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Plectolyngbya hodgsonii: a novel filamentous cyanobacterium from Antarctic lakes

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Abstract A special cluster of filamentous, false-branched cyanobacteria, isolated from littoral mat samples in coastal lakes of the Larsemann Hills region (coll. by D. Hodgson) was studied by a polyphasic approach. This morphotype has several characters corresponding to the traditional genera *Leptolyngbya* (morphology of trichomes), *Pseudophormidium* (type of false branching) or *Schizothrix* (occasional multiple arrangement of trichomes in the sheaths). However, this cluster of strains is distinctly isolated according to its phylogenetic position (based on 16S rRNA gene sequences), and thus, a separate generic classification is justified. The cytomorphology of this generic entity is also characteristic. Therefore, a new genus (*Plectolyngbya* with the type species *P. hodgsonii*) was described. The same cyanobacterial morphotype was found in the littoral zone of the partially frozen inland Monolith Lake in the northern, deglaciated area of James Ross Island, in the NW part of the Weddell Sea. *Plectolyngbya hodgsonii* occurs evidently in more Antarctic lakes of the continental type, under very particular conditions (littoral with average temperature below 3°C during the Antarctic summer season, with periodical drying and freezing for more than 8 months in a year). The valid definition, phenotype documentation and ultrastructural characters of this cyanobacterium are presented in this article. Morphologically (and possibly genetically) similar types are common in other habitats in various regions and represent probably different species.

Keywords Cyanobacteria · New species · Antarctica · Benthos of lakes · Polyphasic approach

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Introduction

The ecological specificity and endemism of a major part of the Antarctic cyanobacterial microflora was proven by the identification of genotypes from Antarctic biotopes (Priscu et al. 1998; Gordon et al. 2000; Vincent et al. 2000; Taton et al. 2006), by the recognition of special morphospecies and by the analyses of characteristic cyanobacterial communities in specialized Antarctic habitats (Komárek 1999; Komárek and Komárek 2003; Komárek et al. 2008). During the molecular characterization of Antarctic strains isolated from various microbial mats (Taton et al. 2006), an isolated cluster was observed in the phylogenetic tree based on 16S rRNA gene sequences. The morphology of the strains was related to several other non-heterocytous, filamentous, simple cyanobacteria, classified up to date in the genera *Leptolyngbya, Pseudophormidium* or *Schizothrix*. The trichomes are relatively thin (less than 4 μm),

ensheathed, and they have the characteristic false "plectonematoid" branching. Such morphotypes have been classified mostly into the genus *Leptolyngbya* (cf. Komárek and Anagnostidis 2005). However, this genus is evidently heterogeneous. Moreover, the false branching is generally present in field material but occurs less frequently in cultures.

Five strains corresponding to this morphotype were shown to belong to one tight and isolated phylogenetic cluster, based on 16S rRNA sequences. They were isolated from samples collected by Dominic Hodgson from the microbial mats in three coastal lakes of the Larsemann Hills region (Prydz Bay, Eastern Antarctica) (Sabbe et al. 2004). Because this cluster (with a characteristic morphology) was separated by 3.68–4.24% (*E. coli* positions: 405–780) from the closest sequence of *Leptolyngbya* available in GenBank and 12.46% (*E. coli* positions: 405–780) from the closest *Schizothrix* sequence in GenBank and because it is phenotypically recognizable from all the nearest generic entities and other morphotypes from Antarctica, it deserves to be classified as a special genus (*Plectolyngbya*). The genus *Pseudophormidium,* which is similar particularly by the presence of false branching, has not yet been studied by molecular methods. However, other characters like the morphology of trichomes, cells and the type of false branching are different to such a degree that the generic identity with this new genus does not seem possible.

In this study, we have revised the generic status of the cluster "*Plectolyngbya*" on the basis of a detailed morphological study of the five strains isolated from the Larsemann Hills' lakes and two samples from the James Ross Island. In addition, the ultrastructure of the selected reference strain (ANT.LPR3) was investigated. On the basis of these combined data, a final taxonomic evaluation and validation of the new genus was proposed.

Materials and methods

Sample collection, strain isolation and cultivation

The study is based on five strains (ANT.LPR2, ANT.LPR3, ANT.LG2.1, ANT.LG2.2, ANT.L52B4) isolated from the littoral of three coastal lakes in the Larsemann Hills (type material: Progress Lake; 69°24′S, 76°23′E) and analysed by 16S rRNA and ITS (Internally Transcribed Spacer) sequencing by Taton et al. (2006) who described the methodologies of isolation, cultivation and sequencing. Material that was morphologically almost identical to these five strains was found during the investigation of cyanobacterial assemblages in the northern part of the James Ross Island (Ulu peninsula), in NW part of the Weddell

Sea. It was observed in the assemblages of cyanobacteria growing in shallow water near the frozen shoreline in the inland Monolith Lake (63°54′S, 53°57′W) (Komárek et al. 2008). Thus, the ecological conditions were very similar to the morphotypes from Larsemann Hills. The populations from James Ross Island were compared with Taton's strains, and the morphology of all these Antarctic populations was found to be almost identical.

Ultrastructure

For electron (transmission) microscopy of the strain ANT.LPR3, trichomes from fresh liquid cultures, cultivated in the BG11 and Z media (Bischoff and Bold 1963, Zehnder in Staub 1961), were used. The material was fixed (without washing) in two ways, with aldehydes and with osmium:

(a) Cytochemical fixation following a modified version of Karnovsky (1965): Concentrated trichomes were fixed for 3 h with a mixture of 2.5% (v/v) glutaraldehyde and 2% (w/v) paraformaldehyde in a 0.1 M cacodylate-HCl buffer, pH 7.2. Paraformaldehyde was dissolved separately at 60°C. This procedure better preserves the cellular membranes.
(b) Bacteriological fixation following Kellenberger et al. (1958): Trichomes and cells (concentrated by centrifugation) were fixed with 1% (w/v) osmium tetroxide in 2% (w/v) veronal-acetate buffer, pH 6.5, enriched with traces of $CaCl_2$ and NaCl, followed by 0.5% (w/v) uranyl acetate fixation by washing repeatedly 3 times in the same (just in 1%, w/v, concentration) buffer. This fixation procedure is especially apt to reveal DNA complexed in nucleoids.

The material fixed in both ways was dehydrated by a gradual series of ethanol baths of concentrations increasing from 40% to 96.6 (w/v), for 3 h each. After that, the material was infiltrated with LR White resin in 96.6% ethanol for 4 h, then embedded in pure LR White for 3 days and encapsulated. The resin was polymerized for 2 days at 60°C. Sections were cut using a Reichert-Jung microtome Ultracut E and subsequently contrasted with 2.5% (w/v) uranyl acetate and, after washing, with the alkaline Reynolds solution (3%, w/v, lead nitrate and 3%, w/v, natrium citrate). Sections were observed and photographed in a digital transmission electron microscope Cei Morgagni 268D.

Phylogenetic analyses

The 16S rRNA gene sequences (*E. coli* positions: 101–1449 and 405–780) of the strains considered in this study were initially analysed by similarity search using the

basic local alignment search tool (BLAST) software, widely available on Internet. Our sequences were included in the database of the ARB software package (Ludwig et al. 2004) and aligned with the reference alignment 'SILVA SSU Ref 94' (Pruesse et al. 2007). Closely related sequences indicated by BLAST that were not in the 'SILVA SSU Ref 94' database were included in the database of ARB and aligned subsequently. Phylogenetic trees were constructed using (1) the neighbour-joining method on a Jukes and Cantor distance matrix implemented in ARB, indels and ambiguous nucleotides were not taken into account in the distance matrix calculation; (2) the Wagner parsimony of DNAPARS (Felsenstein 1989) implemented in ARB; (3) the maximum likelihood of PHYML (Guindon and Gascuel 2003) based on a GTR+I+G model using 4 categories of substitution rate and a Gamma distribution parameter estimated by PHYML from the dataset; the GTR+I+G model was determined to be the most appropriate to our data set according to the Perl script MrAIC1.4.3 (Nylander 2004); (4) the maximum likelihood of TREE-PUZZLE 5.2 (Schmidt et al. 2002) using the quartet puzzling algorithm (Strimmer and von Haeseler 1996) to reconstruct the tree topologies based on 50,000 puzzling steps and the GTR model of substitution (Lanave et al. 1984), substitution rates were determined beforehand by PHYML, 4 categories of substitution rate were used and the Gamma distribution parameter was estimated by TREE-PUZZLE from the data set. Bootstrap analyses involving the construction of 1000 neighbour-joining trees, 500 parsimony trees and 100 maximum likelihood trees (PHYML) were performed. Comparisons of conserved nodes between the 4 tree construction methods were facilitated by the software package ARB and specific scripts written with the Biological Integrated Knowledge and Programming Environment BioBIKE (Elhai et al. 2009).

The trees comprised the sequences determined in this study together with their nearest neighbours, indicated by BLAST, that contained the same positions (*E. coli* positions: 101–1449) and shared more than 95% similarity. Furthermore, our strain sequences had to be integrated with the diversity of Oscillatorian strains actually known at the 16S rRNA gene level. Therefore, from a set of sequences that included all the Oscillatorian strains available within the reference database 'SILVA SSU Ref 94' (sequences longer than 1,200 nt, quality sequence threshold of 50 and alignment quality threshold of 75), the sequences that covered the *E. coli* positions 101–1449 were grouped in operational taxonomic units (OTUs) with a minimal threshold of 97.5% similarity. Seventy-six OTUs were distinguished, 42 sequences were the only representatives of the OTUs to which they belonged and were selected to

be included in the alignment. When there were several sequences in the same OTU, only the 2 most dissimilar sequences per OTU were selected for inclusion in the alignment. Unnamed strains were ignored, and *Gloeobacter violaceus* PCC7421 served as the outgroup.

Taxonomic evaluation

The studied *Plectolyngbya* strains were grouped based on the 16S rRNA gene in a well-defined cluster clearly isolated from any other cyanobacteria. Moreover, they had enough morphological differences to be defined as a new special cyanobacterial generic entity. For a valid description, the prescriptions of the Botanical Code (Greuter et al. 2000) were used. In addition, the rules of the Bacteriological Code were respected when they could be applied for the modern, polyphasic taxonomic evaluation of cyanobacterial taxa.

Results

Molecular characterizations

The molecular characterization on the basis of 16S rRNA and internally transcribed spacer (ITS) sequences was published by Taton et al. (2006). The revised phylogenetic tree is given in Fig. 1. The five strains (ANT.LPR2—GenBank acc. no. AY493583, ANT.LPR3—no. AY493617, ANT.LG2.1—no. AY493615, ANT.LG2.2—no. AY493618, ANT.L52B4—no. AY493616) represent an isolated group (*Plectolyngbya*) separated from other related clusters (Figs. 1, 2). They are characterized by almost identical 16S rRNA sequences (99.69% using the *E. coli* positions: 101–1449 and 99.43–100.00% using the *E. coli* positions: 405–780), though they originated from three different lakes. Therefore, Taton et al. (2006) assigned them to the operational taxonomic unit (OTU) 16ST01[New], noting that these sequences had no close relatives in Genbank at the time of publication; indeed, the first hit indicated by BLAST was *Leptolyngbya* sp. PCC73110 with 95.8–96.3% similarity using the *E. coli* positions: 405–780, indels not taken into account. The ITS sequences within this conspecific OTUs were also highly similar with a minimum level of similarity of 99.2% (in comparison with the limit 95% proposed by Stackebrandt and Goebel 1994). The generic separation of the cluster (*Plectolyngbya* genus nova) is therefore justified by both its position in the phylogenetic tree under 95% of similarity (comp. Figs. 1, 2) and the distinct autapomorphic phenotypic characters (morphology of cells and filaments, type of false branching, ultrastructure).

Morphology (Figs. 3, 4, 5, 6)

The new genus *Plectolyngbya* is a filamentous, aheterocytous, thin, cyanobacterial morphotype, which belongs to the "LPP group B" according to modern revisions (Rippka et al. 1979). Thus, it is closely related to the genus *Leptolyngbya* (Anagnostidis and Komárek 1988), though obligatory false branching occurs in the filaments. Vegetative cells are short, more or less isodiametric or slightly elongated before division. Heterocytes and akinetes are lacking (Figs. 3, 6).

Our populations from Monolith Lake, Ulu peninsula, James Ross Island were found only in developed cyanobacterial mats at two localities in seepages in the shore area. The solitary filaments were intermixed with other cyanobacteria in rich assemblages, but monocultures or massive developments were never observed. Morphologically, our specimens corresponded exactly to the strains studied by molecular methods, and they have similar ecologies (Figs. 4, 5).

Ultrastructure (Figs. 7, 8):

Ultrastructure was studied in the type strain of *Plectolyngbya hodgsonii*, ANT.LPR.3. Two procedures of fixation were used to better reveal the thylakoid system (Karnovsky 1965; Fig. 7) or the nucleoids (Kellenberger et al. 1958; Fig. 8).

Two to seven thylakoids (**t**) are localized in cells parietal, separated from each other by 16–72 nm distance, but we noticed also areas of neighbouring thylakoids closely attached to each other, without any measurable distance. In several cells, the additional formation of 2–6 circular thylakoids, concentrically arranged around inclusions (cyanophycin or polyphosphate granules) can be observed (Fig. 7a, b, d arrows). In the cell cytoplasm, numerous small spherical to slightly ovoid polyphosphate granules (**p**—the largest ones of 220 nm in diameter), larger cyanophycin granules (**c**—up to 390 nm in diameter in the longer axis) and ribosomes are to be seen. The area of nucleoids (**n**) of irregular form is distinctly separated from the thylakoid area and is usually clearly limited by the innermost parietal thylakoid (Fig. 7e–f). Nucleoids have a clear net-like structure, typical of nucleoproteins (Fig. 8). Cell walls are thin; sheaths (thin and more or less firm while living) are widened and with a net-like structure, as seen after fixation in electron microscope (**s**). This sheath structure is particularly distinct after Kellenberger's et al. (1958) fixation (Fig. 8).

Formal description

Plectolyngbya genus nova: Filaments solitary, in clusters or forming mats, 0.8–4 μm wide, without heterocytes and

Fig. 1 Phylogenetic tree inferred from 16S rRNA gene sequences ▶ (*Escherichia coli* positions 101–1449) by Neighbour joining using the Jukes and Cantor model for multiple nucleic acid substitutions. Bootstrap values obtained using Neighbour joining (1,000 trees), parsimony (500 trees) and maximum likelihood (100 trees) are indicated at the nodes when equal to or greater than 50%. An *asterisk* indicates nodes with a TREE-PUZZLE (50,000 puzzling steps) support value equal to or greater than 50. The evolutionary distance between two sequences is obtained by adding the lengths of the horizontal branches connecting them and using the scale bar (0.1 mutation per position). The *Plectolyngbya* cluster is indicated in bold. The other sequences are the Oscillatorian representatives selected as described in the text and the outgroup *Gloeobacter violaceus* PCC7421

akinetes, with thin sheaths, with false branching of both tolypotrichoid and scytonematoid types. Trichomes cylindrical, not or slightly attenuated towards the ends, not or slightly constricted at cross-walls. Cells isodiametric or slightly shorter or longer than wide, with thin cross-walls, without gas vesicles, heterocytes and akinetes. Arrangement of thylakoids is parietal with facultative circular formations. Reproduction by fragmentation of trichomes and hormocytes (immotile hormogonia). Molecular position: see Fig. 1.—Latin diagnosis: *Filamenta solitaria, plus minusve cylindrica, irregulariter flexuosa vel subrecta, in coloniis aggregata, 0.8–4 μm lata, cum vaginis tenuibus, cum ramificationibus falsis sparsis; heterocytae akinetesque carentes. Trichoma cylindrica, ad apices non vel paucim attenuata, ad dissepimenta non vel paucim constricta. Vaginae tenues, firmae, sine colore. Cellulae isodiametricae vel paucim brevior vel longior quam latae, cum parietibus tenuis, sine vesiculis gaseosis. Thylakoidae plus minusve parietales, vel spiraliter circulariterque contortae. Reproductio trichomatibus fragmentatione et cum hormogoniis non motilibus.*—Type species: *Plectolyngbya hodgsonii* species nova.

Plectolyngbya hodgsonii species nova (Figs. 3, 4, 5, 6)

Description: Filaments solitary or in small, free clusters, thin, slightly or irregularly coiled, longer filaments with obligatory false branching (in nature). Trichomes are cylindrical, pale greyish or blue-green, not or slightly constricted at cross-walls, but visible in OM as constricted at cross-walls (compare photos from OM with EM), not attenuated towards the ends, 1–2.5(3) μm wide. Cells cylindrical with homogeneous content, ± isodiametric or slightly longer or shorter than wide, but with almost invisible cross-walls in LM without staining; end cells rounded without calyptra. Sheaths very thin, colourless, attached or slightly widened from trichomes, diffluent. False branching obligatory, branches solitary or in pairs.—Type: BRNM/HY 1406, typical figure: our Fig. 3.—Locality: periphytic and metaphytic among cyanobacteria; locality of the type and reference strain: Lake
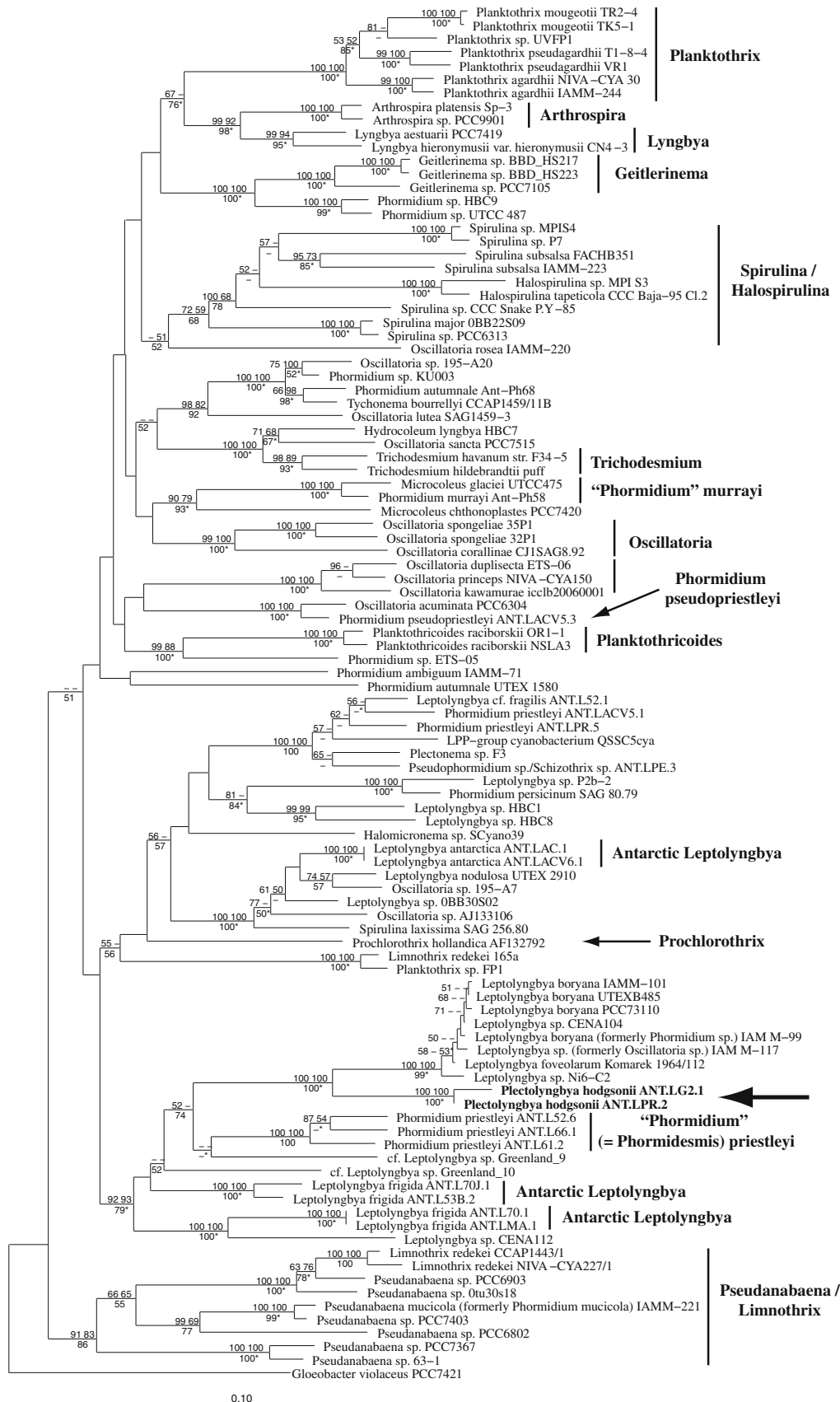
Fig. 2 Relation of *Plectolyngbya* cluster to other taxa and strains (on the generic level) isolated from Antarctic habitats. Antarctic isolates are printed in bold (GenBank acc. nos : ANT.LG2.1 = AY493615; ANT.LPR2 = AY493583). Phylogenetic tree is derived from Taton et al. (2006)
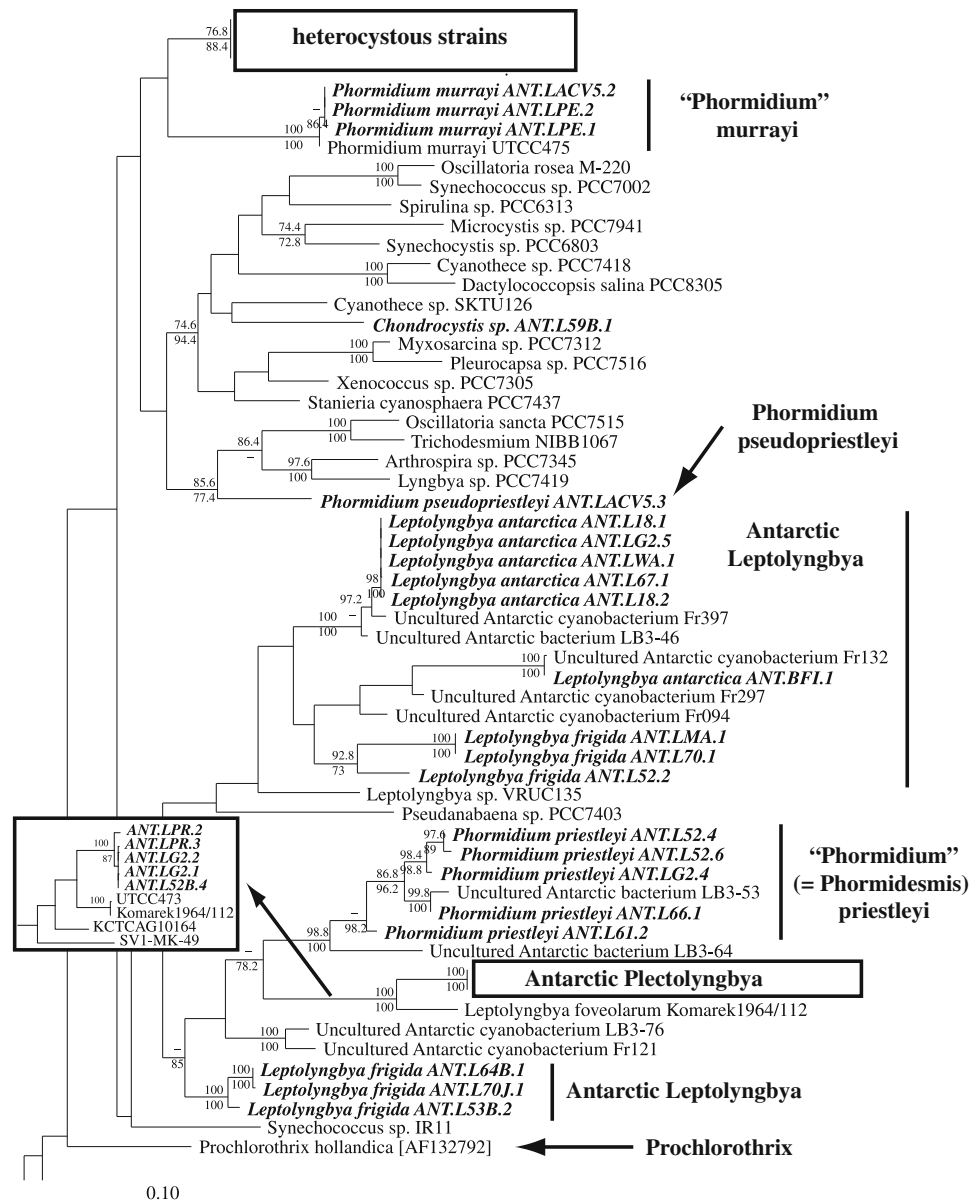
Progress, Larsemann Hills, Antarctica, coll. in the season of Antarctic spring 1997/1998; other localities: Monolith Lake, James Ross Island (NW Weddell Sea), coll. II. 2006.—Type and reference strain: ANT.LPR2 (acc. no. in GenBank = AY493583).

Latin diagnosis: *Filamenta solitaria, vel in coloniis metaphyticis parvis, tenues, paucim irregulariter flexuosa, despues false ramificata, praecipue in conditiones naturales. Trichoma cylindrica, pallide griseo-aeruginosa, ad dissepimenta paucim constricta, ad apices non attenuata, 1–2.5(3) μm lata. Cellulae cylindricae, cum contentu homogeneo, plus minusve isodiametricae vel paucim longior vel brevior quam latae; cellulae apicales sine calyptra. Vaginae tenues, sine colore, diffluentes. Ramificatio falsa cum ramis solitariis vel binis.—Typus: exsiccatum no.*

BRNM/HY 1406; icona typica: icona nostra 2.—Habitatio (locus classicus): periphytice metaphyticeque inter cyanobacteriis alliis in lacu Progress dicto (prope montes Larsemann), Antarctica (coll. D. Hodgson, XI–XII 1997).

Discussion

The revisions and descriptions of newly discovered cyanobacterial taxa should be based on a molecular (genetic) characterization combined with the determination of clear autapomorphic, phenotypic and/or cytological and ecological markers. These requirements are fulfilled in the case of the genus *Plectolyngbya*. Although the number of cyanobacterial 16S rRNA gene sequences in GenBank has

Fig. 3–5 *Plectolyngbya hodgsonii*: **3** = drawing of type material from the strain ANT.LPR3 (**a** detail of trichomes; **b–c** false branching of filaments; **d** simple filament); **4–5** = two different populations from Monolith Lake, James Ross Island (**a** detail of trichomes; **b–d** filaments, mostly with false branching; **e** germinating hormogonium). (Orig.)
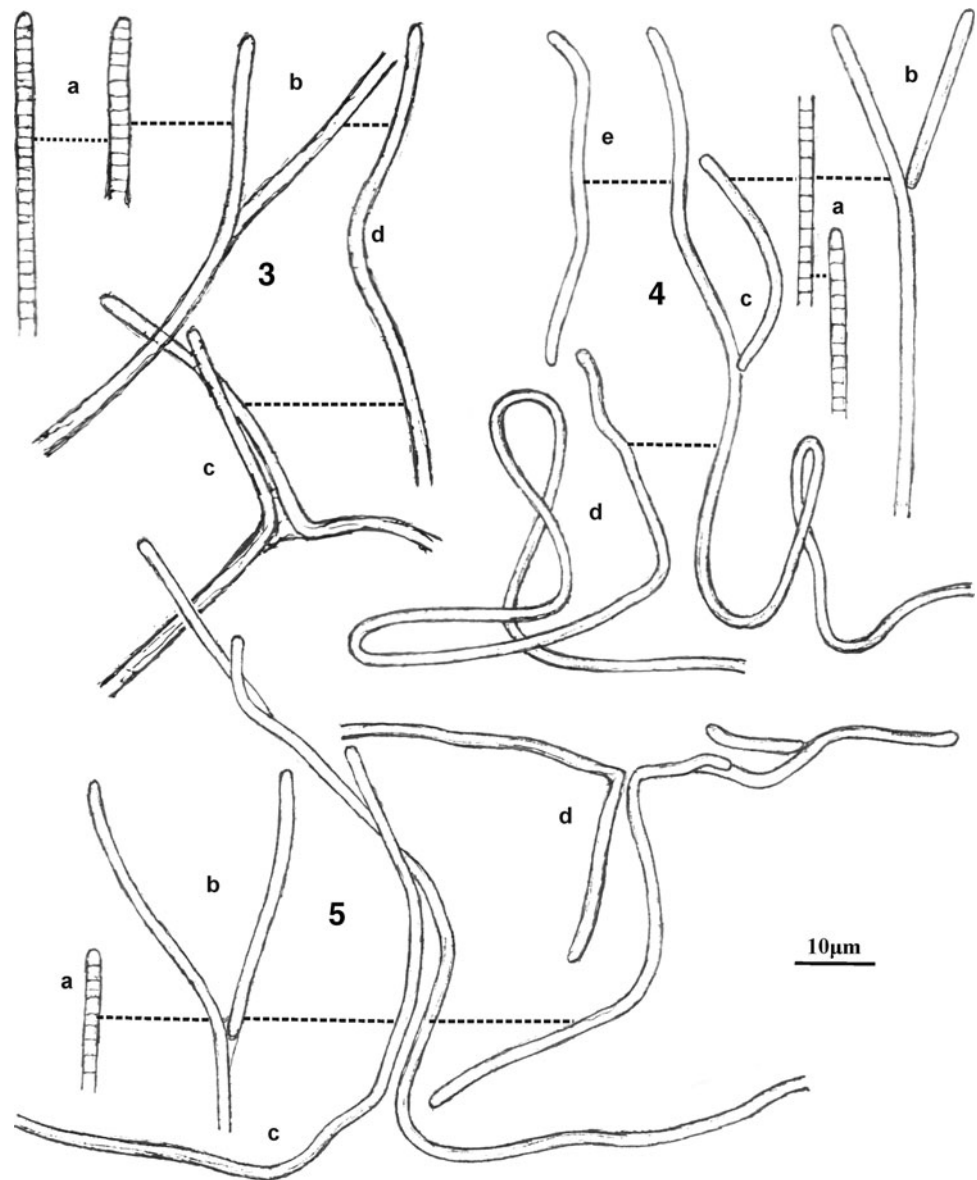
considerably increased these last few years, the studied strains still (February 24, 2010) represent a special genetic cluster (Figs. 1, 2), which is separated from the nearest related clade by at least 4.5% of dissimilarity using the *E. coli* positions 101–1449 and 3.7% using the *E. coli* positions 405–780. The main phenotypic diacritical and diagnostic morphological features of the genus *Plectolyngbya* are thin filaments (up to 4 μm) with facultative sheaths, the occurrence and ability of false branching, the structure of filaments, the quadratic (± isodiametric or slightly longer or shorter) vegetative cells, the absence of a calyptra at the end of trichomes and the special ultrastructure within cells (Figs. 7, 8). The morphology of several other morphospecies with more or less isodiametric cells and false branching, previously identified as *Leptolyngbya* (including the strain

CCALA 83 (Komarek 1964/112)), indicates that *Plectolyngbya* could be distributed over the world and contain also other species from various habitats.

The cluster of *Plectolyngbya* is morphologically similar also to other strains (e.g. CCALA 83 = "Komarek 1964/112"), originally identified as "*Plectonema* sp." or different species of "*Leptolyngbya*" with facultative plectonematoid false branching (Anagnostidis and Komárek 1988; Komárek and Anagnostidis 2005). These strains are more or less false branched, like *Plectolyngbya hodgsonii*, but their phylogenetic position is on a different branch but closely related. This could indicate that the genus *Plectolyngbya* is probably more widely distributed and diversified. Several eco- and morphotypes could exist in this generic cluster in other regions and in various biotopes of
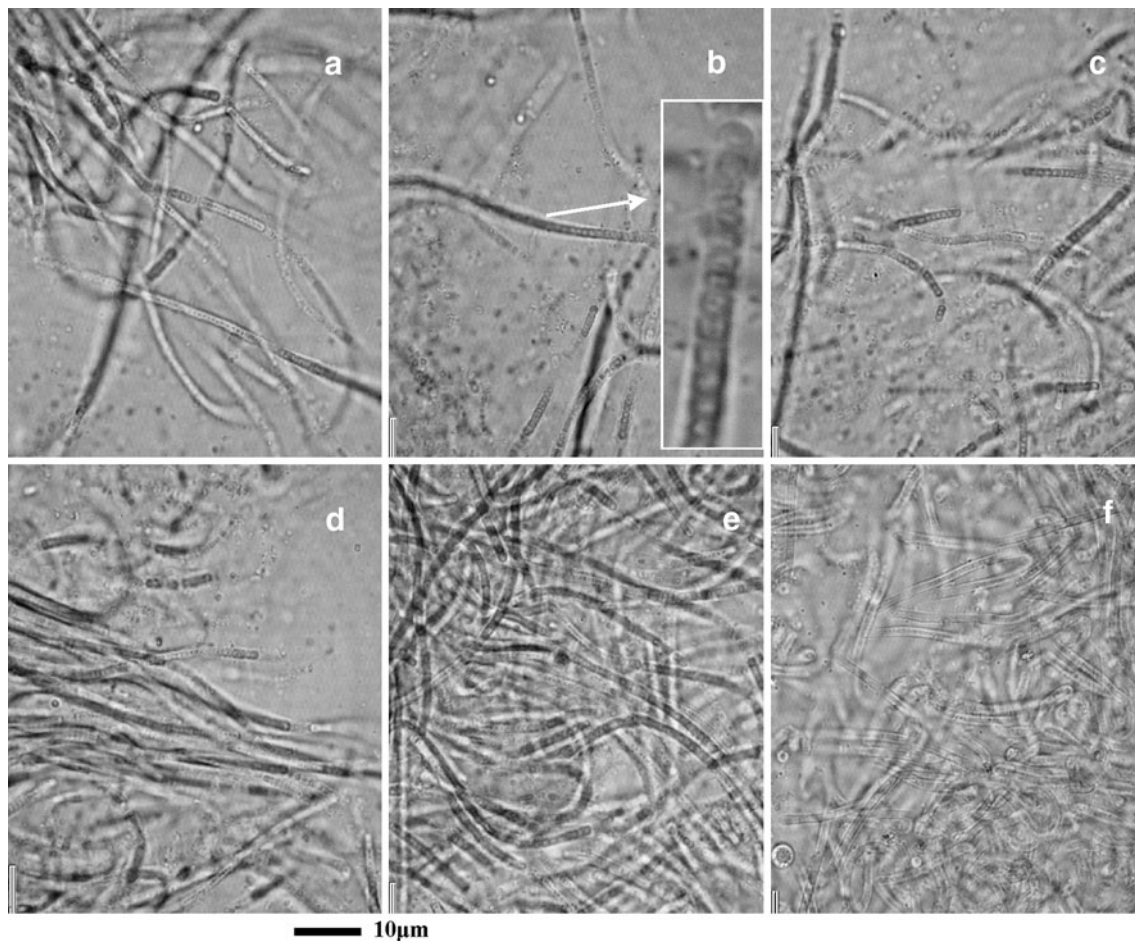
Fig. 6 *Plectolyngbya hodgsonii*: population from the type strain ANT.LPR3; **f** = empty sheaths

the world but already diversified in specially adapted clades (morpho- and ecospecies, cryptospecies). The same conclusion results from the comparison of our cluster with the GenBank database.

Plectolyngbya belongs to the family Pseudanabaenaceae according to its phylogenetic position, ultrastructure and morphology of filaments. Its ultrastructure is also special. In contrast to the majority of other pseudanabaenacean genera, which have strictly parietally arranged thylakoids (cf. Fig. 7 in Komárek and Anagnostidis 2005), formation of circular and concentric thylakoids (Fig. 7, arrows) occurs in *Plectolyngbya*. In addition, the sheath morphology (irregularly net-like after EM treatment) is peculiar. However, the cytological specificities were observed in the type strain of *P. hodgsonii* and must be confirmed by studying other species and strains of this genus.

The nearest genera are *Pseudanabaena* (differs by the obligatory absence of sheaths and false branching, thylakoids always situated parietally), *Leptolyngbya* and *Halomicronema* (false branching, cell ultrastructure), *Planktolyngbya* (solitary form of life, ultrastructure) or

Pseudophormidium from the family Phormidiaceae (with a different type of false branching and a distinctive ultrastructure—cf. Anagnostidis and Komárek 1988). The heterogeneous genus *Leptolyngbya* in its present concept (see Komárek and Anagnostidis 2005) contains several described morphospecies, which also possess the characters typical for *Plectolyngbya*. Therefore, it is very probable that these morphospecies belong to *Plectolyngbya* rather than to *Leptolyngbya*. The following species can be concerned: *Leptolyngbya battersii, L. calotrichoides, L. carnea, L. crispata, L. dangeardii, L. gracillima, L. muralis, L. norvegica* and the "branched" morphotypes of *L. boryanum* and *L. foveolarum*. However, their final taxonomic position must be solved by a combined (polyphasic) study based on molecular analyses together with careful morphological, ecological and ultrastructural analyses.

The molecular detection of novel and endemic species (and genera) from Antarctica, together with morphological and ecological data, supports the hypothesis that a special autochthonous cyanobacterial Antarctic microflora exists (Komárek and Komárek 2009). *Plectolyngbya*
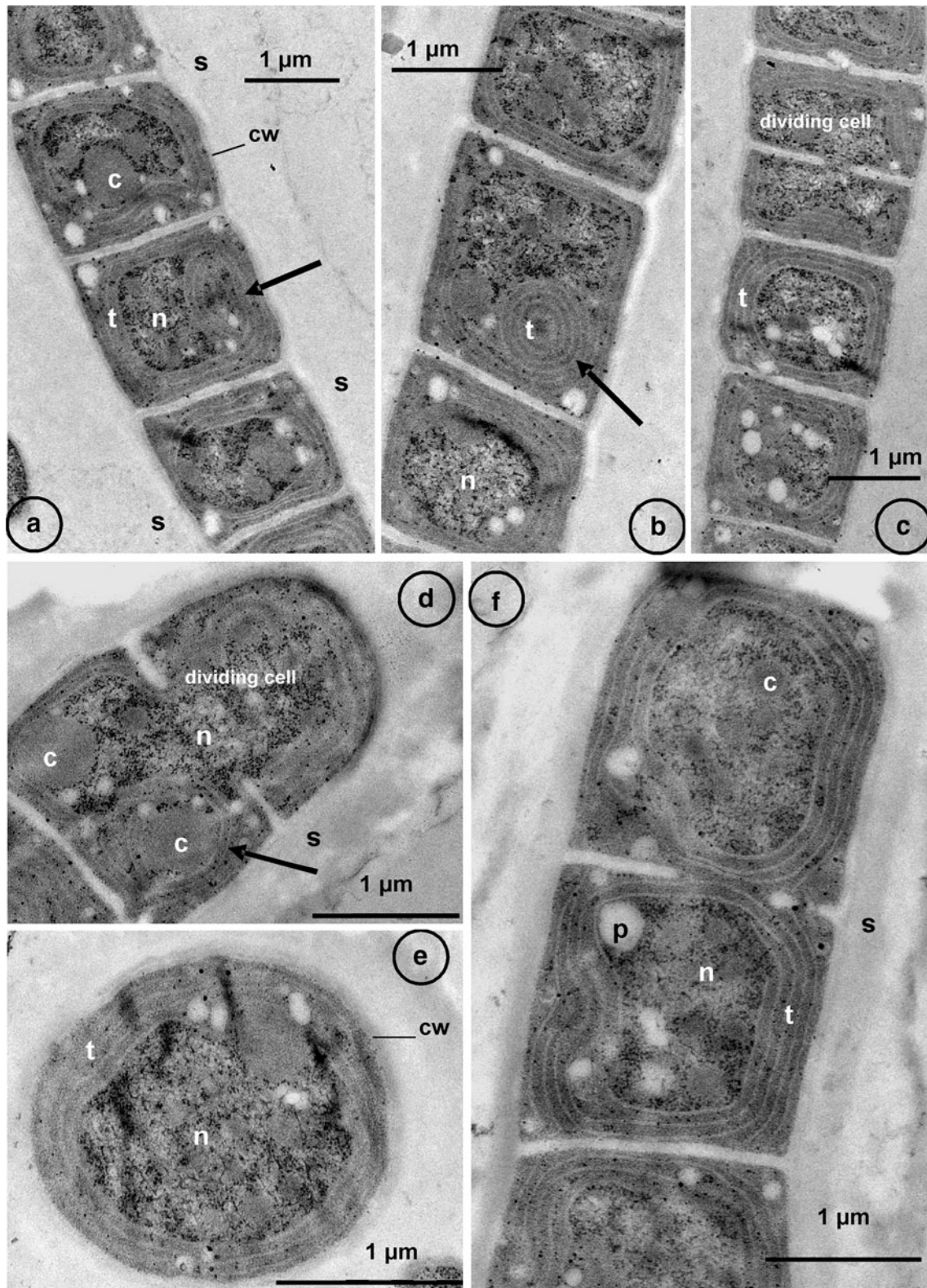
Fig. 7 Ultrastructure of filaments and cells of the type strain ANT.LPR3 of *Plectolyngbya hodgsonii* after fixation according to Karnovsky (1965): **cw** = cell wall, **t** = thylakoids, **p** = polyphosphate granules, **c** = cyanophycin granules, **n** = nucleoids, **s** = mucilaginous sheath, arrows = circular formations of thylakoids

Fig. 8 Ultrastructure of filaments and cells of the type strain ANT.LPR3 of *Plectolyngbya hodgsonii* after fixation according to Kellenberger et al. (1958): **cw** = cell wall, **t** = thylakoidal region, **p** = polyphosphate granules, **n** = nucleoids, **s** = mucilaginous sheaths
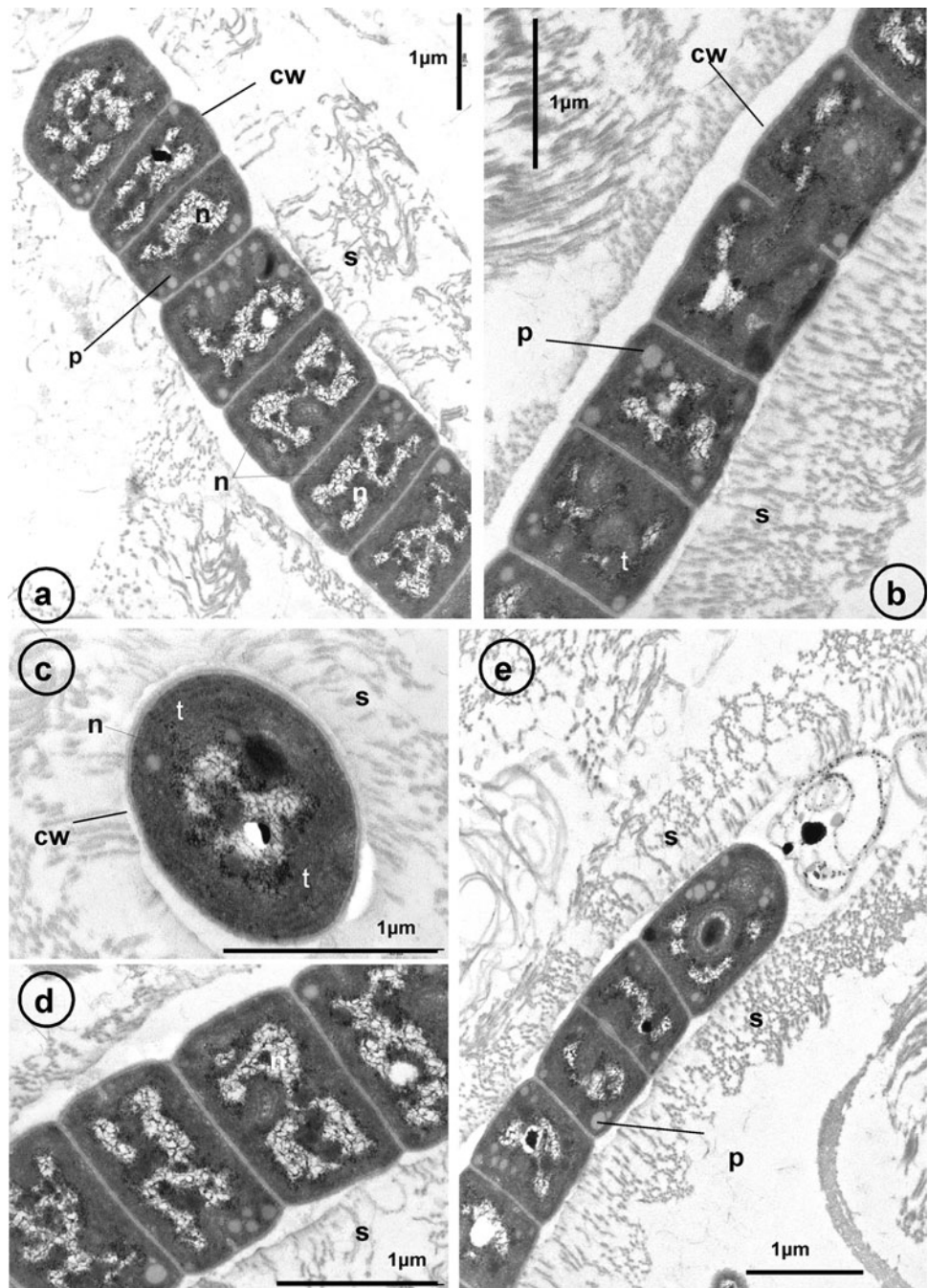
hodgsonii has also special ecology. It was found only in periphyton and metaphyton in mats among other cyanobacteria in the littoral of Antarctic lakes of the continental type.

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