

## ***Anabaena fuscovaginata* (Nostocales), a new cyanobacterial species from periphyton of the freshwater alkaline marsh of Everglades, South Florida, USA.**

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**Abstract:** A new species of the nostocalean genus *Anabaena* was discovered in periphytic cyanobacterial mats of the Everglades marsh, South Florida, USA. In some of the 10 samples where it occurred, the species dominated the cyanobacterial community, however, it was always part of a rich assemblage of microbiota, including many types characteristic for alkaline herbaceous wetlands of Caribbean America or freshwater tropical marshes. On the basis of typical morphological traits and occurrence in a periphytic habitat, I suggest that the species is a member of the genus *Anabaena* in its original sense, valid after recent separation of planktic taxa. As the most distinctive morphological feature, it has firm, coloured sheaths that have never been reported for this genus before.

**Key words:** alkaline marshes, *Anabaena*, cyanobacteria, Everglades, morphology, periphyton, taxonomy

### **Introduction**

The concept of a species in Cyanobacteria and characters necessary for taxonomic description of new taxa have been a subject of continual debate among concerned researchers. The modern polyphasic approach, introduced for cyanobacteria by ANAGNOSTIDIS & KOMÁREK (1985, 1988, 1990), KOMÁREK & ANAGNOSTIDIS (1986, 1989) and since then further developed and applied by scientists all over the world (recently e.g. CASAMATTA et al. 2006; FIORE et al. 2007; FINSINGER et al. 2008; MCGREGOR & RASMUSSEN 2008; PALINSKA & MARQUARDT 2008, and many others), has proved to be the best available tool for reliable and objective taxonomical conclusions. This approach requires the synthesis of detailed description of classical morphological traits, ecology, life history, cell ultrastructure and molecular phylogeny into a congruent picture of a species defined by both unique genetic and phenotypic markers.

Unfortunately, in some cases it is not possible to fulfil all these expectations and provide complete characterization of a species within a single study. For various reasons, some cyanobacterial taxa do not grow well in cultures or cannot be returned to the laboratory alive for cultivation and molecular studies. In our experience, this especially applies

to material from either countries with few laboratory facilities, inaccessible sites, or highly specific habitats. A phycologist has to answer the question: Is it worth describing the morphological diversity of the material even if I cannot characterize its genetic identity? In my opinion, it is always useful to record the biodiversity as well as possible, thus providing at least basic data for improvements by further researchers. This approach is important in taxonomically little explored and ecologically distinct habitats where there is a great number of unknown (but characteristic) types of cyanobacteria. Examples of such environments are wetlands and forests of the tropics, high mountains, and other centers of biodiversity. In these situations, I suggest that new cyanobacterial taxa should be described according to the International Code of Botanical Nomenclature (McNEILL et al. 2006), based exclusively on morphological and ecological characteristics. It should be emphasised that the descriptions should be as precise and detailed as possible, and always accompanied by good-quality images of the material for further comparisons.

One habitat that fits these considerations is the alkaline (sub)tropical shallow marsh. Such wetlands are largely found in warm parts of America, especially the Caribbean

region: Yucatán Peninsula and South Florida (Everglades), Cuba, Venezuela, Puerto Rico, and at minor sites elsewhere. This type of marsh is characterised by raised pH (e.g. in Belize pH = 7.5 – 8.8, after REJMÁNKOVÁ et al. 2004), usually a nutrient-poor environment, fluctuating water levels, and characteristic communities of aquatic plants with extensive accumulations of calcified cyanobacteria-dominated periphytic mats. Thorough observations of the ecology, hydrology, geology and biota and possible means of protection of this peculiar type of wetland can be found in DAVIS & OGDEN (1994), REJMÁNKOVÁ et al. (1996), REJMÁNKOVÁ & KOMÁRKOVÁ (2000) and RICHARDSON (2008).

The species composition of cyanobacteria at several sites has been studied using modern taxonomic approaches. For instance, more than 80 species were found in the wetlands of Cuba (14 new taxa to science, cf. KOMÁREK 1984a,b, 1989a,b, 1995, 2005) and 65 species in the alkaline marshes of northern Belize (19 new to science, cf. KOMÁREK & KOMÁRKOVÁ-LEGNEROVÁ 2007; TURICCHIA et al. 2009). On the other hand, the systematics of the periphytic cyanobacteria of Florida Everglades have been little studied, and usually only a few dominant species have been noted (GLEASON 1974; VYMAZAL & RICHARDSON 1995; MCCORMICK & O'DELL 1996). Application of a more comprehensive examination (MAREŠ 2006) revealed great diversity of morphotypes, some of which evidently represented unknown species. So far only one of them has been described as a new species (*Wolskyella floridana* Mareš et Kaštovský, cf. MAREŠ et al. 2008).

In this study, I present a new species of *Anabaena* (Nostocales, Cyanobacteria) from the periphyton of Everglades that in my opinion also deserves valid taxonomic description due to its conspicuous phenotype and unique locality of occurrence.

## Materials and Methods

The samples of cyanobacterial mats were collected in November 1999 inside the borders of the Water Conservation Area 2A of Everglades, South Florida, USA, at the sampling points C3, C4 and C5 (for maps and detailed description of the area see VYMAZAL et al. 2002). These were the rather less impacted or unimpacted sites of a nutrient enrichment gradient caused by long-term agricultural runoff (water column total phosphorus concentration less or equal

to 14 µg.L<sup>-1</sup>). The locality was mostly ecologically similar to the original oligotrophic alkaline wetland, dominated by a community of emergent and submersed macrophytes with abundant periphyton encrusted by calcium carbonate.

The material was preserved in 1.5% formaldehyde and observed by optical microscopy with differential interference contrast. Morphology of the species was documented by means of digital photography and drawings based on examination of 10 different samples (mats).

## Results

The described species of *Anabaena* dominated the periphytic assemblage at least in some of the samples. In material from site C3 it sometimes contributed up to 50% of total biomass according to my estimations. It retained its characteristic features also in samples where it was scarce. The observed set of populations provided sufficient morphological and ecological data to separate it from existing taxa as a new species under the International Code of Botanical Nomenclature.

### *Anabaena fuscovaginata* MAREŠ, sp. nov.

*Diagnosis:* *Stratum* micro– ad macroscopicum, prostratum, submersum. *Fila* in fasciculis dense agglomerata et intricata vel inter cyanobacteria periphytica dispersa; mucilago sine colore, diffusus, interdum promptus. *Trichoma* sinuata, uniseriata, non ramosa, ad dissepimenta constricta, 2.5–5 µm lata, paucim ad extremitatis attenuata, metamerica, cum (2–) 6–18 (–25) cellulis vegetatibus inter heterocytas. *Akinetes* heterocytarum utrinque contiguae, solitariae vel raro 2 (–3)–seriatae. *Vaginae* adsunt vel gelatinosae, laeves, hyalinae vel luteo–fuscae, ad 2 µm latae, praecipue ad heterocytis akinetisque evolutae. *Cellulae* cylindricae ad doliiformae, isodiametricae vel paucim longior quam latae, 2.5–3.5 (–5) × 2.5–7 (–8) µm, aeruginosae, contentu praecipue granuloso; cellula apicalis paucim elongata, plus minusve obtuse conica. *Heterocytas* intercalares, solitariae, sphaerice cylindricae, praecipue duplo longiores quam latae, 3.5–5 (–7) × (5.5–) 7.5–10 (–12) µm. *Akinetes* cylindricae ad cylindricae–ovatae, apicibus rotundatis, 7–10 (–13) × (10–) 14–20 (–25) µm, heterocytas contiguas, episporio laevi, pallide et translucente lutescenti in akinetis maturis.

*Habitatio:* Periphytice metaphyticeque inter algas cyanobacteriasque, interdum in massis, in

*paludibus alkalinis, oligotrophicis, subtropicis cum vegetatio submerso. – Locus classicus: America septentrionalis, Florida – Everglades, in area dicto Water Conservation Area 2A.*

*Collectio conservata deposita in: BRNM HY 1.431 depositus (coll. Jan Kaštovský, 23.09.1999); iconotypus : figura nostra 1.*

Filaments densely agglomerated and entangled in clusters or dispersed among other cyanobacteria in periphyton; trichomes are sometimes enveloped by a fine and diffuent colourless mucilage. Trichomes wavy, constricted at cross walls, somewhat attenuated towards ends, 2.5–5 µm wide, with a regular metameric structure – one heterocyte usually occurs per (2–) 6–18 (–25) vegetative cells, akinetes always on both sides of the heterocyte, single or rarely 2 (–3) in a row. Sheath smooth, gelatinous, brownish–yellow, sometimes colourless, up to 2 µm wide, usually covering the heterocyte, the neighbouring akinetes and a few adjacent cells, rarely absent. Vegetative cells barrel-shaped, longer than wide or isodiametric, 2.5–3.5 (–5) × 2.5–7 (–8) µm, with blue–green, granulated contents. Apical cells are a little longer, conical or rounded–conical in well–developed trichomes. Heterocytes rounded cylindrical, 3.5–5 (–7) × (5.5–) 7.5–10 (–12) µm, (1.2–) 1.7–2 × longer than wide. Akinetes rounded cylindrical, 7–10 (–13) × (10–) 14–20 (–25) µm, 1.5–2.5 × longer than wide, with smooth translucent yellowish walls.

Freshwater, scattered among other cyanobacteria and algae, sometimes dominant, in periphyton. Recorded from the Florida Everglades, USA, a subtropic alkaline oligotrophic marsh with herbaceous vegetation (Figs. 1, 2).

## Discussion

This new cyanobacterial species, *Anabaena fuscovaginata*, shows many typical characters attributed to the genus in its original sense: benthic types, growing in macroscopic mats, without aerotopes (BORNET & FLAHAULT 1888; KOMÁREK & HAUER 2010). By the regularly metameric structure of filaments (Fig. 2s), with few paraheterocytic akinetes, it can be easily distinguished from similar genera with benthic species like *Nostoc*, *Trichormus*, *Cylindrospermum*, etc. (KOMÁREK & ANAGNOSTIDIS 1989; RAJANIEMI et al. 2005).

Importantly, typical benthic *Anabaena*

(former subg. *Anabaena*) have been shown to have significant genetic differences from planktic types (former subg. *Dolichospermum*) that are phylogenetically closer to *Aphanizomenon* (GUGGER et al. 2002; RAJANIEMI et al. 2005; KOMÁREK & KOMÁRKOVÁ 2006). The taxonomy of planktic cyanobacteria formerly included in the genus *Anabaena* has been further studied, and recently, new genera *Dolichospermum* (KOMÁREK & ZAPOMĚLOVÁ 2007, 2008; WACKLIN et al. 2009) and *Sphaerospermopsis* (ZAPOMĚLOVÁ et al. 2009, 2010) have been separated from *Anabaena* on the basis of polyphasic data. Given the morphological and ecological variability inside the former widely defined genus *Anabaena*, further revisions should be expected, perhaps also including the benthic types.

*Anabaena fuscovaginata* is apparently a member of a group of similar taxa characterized by periphytic or metaphytic origin and morphology of filaments (Table 1). These are somewhat attenuated towards ends (Fig. 2k–q), having akinetes on both sides of the heterocytes (Fig. 2a–j). They have been discussed previously, for example by KOMÁREK (2005) in his study of several periphytic species from Cuba. Morphologically, *A. fuscovaginata* can be distinguished from them by the generally smaller width of trichomes, and by the presence of coloured sheaths (Tables 2, 3, Fig. 2). Interestingly, in my samples two similar taxa occurred, *A. ambigua* C. B. RAO and *A. iyengarii* BHARADWAJ, often accompanying *A. fuscovaginata*. Both were originally described from India, but they probably represent pantropical species that can be found in periphyton of rather unenriched shallow freshwaters and marshes of warm areas all over the world (also reproted from the Caribbean area, Cuba by KOMÁREK 2005). A similar pattern of distribution might be expected in our new species. In support of this contention, the cyanobacterial community in my samples consisted of a considerable number of other morphotypes, many of which could be assigned to known pantropical species (MAREŠ 2006). Alternatively, considering that it has never been reported before from any other tropical or subtropical marshland, it might be restricted to alkaline wetlands of a certain type. Such distribution was already reported for example for *Gomphosphaeria semen–vitis* (KOMÁREK 1989a; KOMÁREK & KOMÁRKOVÁ–LEGNEROVÁ 2007).

Remarkably, I observed a rather firm, gelatinous and intensely coloured sheath,

Table 1. List of morphospecies similar to *A. fuscovaginata* and their distribution.

Species	Reference	Distribution	Habitat
<i>A. ambigua</i> RAO 1937	DESIKACHARY 1959, KOMÁREK 2005	pantropical	periphyton, facultatively plankton
<i>A. bornetiana</i> COLLINS 1896	GEITLER 1932, KOMÁREK 2005	North and Central America	metaphyton, floating clusters
<i>A. fuscovaginata</i> sp. nov.	described here	Everglades, Florida	periphyton
<i>A. iyengarii</i> BHARADWAJA 1935	DESIKACHARY 1959, KOMÁREK 2005	pantropical	metaphyton
<i>A. oblonga</i> DE WILDEMAN 1897	KOMÁREK 1989b, 2005	pantropical	periphyton
<i>A. orientalis</i> DIXIT 1936	DESIKACHARY 1959, KOMÁREK 2005	pantropical	metaphyton
<i>A. turkestanica</i> (A. KISELEV) KOMÁREK 2005	KOMÁREK 2005, ELENKIN 1938, HOLLERBACH et al. 1953	Central Asia, Cuba	metaphyton

Table 2. Comparison of morphospecies similar to *A. fuscovaginata* [ + present; (+) rarely present; ? not known; B brown].

Species	trichome ends narrowed	trichome ends cylindrical	cells barrel-shaped	cells cylindrical	facultative aerotopes	heterocytes spherical	heterocytes cylindrical	akinetes oval	akinetes widely oval	akinetes cylindrical	akinetes at heterocytes	akinetes distant from heterocytes	coloured exospore	endospore	colourless diffuent mucilage	coloured firm sheath on akinetes
<i>A. ambigua</i>	(+)		+		+	+			+		+					?
<i>A. bornetiana</i>		+	+			+				+		+				–
<i>A. fuscovaginata</i>	+		+	+			+			+	+		+		+	+
<i>A. iyengarii</i>		+	+			+	+	+	+	+	+				+	
<i>A. oblonga</i>	+		+				+			+		+	(+)		?	
<i>A. orientalis</i>	+			+			+	+			+		(+)	B	?	
<i>A. turkestanica</i>	+		+			+	(+)		+		+				+	

Table 3. Comparison of dimensions in morphospecies similar to *A. fuscovaginata* [l:w length to width ratio].

Species	cell width ( $\mu\text{m}$ )	heterocyte dimensions ( $\mu\text{m}$ )	akinetes dimensions ( $\mu\text{m}$ )	cell l : w	heterocyte l : w	akinetes l : w
<i>A. ambigua</i>	5.9–9	6.0–11.2 $\times$ 7.0–12.8	13.2–15.0 $\times$ 10.6–12.0	0.4–0.8 : 1	0.8–1.2 : 1	1.1–1.4 : 1
<i>A. bornetiana</i>	7–7.3	7.0–9.5 (diameter)	17.8–24 $\times$ 7.2–9.2	0.6–1 : 1	0.9–1.1 : 1	3.6–4.9 : 1
<i>A. fuscovaginata</i>	2.5–5	5.5–12 $\times$ 3.5–7	10–25 $\times$ 7–13	1.0–1.9 : 1	1.2–2 : 1	1.5–2.0 : 1
<i>A. iyengarii</i>	3.5–5	5.6–9.6 $\times$ 5.6–7.2	12.0–21.4 $\times$ 9.2–10.8	0.4–1 : 1	0.9–1.4 : 1	1.2–2.5 : 1
<i>A. oblonga</i>	4.5–5	7.0–9.6 $\times$ 6.2–7.3	9.0–15.0 $\times$ 5.0–6.0	1–1.5 : 1	1.2–1.8 : 1	2–4 : 1
<i>A. orientalis</i>	3–5.4	5.8–14.3 $\times$ 5.8–7.6	13.0–24 $\times$ 9.2–14.3	0.8–1.4 : 1	1.4–1.9 : 1	1.9–2.3 : 1
<i>A. turkestanica</i>	3.6–4.8	3.2–4.8 $\times$ 4.6–5.0	10.0–16.0 $\times$ 8.0–11.0	0.4–1.3 : 1	0.9–1.1 : 1	1.1–1.4 : 1

covering most of the *A. fuscovaginata* filaments (Fig. 2). No report of this phenomenon has been found for *Anabaena*, yet. According to the generic description, mucilaginous envelopes are quite common in the genus, but usually in a colourless and rather diffuent form (also occasionally visible in *A. fuscovaginata*, Fig. 2u–v) while firm and coloured sheaths are typical for other nostocalean genera (for example *Scytonema*). Whether or not this characteristic phenotypic trait should have taxonomic implications at the generic level, has to be finally resolved by polyphasic approach, including molecular–phylogenetic study. Until then, considering the overall similarity in all other recorded characteristics, I suggest keeping the species within *Anabaena*.

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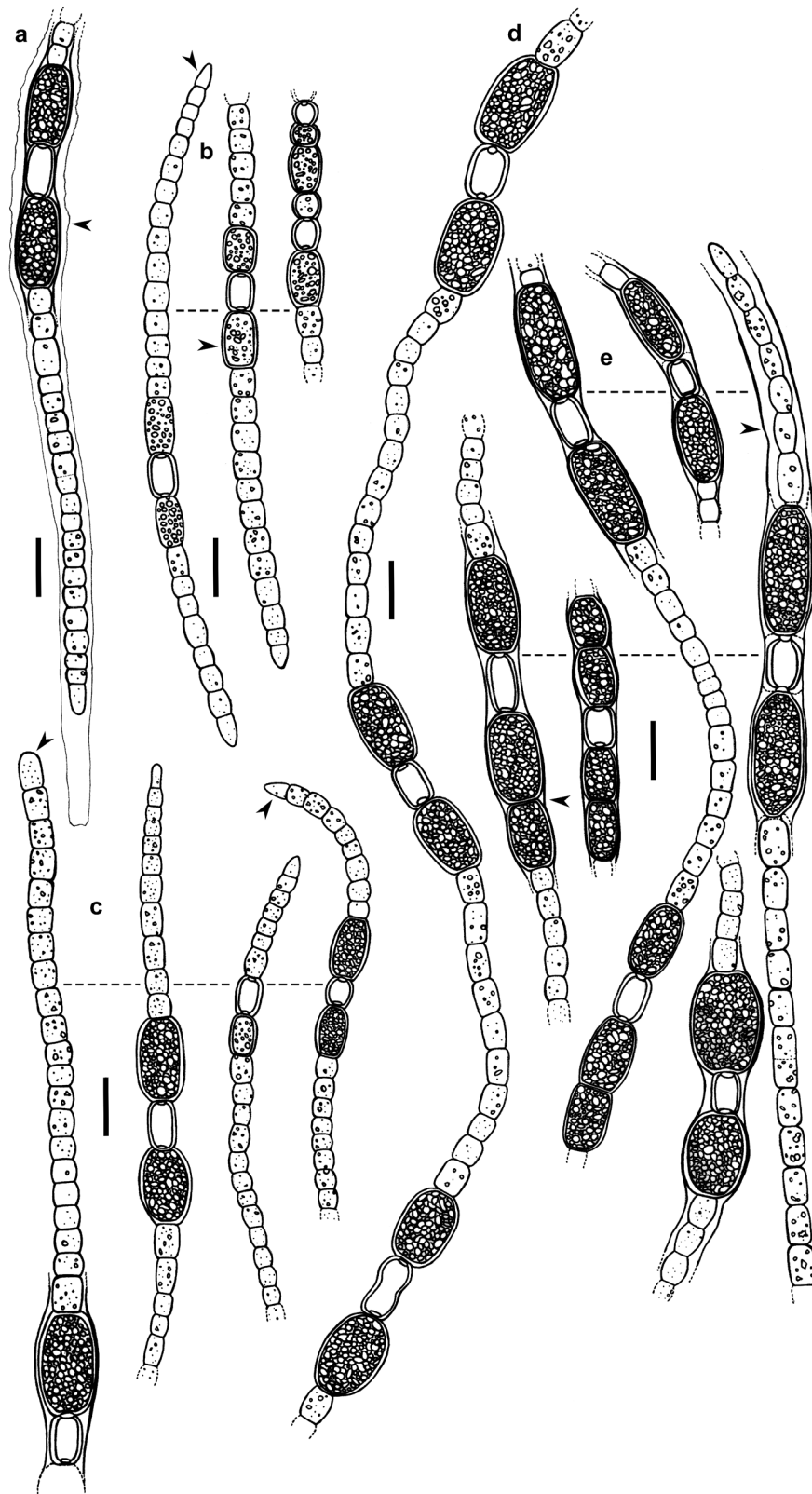


Fig. 1. *Anabaena fuscovaginata*: (a) filament covered by a diffuent mucilaginous envelope; (b) young filaments with emerging akinetes and narrowed ends; (c) variability of trichome ends (cylindrical to conical apical cells); (d) filament of typical metameric structure; (e) variability of ripe akinetes with gelatinous sheaths. Scale bars 10 µm.

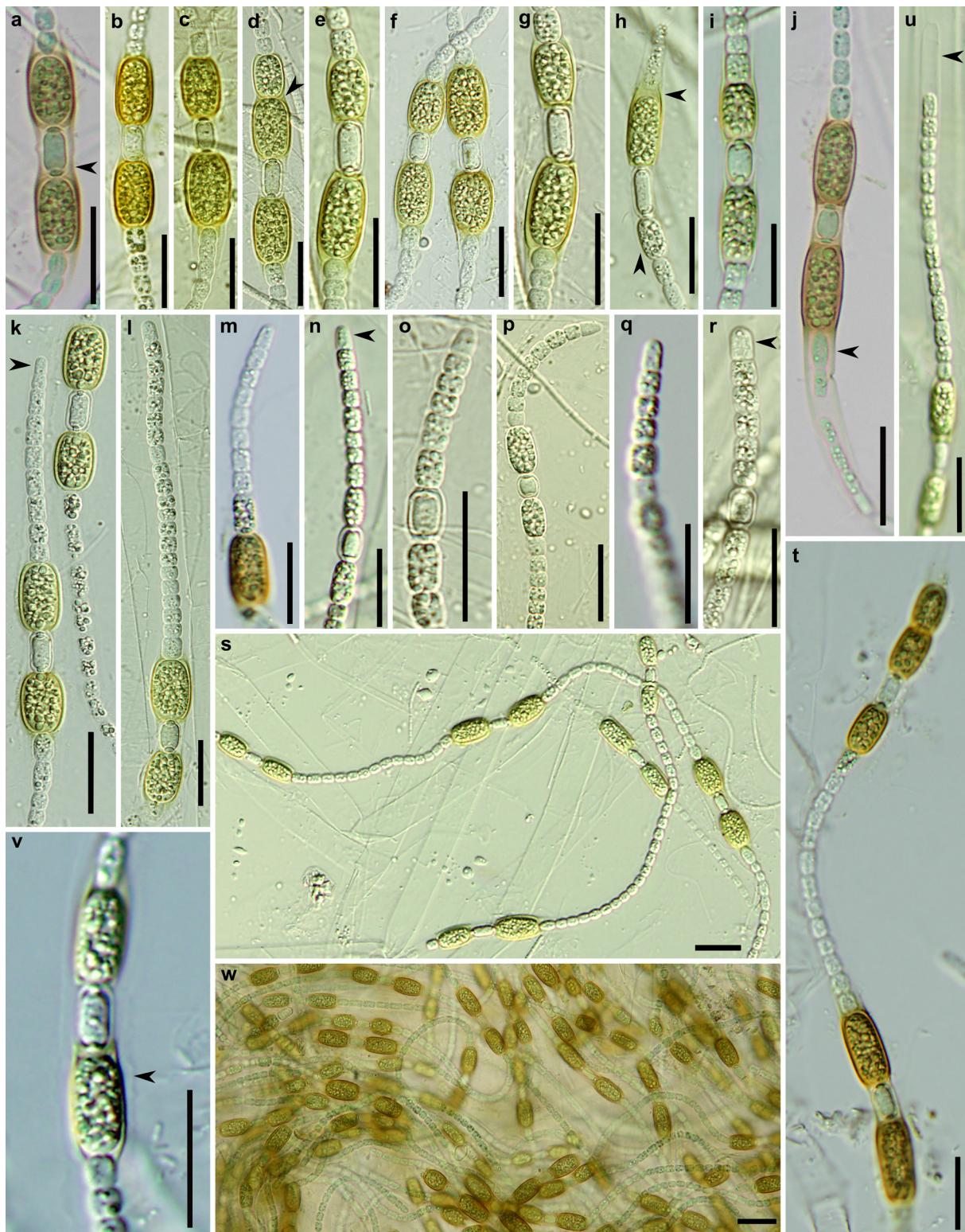


Fig. 2. Microphotographs of *Anabaena fuscovaginata*: (a–j) variability of akinetes and coloured gelatinous sheaths (horizontal arrows: sheaths, skewed arrow: two akinetes in a row); (k–r) variability of trichome ends (arrows – conical to cylindrical apical cells); (s–t) metameric structure of the filaments; (u–v) diffuent mucilaginous envelopes (arrow); (w) filaments densely clustered in a periphytic mat. Scale bars 20  $\mu\text{m}$ .



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