

## Life cycle of *Aulosira epiphytica* comb. nova (Cyanobacteria) just completed

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**Abstract:** Among littoral mats of an irrigation canal in South Slovakia, long filaments of a heterocytous cyanobacterium were found that were longitudinally attached to filaments of *Mougeotia* sp. and the centric diatom *Aulacoseira italica* (Ehrenberg) Simonsen. Similar heterocytous taxa attached to other algae have been described, but without akinetes, so their life cycle is incompletely known and their taxonomic position remains unclear. The presence of a sheath and the observation of solitary akinetes allowed us to classify our specimens as a representative of the genus *Aulosira* Bornet et Flahault. Our study lead us to propose a new combination – *Aulosira epiphytica* (Gardner) Hindáková et Hindák, comb. nova, using *Nodularia epiphytica* Gardner 1927 as basionym.

**Key words:** akinetes; algal mats; heterocytous cyanobacteria; irrigation canal; new combination; S Slovakia.

### Introduction

In littoral algal mats of an irrigation canal at Čiližská Radvaň near Medveďov, South Slovakia, many conspicuous long heterocytous cyanobacteria were found to be firmly attached to filaments of *Mougeotia* sp. (Figs 1, 2, 6) and the centric diatom *Aulacoseira italica* (Figs 3, 4, 20, 21). Filaments were solitary,  $\pm$  straight to slightly loosely coiled, with fine hyaline (Fig. 9) to brownish sheaths (Fig. 11) and regularly metameric trichomes. No akinetes were found in the field samples. At a first look, they resembled some heterocytous taxa with attached filaments on algae or other plants, e.g. *Nodularia epiphytica* Gardner 1927 (his Pl. 12: 16, our Fig. 19), *Anabaena epiphytica* Gardner 1927, or *A. huberi* De-Toni 1934 (= *A. epiphytica* Huber-Pestalozzi 1930). However, in these taxa akinetes were never found, thus their correct taxonomic status has remained unclear (Komárek 2013).

When the material was kept under laboratory conditions, within two weeks akinetes appeared in trichomes, initially solitary (Figs 15, 16, 18) but later also two joined akinetes were observed (Fig. 17). They were long, cylindrical, with smooth exospores, and distant from heterocytes. These new observations have enabled us to conclusively identify this cyanophyte.

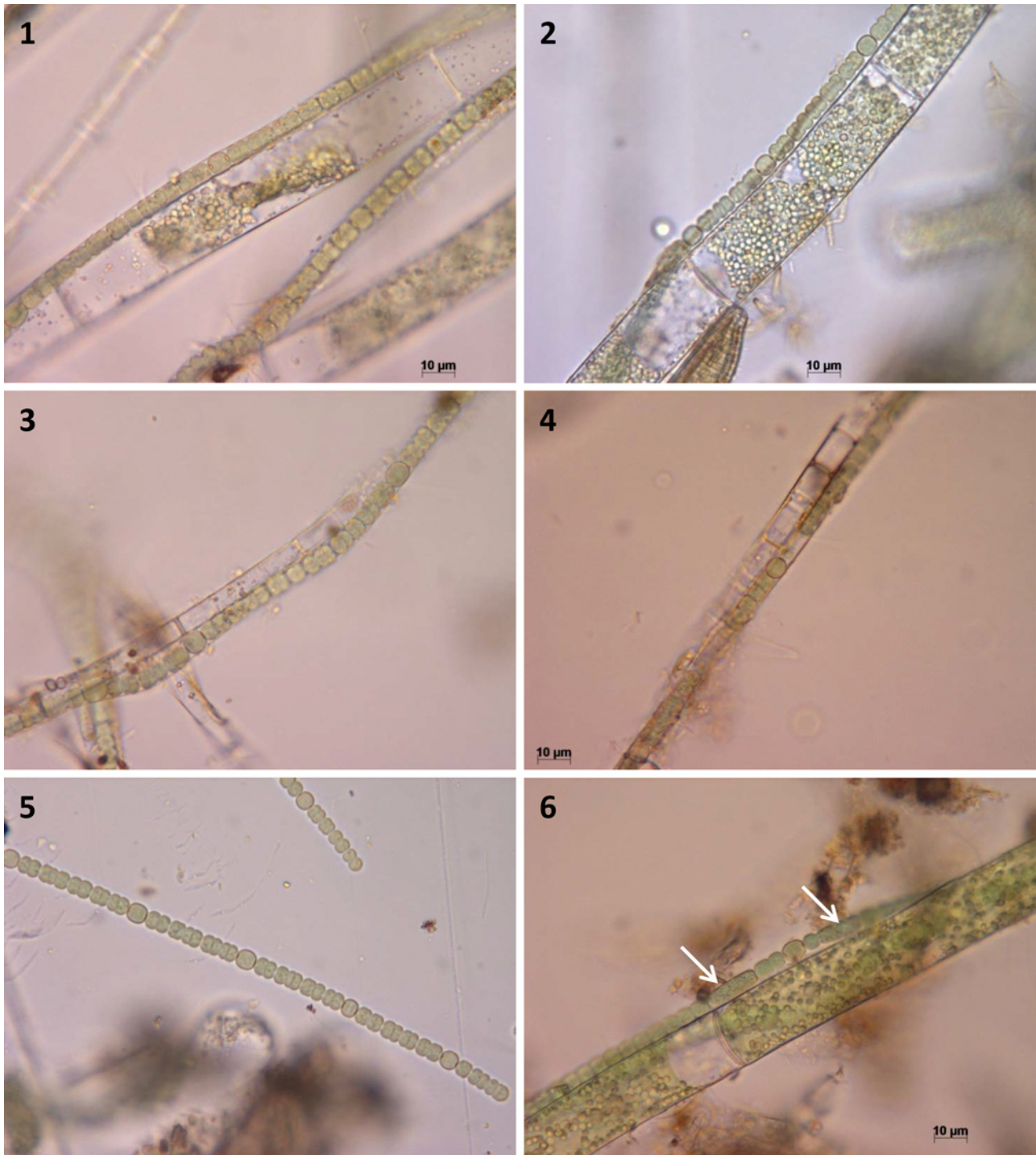
Because of the morphology, especially the presence of a sheath in filaments, and akinetes positioned distant from the heterocytes, it was evident that it is a representative of the genus *Aulosira* Bornet et Flahault (Bornet et Flahault 1886-1888; Starmach 1966; Kondrateva 1968; Komárek 2013). A possible affinity with other morphologically similar genera such as *Anabaena*

or *Nodularia* could be ruled out: the first genus has no sheaths around single trichomes, and the strategy of formation of the akinetes is different in the second genus in which all vegetative cells are able to transform into akinetes, as in the genus *Nostoc* (Komárek 2013).

Another problem that had to be addressed was to find a correct basionym. Gardner (1927) published simultaneously in the same study two heterocytous species with filaments attached to other algae or plants: *Anabaena epiphytica* Gardner (p. 61) and *Nodularia epiphytica* Gardner (p. 65). The diagnoses concerning the morphology of filaments, cells and heterocytes differ from each other only slightly. In both species akinetes were not observed (cf. also Komárek 2013). Although the description of *Anabaena epiphytica* Gardner appeared four pages before that of *Nodularia epiphytica*, albeit without figures, we prefer *Nodularia epiphytica* Gardner as basionym, since sheaths in filaments were both mentioned in the diagnosis and depicted in Pl. 12, Fig. 16.

### Material and methods

Samples were collected on July 19, 2016 from the littoral of macroscopic green mats of *Mougeotia* sp. steril. in the drainage Červený canal at Čiližská Radvaň (47.8135N, 17.70941E). The plain field is located between Medveďovo and Veľký Meder, at 111–122 m a.s.l.; and is a part of the Danube Lowland (Podunajská nížina), S Slovakia (water temperature 18.5°C; pH 7.8; conductivity 442 ms/cm; salinity 0.22 PSU; orig. M. Ilýová). Subcultures of the original field sample were maintained in the phycological laboratory of the Institute of Botany SAS in Bratislava. Light microscope studies were performed on living material with a Leitz



Figs 1–6. Cyanobacterium *Aulosira epiphytica* (Gardner) Hindáková et Hindák, comb. nova. Slightly coiled filaments attached to *Zygnema* sp. steril. (1, 2, 6) or to the centric diatom *Aulacoseira italica* (3, 4); free moving filaments (5), akinetes (arrows, 6). LM, scale 10 µm.

Diaplan microscope and documented with a Zeiss Axio Cam ICc3 digital camera. The diatoms were determined from a permanent slide (live sample boiled with 30% H<sub>2</sub>O<sub>2</sub> for 30 min., thereafter washed 3 times with distilled water and mounted with Naphrax).

## Results

### Taxonomy

*Aulosira epiphytica* (Gardner) Hindáková et Hindák, comb. nova

Basionym: *Nodularia epiphytica* Gardner 1927

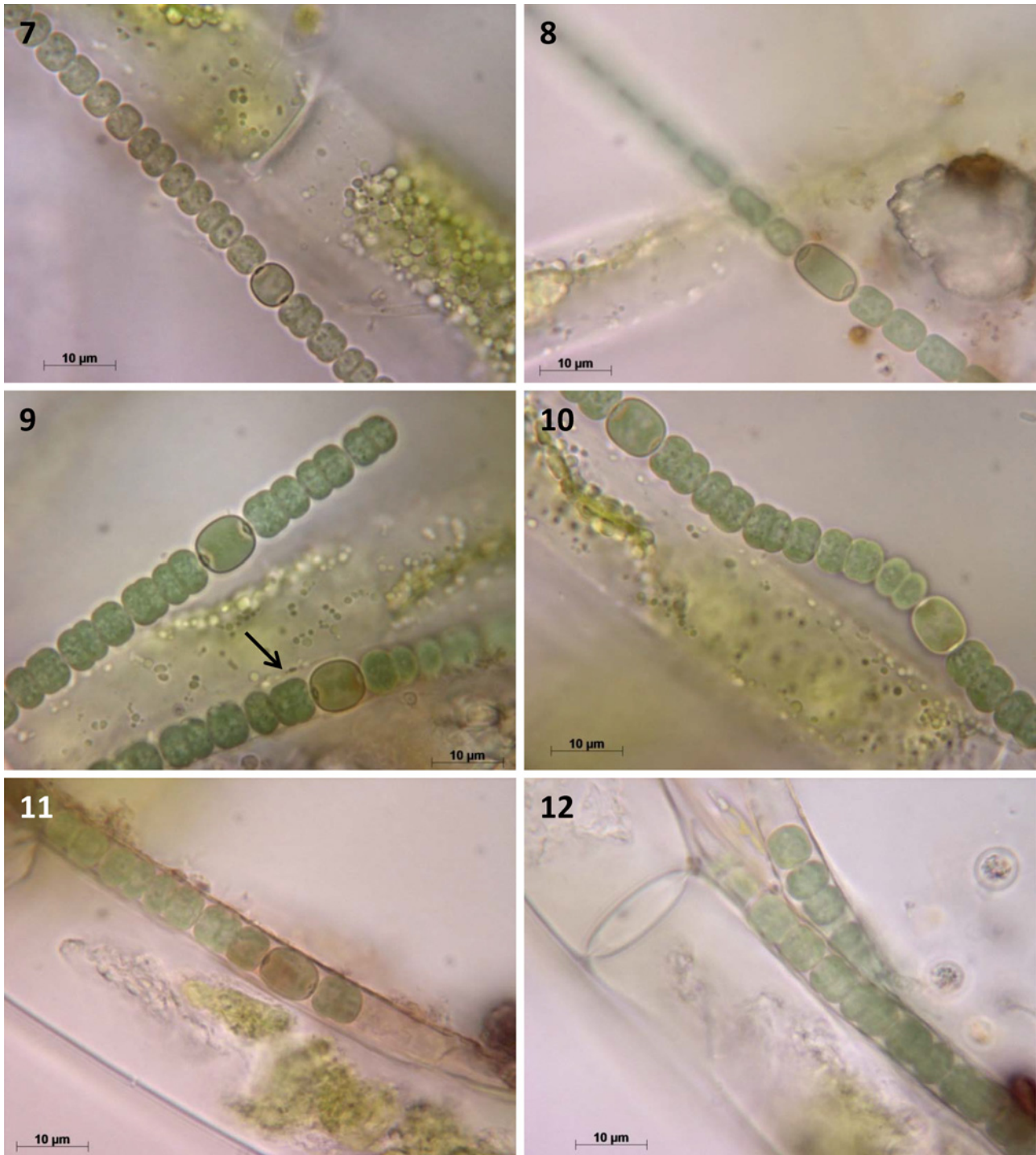
Synonyms:

*Anabaena epiphytica* Gardner 1927 (see also Komárek 2013, p. 835)

?*Anabaena huberi* De-Toni 1934 (= *Anabaena epiphytica* Huber-Pestalozzi 1930) (Komárek 2013, p. 835)

?*Anabaena repens* Behre 1956 (Komárek 2013, p.819)

?*Anabaena* cf. *verrucosa* Boye-Petersen sensu Prescott 1962 (Komárek 2013, p. 838)



Figs 7–12. Cyanobacterium *Aulosira epiphytica* (Gardner) Hindáková et Hindák, comb. nova. Filaments with spherical (7), oval (8) to short cylindrical heterocytes (9–11), with colourless sheaths (9, arrow), or prominent brownish sheaths (11,12), hormogonia escaping from sheaths (12). LM, scale 10 µm.

*Description of investigated material*

**Filaments:** solitary, straight to slightly irregularly coiled, firmly attached to algae along the whole length, up to 500 µm long or longer, (4)–4.5–6.5 µm wide, with a fine, distinct, colourless, unlamellated sheath enveloping one trichome, open at the ends or perforated after formation of hormogonia, without mucilaginous envelope; young filaments without visible sheaths.

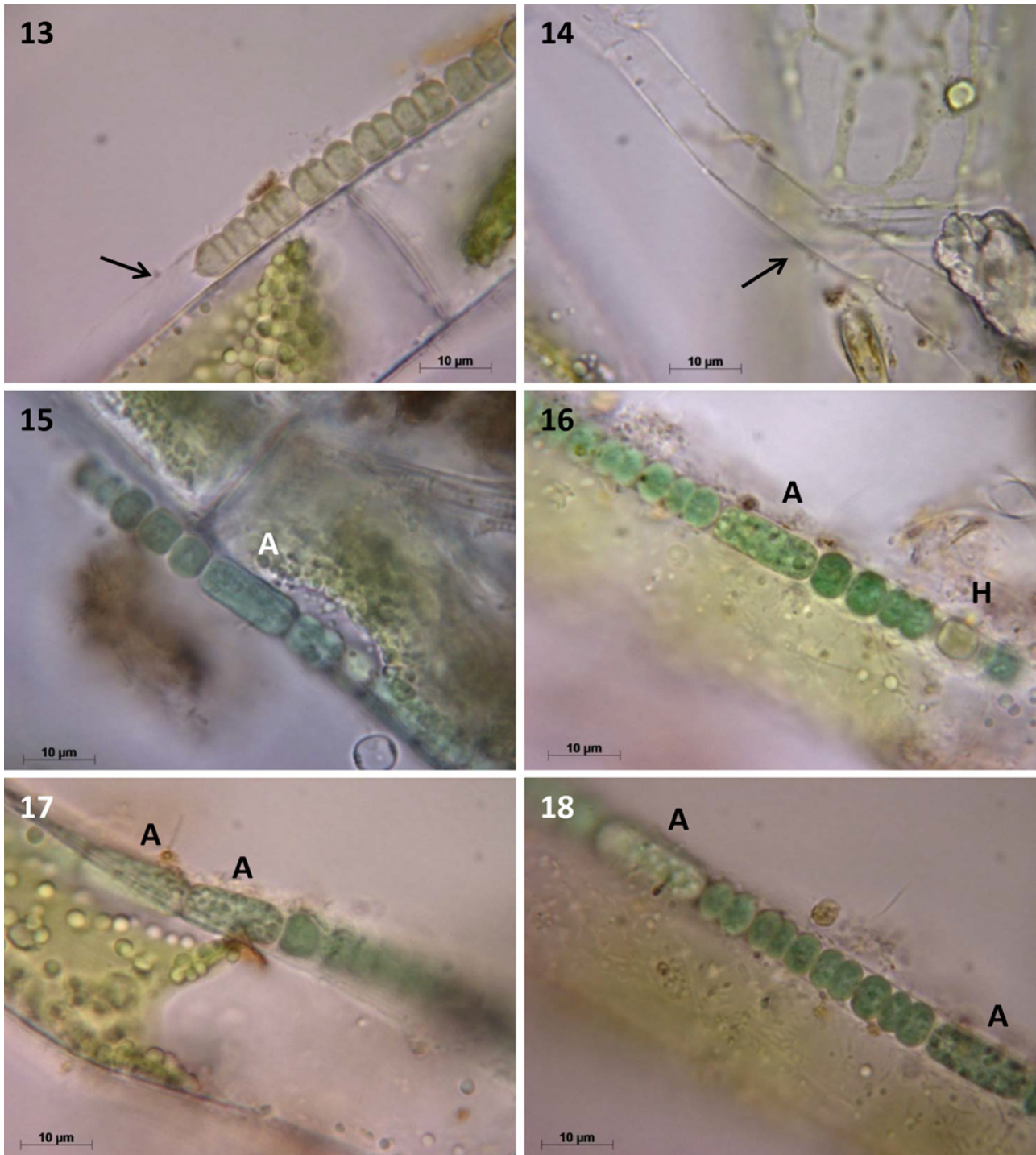
**Trichomes:** uniseriate, isopolar, always metameric, of the same width along the whole length, not attenuated to-

wards the ends, (4)–4.5–6 µm wide, constricted at the cross walls, in a developed trichome with 3–6 (or more) solitary heterocytes.

**Hormogonia:** short to long, without visible sheaths, markedly moving up and down within the sheath of “old” filaments, liberated through openings in the sheaths and moving free for some time.

**Cells:** spherical to barrel-shaped, (4)–5–6–(6.5) µm in diam., young cells hemispherical, terminal cells not elongated, rounded or shortly conical, blue-green or





Figs 13–18. Cyanobacterium *Aulosira epiphytica* (Gardner) Hindáková et Hindák, comb. nova. Terminal cells within colourless sheaths (arrow, 13), empty sheath (14), filaments with single akinetes (15, 16), and with two akinetes side-by-side (17) or apart (18), A – akinete, H – heterocyst. LM, scale 10 µm.

pale olive-green, without aerotopes, usually with prominent granules.

**Heterocytes:** spherical, oval or rarely shortly cylindrical, usually of the same width or slightly greater than vegetative cells, (5)–5.5–7–(12) µm long, intercalary, developing regularly at certain distances (30–90 µm) from one another along the trichome.

**Akinetes:** long cylindrical, 11–18 × 5–5–(7) µm, apoheterocytic (developing between two heterocytes), solitary or in pairs, with smooth exospores, with many

granules of different size.

**Cells:** dividing cross-wise and growing to the original size before the next division; without meristematic zones.

**Reproduction** by hormogonia escaping from sheaths and by akinetes.

**Ecology:** in littoral algal mats, attached to filamentous green algae (*Mougeotia* sp. steril.) or to diatoms (*Aulacoseira italica*) in the irrigation Červený canal at Čiližská Radvaň, South Slovakia.

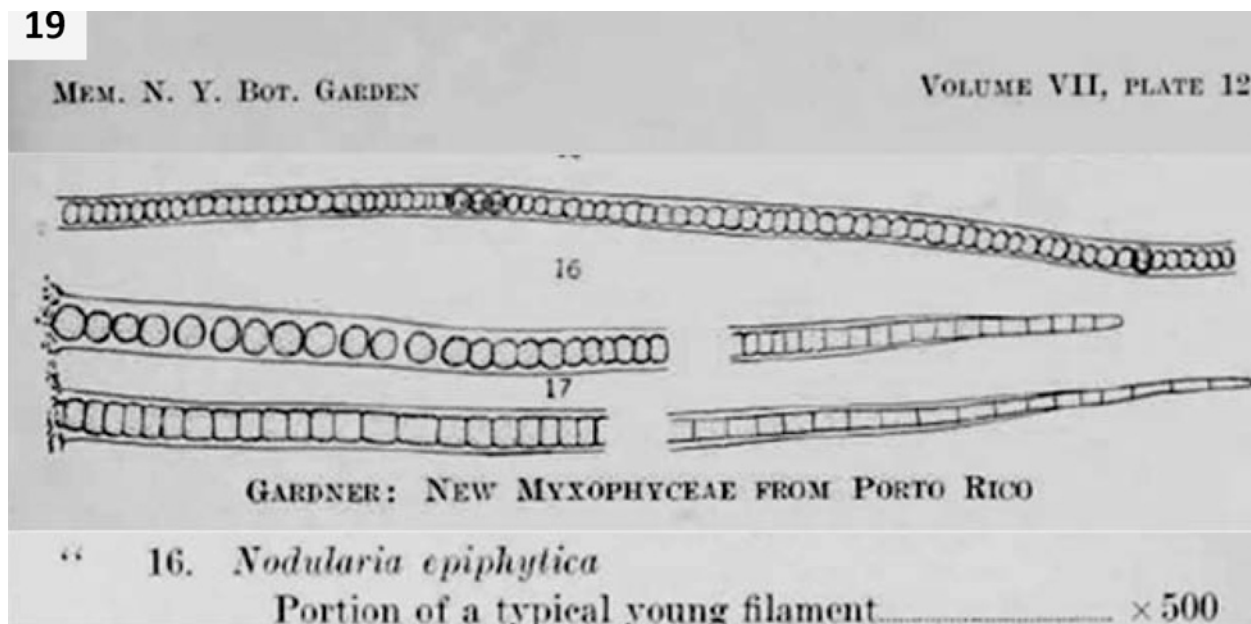


Fig. 19. Gardner's (1927) original drawing of *Nodularia epiphytica*.

## Discussion

The main criteria used for the taxonomic identification of the studied heterocytous cyanobacterium as *Aulosira epiphytica* were attached filaments, presence of a sheath of filaments and formation of akinetes. Studied material of *Aulosira epiphytica* was unique by long slightly coiled filaments, longitudinally firmly attached selectively to filaments of *Mougeotia* sp. steril. and to long chains of the diatom *Aulacoseira italica*, although other filamentous algae (especially of the genus *Oedogonium*) were also present. Hormogonia or young filaments in subcultures were free and able to move (e.g. Figs 7–10). In this very stadium, when a sheath was invisible and filaments were immotile, the filaments resembled a representative of the genus *Anabaena*. Probably, it was a case of *Anabaena epiphytica* Gardner 1927 or *A. huberi* De-Toni 1934 (= *Anabaena epiphytica* Huber-Pestalozzi 1930), in which the authors observed filaments in the stadium without visible sheaths. When aging, the hyaline sheaths (Figs 9, 13) gradually became yellowish to dark brown (Fig. 4). No specific incrustation elements on the surface of the sheaths were observed, but in old filaments the incrustation was irregular (Fig. 11). Occasionally, very fine longitudinal striation was faintly visible on the sheath surface. Trichomes regularly fragmented within old filaments and transformed into hormogonia which were able to move within the sheaths (Figs 4, 11, 12) and were liberated through an opening of filaments. Terminal cells of hormogonia were hemispherical, obtuse to shortly conical (Fig. 13).

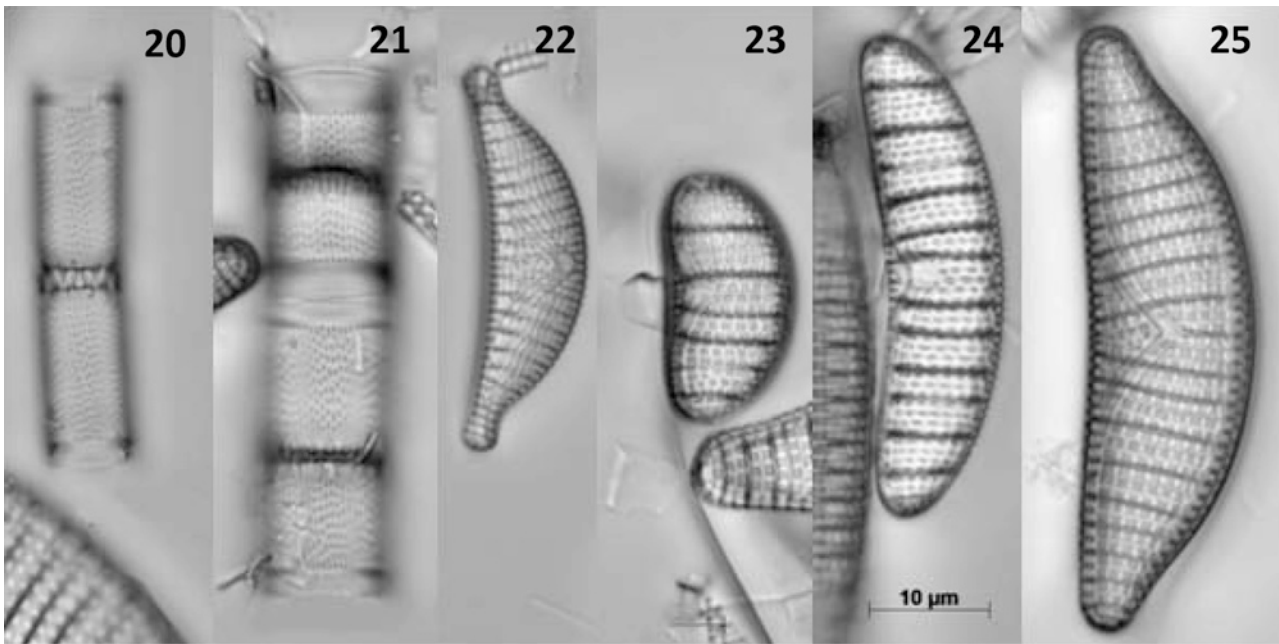
Heterocytes developed always solitary and intercalary more or less at regular distances (Fig. 5). In field material they were mostly spherical (Fig. 7), but in subcultures slightly oval (Figs 8–10). The akinetes were regularly cylindrical and with many conspicu-

ous granules (Fig. 16–18). They were formed between heterocytes, never in their vicinity, separated by 4–10 vegetative cells. Paired akinetes were rarely observed (Fig. 17). Their cell content was bright blue-green, homogenous, with parietal chromatoplasm.

*Anabaena huberi* De-Toni 1934 (syn. *Anabaena epiphytica* Huber-Pestalozzi 1930), described from Switzerland, is most likely a synonym of *Aulosira epiphytica*. Filaments grew epiphytically on green filamentous algae, ± straight, cells barrel-shaped, 2.6–3.8 × 2 µm, heterocytes elliptic, 3–5 × 2 µm, akinetes unknown.

No other species of *Aulosira* are known to grow epiphytically on filamentous algae. In the genus *Anabaena* at least two taxa bear some resemblance to our *Aulosira epiphytica*. In the first, *A. cf. verrucosa* sensu Prescott 1962 (Komárek 2013, p. 836) in which sheaths were depicted (probably a member of the genus *Aulosira*), akinetes were long cylindrical, with rounded ends, 2–3 in a row. The second is *A. repens* Behre 1956 (Komárek 2013, p. 819) with wide mucilaginous envelopes around filaments, cells 3–4 × 1.8–3 µm and cylindrical akinetes, 15–20 × 4.5–7 µm, i.e. the akinetes were similar in shape and size as in our material of *Aulosira epiphytica*.

In the algal mats of the drainage canal that we investigated, we determined some accompanying cyanophytes and algae. According to the literature (Hofmann et al. 2013; Houk 2003; Starmach 1985), they are common and cosmopolitan taxa typical for such type of biotope, e.g. cyanobacteria from the genera *Calothrix*, *Leptolyngbya*, *Nostoc*, *Pseudanabaena*, the chrysophytes *Dinobryon sertularia*, *Epipyxis socialis*, *E. utriculus*, *Lagynion* sp., diatoms including *Aulacoseira italica* (Figs 20, 21), *Melosira varians*, *Navicula radiosa*, *Cocconeis placentula*, *Cymbella cymbiformis*, *Encyonema silesiacum*, *Epithemia sorex* (Fig. 22), *E. frickei* (Figs 23, 24), *E. turgida*



Figs 20–25. Chains of the centric diatom *Aulacoseira italica* (20, 21) as a substrate for the studied cyanobacterium. Pennate diatoms of the genera *Epithemia* which were frequently attached on filaments of *Zygnema* sp. together with studied cyanobacterium: *Epithemia sorex* (22), *E. frickei* (23, 24), *E. turgida* (25). LM, permanent slide, scale 10 µm.

(Fig. 25), *Fragilaria acus*, *F. brevistriata*, *F. ulna*, *Gomphonema acuminatum*, *G. parvulum*, *Halamphora veneta*, *Nitzschia gracilis*, *Nitzschia* spp., *Rhopalodia gibba*, and the green algae *Ankistrodesmus fusiformis*, *Coelastrum astroideum*, *Didymocystis inconspicua*, *Pediastrum boryanum*, *P. tetras*, *Scenedesmus abundans*, *S. acuminatus*, *S. armatus*, *S. maximus*, *S. obliquus*, *Oedogonium* sp. div., *Cylindrocapsa* sp., *Cosmarium laeve*, *Closterium moniliferum*, *Zygnema* sp. steril., etc.

For a comparison, we cite here the original diagnosis of Gardner (1927, p. 65) of *Nodularia epiphytica* which was chosen as a basionym for our new combination *Aulosira epiphytica*: “Filaments aggregated into small masses or scattered more or less upon the host; mostly relatively straight but in part curved, 60–125 µm long, 4.7–5 µm diam.; trichomes 3–3.8 µm diam.; cells bright aeruginous, homogeneous, in part showing a clear central area, about one half the diameter long, deeply constricted at dissepiments, making them short-dolioform; apical cells subspherical; heterocysts of the same shape and size as the cells, or in part larger, sparse, at times 2–3 seriate; sheath very nearly transparent, thin; spores unknown. Growing on a very small species of Hepaticae on limestone, Hato Arriba, Arecibo, no. 1415 a, type; Coamo Springs, no. 282 d.”

Our investigated material of *Aulosira epiphytica* deviates from the original diagnosis of *Nodularia epiphytica* only in two respects: (i) heterocysts were formed always solitary, never (at times) in 2–3 in a rows, (ii) the substrate of the filaments were algae, not liverworts. However, Gardner’s type locality in Porto Rico is geographically rather far from our collection site (Central European Čiližská Radvaň), so a more detailed ecological comparison of these two habitats would be useful.

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