

# Conspicuous forms of heterocytes and hormogonia in *Rivularia mesenterica* (Cyanophyta/Cyanobacteria)

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*Dedicated to Assoc. Prof. Dr Stefan Draganov in honour of his 75<sup>th</sup> anniversary*

**Abstract.** Formation of heterocytes and hormogonia in the marine macroscopic nostocalean cyanophyte/cyanobacterium, *Rivularia mesenterica*, was studied. Radial filaments in macroscopic, hollow, spherical to hemispherical thalli attached to stones in the intertidal region of the Adriatic Sea, Croatia, were heteropolar, *Dichothrix*-like false branched, with 1–3 basal heterocytes. Trichomes were divided into two parts in the median enlarged portion. After division, the lower part of a trichome developed as its main axis, and its upper (originally apical) part became a lateral axis attached to the main axis by a spherical heterocyte. Afterwards, spherical heterocytes died off, and consequently the lateral filament became separated from the main axis. One or two conical, cylindrical, spherical, or discoid heterocytes adjacent to the spherical heterocyte arose subsequently. Terminal hormogonia and hormogonia originating from the old parts of filaments were observed in the laboratory cultured material.

**Key words:** Adriatic Sea, Croatia, *Cyanophyta/Cyanobacteria*, heterocytes, hormogonia, *Nostocales*, *Rivularia mesenterica*

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## Introduction

According to the latest classification of the *Nostocales* by Komárek & Anagnostidis (1989), the genus *Rivularia* J. Agardh ex Bornet & Flahault is placed in the family *Rivulariaceae* Kütz., in the neighbourhood of the genera *Dichothrix* Zanardini ex Bornet & Flahault and *Gloeotrichia* J. Agardh ex Bornet & Flahault. It differs from the first genus by the production of macroscopic spherical to hemispherical thalli with radially positioned filaments, and from the second genus by the absence of akinetes. Geitler (1932) introduced 20 species of the genus, of which eight taxa were marine. Desikachary (1959) reported seven species from India, while Starmach (1966)

in his Polish flora and John & al. (2002) in the flora of the British Isles both listed ten species. Komárek & al. (2003) accepted the same number of species (but not the same species) as Geitler did in 1932.

The present paper offers results based on observations of the thallus morphology of *Rivularia mesenterica* Thur. (nomenclature following Geitler 1932) in natural, as well as in laboratory conditions. Special attention is paid to the modes of formation of heterocytes and hormogonia that are in some respects unusual in comparison to the representatives of this group of nostocalean cyanophytes.

## Material and methods

The investigated material was collected by Alica Hindáková, SAS Institute of Botany, Bratislava, from rock surfaces in the Adriatic Sea, in the uppermost part of the intertidal region at Zambratia, Istria Peninsula, Croatia, on July 11, 2006. The species formed macroscopic hollow spherical to hemispherical thalli (Fig. 1).

In the laboratory, natural material was transferred into Petri dishes with a salt medium and put in a cultivation box. The cyanophytes were grown in liquid and on agarized marine medium (Red Sea Salt used for aquaria), under standard laboratory conditions, i.e. illuminated by fluorescent tubes and at a temperature of about 25–30 °C. The collected material refused to grow in any freshwater media. Several subcultures were isolated but permanently other cyanophytes (namely from the genus *Leptolyngbya* Anagn. & Komárek) contaminated the colonial mucilage of *R. mesenterica*.

A Leitz Diaplan microscope equipped with a Wild Photoautomat MPS 45 was used for LM investigation. The preserved samples are stored at the Institute of Botany of the Slovak Academy of Sciences in Bratislava, Slovakia.

## Results

### Natural material

Description: Colonies compact, hollow, spherical to hemispherical, macroscopic, up to 4 cm in diameter, attached basally to periodically submerged stones in the tidal zone, dark-green in colour, solitary or confluent (Fig. 1). Colonies formed by radially positioned filaments, embedded in firm mucilaginous envelopes. Filaments with false branches, heteropolar, with 1–3 basal heterocytes and hair-like apical ends, not extending beyond the surface of the mucilage. No calcite crystals deposited in mucilage were observed. Branches of the *Dichothrix*-type, solitary, multiaxial (Figs 2–4). Filaments densely and evenly distributed in the colony, perpendicular to the outer surface. Sheath firm, mucilaginous and hyaline, up to 12 µm thick, at the base of filaments as wide as spherical heterocytes, longitudinally layered, reaching the end in old filaments. Trichomes not, or slightly constricted at the cross walls, at widest points 10–12 µm. Heterocytes basal, 1–3 in number; no akinetes formed. Trichomes divided into two parts in

the intercalary meristematic zone, approximately in the middle part of the branch axes.

Division of the trichomes was similar to other species of the genus *Rivularia* (Geitler 1930, 1960; Kosinskaya 1948; Komárek & Anagnostidis 1989; Komárek & al. 2003), but some peculiarities were observed. Trichomes were divided into two approximately equal parts at the widest point of their enlarged median portion, i.e. before formation of heterocytes at the base of the new branch axes. The dividing zones of trichomes became gradually and markedly widened into a long fusiform shape, similarly as in *R. polyotis* (J. Agardh) Bornet & Flahault (cf. Geitler 1932, Fig. 40b or 1960, Fig. 93b). Thus these so-called meristematic zones differed from other parts of the trichomes by size, more intensive blue-green colour, abundant presence of granules and conspicuous constriction at the cross walls (Figs 5–8). After division of the trichomes, their lower (originally basal) part developed into a main axis of the trichomes, while the upper (originally apical) part changed into a lateral axis. The lateral axis remained attached to the main axis by a newly formed basal heterocyte (Figs 6–8, 10, 11, 19a, b). Adult heterocytes were regularly spherical and slightly thickened at the connection point with a neighbouring vegetative cell. Their contents were not homogenous, but irregularly granulated and greyish, 12–15 µm in diameter and, hence, remarkably different from the heterocytes developed later in their neighbourhood. Spherical heterocytes were connected to the main axis by a widened hemispherical outgrowth of the sheath (Figs 6, 7, 10). Subsequently, spherical heterocytes died off, and thus the lateral filaments became detached from the main axis and started to grow as an independent free main axis.

“Typical” heterocytes were formed secondarily, adjacent to the primary originating spherical heterocytes, 1–2 in number. They were as wide as vegetative cells, but smaller than the primary heterocytes, and broadly oval to conical, cylindrical, spherical, or discoid, with homogenous content and yellowish-green to yellow in colour, and as wide as their adjacent vegetative cells (Figs 10, 11). In natural material, neither necridic cells during division of filaments, nor intercalary heterocytes were observed.

Vegetative cells were elongated, cylindrical to discoid, bright blue-green, at the basal part of the trichomes, 5–8–(12) µm wide and with hair-like tips, without aerotopes. In colonies, no apical hormogonia were observed.

### Laboratory cultivated material

Natural material kept under laboratory conditions gradually lost some of its typical features. Colonies disintegrated into smaller segments or solitary filaments. Basal spherical heterocytes were not formed anymore, but production of intercalary heterocytes and hormogonia was commonly observed. Hormogonia originated in two types: from attenuated trichome ends in the upper part of the colonies (Figs 12, 13) and from old parts of trichomes (Figs 14, 15).

Terminal hormogonia are known in many species of the genus *Rivularia*. Trichomes of *R. mesenterica* were segmented into one or several parts and hormogonia were finally released from the sheath. Some hormogonia were fusiform in the beginning (Fig. 13), then developed into sausage-like filaments with cells of equal width, but markedly constricted at the cross walls (Fig. 12). Subsequently, one heterocyte was formed at one end of the hormogonium. Hormogonia exhibited a slow gliding movement.

The second type is represented by hormogonia arising from an old fragment of the filaments, usually in their basal parts. A group of cells or an isolated cell (Fig. 14, 15) grew into short hormogonia with a heterocyte at one end. Hormogonia germinated either in monopolar *Calothrix*-like filaments (cf. *C. nidulans* in Kondrateva 1968, Fig. 222), or bipolar *Scytonematopsis*- or *Camptylonemopsis*-like filaments (cf. Komárek & Anagnostidis 1989, Tables 1, 8). Occasionally, one or two large heterocytes were produced in the central part of the hormogonia and the vegetative cells of the hormogonia grew in both directions of the tapered ends, horn-like in shape (Fig. 17), or resembling a long letter U (Fig. 18). In old subcultures, hormogonia aggregated together resembling filaments in some species of the genus *Calothrix*, which have conspicuously wider (to 20 µm) basal parts (Fig. 16).

### Discussion

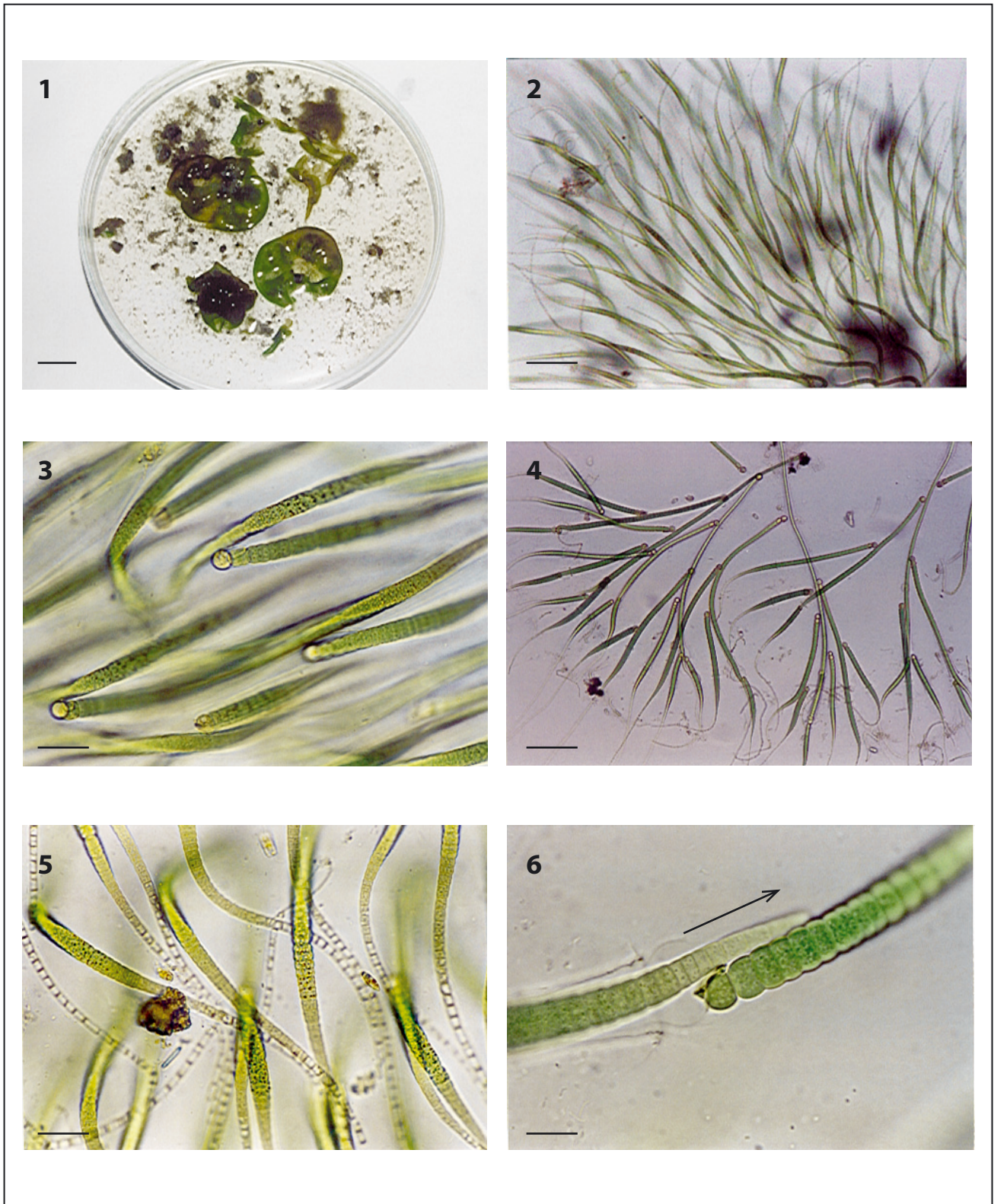
Using Geitler's (1932) monograph on Cyanophytes, the investigated colonies from the Adriatic Sea can be assigned to the species *R. mesenterica*. Unfortunately, no illustrations were included in the above-mentioned book, but the description of colonies, filaments and cells is in good agreement with our obser-

vations. Another point in favour is the original place of its occurrence: the Mediterranean Sea in Adria. The species was recently documented on colour photos by Kaštovský (2006) and his finding also comes from Croatia: at Sumartin, Island of Brač. No data on this species outside the Mediterranean region were found in the cited cyanophyte monographs. A similar species with cosmopolitan distribution is *R. bullata* (Poir.) Berk. ex Bornet & Flahault, with filaments 5–8–(10) µm wide (Geitler 1932; Komárek 1956; Desikachary 1959), but little is known about its morphology and ecology (John & al. 2002). However, persistence of the daughter trichome in the sheath of mother filament, as described by Komárek (1956) in *R. bullata*, was not observed in *R. mesenterica*, and correspondingly no formation of intercalary heterocytes (Fig. 19).

In our material two peculiar phenomena were found: the formation of a special type of basal heterocyte in natural material, and the production of hormogonia from old parts of the trichomes in laboratory subcultures.

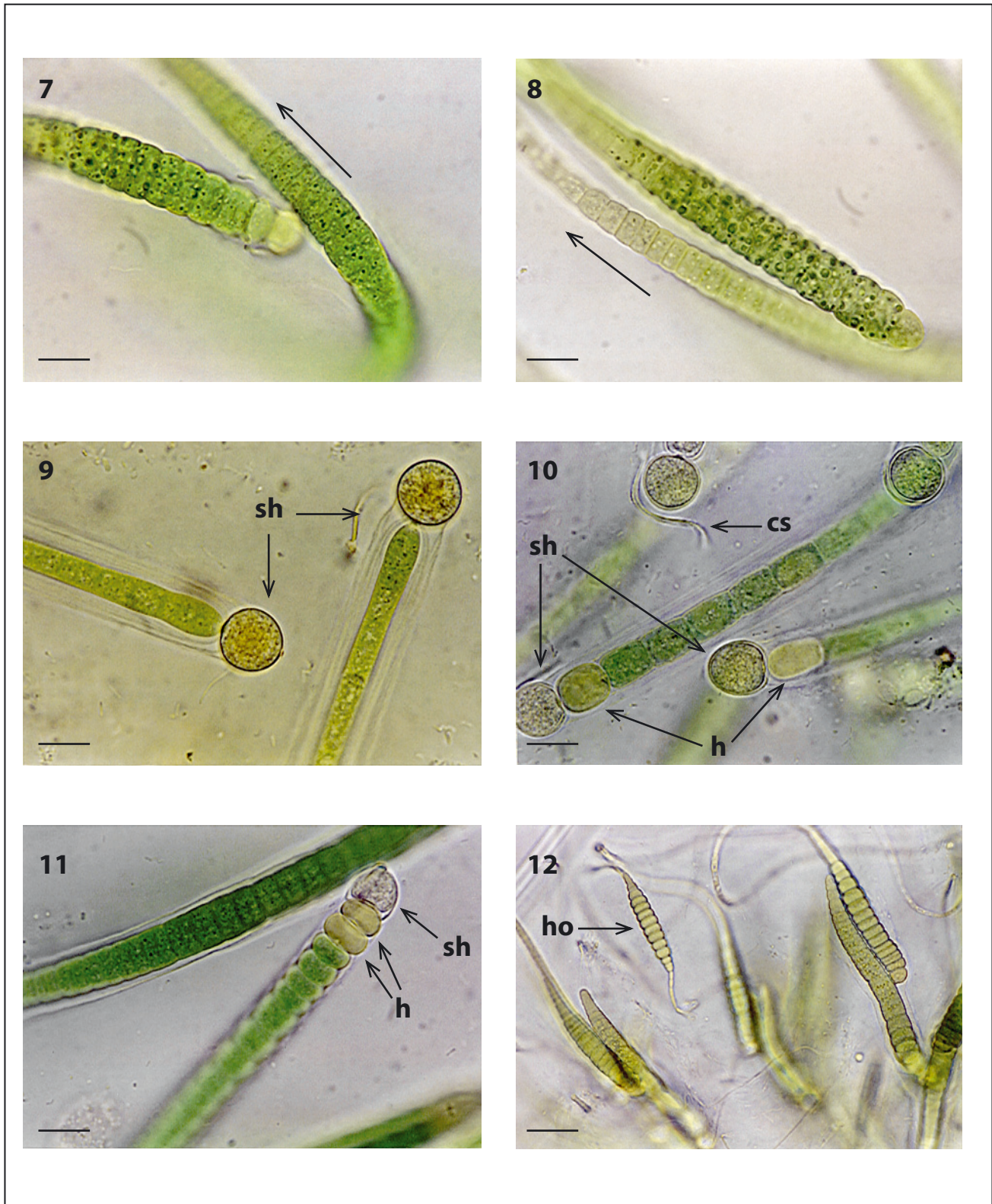
The production of basal heterocytes is a rather common feature in the families *Scytonemataceae*, *Microchataceae* and *Rivulariaceae* (cf. Komárek & Anagnostidis 1989; Hindák 2001). Like in the other members of the *Nostocales*, these heterocytes can be designated as "typical" in their origin and function. However, spherical heterocytes in *R. mesenterica* that are formed at the base of a new lateral axis after the division of trichomes resemble the typical heterocytes only superficially. Their main function is apparently connected with the growth and enlargement of colonies: death of a heterocyte interrupts the connection between the main axis and the lateral axis and the young filaments are separated and start to grow as a main axis (Fig. 19a, b). This is evident from the peculiar colour and structure of their contents and from the fact that the function of spherical heterocytes is temporally limited. Further studies (especially EM) are needed for the elucidation of this phenomenon unobserved in nostocalean cyanophytes.

Formation of hormogonia by disintegration of trichomes directly or via a necridial disc, or necridial cell is known in many cyanophytes. In some cases the germination of hormogonia in *R. mesenterica* resembles the germination of akinetes (Figs 14, 15). However, the genus *Rivularia* has no akinetes and the old cells in the filaments have no structured cell walls.



**Figs 1–6.** *Rivularia mesenterica*:

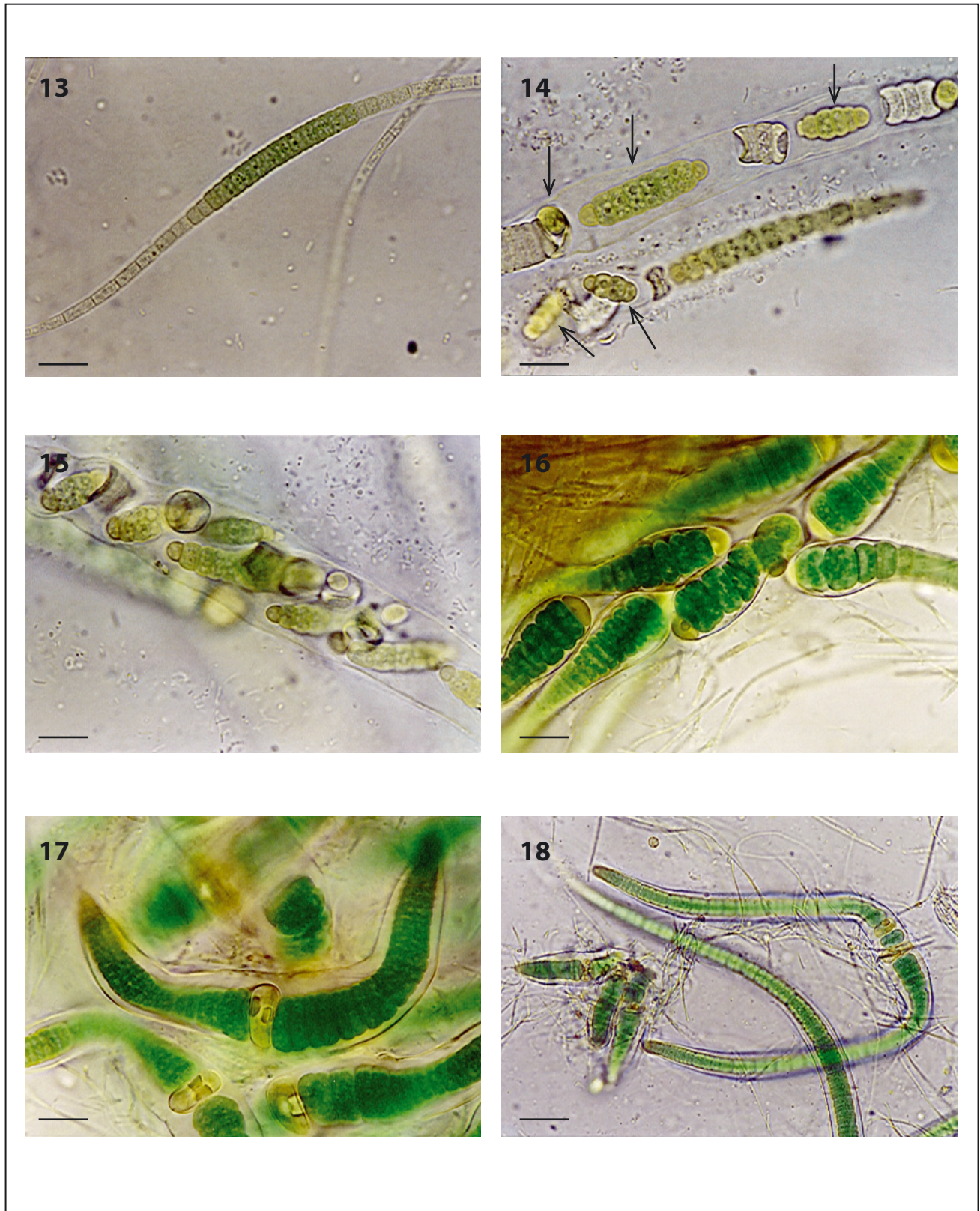
1, macroscopic colonies from stones at Zambratia, Istria. Scale = 1 cm; 2–4, ramification of filaments in the colonies; 5, meristematic zones in filaments; 6, formation of a lateral and main (arrow) axis after the filament division. Scale = 10  $\mu$ m.



**Figs 7–12.** *Rivularia mesenterica*:

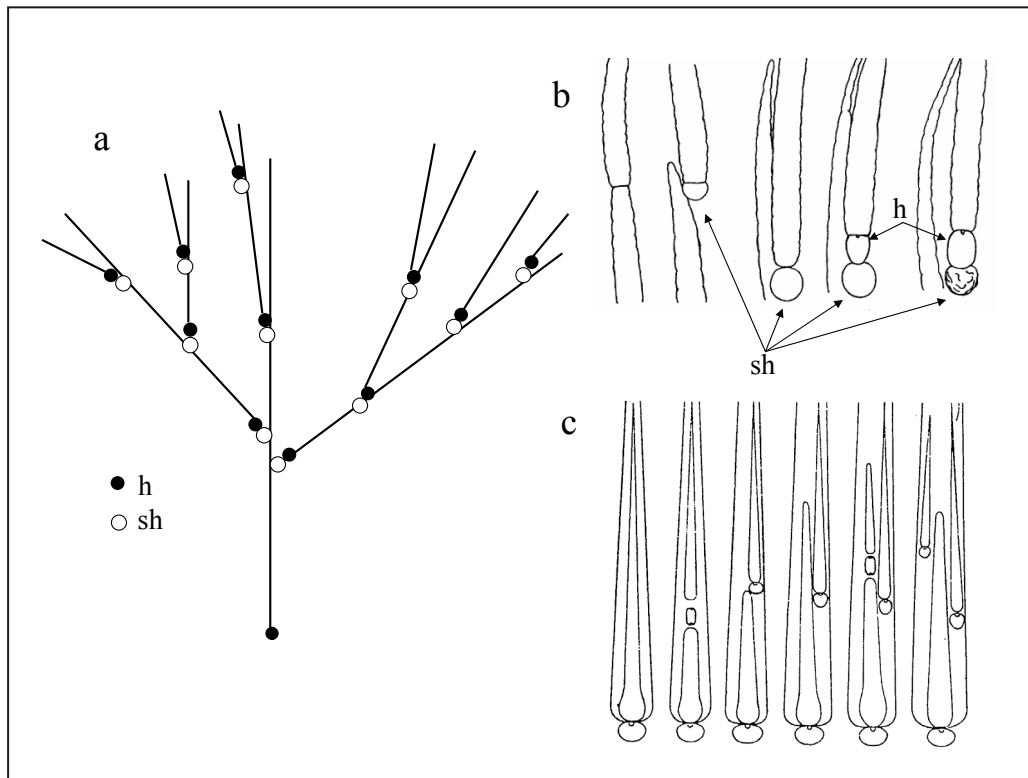
7–8, formation of heterocytes at the base of lateral filaments, arrows indicate the main axes of filaments; 9, trichomes with laminated sheath and basal spherical (primary) heterocyte; 10–11, filaments with basal spherical heterocyte and adjacent heterocytes; 12, formation of a hormogonium in the upper part of the colony.

**Abbreviations:** **h** – heterocyte, **ho** – hormogonium, **cs** – connecting sheath, **sh** – spherical heterocyte. Scale = 10  $\mu$ m.



**Figs 13–18.** *Rivularia mesenterica*:

13, formation of a hormogonium in the upper part of the trichome; 14–15, formation hormogonia (arrows) in old parts of trichomes, 16, monopolar germination of hormogonia into *Calothrix*-like filaments; 17–18, bipolar germination of hormogonia into *Scytonematopsis*/*Camptylonemopsis*-like filaments. Scale = 10  $\mu$ m.



**Fig. 19a, b.** Scheme of development of young trichomes in *Rivularia mesenterica* from the studied material: **a** – *Dichothrix*-like ramification of filaments; **b** – formation of primary spherical heterocysts and typical heterocysts; **c** – scheme of development of young trichomes in *R. bullata* (after Komárek 1956).  
Abbreviations: **sh** – spherical heterocysts; **h** – heterocysts.

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