

Morphological characterization of three subaerial *Calothrix* species (Nostocales, Cyanobacteria)

Bohuslav UHER

Department of Botany, Faculty of Natural Sciences, Comenius University in Bratislava, Révová 39, SK-81102 Bratislava, Slovakia

Abstract: This paper characterizes and discusses the concept of species *Calothrix braunii*, *C. capitularis* spec. nova and *C. parietina* which were identified on subaerial rocky substrates in gorges of the Slovenský raj National Park in Slovakia. In this paper, we focused on phenotypic characters, i.e. natural and cultural morphological variability. Their variable and stabile phenotypic markers are discussed.

Key words: cyanobacteria, cyanophytes, subaerial habitats, *Calothrix*, Slovenský raj National park, Slovakia

Introduction

The genus *Calothrix* was firstly described by C. AGARDH (1824). It is a polymorphic genus with the inner taxonomy which is unclear yet. The common features are mainly these: hormogonia give rise to young filaments with terminal heterocyte at only one end of the trichome; mature trichome tapers from base, which bears a terminal heterocyte, to apex; vegetative cells disc-shaped, isodiametric or cylindrical (KOMÁREK & ANAGNOSTIDIS 1989).

The purpose of this research was to examine the morphological variability and ontogeny of three species within genus *Calothrix* C. AGARDH ex GOMONT et FLAHAULT 1886 and the congruence of the natural and culture material. Autecology of species is discussed.

Material and methods

The Slovenský raj National park (HRIC 1982) is situated between 20°16'15"-20°31'40"E and 48°57'35"-48°57'30"N (TULIS 1985). On June 23rd 1999, the samples were scraped from the calcareous rocky walls in the Kysel' Gorge (scraped from KAROLÍNY's Waterfall; No. 1) and in the Kláštorská Gorge (scraped from ANTON STRAKA's Waterfall; No. 2). Samples were taken from the wet limestone walls near the waterfalls and they were kept in plastic bags at low temperature (4°C) before examination. The morphology of the species was studied from both natural and cultured material using LM Zeiss. Isolated strains: UHER 2000/6527, UHER 2000/5536 and UHER 1999/011 are deposited in Department of Botany, Faculty of Natural

Sciences, at Comenius University in Bratislava, Slovakia. The strains were maintained in the media BG11 (RIPPKA et al. 1979) and BG11₀ (RIPPKA 1988) at temperature 20 °C, illumination 500 lx, continuous light. The cultivation was necessary for detailed taxonomic studies of these subaerial cyanobacteria. Species identification follow KOMÁREK & ANAGNOSTIDIS (1989), nomenclature unified according to BRUMITT & POWELL (1992).

Results and discussion

Calothrix braunii BORNET et FLAHAULT (Fig. 1)

Thallus brown-green, microscopic or macroscopic, fascicular, bushy. Filaments rarely branched, up to 500 µm, at the basis bulbously wide (7.5)9–12.5(15) µm, in the middle of filament 4.5–8.5 µm wide, parallelly arranged, creating macroscopic lines on substrate. Sheaths yellowish, brownish, not-layered. Trichomes not clearly constricted at the cross walls, ended by hairs. Cell sizes at the basis are (4.5)5–7 × 5–12.5 µm, in the middle zone of trichomes 5–7 × 2.5–5 µm. Heterocytes hemispherical, basal, 5–7 µm in diameter.

This taxon was found at the site No. 1 and its detail ontogeny was studied from the strain UHER 2000/6527. *C. braunii* was recorded from submerged stones, shells (conches) and plants in Europe and North America (FRÉMY 1927, GEITLER 1932, COPELAND 1936, KOSTER 1960, STARMACH 1966), especially from submerged plants of the genus *Carex* (WHELDEN 1947); found on the wall of a house (described as var. nov. *concorda*) and the

soil (described as var. *nova mollis*; GARDNER 1927); from moss tufts on rocks associated with *Nostoc microscopicum* (CARMICH.) ELENKIN (NAKANO 1971). It was also recorded from rock-pools by KANN (1978), from dead submersed plants by DROUET (1943) and on rocks in streams by LÓPEZ & PENALTA (2004) and UHER & KOVÁČIK (2002). Records published by EL-AYOUTY et al. (1977), WURTZ (1947, p. 105), THOMPSON (1938) and COM-PÈRE (1967, 1974), seem to represent other species, because of differences in thallus habit, character of cells, basal trichome parts and sheaths. These data show that the habitats typical of *C. braunii* are surfaces of wet or submerged rocks and plants in relatively clear water (especially dead plants or wood).

***Calothrix capitularis* spec. nova** (Fig. 2)

Latin diagnosis:

Thallus macroscopicus, formae linearis, fascicularis, olivaceo-viridis aut fusco-viridis; filamentis heteropolaribus, singulis aut 2–3(5) consociatis, raro ramosis, parte basali incrassato concretescentibus, 400–800 µm longis, 7.5–25 µm crassis, ad apicem versus attenuatis, in pilum articulatum productis, pilis 50–150 µm longis, 2–3 µm crassis. Cellulae infima basi 15–25 µm longae, crassae, diametro 2-3-plo breviores, ad septa constrictae, cellula basali rotundato-subglobosa. Trichomata aeruginea vel intense glauco-viridia, in media parte 5,5–7,5 µm crassa, ad septa plus minusve (interdum valde) constricta. Sporae ad filorum basin solitariae; heterocytis basalibus (1), rarius intercalariibus, rotundato-conicis, incoloratis aut flavis. Vaginae tenues homogeneae hyalinae. Reproductio per sporas et hormogonia heteropolaria.

Locus classicus: in Karoliny cataracta territorii Slovenský Raj dicti, Slovakia.

Habitatio: Inter petras calcareas ad parietes humidus territorii Slovenský Raj (“Paradisus Slovacus”) dicti.

Typus: UHER 1999/011 (Herbarium SLO, Bratislava, SVK), Icona typica (Holotypus): Figura Nostra 3.

Thallus macroscopic, lines forming, fascicular, olive or brownish green; created by filaments arranged parallelly or solitary, rarely branched. Filaments olive-green, blue-green, up to 800 µm long, at the basis expressively cudgel-shaped or elliptically widespread, up to 25 µm

wide, after abruptly narrowed continuing by trichome 7.5–9(10) µm wide. Filaments ended by long hyaline hair, up to 150 µm long, 2–3 µm in diameter. Trichomes heteropolar, at the basis with hemispherical terminal heterocyte, 7.5–10 µm in diameter. Cells at basis narrow-elliptic or narrow-cylindrical creating spore, often with clear constrictions by cross walls, (10)12.5–15(17) µm in diameter. Cells in the middle zone of trichome wider than longer or isodiametric, 5.5–7.5 µm in diameter. Initial stages 6-10-celled, heteropolar, later creating specific basal wideness and apical hair. Reproduction by 6-16 celled hormogonia.

This species was found at the sampling places Nos 1 and 2. Its detail life cycle was studied from the strain UHER 1999/011. *C. capitularis* spec. nova was compared with all the validly described *Calothrix* spp. In particular, stable characteristics were compared (not changed by cultivation): shape and localisation of heterocytes, presence/absence of hair, terminal part of trichomes (spore/akinete creation), character of sheath (lamellate or non-lamellate). The autecological characters were compared too (see Tab. 1). The ontogeny was not compared because of lack of ontogenetic observations in literature. Autecologically close to *C. capitularis* are the following epilithic species: *C. braunii* f. *concorda* N.L.GARDNER (GARDNER 1927), *C. breviararticulata* f. *tatrensis* STARMACH (STARMACH 1966), *C. clausa* SETCH. et N.L.GARDNER (GEITLER 1932), *C. clavata* G.S. WEST (WEST 1914), *C. clavatoidea* S.L.GHOSE (GHOSE 1927), *C. contarenii* (ZANARDINI) BORNET et FLAHAULT (BORNET & FLAHAULT 1886), *C. estonica* KUKK (KUKK 1959), *C. fonticola* BRABEZ (BRABEZ 1941), *C. fusca* f. *durabilis* STARMACH (STARMACH 1966; alkaline substrate), *C. parietina* (NÄGELI) THUR. (THURET 1875), *C. pulvinata* (MERT.) C. AGARDH (AGARDH 1824), *C. simplex* N.L.GARDNER (GARDNER 1927), *C. violacea* JAAG (JAAG 1938; alkaline substrate). We observed *C. capitularis* sp. nov. on limestone (alkaline substrate). If the stable morphological characteristics were compared, then the closest species are: *C. braunii* f. *concorda* N.L.GARDNER, *C. clausa* SETCH. et N.L.GARDNER, *C. codicola* SETCH. et N.L.GARDNER (SETCHEL & GARDNER 1930) and *C. simplex* N.L.Gardner. *C. simplex* resembles some sterile initial stages of our new species (cf. GARDNER 1927). But the typical features of adult habit of

C. capitularis (presence of spores and wide terminal cells) are missing in description of *C. simplex*.

***Calothrix parietina* (NÄGELI) THUR. (Fig. 3)**

Colonies brown, or olive-green, incrusting by carbonates. Filaments up to 1 mm, at the basis (7)9–18.5(23) μm wide, in the middle zone (5.5)7–14(16) μm wide, often branched. Trichomes by the cross walls constricted or not constricted, in hair ended. Cell sizes at the basis of trichome (4)5–12 \times (4)4.5–9(13) μm , in the middle zone 5.5–12.5 \times 5.5–7.5(10.5) μm . Heterocytes terminal, sometimes 2–3 together, with sizes 7.5–12.5 \times 7.5–10.5 μm .

This taxon was found at the site No. 2 and its detail ontogeny was studied from the strain UHER 2000/5536. It is the most recorded species of genus *Calothrix* in the literature and its ecological and taxonomic features, which are in some cases controversial, are often discussed. It was recorded as a cosmopolitan species from rocky streams by DROUET (1943), from limestone streams by DROUET (1939), on wet rocks and in pools by Copeland (1936) and by KANN (1978), from wetted limestone by ERCEGOVIĆ (1925) and by GOLUBIĆ (1967), moist soil, stones, wood and rocks by KOSTER (1960) and on rocks by FRÉMY (1929). But some original drawings and the description published as *C. parietina* are totally different in habit (cf. COMPÈRE 1970, SCHWABE 1962), therefore some floristic data could be lapses. The lack of exact information, such as determination of substrata, details about the habit, makes the published data unclear.

Old records show, that the genus *Calothrix* comprises about 80 freshwater and 14 marine morphotypes from temperate, subtropical and tropical regions. Many of these taxa are difficult to identify, especially in the initial young stage, which is a regular consequence of the cultivation of isolated strains. Even in natural samples, the shape of the colonies (filaments, heterocytes, hormogonia, sheaths) is very variable and if the size of cells does not differ, the decision between the names of the species offered by keys and taxonomic literature (original articles) is sometimes impossible.

Several morphological features were analyzed from natural populations, the comparisons between natural populations and cultivated strains were made to demonstrate stable phenotypic markers.

The obtained data showed that the variability of studied species is high and that it is necessary to analyze a whole life cycle for the exact identification.

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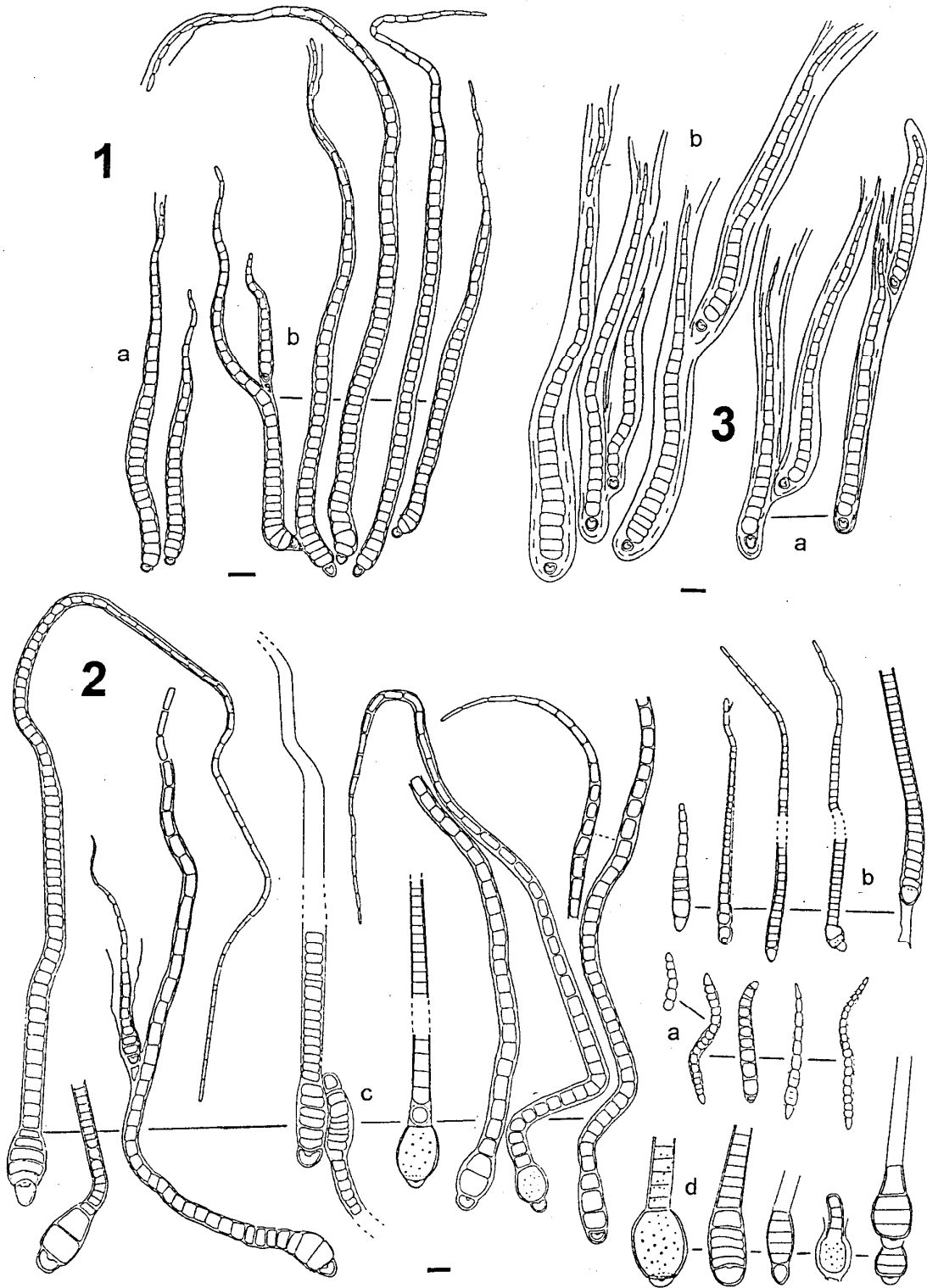
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Table 1. Comparison of cyanobacterial species close to *C. capitularis* spec. nova with respect to their autecology and morphology.

Species of genus <i>Calothrix</i>	Characteristics/features						Heterocyte Diameter [µm]	Habitat
	Filament [µm]		Cell [µm]		Sheath color	Hair P/A		
	length	width	length	width				
<i>C. braunii</i> f. <i>concorda</i> * ⁺	500-800	2,5-7,5	?	?	?	?	P	On the wall of house in Maricao
<i>C. breviaritculata</i> f. <i>tatrensis</i> *	107-357	5-12	?	?	?	yellow-brown	P	On the wet rocks
<i>C. clausa</i> * ⁺	500-600	24(28)	3-4	3-24	?	sinicolor	?	Epilithic
<i>C. clavata</i> *	100	7	4-15	5-5,5	?	sinicolor	P	Epilithic, soil
<i>C. clavatooides</i> *	200	14	6-20	3-13	?	sinicolor	P	Epilithic
<i>C. codicola</i> ⁺	400-600	16	?	10-17	?	sinicolor	P	Epiphytic, Codium sp.
<i>C. contarenii</i> *	1000	9-15	?	6-8	?	sinicolor yellowish brown	P	Epilithic
<i>C. estonica</i> *	2000	3-15	?	3-10	?	sinicolor	P	Epilithic in lake
<i>C. fonticola</i> *	62-75	7,5-10	?	?	?	?	?	In springs, aubaquatic
<i>C. fusca</i> f. <i>durabilis</i> *	38-120	7-11,2	?	3,5-11	?	?	P	Alkaline rocks, by <i>Pleurocapsa aurantiaca</i>
<i>C. parietina</i> *	1000	10-12 (18)	7,5-15	5-10	?	yellowish-brown, lamellate	P	Stagnant water, epilithic Converse taxon determined from meny habitats!!! Different iconotypes
<i>C. pulvinata</i> *	2000-3000	15-18	?	8-12	?	hyaline, brownish, lamellate	P	Periphytic, epilithic
<i>C. simplex</i> * ⁺	200-250	To 18	?	10-14	?	hyaline	P	Epilithic in streams
<i>C. violacea</i> *	100-300	?	?	3-6	?	violet	A	Epilithic, calcareous rocks
<i>C. capitularis</i> spec. nova	400-800	to 25	5,5-7,5	7,5-15(17)	?	hyaline	P	Epilithic, calcareous rocks



Figs 1-3. 1 *Calothrix braunii*: a young filaments, b adult colony; 2 *C. capitularis* spec. nova: a initial stages, hormogonia, b young filaments, c adult filaments, d the basal parts with akinetes; 3 *C. parietina*: a young filaments, b adult colony. Scale bars 10 μ m.