Taxonomic revision of the freshwater cyanobacterium „*Phormidium*“ *murrayi* = *Wilmottia murrayi*

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Abstract: The cyanobacterial genus *Phormidium* is polyphyletic, as follows from recent molecular and phenotypic analyses. Several isolated clusters were found also in Antarctic populations (Taton et al. 2006, Taton et al. 2010, Strunecký et al. 2010b). A few of them have already been described or revised on the generic level (e.g., *Phormidesmis, Microcoleus*). One of the separate clusters belongs to a group of strains identified traditionally as *Phormidium murrayi*, described from the Antarctica originally in 1911 as *Lyngbya murrayi* by W. et G.S.West. We evaluated 23 morphologically similar strains or populations resembling *Ph. murrayi* from the Antarctica and few other geographical regions; 6 of them were studied by us by molecular methods. Morphological characteristics and 16S rDNA similarity corresponding with typical *Ph. murrayi* were shared mostly by the specimens from the Antarctica. Molecular analyses confirmed that *Ph. murrayi* represents a special taxonomic group (on the generic level) inhabiting commonly shallow freshwater classified as a special genus in cyanobacterial taxonomy (*Wilmottia* gen. nov.) according to the recent "polyphasic approach". The type species of *Wilmottia, W. murrayi,* was determined up to now to be characteristic for the Antarctica. Other phylogenetically and morphologically similar types from other regions represent possibly different taxa (species) of the same generic unit. The specific status of similar or related strains from other regions should be therefore solved in the future.

Key words: cyanobacteria, *Phormidium*, polyphasic taxonomy, ecology, Antarctica

Introduction

The traditional genus *Phormidium* Kützing ex Gomont 1892 is polyphyletic, as follows from recent molecular analyses (Taton et al. 2006; Marquardt & Palinska 2007; Palinska & Marquardt 2008; Strunecký et al. 2010 a,b), as well as from detailed cytomorphological investigations (e.g., Turicchia et al. 2009). Several phenotypically distinct clusters were also characterized during the study of Antarctic cyanobacterial populations (Taton et al. 2006, 2010; Comte et al. 2007; Strunecký et al. 2010a), the isolated position of which in phylogenetic trees was confirmed. To such separated clusters belong also group of strains identified traditionally as *Phormidium murrayi*, described originally from the Antarctica by W. et G.S.West in 1911 as *Lyngbya murrayi*. Similar types ("*Phormidium murrayi*") evaluated by molecular methods in previous studies (Taton et al. 2006; Strunecký et al. 2010b) represented also always a special, isolated cluster of simple trichal cyanobacterial morphospecies.

We studied 23 morphologically similar strains isolated from Antarctic localities (6 supported by molecular sequencing) and compared them with the corresponding literature. Based on a polyphasic evaluation, which combines genotypic (16S rRNA gene sequencing) and phenotypic (morphological analysis, ultrastructural sections) methods, a special cluster was isolated, which must be separated from the traditional genus *Phormidium* (Geitler 1932; Anagnostidis & Komárek 1988; Komárek & Anagnostidis 2005). In our paper a new genus is classified as *Wilmottia* gen. novum. Comparison with literature sources (Boyer et al. 2002; Šabacká 2004; Casamatta et al. 2005; Taton et al. 2006; Comte et al. 2007; Lokmer 2007, Wood et al. 2009; Heath et al. 2010; Strunecký et al. 2010b) supported this taxonomic transfer.

Methods

Sampling sites and strain preparation. The list of strains and literary data are presented in Table 1. *Phormidium* specimens were collected from various localities of Antarctic habitat types (Fig. 1). Samples were transported frozen to the laboratory. A small quantity of each sample was placed on a Petri dish with agar (solid medium with 1.5 % agar containing the mineral nutrient medium BG–11; RIPPKA et al. 1979). The dilution plate method was used for isolation and culturing of cyanobacteria (ELSTER et al. 1999). This method was repeated several times to obtain isolated unialgal strain colonies. The Petri dishes were placed in an illuminated refrigerator (90 $\mu mol.m^{-2}.s^{-1}$ = cca 20 $W.m^{-2}$ PAR, temperature 5 to 8 °C) with a light regime of 18 h of white fluorescent light, 2 h of UV–B radiation, and 4 h of darkness. Germicidal lamps sterilized the cultivation box repeatedly (UV–B radiation did not penetrate through the glass dish). After a few days of cultivation, visible colonies of *Phormidium* were observed and separately transferred to sterile agar tubes. Subsequently, pure strains (unialgal with low bacterial contamination) were cultured at a temperature of 6 °C and light of 30 $\mu mol.m^{-2}.s^{1}$. In addition to these newly isolated strains, strains originating from CCALA (Culture collection of Algae at the Laboratory of Algology, Třeboň, Czech Republic – http://www.butbn.cas.cz/ccala) with previously sequenced and published 16S rRNA genes (CCALA 852, CCALA 843; STRUNECKÝ et al. 2010a), and sequences from GenBank (Ant–Ph58, COMTE et al. 2007; ANT.ACEV5.2, TATON et al. 2006; B–Tom, LOKMER 2007; CYN38, CYN39, WOOD et al. 2009, HEATH et al. 2010), were compared and used for phylogenetic analyses. All new isolated strains were allocated to the CCALA. The cultivated strains were identified according to ANAGNOSTIDIS & KOMÁREK (1988) and KOMÁREK & ANAGNOSTIDIS (2005). Strain morphologies were analyzed using an Olympus BC51 light microscope up to 1000x magnification. Photomicrographs were taken with an Olympus DP71 Camera, equipped with Quick Photo Micro software.

Molecular analyses. DNA from the unialgal strains was extracted using the modified method of YILMAZ et al. (2009). The available amount of cultivated cells was suspended in 50 µl of TE buffer at pH 7.4. and 750 µl of XS buffer (1% potassium ethyl xanthogenate; 100 mM Tris–HCl, pH 7.4; 20 mM EDTA, pH 8; 800 mM amonium acetate and 1% SDS) in an eppendorf tube with glass beads. The trichomes were manually crushed by a micropestle. Tubes were incubated for 2 h at 70 °C. After incubation, the tubes were vortexed for 30 s and frozen at –70 °C for 30 min. The sample was thawed and shaken for 15 min, centrifuged for 20 min at 15,000g and the supernatant was transferred to a clean microcentrifuge tube. The DNA was precipitated overnight in a 2:3 volume of 100% ethanol with the addition of 1:20 volume of Sodium Acetate (3 M, pH 5.2) followed by centrifugation for 30 min at 15,000g. The supernatant was discarded and the pellet was washed with 100 µl of 70% ethanol followed by centrifugation for 15 min. After discarding the supernatant, the pellet was dried and dissolved in 100 µl of miliQ water.

The 16S rRNA gene with the 16S–23S intergenetic segment was amplified using the primers 359F (GGGGAATYTTCCGCAATGGG (NÜBEL et al. 1997), and 23S30R (CTTCGCCTCTGTGTGCCTAGGT) (WILMOTTE et al. 1993) with the following settings: a starting denaturation step (94 °C, 5 min); 40 cycles of 30 s at 94 °C, 30 s at 55 °C, and 3 minutes at 72 °C; final extension for 7 minutes at 72 °C and cooling to 4 °C. A successful PCR was confirmed by running a sub–sample on a 1 % agarose gel stained with ethidium bromide. PCR products were purified using a QIAquick PCR Purification Kit (QIAgen, Germany). Sequencing of the 16S rRNA gene fragment was performed on an ABI 3100 sequencer, using BD3.1 (Applied Biosystems, Foster City, USA) chemistry, with six primers (27F, 23S30R, CYA_810R–GTTATGGTCCAGCAAAGCGCCTTCGCCA, CYA783F–TGGGATTAGATACCCCAGTAGTC (STRUNECKÝ et al. 2010b), S17*–GGCTACCT-TGTTACGAC and ILE23F– ATTAGCTCA-GGTGGTTAG (WILMOTTE & HERDMAN 2001) to obtain complementary sequences. For examining the molecular phylogeny, 11 sequenced strains were combined with strains studied by previous authors (Table 1).

Phylogenetic analyses. Sequences were aligned in MAFFT (mafft.cbrc.jp) (KATOH & TOH 2010) considering secondary structure and minor changes were done manually with BioEdit 7.0.1 (HALL 1999). For the phylogenetic analysis, a fragment of 1097 nt was used (corresponding to *E. coli* ATCC 11775 16S rRNA residues 302–1,412) except of the New Zealand strains CYN38 and CYN39. Phylogenetic analysis was conducted in Mega 4 (TAMURA et al. 2007) using the Maximum Likelihood method under the Jukes–Cantor method with uniform rates among sites. Topology vas validated by Maximum parsimony under Close–Neighbor–Interchange method in Mega 4 and Bayesian analysis in MrBayes 3.1.2. (HUELSENBECK & RONQUIST 2001) at metacentrum.cz. For the Bayesian analysis, two runs of four Markov chains over 8 000 000 generations, sampling every 100 generations, were employed. The initial 20 000 generations were discarded as burn–in. Sequences for comparison of *W. murrayi* were chosen under extensive evaluation of more than 1100 sequences of oscillatorialean cyanobacteria available in GenBank (www.ncbi.nlm.nih.gov) and Ribosomal database project (rdp.cme.msu.edu) (unpublished). For a synoptic view

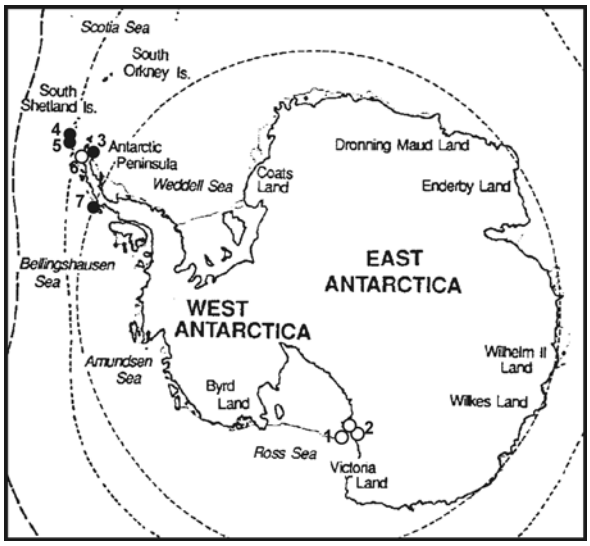

Fig. 1. Confirmed localities of *Wilmottia murrayi* in the Antarctica (black points); in all localities were collected samples and isolated strains from several sites. Localities (selected from literature are marked by circles): (1) Ross Island; (2) vicinity of McMurdo station; (3) James Ross Island; (4) King George Island – Admiralty Bay; (5) King George Island – Jubany; (6) Cierva Point; (7) Adelaide Island (Map of Antarctica modified according to Stonehouse 1989).

of phylogenetic tree only the sequences designated mostly as *Phormidium*–like cyanobacteria available from culture collection were chosen as specimens to the presented phylogenetic tree. Sequence identity matrix was calculated from the alignment in BioEdit (Hall 1999) from all positions including gaps.

Ultrastructure. For the ultrastructural studies, biological material of strains were fixed with 6% glutaraldehyde and kept in this solution for several days at room temperature. After that, samples were washed with 0.05 M phosphate buffer (pH 7.2) and postfixed with 2% osmium tetroxide in the same buffer for 2 hours at room temperature. After washing with 0.05 M phosphate buffer, the cells were dehydrated with a graded isopropanol series and embedded in Spurr's resin (Spurr 1969) using propylene oxide as an intermediate stage. Thin sections were stained with uranyl acetate and lead citrate and observed in a Jeol JEN 1010 transmission electron microscope at 80 kV.

Results

Genotypic characters

Strains identified as *"Phormidium murrayi*" and analysed by 16S rRNA gene sequencing from Antarctic habitats form a concise separated cluster. This result confirms the previous published studies (Taton et al. 2006; Strunecký et al. 2010). The genetic similarity between 11 strains, identified or as *"Phormidium murrayi",* or "*Microcoleus glaciei"* was 98–100% (Fig. 2; Table 2B). Similar types to *"Ph. murrayi"* isolated from other habitats and from localities outside Antarctica (strains B–Tom from Brazil – Lokmer 2007; strains CYN 38 and CYN 39 from New Zealand – Wood et al. 2009, Heath et al. 2010) belong to the same clade (Fig. 2). However the sequences of the New Zealand strains of *Phormidium murrayi* were available only in a 659 bp fragment that was approximately one–half the length of the sequences from this study. Therefore, the Antarctic *"Ph. murrayi"* represents a special unique taxon, distinct from other cyanobacterial clades by always more than 7.1% and representing therefore a special generic taxon, well separable from the genus *Phormidium*. The strains from Brazil and New Zealand (B–Tom, CYN38, CYN39) could be probably considered as special species from the same generic unit, separating also ecologically and phytogeographically from the Antarctic populations.

We have constructed large amount of phylogenetic trees from more than 1100 sequences of oscillatorialean cyanobacteria as starting point by various methods (unpublished). *Geitlerinema* came close to *W. murrayi* in the most cases hence we propose that *Geitlerinema* is the most related taxon to the new genus. The positions of both generic entities in the phylogenetic tree and also other markers (ultrastructure) indicate the classification of these genera rather in the vicinity of pseudanabaenacean cyanobacteria than to the cluster of the phormidiacean clade. Our results are also in agreement with autapomorphic morphological features (*Wilmottia* was sooner classified into the genera *Phormidium* and *Microcoleus*, i.e., distant from *Geitlerinema* and from Pseudanabaenaceae). Value of 70% in the bootstrap could have been considered as low, however under the current availability of related sequences in nucleotide databases we consider it sufficiently high for our statement and separation of *Wilmottia* from *Geitlerinema*.

Phenotypic characters

All strains included in the Antarctic cluster "*Ph. murrayi"* have more or less the same morphology, which is distant from typical *Phormidium* (based on the type species *P. lucidum*) and *Microcoleus* (based on *M. vaginatus*), and can serve to characterize this taxon on the generic level.

Table 1. List of cyanobacterial strains examined in this study and their taxonomic assignments. Accessions numbers of strains, which were firstly sequenced in our study, are included in "Notes" (in bold). Explanations: (T) type strain of *Wilmottia murrayi*; (x) strains from outside of the Antarctica.

Strain/sample	Isolation	Habitat	Locality	References	Used names	Notes
Original descriptions		stagnant, slightly salty waters + soils	Ant., Ross. Isl. Green Lake	W. et G.S.West 1911	*Lyngbya murrayi*	orig. description
				Anagnostidis & Komárek 1988	*Phormidium murrayi*	recombination to *Phormidium*
ANT.ACEV 5.2		periphyton of a pool	Ant., Bratina Isl.	Taton et al. 2006	*Phormidium* cf. *murrayi*	S-40; ANT. LACEV.5.2
ANT.PH 16	Šabacká /Elster 2004	ornithogenic soil	Ant., Adelaide Isl.	Šabacká 2004; Comte et al. 2008	*Phormidium* cf. *murrayi*	
ANT.PH 53	Šabacká /Elster 2004	seepages	Ant., King George Isl., Jubany	Šabacká 2004; Comte et al. 2008	*Phormidium* cf. *murrayi*	
ANT.PH 58	Šabacká /Elster 2004	dried pool	Ant., King George Isl., Jubany	Šabacká 2004; Comte & al. 2008	*Phormidium* cf. *murrayi*	
ANT. PENDANT 1.16.5				Taton et al. 2006		ANT.LPE1
ANT. PENDANT 2.16.5				Taton et al. 2006		ANT.LPE2
CCALA 843 **T**	Kováčik 2007/1	aerophytic, whale bones	Ant., King George Isl.	Strunecký et al. 2010	*Phormidium* cf. *murrayi*	KGI 28; 28-KGI **HQ873481**
UTCC 475	Vincent	benthic substrate of a lake	Ant., McMurdo ice shelf	Casamatta et al. 2005; Taton et al. 2006	*Phormidium murrayi, Microcoleus glaciei*	orig. description of *M. glaciei;* BRY C-37700
26 GL 17	Šnokhousová / Elster 2008		Ant., James Ross Isl., Green Lake		*Phormidium murrayi*	6; 6 ZL 17 **HQ873484**
26 GL 20	Šnokhousová / Elster 2008	lake, periphyton	Ant., James Ross Isl., Green Lake		*Phormidium* cf. *murrayi*	26; 7 **HQ873483**

Table 1 Cont.

A 21		Lukešová/ Komárek O. 2007	ornithogenic soil	Ant., King George Isl.		*Phormidium* sp.	O 21-rook.; L 4/96; L21 **HQ873485**
A 27		Lukešová/ Komárek O. 2007	soil (*Deschampsia*, mosses, lichens)	Ant., King George Isl.		*Phormidium* sp.	**HQ873486**
29 RL-C		Šnokhousová / Elster 2008	lake, periphyton	Ant., James Ross Isl., Red Lake		*Phormidium* cf. *murrayi*	"C" **HQ873482**
JC1-HC1 177-7a					Boyer et al. 2002	*Microcoleus steenstrupii*	
CYN 38	**x**		river, periphyton	New Zealand	Wood et al. 2009; Heath et al. 2010	*Phormidium murrayi*	
CYN 39	**x**		river, periphyton	New Zealand	Wood et al. 2009; Heath et al. 2010	*Phormidium murrayi*	
SC 7-1						uncultured cyanobacterium	
SAG 2212						*Microcoleus* sp.	
JR 8		natural sample, 2006	wet crusts on soils	Ant., James Ross Isl.		*Phormidium murrayi*	
JR 49		natural sample, 2006	seepages near lakes	Ant., James Ross Isl.		*Phormidium murrayi*	
JR 119		natural sample, 2006	wet rocks near the stream	Ant., James Ross Isl.		*Phormidium murrayi*	
B-Tom	**x**		wet rock on the sea shore	Brazil, Tominhos	Lokmer 2007	*Phormidium* cf. *murrayi*	
CD 29						uncultured cyanobacterium	

Filaments are solitary or with several to many trichomes associated parallely (in fascicles), without common firm sheaths, but enveloped by wide, colorless, unlamellated, diffluent slime. The tendency to form fascicles enveloped by common slime occurs in all populations. Trichomes are simple, cylindrical along the whole length (up to the end), uniseriate, pale blue–green, not or only slightly constricted at cross–walls, (2)3.2–6.3 µm wide. Cells are longer than wide or ± isodiametric, end cells are rounded or slightly conical, without calyptra. All cells are capable of division (without meristematic zones). The cell content is either homogeneous or with slightly distinct peripheral chromatoplasma. Solitary granules are found in the cells, sometimes more concentrated near the cross–walls (Figs 3, 4).

Ultrastructure

The ultrastructure of cells corresponds in principle to the scheme of pseudanabaenacean cells (Fig. 5). The thylakoidal system is localized more or less parietally, but the parietal position is not quite regular, and sometimes the solitary thylakoids or their small fascicles pass through the central or inner part of cells. Fascicles contain 3–8 parallel thylakoids. Thylakoids are sometimes interrupted and occasionally form a triangular pattern in cross section (similarly as in several species of *Geitlerinema*). The solitary granules are polyphosphates, cyanophycin and carboxysomes.

Formal description

From the genetic, morphological and cytological (ultrastructural) analyses follows that the Antarctic cyanobacterium, described originally as "*Lyngbya murrayi* W. et G.S.WEST 1911" and designated later as *"Phormidium murrayi* (W. et G.S.WEST) ANAGNOSTIDIS et KOMÁREK 1988" or "*Microcoleus glaciei* CASAMATTA et JOHANSEN 2005", represents a special genus according to the modern criteria of cyanobacterial taxonomy. The formal taxonomic description is as follows:

Description of the genus

***Wilmottia* gen. nov. (Figs 3–4)**

Diagnosis: Filamenta cylindrica, plus minusve recta vel paucim flexuousa, arcuata et circinata, non ramosa, solitaria vel ad multis consociata, plus minusve paralleliter in fasciculis repentes, in muco diffluenti et sine colore, aggregata; fila singularia cum vaginis facultativis, tenuis, firmis, apice apertis, non ramosis, hyalinis, incoloratis, non lamellosis. Trichoma recta vel leviter curva et flexuosa, cylindrica, ad septa non vel paucim constricta, ad apices non attenuata, 3.0–6.0 µm lata; heterocytis vel akinetis carentibus. Cellulae cylindricae, aeruginosae, saepe cum granulis sparsis sed distinctis, dispersis impletae; granula interdum ad septa transversalia agglomerata; cellulae plus minusve quadraticae vel paucim brevior vel longior quam latae; cellula apicalis cylindrica, cum cellulis vegetativis similis, terminaliter obtusa, rotundata vel oblonge–conica, sine calyptra. Thylakoides fasciculatae, praecipue parietales vel plus minusve paralleliter vel paucim irregulariter dispositae. Cellulae perpendiculariter dividuntur, zonis meristematicis absentibus. Reproductio disintegratione trichomatibus in partes immobilibus, necridis carentibus. – Typus generis: Wilmottia murrayi (W. et G.S.WEST) comb. nov.

Filaments solitary or in clusters, cylindrical along the whole length, ± straight or slightly coiled, with facultative sheath, sometimes in dense, irregular fascicles with colorless and diffluent slime, not enveloped together by a firm, wide sheath. Sheaths around single trichomes thin, ± fine but firm, colorless, sometimes diffluent, attached to trichomes. Never falsely branched. Trichomes cylindrical, not or only very slightly (exceptionally) constricted at cross walls, not attenuated or widened towards ends, without calyptra. Cells isodiametric, rarely shortened to ½ of their width, or up to 2× longer than wide, sometimes with different length in one and the same trichome, cylindrical, pale blue–green, usually with scattered larger granules, which are sometimes gathered more at cross–walls; apical cells cylindrical, rounded at the apex, without calyptra. Position of thylakoids in cells mostly parietal with few occasional irregularities (dependent on environmental conditions). Reproduction: Cells divide in the trichome perpendicularly by binary fission; all cells (including apical) are able to divide, without meristematic zones; disintegration of trichomes into immotile segments, without necridic cells. Phylogenetic similarity is about 90% against the *Geitlerinema* and 87.7–96.9 % to the nearest *Phormidium* clusters (Fig. 2). – Type species: *Wilmottia murrayi* (W. et G.S.WEST) comb. nov. – Etymology: The new genus was named to honor our colleague and friend Professor ANNICK WILMOTTE (Liège), who has contributed substantially to the knowledge of the ecology and diversity of Antarctic cyanobacterial microflora.

***Wilmottia murrayi* (W. et G.S.West) comb. nov.**

Basionym: *Lyngbya murrayi* W. et G.S.West, Brit. Antarct. Exp. 1: 289, 1911.

Synonyms: *Phormidium murrayi* (W. et G.S.West) Anagnostidis et Komárek, Algological Studies 50–53: 408, 1988.

Microcoleus glaciei Casamatta, Johansen, Vis et Broadwater, J. Phycol. 41: 432–433, 2005.

Type strain: CCALA 843 (KGI28) deposited in collections CCALA and PCC; isolated by Kováčik from King George Island, South Shetlands, Antarctica, 2007; accession number HQ873481.

Description: Solitary trichomes, in cluster or fine mats, blue–green or dark blue–green (on agar). Filaments solitary or several to many ± parallely arranged, fasciculated, associated in common, colorless, very fine and diffluent slimy envelope; fascicles up to more than 50 μm wide. Single trichomes have facultatively delicate, but firm, colorless, unlamellated sheaths, open at the end. Trichomes straight or slightly curved, not or only slightly constricted at cross walls, (3.1)3.6–5.4 μm wide. Cells quadratic or longer, rarely also shorter than wide, (2.6)3.4–7.2(11) μm long, pale blue–green, with homogeneous, finely granular content, usually with scattered large granules which are sometimes constricted near cross–walls; cross–walls sometimes indistinct. Terminal cells rounded at the ends. Thylakoids ± parietal, fasciculated.

Typus (holotypus): exsiccate No. 2334HY, deposited in herbarium of Moravian Museum in Brno (BRNM); icona typica = figura nostra 3a.

Other strains: see in Table 1 and Fig. 2.

Generic position: classification among pseudanabaenacean genera according to phylogenetic position, morphology of trichomes, absence of necridic cells and ultrastructure (± parietal position of thylakoids).

Autapomorphic characters: Morphology of cells and cylindrical trichomes; fasciculation of trichomes without firmly delimited sheath, absence of necridic cells, absence of branching, ultrastructure.

Habitat: Benthic substrates in littoral of lakes, shores of glacial streams or water seepages, less frequently on wet soils usually among mosses, other algae and cyanobacteria. Broady (2005) records "*Ph. murrayi*" from similar localities and also from epilithic habitats and from cryoconits.

Distribution: Known mainly from Antarctica (Fig. 1). Strains CYN 38 and CYN 39 from New Zealand are included into the same cluster as *Wilmottia murrayi,* but only a fragment 659 bp was available from both strains and their systematic position (and distribution of *W. murrayi*) must be therefore confirmed. Known localities:

– Antarctic peninsula, Cierva Point (Mataloni & Pose 2001; Mataloni & Tell 2002);
– Bratina Island, SW from McMurdo Station (Taton et al. 2003, 2006);
– James Ross Island, Green Lake, Red Lake; seepages below Berry Hill; (strains isol. by Elster); natural samples from Ulu peninsula (coll. Komárek);
– McMurdo Ice Shelf (isol. by Vincent; in Quesada & Vincent 1997, as *Phormidium murrayi*; Casamatta et al. 2005, as *Microcoleus glaciei*);
– Ross Island, Green Lake (orig. locality, locus classicus); (West & West 1911; Broady & Kibblewhite 1991, as "oscillatorialean morphotype J");
– South Shetlands, King George Island (Admiralty Bay) (strains isol. by Lukešová/O. Komárek and Kováčik; Strunecký et al. 2010); (Jubany Station; Šabacká 2004);
– SW part of Antarctic Peninsula (Adelaide Island) near Rothera Station (Šabacká 2004; Comte et al. 2007).

Discussion

The modern taxonomic revision and re–classification of cyanobacteria is based on the "polyphasic approach". It is a complex evaluation of cyanobacterial diversity, in which the determination of the phylogenetic position of various clades (taxa) is the first criterion for the classification process. Numerous traditional genera appeared to be heterogeneous and must be divided in different taxonomic clusters. This concerns also the genus *Phormidium*, in which several groups were already separated and reorganized. Usually, a difference as large as 95% in genetic similarity is considered as the limit between different genera (Stackebrandt & Goebel 1994). It is impossible to accept strictly this limit for generic separation, because other markers (ecology, morphology – autapomorphic features) must also be included into the classification criteria. However, usually a similarity of about 95% or lower indicates separation into various generic entities.

In all taxonomic units (genera), which were separated by modern revisions were found also corresponding morphological characters that were connected with the genetic unification. Only in phenotypically very simple types (*Synechococcus, Pseudanabaena, Leptolyngbya* and others) is the morphological characterization difficult; the status of "cryptic" taxa (cryptic genera) is accepted for such taxonomic units. In the relatively simple traditional genus *Phormidium*, certain phenotypic features are recognizable, which were usually neglected or considered as variable and not important for characterization of taxa on the supraspecific level (cell width to length ratio, narrowed trichomes towards ends, absence of calyptra, absence/presence of necridic cells, constrictions at cross–walls, limits of dimensions, position of thylakoids in cells, etc.).

In the case of "*Phormidium murrayi*" (= *Wilmottia murrayi*), the group of all strains and populations corresponding morphologically to this species is unified in one phylogenetic cluster. Nearest to *Wilmottia* are the genera *Geitlerinema* and partly also *Phormidesmis, Leptolyngbya* and *Halomicronema*. However, *Wilmottia* is clearly separated genetically (position in phylogenetic trees) from all related oscillatorialean genera. This separate cluster, which must be considered as decisive for genetic definition is demonstrated already in Taton et al. (2006) under the clade *Phormidium murrayi*. The various phenotype features can overlap slightly with few morphologically similar genera. From this point of view *Wilmottia* can be included in numerous other "cryptogenera" described and accepted recently as *Halomicronema* Abed et al., *Halospirulina* Nübel et al., *Coleofasciculus* Siegesmund et al. and others. However, the autapomorphic characters of *Wilmottia* can discriminate it in such a degree that the phenotypic identification is also possible. *Wilmottia* was sooner classified to *Phormidium* or *Microcoleus*, but it is distinctly distant from these genera by morphology and size of trichomes (narrower trichomes, not attenuated trichome ends, absence of any calyptra, absence of distinct constrictions at cross–walls), and particularly by ultrastructure (parietal thylakoids). The diacritical morphological features of related fine filamentous genera (which are, however, genetically also different) are as follows:

Geitlerinema – without sheaths, motility, narrowed and sometimes morphologically distinct ends of trichomes; *Phormidesmis* – uniform trichomes with ± isodiametric cells, distinct constrictions at cross–walls, firm sheaths around tight fascicles of trichomes; *Trichocoleus* – morphology of cells and terminal cells, morphology of sheaths, type of fasciculation; *Halomicronema* – absence of sheaths, smaller dimensions of trichomes, ecology; *Leptolyngbya* – facultative occurrence of firm sheaths around solitary trichomes, absence of fasciculation.

However, almost all these genera are heterogeneous and the cited diacritical characters concern mainly the type species. Many taxa classified into these generic clusters must be revised and numerous transfers of species between revised genera are expected.

Up to now, only one species, *W. murrayi*, should be evidently classified into the genus *Wilmottia*. No distinct groups were recognized among all Antarctic populations and strains included in these clusters. It concerns also the species *Microcoleus glaciei*, originally isolated under the name *Phormidium murrayi* by Vincent (cf. Casamatta et al. 2005). The morphology of this species is quite the same as in "*Lyngbya murrayi*". The fasciculation of trichomes is an interesting marker, which was recognized in all strains of *Wilmottia murrayi* and represents probably one of the specific characters of this genus. In comparison with *Microcoleus*, the common mucilaginous sheath is not strictly delimited and the parallel fasciculation of trichomes is a little looser than in *Microcoleus.*

All confirmed strains of *Wilmottia murrayi* were isolated from coastal Antarctica (Fig. 1) and the ecology of this species seems to be very distinct (benthic, periphyton in lake littoral, margin of streams, watery seepages, with low temperature and long frozen periods). This genotype may occur in other regions, but, up to now, it is necessary to consider *W. murrayi* as endemic for the Antarctica. On the other hand, the genus *Wilmottia* can contain more species, which differ from the type also ecologically and geographically. From comparison in the GenBank, we found morphologically similar populations from Brazil (Lokmer 2007; strain B–Tom) and New Zealand (Wood et al. 2009; Heath et al. 2010; strains CYN38, CYN39), which have also slightly different ecology and can represent a special species from *Wilmottia*. Morphologically, several described species from the widely conceived, traditional genera *Phormidium* and *Lyngbya* are conspicuously similar to *W. murrayi* and can be

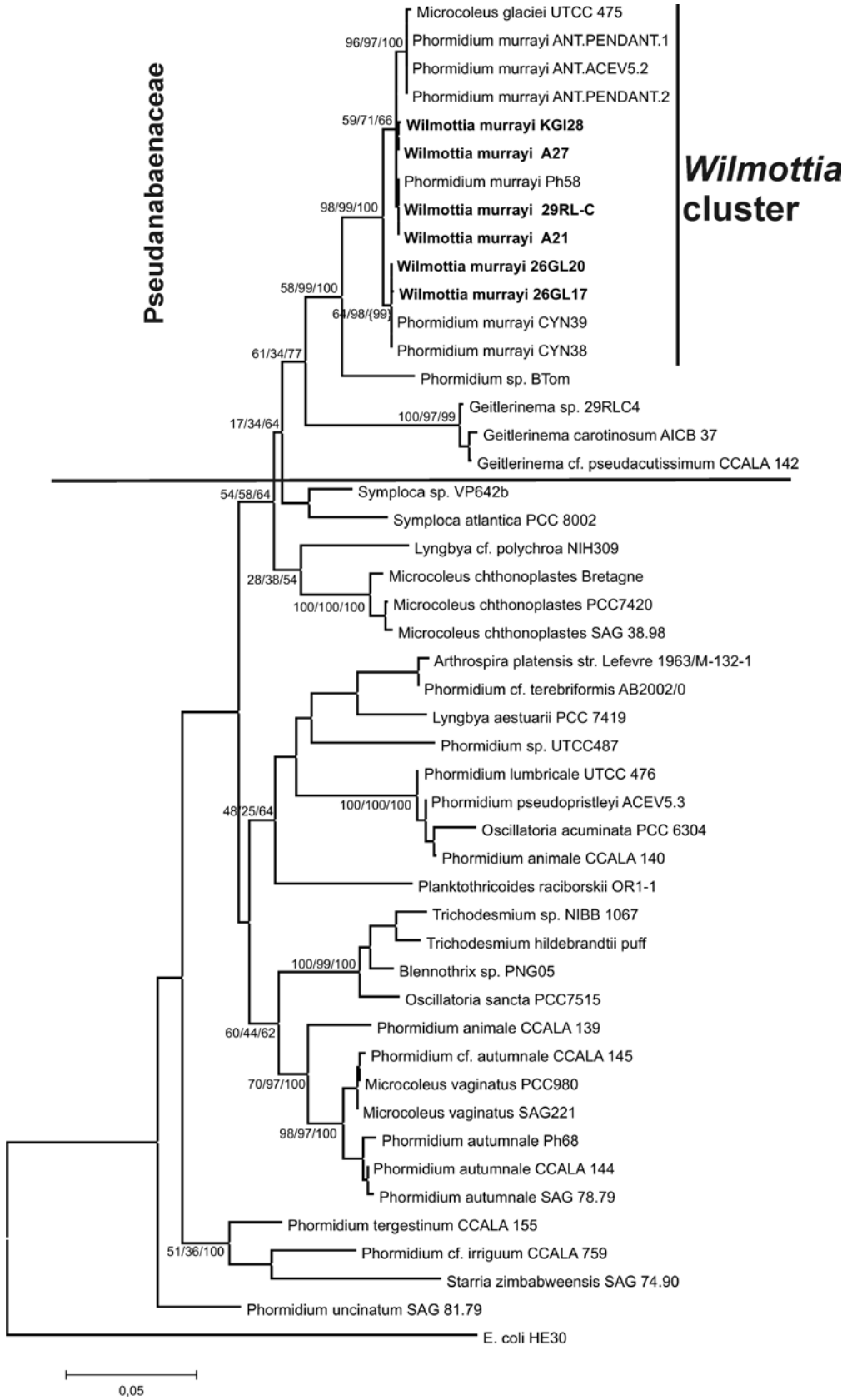

Fig. 2. Position of our *Wilmottia*–strains (printed by bold font) in the phylogenetic tree in comparison with other *Phormidium* strains, selected from GenBank. Accessions numbers of our strains see in Table 1. The evolutionary (molecular phylogenetic) analyses were conducted in MEGA4 (Tamura et al., 2007), using the Maximum Likelihood method based on the Jukes–Cantor model [1]. The bootstrap consensus tree inferred from 1000 replicates. Values corresponding to partitions reproduced in less than 50% bootstrap replicates are not shown.

Table 2. Percent similarity matrix in partial 16S rDNA sequence (1072 bp compared) for *Wilmottia murrayi* and other selected oscillatorialean strains from related genera (A) and inside *W. murrayi* clade (B).

A

		2	3	4	5	6	7	8	9	10	11	12	13	14
1	*Wilmottia murrayi* KGI28	92.7	92.4	92.9	92.7	91.8	91.2	90.6	91.0	92.8	91.6	92.7	91.9	87.6
2	*Microcoleus vaginatus* SAG221		99.4	96.8	94.8	93.3	91.8	92.0	92.3	90.8	89.8	91.7	89.8	87.6
3	*Phormidium* cf. *autumnale* CCALA 145			96.9	94.7	92.9	92.1	91.9	92.2	90.9	89.9	91.8	89.8	87.7
4	*Phormidium autumnale* Ph68				95.2	93.6	92.9	92.3	92.6	91.5	90.6	91.7	90.5	88.5
5	*Phormidium animale* CCALA 139					93.5	92.1	91.9	91.9	92.6	91.8	92.2	90,4	88,1
6	*Blennothrix* sp. PNG05						91.7	91.6	91.5	91.5	89.9	91.6	88,8	87,6
7	*Arthrospira platensis* str. Lefevre 1963/M-132-1							93.1	93.2	91.9	91.1	91.1	89.1	89.4
8	*Phormidium animale* CCALA 140								99.0	90.9	90.4	90.6	88.4	88.0
9	*Phormidium pseudopriestleyi* ACEV5.3									91.0	90.5	90.8	88.9	88.2
10	*Microcoleus chthonoplastes* PCC7420										92.5	92.5	90.8	88.0
11	*Phormidium tergestinum* CCALA 155											90.4	89.0	88.2
12	*Symploca atlantica* PCC 8002												90.7	88.3
13	*Geitlerinema* cf. *pseudacutissimum* CCALA 142													85.9
14	*Planktothricoides raciborskii* OR1-1													

B

		2	3	4	5	6	7	8	9	10	11	12
1	*Phormidium sp.* B-Tom	94.6	95.2	95.2	94.5	95.1	95.2	95.1	94.8	94.8	94.8	95.1
2	*Microcoleus glaciei* UTCC475		99.2	99.2	99.5	98.1	98.2	98.1	99.8	99.8	99.8	99.1
3	*Wilmottia murrayi* CCALA 843			99.6	99.1	98.5	98.6	98.5	99.4	99.4	99.4	99.5
4	*Wilmottia murrayi* 29RL-C				99.1	98.7	98.8	98.7	99.4	99.4	99.4	99.9
5	*Wilmottia murrayi* 26GL20					98.0	98.1	98.0	99.7	99.7	99.7	99.0
6	*Wilmottia murrayi* 26GL17						99.9	99.8	98.3	98.3	98.3	98.6
7	*Wilmottia murrayi* ANT.PENDANT.2							99.9	98.4	98.4	98.4	98.7
8	*Wilmottia murrayi* ANT.ACEV5.2								98.3	98.3	98.3	98.6
9	*Wilmottia murrayi* ANT.PENDANT.1									100	100	99.3
10	*Phormidium murrayi* Ph58										100	99.3
11	*Wilmottia murrayi* A21											99.3
12	*Wilmottia murrayi* A27											

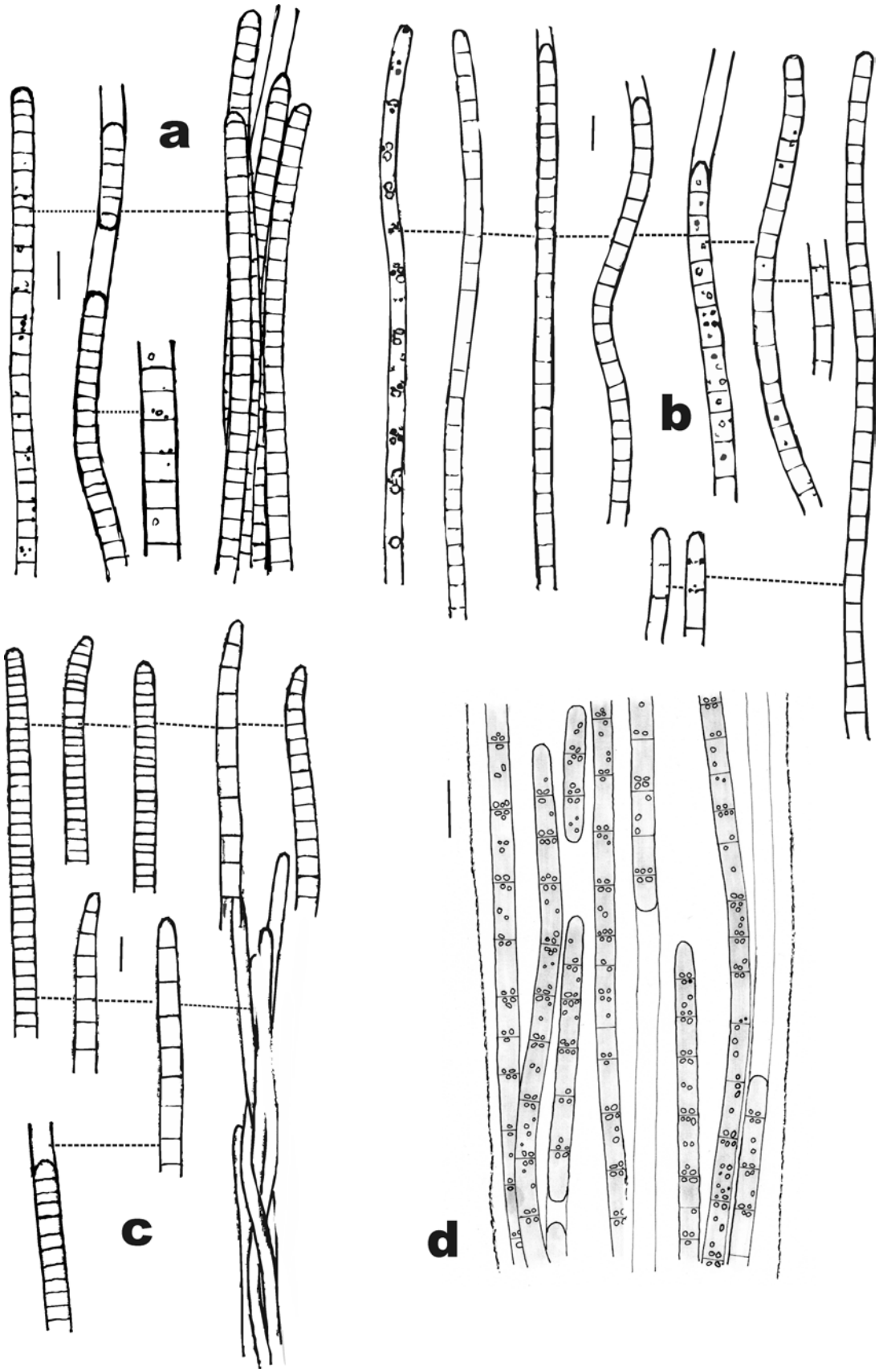

Fig. 3. *Wilmottia murrayi* : (a) from the type strain CCALA 843 (icona typica); (b) from littoral of small stream bellow Devils Rocks, James Ross Island; (c) from seepages near Lachman lakes, James Ross Island; (a–c) orig., (d) orig. JOHANSEN (specimen corresponding to *Microcoleus glaciei*, strain UTCC 475). Scale bars 10 µm.

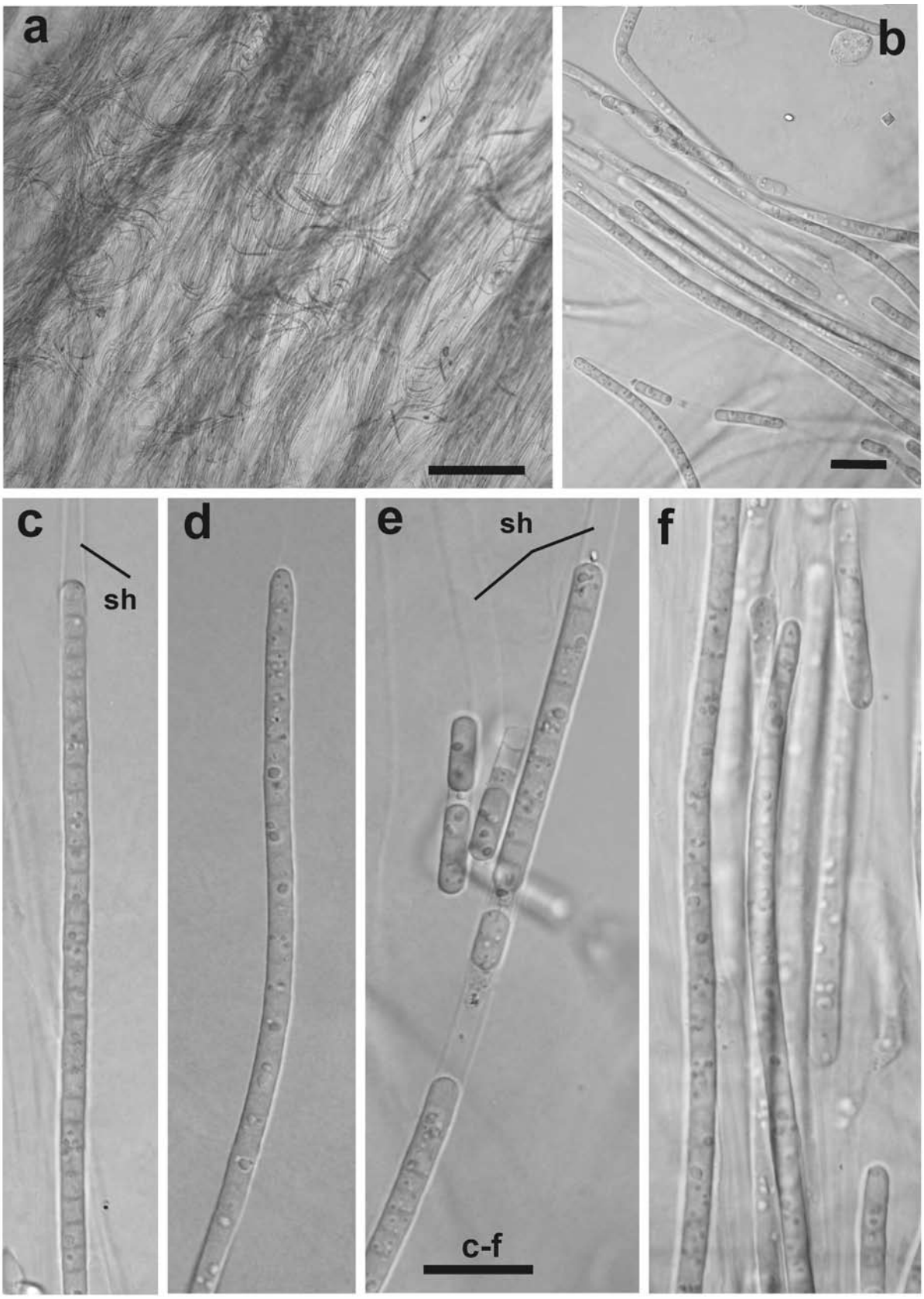

Fig. 4. *Wilmottia murrayi* from the type strain CCALA 843: (a–b) fasciculated growth of filaments, (c–f) details of trichomes, (e) production of hormogonia; (sh) individual sheaths around trichomes. Scale bars a 200 μm; b–f 20 μm. Orig.

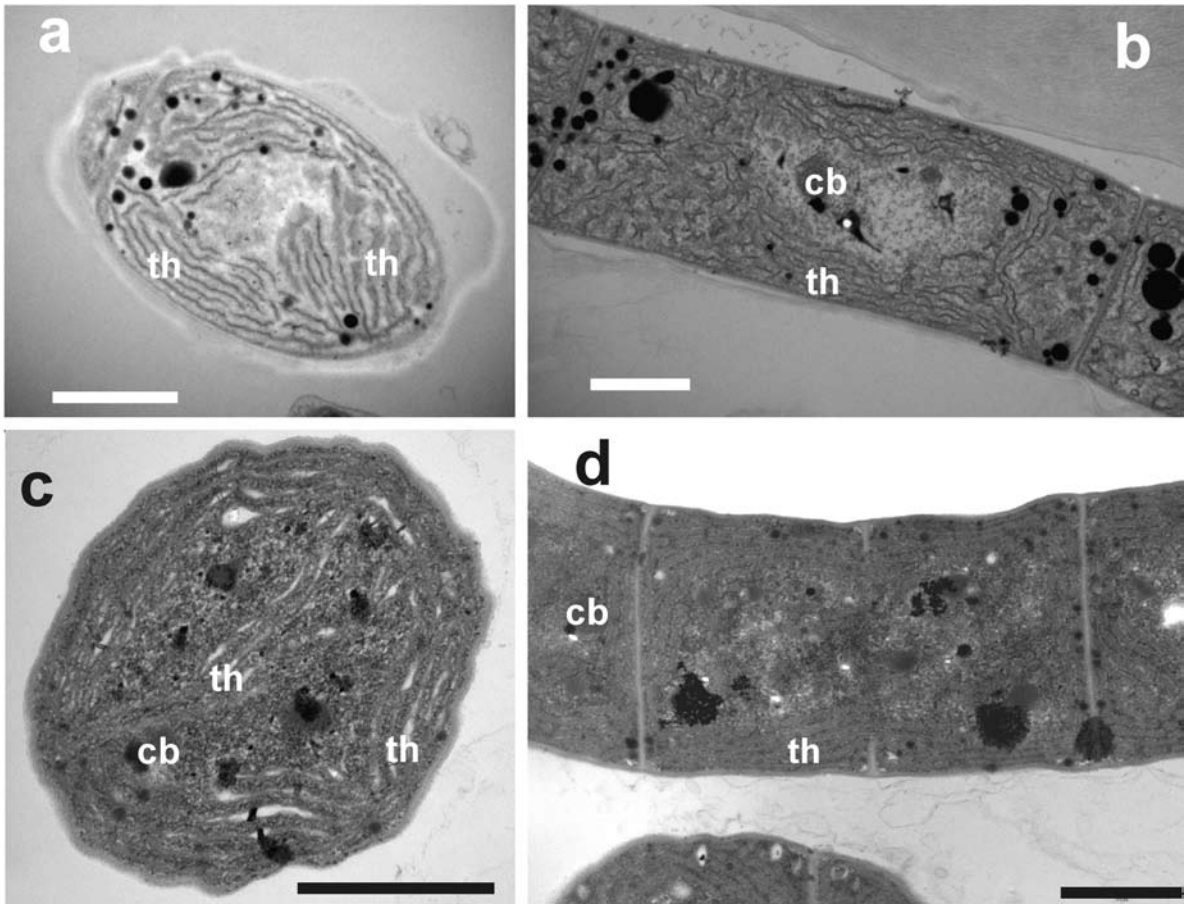

Fig. 5. Ultrastructure of two strains of *Wilmottia murrayi*: (a–b) strain ANT.ACEV 5.2; (c–d) strain CCALA 843 (type strain). Explanations: (th) thylakoids, (cb) carboxysomes; the agglomeration of cyanophycin and polyphosphate granules near the cross–walls is remarkable (a,b,f). Scale bar 2 µm. Orig.

generically identical with *Wilmottia.* They are, e.g., the Antarctic *Lyngbya scottii* Fritsch 1912 [= *Leptolyngbya scottii* (Fritsch) Anagnostidis et Komárek 1988] and (from other regions and other habitats) *Phormidium corium* Gomont 1892, *Ph. crouanii* Gomont 1892, *Ph. incrustatum* Gomont ex Gomont 1892, *Ph. inundatum* Kützing ex Gomont 1892, *Ph. macedonicum* Čado 1959, *Ph. papyraceum* Gomont ex Gomont 1892, *Ph. rimosum* (Komárek) Anagnostidis et Komárek 1988 and others (cf. Anagnostidis & Komárek 1988).

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