT ex BORN. et FLAH. and *N. commune* VAUCHER ex BORN. et FLAH. according to BORNET & FLAHAULT (1888)[1], FORTI (1907)[2], FRÉMY (1930)[3], GEITLER (1932)[4], DESIKACHARY (1959)[5], STARMACH (1966)[6] and VIGNA & WENZEL[7] (this paper).

Species	*Nostoc foliaceum* (1, 2, 4, 6, 7)	*Nostoc commune* (1, 2, 3, 4, 5, 6)
Geographic distribution	North America (USA), Europe, South America	Cosmopolitan
Environment	continental: terrestrial	continental: terrestrial and aquatic
Habitat	terrestrial, on moist soils among moss and grass, on wet sandy loam soils, on wet rocks.	terrestrial, on moist soils, sometimes on dry soils, rock cavities, cultivated lands, edge of brackish marshes, stagnant water, puddles, littoral of lakes, on wet wood, rice paddies
Thallus	Macroscopic	macroscopic, from 1 to many cm in length
Colours	light green, olive green to yellowish brown	bluish-green, olive to yellowish brown to almost black
Shapes	from spherical to irregular, flattened, folded, concave, to hollow or lacunose, reticulate (when highly perforated)	from spherical to flattened and undulated, fleshy or membranous, sometimes torn or split or perforated sheets
Consistency	firm, mucilaginous and spongy	very firm, on the surface, leather-like
Sheath (position, colour)	at thallus periphery, thick, no lamellated yellowish brown	at thallus periphery, thick, sometimes lamellated yellowish brown
Trichomes	long, straight, flexuous to contorted	flexuous to very entangled
Cells (shape, dimensions)	compressed, spherical to cylindrical, 4–5 x 4–7 µm	compressed to barrel-shaped, spherical to oval, 4.5–6 µm
Heterocysts (position, shape, dimensions)	terminal: spherical, 4–5 µm intercalar: spherical to elongated, 4 x 4–8 µm	almost spherical, 7 µm single or in series, 7 µm
Akinetes (shape, dimensions, epispore)	spherical to oval, 4 x 7–10 µm, smooth, colourless to yellowish	Observed only once, of same diameter as vegetative cells smooth, colourless

the polar nodules obliterated the pores between the heterocyst and vegetative cells.

Akinetes

Mature akinetes were covered by a wide outer layer of fibrillar structures (0.4–0.5 µm in width) and an inner homogeneous layer (0.2 µm in width).

Thylakoids with phycobilisomes filling almost all the cell lumen could be observed. There were abundant α granules between the thylakoids, and massive deposition of cyanophycin in the form of more or less spherical bodies (Fig. 12).

Conclusions

To conclude, we highlight the following aspects of our study:

- Our knowledge of this species has been improved by observations and illustrations based on material obtained from the field and cultures (Figs 1–8).
- The new information provided by our study includes the development and ultrastructure of akinetes. In addition, morphological and ultramorphological studies were completed by the analysis of vegetative cells and heterocysts (Figs 9–12).
- On the basis of observations made by us and other authors (Table 1), we compare *N. foliaceum* with *N. commune* by pointing out their similarities and differences concerning the types and aspects of the thallus, cell characteristics and environmental characteristics of the habitat. We remark that many of the characters used to separate these species fall in the same range, except the presence of akinetes that in *N. commune* was mentioned only once (GEITLER 1932, DESIKACHARY 1959, STARMACH 1966).

For this reason we suggest to analyze in a future a possible synonymy of *N. foliaceum* with *N. commune* on the base of a proper molecular phylogenetic analysis.

References

ARCHIBALD, P. A. & BOLD, H. C. (1970): The genus *Chlorococcum* MENEGHINI. – Phyc. Stud. **XI**. University of Texas Public. **7015**: 1–115.

BORNET, E. & FLAHAULT, C. (1888): Révision des Nostocacées héterocystées. – Ann. Sci. Nat. Bot. 7ème sér., **7**: 177–262.

BROWN, D. L. & BISALPUTRA, T. (1969): Fine structure of the blue-green alga *Nostoc sphaericum*: the structured granule. – Phycologia **8**: 119–126.

DESIKACHARY, T. V. (1959): Cyanophyta. – I.C.A.R. Monographs on Algae, 1–686, New Dehli.

FORTI, A. (1907): Sylloge Myxophycearum. – In: DE TONI, J. B, Sylloge Algarum **5**: 1–761.

FRÉMY, P. (1930): Les Myxophycées de l'Afrique équatoriale française. – Mém. Arch. Bot. **3**: 1–508.

GEITLER, L. (1932): Cyanophyceae. – In: RABENHORST, L. (Ed.): Kryptogamen Flora **14**: 1–1196. Akad. Verlagsgesellschaft, Leipzig.

JENSEN, T. E. (1979): Occurrence of tri-lamellar bodies in isolates of *Nostoc* (Cyanophyceae). – J. Phycol. **15**: 112–114.

JENSEN, T. E. (1985): Cell inclusion in the Cyanobacteria. – Algological Studies **38–39**: 33–73.

KOMÁREK, J. & ANAGNOSTIDIS, K. (1989): Modern approach to the classification system of Cyanophytes 4– Nostocales. – Algological Studies **56**: 247–345.
MC GUIRE, R. F. (1984): A numerical taxonomic study of *Nostoc* and *Anabaena*. – J. Phycol. **20**: 454–460.
PRINGSHEIM, E. G. (1946): The biphasic or soil-water culture medium for growing algae and flagellata. – J. Ecol. **33**: 193–204.
SKUJA, H. (1964): Grundzüge der Algenflora und der Algenvegetation der Fjelgegenden um Abisko in Schwedisch-Lappland. – Nova Acta R. Soc. Scient. Upsal. Ser. 4, **18**: 1–465.
STARMACH, K. (1966.): Cyanophyta – Glaukophyta (Sinice, Glaukofity). – In: Flora Slodkowodna Polski **2**: 1–807. Polska Akad. Nauk, Warzawa.
TILDEN, J. (1910): The Myxophyceae of North America and adjacent regions. Minnesota Algae **1**: 1–319, Minneapolis.

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