

## Note

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# First record of the cyanobacterial genus *Wilmottia* (Coleofasciculaeae, Oscillatoriales) from the South Orkney Islands (Antarctica)

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Two cyanobacterial morphotypes isolated from Signy Island, South Orkney Islands, maritime Antarctica were characterised using a polyphasic approach combining morphological, cytological and molecular analyses. These analyses showed that the strains grouped with members of the genus *Wilmottia*. This genus has three species, *W. murrayi*, *W. stricta*, and *W. koreana*. Both morphotypes analysed in this study were placed within the clade of *W. murrayi*. This clade showed a well-supported separation from Antarctica and New Zealand strains, as well as the strains from other regions. *W. murrayi* was first described from Antarctica and is now known from several Antarctic regions. Confirmation of the occurrence of *W. murrayi* at Signy Island significantly extends its known distribution in Antarctica. In addition, a new combination, *W. arthurensis*, is suggested for *Phormidium arthurensis*.

**Key Words:** Antarctic cyanobacteria; distribution; polyphasic analysis; taxonomy; 16S rDNA

## INTRODUCTION

The genus *Wilmottia* O. Strunecky, J. Elster & J. Komárek was established from material originally referred to *Phormidium* Kützing ex Gomont that originated from Antarctica. According to Strunecký et al. (2011), the genus *Wilmottia* originally comprised a single species, *W. murrayi* (West & G. S. West) Strunecký, Elster & Komárek, which was described based on a polyphasic evaluation of *Phormidium*-like strains isolated from Antarctic localities. Subsequently, *W. murrayi* has been recorded from diverse habitats around the Antarctic, ranging from freshwater lakes (Boyer et al. 2002, Šabacká and Elster

2004, Casamatta et al. 2005, Taton et al. 2006, Comte et al. 2007) to various soil conditions (Šabacká and Elster 2004, Komárek and Anagnostidis 2005, Comte et al. 2007), stagnant to slightly saline water (Komárek and Anagnostidis 2005), seepages (Šabacká and Elster 2004, Comte et al. 2007), and even on whale bones (Strunecký et al. 2010). The species has also been recorded from New Zealand rivers (Wood et al. 2009, Heath et al. 2010) and on wet rocks on the seashore in Brazil (Lokmer 2007). A second species, *W. stricta* Machado-de-Lima, Martins et Branco has been described from streams of São Paulo, Brazil



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(Machado-de-Lima et al. 2017).

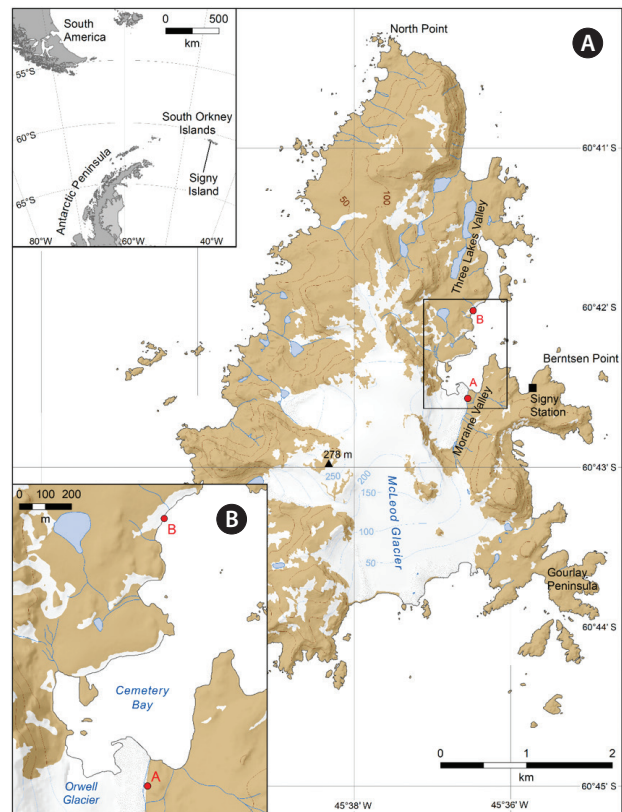
The genus *Wilmottia* was initially included within the family Phormidiaceae, but it was later transferred to Coleofasciculaceae based on molecular and ultrastructural evidence (Komárek et al. 2014). Species are described as having solitary trichomes or with several to many trichomes forming fascicles, presenting cylindrical trichome with cells almost isodiametric or longer than wide that are not or only slightly constricted at cross-walls (Komárek and Anagnostidis 2005). Solitary granules are present in vegetative cells and are occasionally placed near the cross-walls (Komárek and Anagnostidis 2005). The apical cell is rounded or slightly conical without any distinctive feature, which conforms to *Phormidium* group IV (Komárek and Anagnostidis 2005). The genus as currently described possesses characteristics that are morphologically identical to those of *Phormidium*, thus hindering reliable morphological assessment. Hence, representatives of this genera can only be distinguished with confidence based on the molecular evaluation.

In a broad-scale, floristic survey conducted on Signy Island (South Orkney Islands, 60.7170° S, 45.6000° W) by Broady (1979) recorded the occurrence of five *Phormidium* morphospecies: *P. autumnale* Gomont, *P. foveolarum* Gomont, *P. frigidum* F. E. Fritsch, *P. priestleyi* F. E. Fritsch, and *P. cf. rubroterricola* N. L. Gardner. These identifications were based solely on morphological features and Signy Island isolates have not been subjected to any subsequent molecular assessment. However, recent molecular evaluation of *P. priestleyi* resulted in the proposition of the new genus *Phormidesmis* Turicchia, Ventura, Komárková & Komárek with *Phormidesmis priestleyi* (F. E. Fritsch) Komárek, Kastovský, Ventura, Turicchia & Smarda as the type species (Komárek et al. 2009). This study illustrated the potential of genetic data to complement the morphological approach in determining which species belong to the genus *Phormidium*. In the current study, we report the polyphasic characterization of two cyanobacterial morphotypes that resemble *P. cf. rubroterricola* as described by Broady (1979).

## MATERIALS AND METHODS

### Sample origin and culture conditions

Two samples were obtained from Signy Island during the austral summer of 2015/16: (1) sample A (60°42.570' S, 45°36.550' W) at the northern end of the lateral moraine of Orwell Glacier in Cemetery Bay; and (2) sample



**Fig. 1.** Map showing the sample locations on Signy Island, South Orkney Islands, Antarctica. (A) Location of Signy Island, South Orkney Islands, in relation to the Antarctic Peninsula and southern South America. (B) Enlargement which more accurately locates sample locations that are also shown within the box on the main figure. Samples A and B were obtained at the northern end of the lateral moraine of Orwell Glacier in Cemetery Bay and on the slope north-west of Waterpipe Beach, respectively. Map prepared by Laura Gerrish (BAS Mapping and Geographic Information Centre).

B (60°42.020' S, 45°36.480' W) on the slope north-west of Waterpipe Beach (Fig. 1). Both samples were collected from rock surfaces using sterile equipment. Sample A comprised of moss while sample B comprised of lichens and moss. They were frozen soon after collection at the research station of the British Antarctic Survey on Signy Island and transported frozen (-20°C) in sterile containers to Universiti Sains Malaysia, Penang, Malaysia. A small amount of each sample was streak inoculated onto 1% agarised full-strength BG-11 medium in Petri plates (Rippka et al. 1979), supplemented with 1% cycloheximide to prevent undesirable growth of eukaryotes (Bolch and Blackburn 1996). Cultures were incubated for three weeks under 24 h light provided by a cool white fluorescent lamp at 27  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at 15  $\pm$  2°C. Unialgal cultures of filamentous cyanobacteria were established by inocu-

lation onto fresh medium of small amounts of filaments removed from the edges of developing colonies.

### Morphological characterization

Morphological examination was carried out on the strains in cultures using an Olympus BX-53 bright field microscope (Olympus America Inc., Center Valley, PA, USA) at 100–2,000× magnification. Photomicrographs were taken. A camera lucida was used for making the illustrations. The following morphological features were recorded for both morphotypes: filament and trichome width, cell shape, apical cell shape, sheath morphology, cell colour, and presence of granules. Measurements were taken from 30 replicates of randomly chosen for every specimens. The characteristics of the studied morphotypes were compared with data presented in Broady (1979), Komárek and Anagnostidis (2005), Strunecký et al. (2011), and Machado-de-Lima et al. (2017).

### Transmission electron microscopy

Filaments taken from week three cultures of both morphotypes were fixed for 24 h in McDowell-Trump fixative solution (McDowell and Trump 1976) prepared in 0.1 M phosphate buffer, and then post-fixed with 1% osmium tetroxide. Upon fixation, the samples were dehydrated in an ethanol series (50, 75, 95, and 100%), 50 and 75% ethanol for 15 min each, followed by 2 times dehydration in the following; 95% ethanol for 15 min, 100% ethanol for 30 min and 100% acetone for 10 min. The samples were then embedded in Spurr's Low Viscosity epoxy resin (Spurr 1969) mixed in a rotator overnight. Ultrathin sections of specimens (<0.1 µm) were prepared using a PowerTome XL ultramicrotome (Labtech, Quebec, Canada) with a glass knife. To improve contrast, these sections were treated with uranyl acetate and lead citrate (Reynolds 1963). Thin sections were collected on copper grids. A JEM 2000FX transmission electron microscope (JEOL, Tokyo, Japan) was used to observe the samples operating at 100 kV.

### Molecular analyses

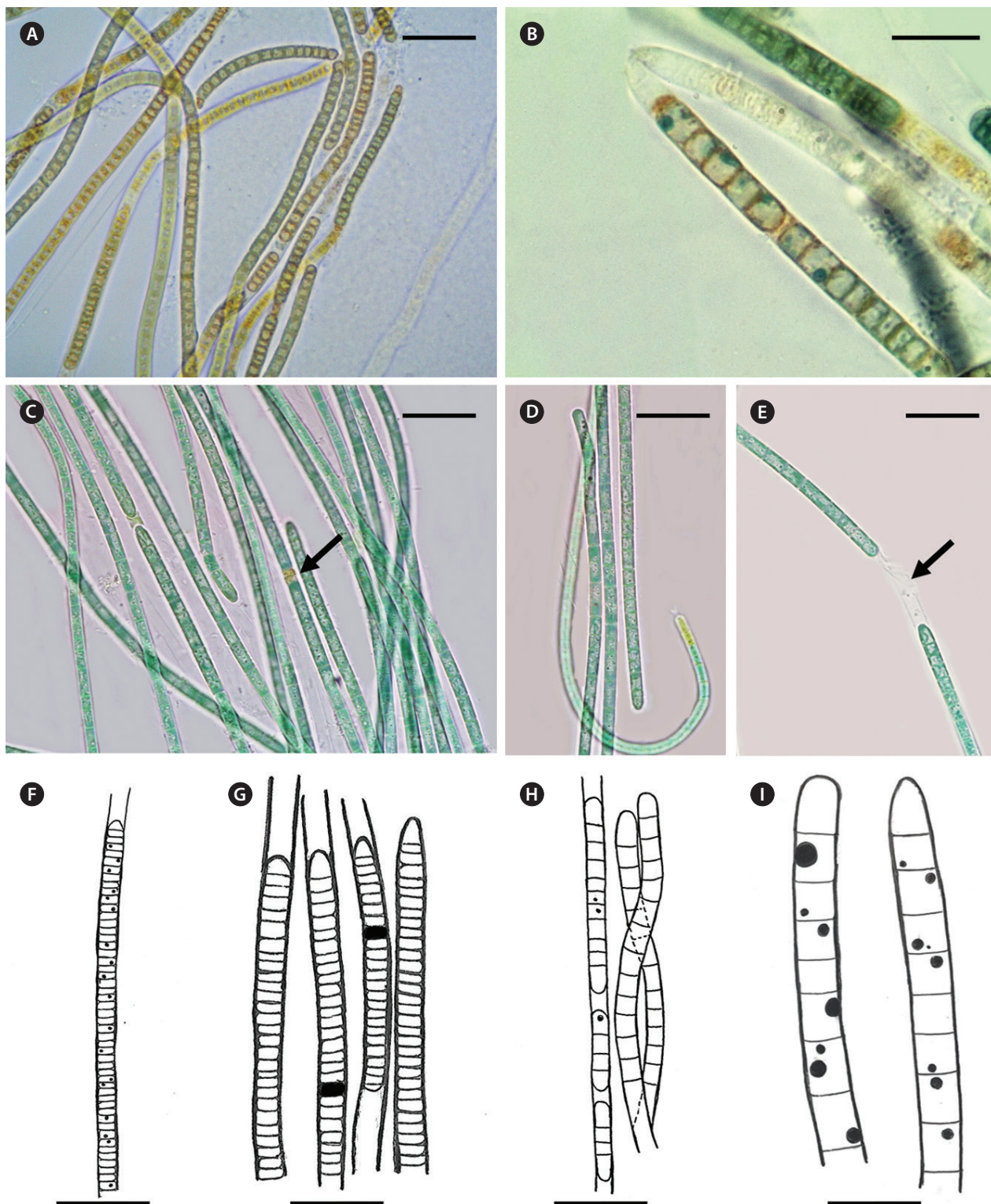
DNA was extracted using the G-spin for bacterial genomic DNA extraction kit (iNtRON Biotechnology, Seongnam, Korea) according to the manufacturer's protocol. The quality of extracted DNA was determined using a NanoDrop Quawell UV Spectrophotometer Q3000 (Thermo Fisher Scientific, Waltham, MA, USA) for ±1.5

µg mL<sup>-1</sup> between 12 and 24 h. The 16S rDNA gene sequences were amplified using the combination of primers 2 (5'-GGG GGA TTT TCC GCA ATG GG-3') and 3 (5'-CGC TCT ACC AAC TGA GCT A-3') (Boyer et al. 2001). The reaction mix comprised 25 µL of MyTaq™ Red Mix (Meridian Bioscience, Cincinnati, OH, USA), 1 µL of each forward and reverse primer, 2 µL of DNA template, and 21 µL of ultra-pure water, giving a final volume of 50 µL. Polymerase chain reaction (PCR) was carried out using a Thermal Cycler (Bio-Rad, Hercules, CA, USA). Thermal cycling conditions were set as follows: 95°C for 2 min, 95°C for 15 s, 55°C for 15 s (30 cycles), 72°C for 20 s followed by 7 min extension at 72°C. Once the reaction was completed, the integrity of the PCR product was verified using 2% agarose gel. PCR products were sequenced at NHK Bioscience Solutions Sdn. Bhd. (Korea) using the Sanger sequencing method and the same primer pair used for PCR reactions.

### Phylogenetic analyses

The chromatograms were checked, edited, and trimmed manually to achieve maximum homology. The 16S rDNA consensus sequences were built in by assembling the forward and reverse sequences. Sequence alignments between newly determined sequences and a selection of GenBank sequences, were made using MUSCLE algorithm in the Geneious 11.0 software package (<http://www.geneious.com>) (Kearse et al. 2012). The data set included 60 operational taxonomic units, consisting of two sequences newly obtained in this study together with additional sequences retrieved from GenBank of closely related species of *Wilmottia*, more distantly related species of *Phormidium sensu stricto* and one outgroup taxon (*Gloeobacter violaceus* FR798924). New sequences generated in this study have been deposited in GenBank under accession numbers USMFMS1 MN656000 and USMFMS2 MN656001.

Phylogenetic analyses were performed using two different methods: maximum likelihood (ML) and Bayesian inference (BI). The program Kakusan4 determined the best-fit model of DNA substitution (Kanehisa et al. 2011). ML analyses were performed with W-IQ-TREE (<http://iqtree.cibiv.univie.ac.at>) (Trifinopoulos et al. 2016). The analysis used the general time-reversible invariant-sites nucleotide substitution model with the default parameters. Bootstrap proportions were calculated (1,000 replicates) to assess the robustness of each node. BI analyses were performed using MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003). Two independent analyses, each



**Fig. 2.** Detailed morphology of cultured strains. *Wilmottia murrayi* USMFMS1 (A, B, F & G). Light microscopy: trichomes olive green to brownish with distinctly constricted cell walls (A) and cells with granules, sheath firm and colourless (B). Illustrations: cells with granules, sheath firm and colourless (F) and apical cells round to cylindrical, necridic cells with dense contents (G). *Wilmottia murrayi* USMFMS2 (C–E, H & I). Light microscopy: filaments bright blue-green to pale blue, necridic cell (arrow) (C), apical cells rounded to cylindrically round (D), and sheath, thin and colourless (arrow) (E). Illustration: longer than wide cells, granulated at cross-walls (H) and trichomes not constricted at cross walls, rounded apical cells (I). Scale bars represent: A, C–F & H, 10  $\mu$ m; B, G & I, 5  $\mu$ m.

consisting of four Markov chains, were run simultaneously for 1,000,000 generations, sampling every 100 generations. Bayesian posterior probability values were calculated using the remaining trees after a burn in of 25% of saved trees was removed. ML and BI trees were edited with the program FigTree v1.3.1 (Rambaut 2009). We rooted both phylogenetic trees with the same outgroup (*Gloeobacter violaceus* FR798924). The uncorrected pairwise genetic distances ( $p$ -distance) in MEGA X (Stecher et al. 2020) was also calculated.

## RESULTS

### Light microscopy

*Wilmottia murrayi* USMFMS1. Fig. 2A, B, F & G was isolated from sample associated with moss. Macroscopically forming pale blue-green, thin, flat mats spreading over agar surface. Filaments olive-green to slightly brownish, often somewhat curved, rarely straight, long. Sheath thin, firm, colourless (Fig. 2F & G). Trichomes immotile, distinctly constricted at cross-walls (Fig. 2A). Cells distinctly shorter than wide, (1.9) 2.5–4.4  $\mu\text{m}$  wide, 1.2–3.1 (3.3)  $\mu\text{m}$  long, containing small, scattered granules sometimes gathered at cross-walls (Fig. 2B). Apical cell rounded, not attenuated and without calyptra (Fig. 2G). Reproduction by fragmentation of trichomes at necridic cells (Fig. 2G).

*Wilmottia murrayi* USMFMS2. Fig. 2C–E, H & I was isolated from sample associated with lichen and moss. Macroscopically forming bright blue-green, thin, flat mats spreading over agar surface. Filaments bright blue green, variously bent, densely entangled or nearly straight and in parallel arrangement, long (Fig. 2C). Sheath thin, firm, and colourless (Fig. 2E). Trichomes motile with slow jerky gliding, not constricted at the cross walls (Fig. 2H). Cells mostly almost isodiametric or longer than wide, (2.5) 3.0–4.0 (4.5)  $\mu\text{m}$  wide, 3.0–5.0 (5.6)  $\mu\text{m}$  long, often containing one or two large granules at various positions within the cells (Fig. 2I). Apical cell cylindrical to somewhat conical, rounded, not attenuated, without calyptra (Fig. 2D & I). Reproduction by fragmentation of trichomes at necridic cells (Fig. 2C).

Differences were evident in morphological characteristics between our strains grown under the same culture conditions and also with previous descriptions of the species (Table 1). Apical cell shape, trichome pigmentation and sheath development were consistent among our strains. Other characteristics were inconsistent, as the

cell width and length, which were smallest in the *Wilmottia murrayi* USMFMS1. This strain also had trichomes with distinct constrictions at the cross-walls, all other strains were either not constricted or slightly constricted (Table 1). Fascicle formation was absent in the current strains but occurred in two of the four previously studied strains. Necridic cells were present in the current two strains and have not been recorded previously.

### Transmission electron microscopy

Both morphotypes were examined with transmission electron microscopy (Fig. 3A–D) to visualise cytomorphological arrangement and granular inclusions. Thylakoids (3–8 per cell) showed parietal and irregular arrangement when viewed in transverse and longitudinal sections of both morphotypes (Fig. 3A & B), but were not clearly seen in longitudinal section of USMFMS2 (Fig. 3D). Occasional polyphosphate granules were visible in both morphotypes (Fig. 3A & C). Carboxysomes were seen only in Morphotype 2 (Fig. 3C & D). Cell walls were simple (Fig. 3B) and trichomes were enclosed within distinct sheaths (Fig. 3B & D) for both morphotypes.

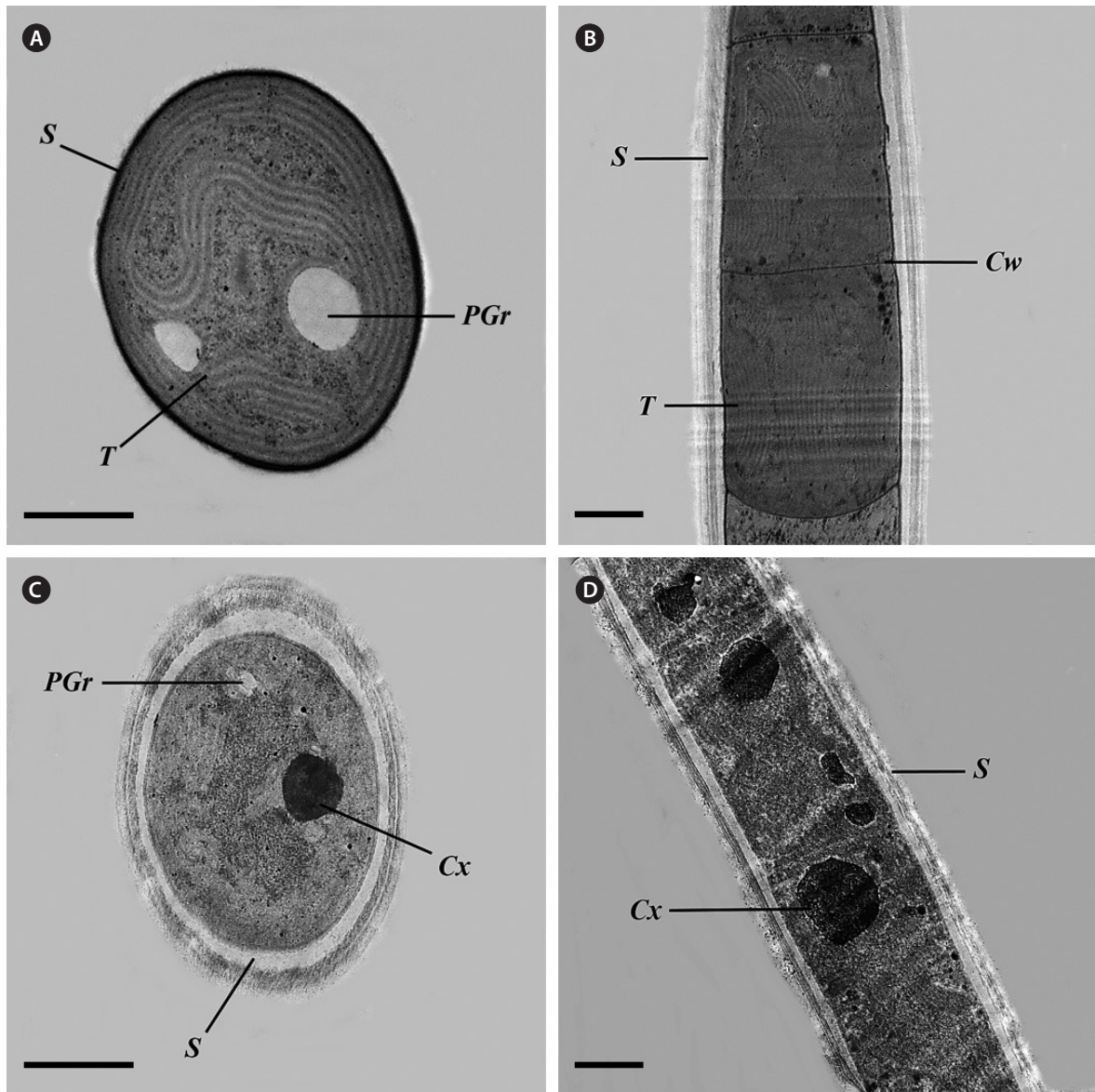
### Phylogenetic analysis

The 16S rDNA sequence dataset consisted of 1,472 base pairs (bp), including gaps. Both ML and BI analyses yielded a congruent topology and therefore only the ML tree is presented (Fig. 4), and  $p$ -distance is given in Supplementary Table S1. ML tree clearly indicate the monophyletic clade of genus *Wilmottia*, including our two samples (86% ML bootstrap percentage and 0.98% BI posterior probability). The genetic divergences ( $p$ -distance) within *Wilmottia* clade range from 0–3.9% (Supplementary Table S1). *W. murrayi* USMFMS1 and *W. murrayi* USMFMS2 are placed within a clade that contains all sequences of *W. murrayi* (98% ML bootstrap percentage and 100% BI posterior probability) with small genetic differences ( $p$ -distances 0–1.4%) (Supplementary Table S1). The largest clade within this includes all sequences of *W. murrayi* as well as three strains labelled *Wilmottia* sp. and two labelled *Phormidium* sp. *W. murrayi* clade is sister to the clade containing *W. stricta* (KY288996), *Wilmottia* sp. (KU219760), and *Phormidium arthurensis* (HQ012544), and the genetic divergences between these two clades range from 1.8–3% (Supplementary Table S1).

Sequences of *W. murrayi* that were retrieved from GenBank and the study strains were clustered in two sister sub clades, 1 and 2. Sub clade 1 (S1) ( $p$ -distance

**Table 1.** Comparison of the morphological characteristics among *Wilmottia murrayi*, *W. stricta*, and *W. koreana* (Komárek and Anagnostidis 2005, Strunecký et al. 2011, Machado-de-Lima et al. 2017, Lee et al. 2020) and the two morphotypes examined in this study

Character	<i>W. murrayi</i> (Strunecký et al. 2011)	<i>W. murrayi</i> (Machado-de-Lima et al. 2017)	<i>W. stricta</i> Machado-de-Lima, Martins & Branco	<i>W. koreana</i> N. Lee, Y. Seo, J. Ki & O. Lee	<i>W. murrayi</i> USMFMS1	<i>W. murrayi</i> USMFMS2
Cell width (µm)	(3.1) 3.6–5.4	3.2–5.6	3.2–5.5	3.4–4.2 (4.3)	(1.5) 2.5–4.4	(2.5) 3.0–4.0 (4.5)
Cell length (µm)	(2.6) 3.4–7.2 (11)	2.4–8.8	2.4–8.8	2.6–5.4	1.2–3.1 (3.3)	3.0–5.0 (5.6)
Cell shape	Isodiametric; longer than wide	Isodiametric; slightly shorter; longer than wide	Isodiametric; longer than wide; shorter than wide	Isodiametric; longer than wide; shorter than wide	Shorter than wide	Isodiametric; longer than wide
Cross-wall	Not or very slightly constricted	Slightly constricted	Slightly constricted	Not or slightly constricted	Distinctly constricted	Not constricted
Colour	Blue-green; dark blue-green	Blue-green; dark blue-green	Blue-green	Blue-green	Olive green	Bright blue-green
Filaments	Solitary; several to many; parallel arranged; fasciculated	Straight	Entangled; fasciculate; straight	Solitary; straight	Solitary; straight	Densely entangled; straight; slightly flexuous
Fascicle formation	Present	Absent	Present	Absent	Absent	Absent
Apical cells	Rounded	Rounded conical	Rounded	Rounded	Rounded	Cylindrical rounded
Granulation	Scattered granules	Ungranulated	Ungranulated	Scattered granules	Scattered granules; sometimes gathered at cross-walls	Scattered granules
Sheaths	Colourless; very fine, diffluent; attached to trichome	Colourless; firm; thin; facultative; attached to trichome	Thin; homogenous; colourless	Thin; hyaline; colourless	Colourless; firm; thin	Colourless; firm; thin
Reproduction	Cells divide in the trichome perpendicularly by binary fission	Disintegration of trichomes into hormogonia	Disintegration of trichomes without necridic cells	Disintegration of trichomes without necridic cells	Necridic cells	Necridic cells
Occurrence	Antarctic ponds, terrestrial habitats and New Zealand river	Aquatic; aerophytic habitats, São Paulo, Brazil	Talhadinho stream, São Paulo, Brazil	On tree bark, Mount Gwanggyo, Korea	Terrestrial habitat Signy Island, associated with moss	Terrestrial habitat Signy Island, associated with lichens and moss



**Fig. 3.** Transmission electron micrographs. *Wilmottia murrayi* USMFMS1 (A & B). Cross-section (A) and longitudinal section (B). *Wilmottia murrayi* USMFMS2 (C & D). Cross-section (C) and longitudinal section (D). S, sheath; T, thylakoids; PGr, polyphosphate granules; Cw, cross-wall; Cx, carboxysome. Scale bars represent: A & C, 500 nm; B & D, 2  $\mu$ m.

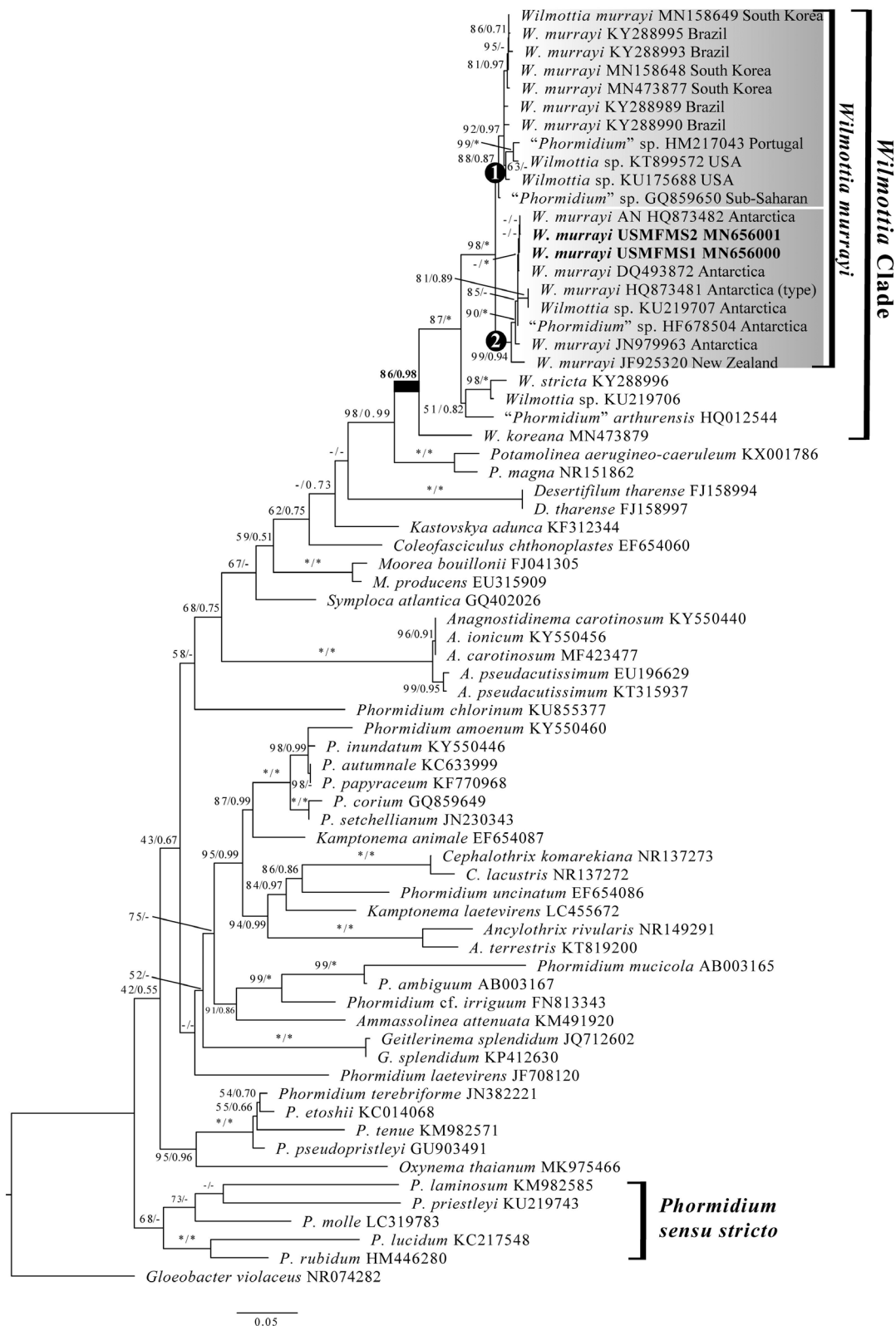
0–1.1%) comprised of strains from Korea (MN158649 and MN158648), Brazil (KY288995, KY288993, KY288989, and KY288990), Russia (KU175688), the USA (KT899572), Portugal (HM217043), and Sub-Saharan Africa (GQ859650). On the other hand, sub clade 2 (S2) ( $p$ -distance 0–0.6%) included all the Antarctic strains (HQ873482, KU219707, DQ493872, HF678504, and JN979963), one strain from New Zealand (JF925320) and the studied strains (MN656000 and MN656001). The genetic divergences between these two sub clades range from 0.6–1.4% (Supplementary Table S1). Our phylogenetic analysis places

*P. arthurensis* (HQ012544) in the *Wilmottia* clade ( $p$ -distance 1.7–3.4%).

***Wilmottia arthurensis* (Novis and Visnovsky)  
Radzi and Merican comb. nov.**

**Basionym.** *Phormidium arthurensis* Novis and Visnovsky, Phytotaxa 22: 19, fig. 7G–M ('*arthurensis*').

**Type locality.** New Zealand, Westland, Mt. Philistine, Sep 30, 2007, CHR610795.



**Fig. 4.** Phylogenetic analysis. 16S rDNA gene-based maximum likelihood (ML) phylogenetic tree showing the phylogenetic positions of *Wilmottia murrayi* USMFMS1 (MN656000) and *Wilmottia murrayi* USMFMS2 (MN656001). ML bootstrap values (left) followed by Bayesian inference bootstrap values (right) on branches. Dashes indicate support values less than 50%. The sequences generated in this study are in boldface. The tree is rooted with *Gloeobacter violaceus* (NR074282).



## DISCUSSION

Classification based solely on morphological characteristics can be challenging, therefore identification using polyphasic approaches is strongly recommended (Komárek 2016). The 16S rDNA phylogenetic analysis confirms that *W. murrayi* USMFMS1 and *W. murrayi* USMFMS2 are referable to *W. murrayi* despite the differences observed in their morphological characteristics. Our major clade of *W. murrayi* showed a well-supported separation of strains from Antarctica and New Zealand from those from other regions.

We suggest that the identity of the three *Phormidium* sequences (HM217043, GQ859650, and HF678504) that were retrieved from GenBank and clustered with *Wilmottia* should be renamed as *Wilmottia* (Fig. 4). The name *Phormidium* was applied for strains HM217043 and GQ859650 before the genus *Wilmottia* was erected by Strunecký et al. (2011). For strain HF678504, the name *Phormidium* has quite likely been associated with the strain due to lack of critical evaluation of its morphology. If isolates in culture are still available, then the morphology of these could be checked for conformity to *W. murrayi*. Incorrect taxonomic designation of sequence data from isolated cyanobacteria strains can be highly problematic in modern cyanobacterial taxonomy (Komárek 2016). The morphology and molecular phylogenetic data for *P. arthurensis* as described by Novis and Visnovsky (2011) also suggests that this species would be best placed within *Wilmottia* (Strunecký et al. 2011) (Fig. 4). We suggest that the genus *Wilmottia* includes four species: *W. murrayi* (type species), *W. stricta*, *W. koreana*, and a new combination *W. arthurensis*.

Morphological differences were evident between our two morphotypes when compared under the same culture conditions and also with previous descriptions of *W. murrayi*. Both morphotypes were consistent with *W. murrayi* (West and G. S. West) Strunecký, Elster and Komárek (2011) regarding the apical cell shape and attenuation, granulation, trichome pigmentation, and sheath development, but both had narrower trichomes than the previously recorded minimum of the range for *W. murrayi* (Strunecký et al. 2011, Machado-de-Lima et al. 2017). Cell shape differed between both morphotypes with cells of *W. murrayi* USMFMS1 being distinctly shorter than wide and those of *W. murrayi* USMFMS2, ranging from isodiametric to shorter and longer than wide (Fig. 2). In previous descriptions, cell shape of *W. murrayi* has been described as isodiametric to longer than wide. Both morphotypes formed necridic cells, but this had not been

recorded previously. Fascicle formation was absent in both morphotypes, although Strunecký et al. (2011) considered fasciculation of trichomes to be a characteristic of the genus. Lack of fasciculation has also been reported for Brazilian strains of *W. murrayi*, but fascicles did occur in *W. stricta* (Machado-de-Lima et al. 2017).

Previous records of *W. murrayi* showed varying degrees of constriction from unconstricted to slightly constricted (Strunecký et al. 2011). Our *W. murrayi* USMFMS1 was distinctly constricted at cross walls whilst *W. murrayi* USMFMS2 lacked constrictions (Fig. 2). The Brazilian strains of *W. murrayi* also had constrictions (Machado-de-Lima et al. 2017). Although the absence of distinct constrictions was proposed by Strunecký et al. (2011) to be diagnostic for the genus the evidence now suggests otherwise. Additionally, this characteristic cannot be diagnostic for the Family Coleofasciculaceae (Komárek et al. 2014). Phenotypic identification of *Wilmottia* is said to be possible based on trichome width, absence of attenuation, constriction and a calyptra (Strunecký et al. 2011). However, constriction of trichomes at cross walls, apical cell shape and cell dimensions differed between our two strains when cultured under identical conditions. Therefore, we conclude that separation of the genus *Wilmottia* based solely on morphological traits is not recommended and can result in unreliable and erroneous identification. Furthermore, the levels of differentiation apparent between the different strains examined in this study, and currently assigned to *W. murrayi*, suggest that a reassessment of the taxonomic status of these sub-clades would be appropriate, and that the current taxon may represent a species group rather than a single species.

In the ultrastructure analysis, the arrangement of parietal thylakoids in concentric layers and with some thylakoid stacks irregularly penetrating the interior of the cell for both morphotypes conformed to that found in other strains of *Wilmottia* as well as in other species in the Coleofasciculaceae (Strunecký et al. 2011, Komárek et al. 2014). The solitary granules are polyphosphates and carboxysomes (Fig. 3C).

Our study of two cyanobacterial morphotypes isolated from terrestrial habitats in Signy Island resulted in the identification of the species *W. murrayi*. This species was originally described from Antarctica (Taton et al. 2006, Strunecký et al. 2010) and our results extend its distribution within the region. Recent records of its occurrence in New Zealand (Strunecký et al. 2011) and Brazil (Machado-de-Lima et al. 2017) show a more cosmopolitan distribution.

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## CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

## SUPPLEMENTARY MATERIALS

**Supplementary Table S1.** Un-corrected genetic distance (*p*-distance) within clades of *Wilmottia* (<https://www.e-algae.org>).

## REFERENCES

- Bolch, C. J. S. & Blackburn, S. I. 1996. Isolation and purification of Australian isolates of the toxic cyanobacterium *Microcystis aeruginosa* Kütz. J. Appl. Phycol. 8:5–13.
- Boyer, S. L., Flechtner, V. R. & Johansen, J. R. 2001. Is the 16S-23S rRNA internal transcribed spacer region a good tool for use in molecular systematics and population genetics? A case study in cyanobacteria. Mol. Biol. Evol. 18:1057–1069.
- Boyer, S. L., Johansen, J. R., Flechtner, V. R. & Howard, G. L. 2002. Phylogeny and genetic variance in terrestrial *Microcoleus* (Cyanophyceae) species based on sequence analysis of the 16S rDNA gene and associated 16S-23S ITS region. J. Phycol. 38:1222–1235.
- Broady, P. A. 1979. *The terrestrial algae of Signy Island, South Orkney Islands, Vol. 98. British Antarctic Survey Scientific Reports*. British Antarctic Survey, Cambridge, 117 pp.
- Casamatta, D. A., Johansen, J. R., Vis, M. L. & Broadwater, S. T. 2005. Molecular and morphological characterization of ten polar and near-polar strains within the Oscillatoriales (Cyanobacteria). J. Phycol. 41:421–438.
- Comte, K., Šabacká, M., Carré-Mlouka, A., Elster, J. & Komárek, J. 2007. Relationships between the Arctic and the Antarctic cyanobacteria; three *Phormidium*-like strains evaluated by a polyphasic approach. FEMS Microbiol. Ecol. 59:366–376.
- Heath, M. W., Wood, S. A. & Ryan, K. G. 2010. Polyphasic assessment of fresh-water benthic mat-forming cyanobacteria isolated from New Zealand. FEMS Microbiol. Ecol. 73:95–109.
- Kanehisa, M., Goto, S., Sato, Y., Furumichi, M. & Tanabe, M. 2011. KEGG for integration and interpretation of large-scale molecular data sets. Nucl. Acids Res. 40:109–114.
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. & Drummond, A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649.
- Komárek, J. 2016. A polyphasic approach for the taxonomy of cyanobacteria: principles and applications. Eur. J. Phycol. 51:346–353.
- Komárek, J. & Anagnostidis, K. 2005. Cyanoprokaryota. Teil 2: Oscillatoriales. In Budel, B., Gartner, G., Krienitz, L. & Schagerl, M. (Eds.) *Süßwasserflora von Mitteleuropa; Band 19/2*. Spektrum Akademischer Verlag, Heidelberg, pp. 1–759.
- Komárek, J., Kaštovský, J., Mareš, J. & Johansen, J. R. 2014. Taxonomic classification of cyanoprokaryotes (cyanobacterial genera) 2014, using a polyphasic approach. Preslia 86:295–335.
- Komárek, J., Kaštovský, J., Ventura, S., Turicchia, S. & Šmarda, J. 2009. The cyanobacterial genus *Phormidesmis*. Algol. Stud. 129:41–59.
- Lee, N. -J., Seo, Y., Ki, J. -S. & Lee, O. -M. 2020. Morphology and molecular description of *Wilmottia koreana* sp. nov. (Oscillatoriales, Cyanobacteria) isolated from the Republic of Korea. Phytotaxa 447:237–251.
- Lokmer, A. 2007. *Polyphasic approach to the taxonomy of the selected oscillatorian strains (Cyanobacteria)*. M.S. thesis, Faculty of Sciences, University of South Bohemia, České Budějovice, 40 pp.
- Machado-de-Lima, N. M., Martins, M. D. & Branco, L. H. Z. 2017. Description of a tropical new species of *Wilmottia* (Oscillatoriales, Cyanobacteria) and considerations about the monophyly of *W. murrayi*. Phytotaxa 307:43–54.

- McDowell, E. M. & Trump, B. F. 1976. Histologic fixatives suitable for diagnostic light and electron microscopy. *Arch. Pathol. Lab. Med.* 100:405–414.
- Novis, P. M. & Visnovsky, G. 2011. Novel alpine algae from New Zealand: cyanobacteria. *Phytotaxa* 22:1–24.
- Rambaut, A. 2009. FigTree version 1.3.1. Computer program distributed by the author. Available from: <http://tree.bio.ed.ac.uk/software/figtree>. Accessed Oct 30, 2019.
- Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* 17:208–212.
- Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M. & Stanier, R. Y. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol.* 111:1–61.
- Ronquist, F. & Huelsenbeck, J. P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574.
- Šabacká, M. & Elster, J. 2004. *Response of algal and cyanobacterial communities from Arctic and Antarctic wetland habitats to freezing and desiccation stress.* In XXX Międzynarodowe Sympozjum Polarne, Gdynia, pp. 181–182.
- Spurr, A. R. 1969. A low-viscosity epoxy resin-embedding medium for electron microscopy. *J. Ultrastruct. Res.* 26:31–43.
- Stecher, G., Tamura, K. & Kumar, S. 2020. Molecular Evolutionary Genetics Analysis (MEGA) for macOS. *Mol. Biol. Evol.* 37:1237–1239.
- Strunecký, O., Elster, J. & Komárek, J. 2010. Phylogenetic relationships between geographically separate *Phormidium* cyanobacteria: is there a link between north and south polar regions? *Polar Biol.* 33:1419–1428.
- Strunecký, O., Elster, J. & Komárek, J. 2011. Taxonomic revision of the freshwater cyanobacterium “*Phormidium*” *murrayi* = *Wilmottia murrayi*. *Fottea* 11:57–71.
- Taton, A., Grubisic, S., Ertz, D., Hodgson, D. A., Piccardi, R., Biondi, N., Tredici, M. R., Mainini, M., Losi, D., Marinelli, F. & Wilmotte, A. 2006. Polyphasic study of Antarctic cyanobacterial strains. *J. Phycol.* 42:1257–1270.
- Trifinopoulos, J., Nguyen, L. -T., von Haeseler, A. & Minh, B. Q. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Res.* 44:W232–W235.
- West, W. & West, G. S. 1911. Freshwater algae. In Murray, J. (Ed.) *Biology, Vol. 1. Reports on the Scientific Investigation, British Antarctica Expedition 1907-09.* Heinemann, London, pp. 263–298.
- Wood, S. A., Hamilton, D. P., Paul, W. J., Safi, K. A. & Williamson, W. M. 2009. *New Zealand Guidelines for cyanobacteria in recreational fresh waters: interim guidelines.* Ministry for the Environment and Ministry of Health, Wellington, 82 pp.