Fottea, Olomouc, 25(1): 91–104, 2025
DOI: 10.5507/fot.2024.013

Desmonostoc cockrellii sp. nov. (Nostocales, Cyanobacteria): a new record of a subaerophytic species from The Habitat Penang Hill, Malaysia

Billy Lim Chun Ginn[1,6], Choi Yi Lau[1], Nur Afiqah Asma[2], Noor Aziah Serri[2], Paul Broady[3], Peter Convey[4,5], Narongrit Muangmai[6,7,8] & Faradina Merican[1*]

[1]*School of Biological Sciences, Universiti Sains Malaysia, Minden 11800, Penang, Malaysia; * Corresponding author e–mail: faradina@usm.my*
[2] *Bioprocess Technology Division, School of Industrial Technology, Universiti Sains Malaysia, Minden 11800, Penang, Malaysia*
[3]*School of Biological Sciences, University of Canterbury, Christchurch 8041, New Zealand*
[4]*British Antarctic Survey, NERC, High Cross, Madingley Road, Cambridge CB3 0ET, United Kingdom*
[5]*Department of Zoology, University of Johannesburg, Auckland Park 2006, South Africa*
[6]*Department of Fishery Biology, Faculty of Fisheries, Kasetsart University, Chatuchak, Bangkok 10900, Thailand*
[7]*Biodiversity Center Kasetsart University (BDCKU), Chatuchak, Bangkok 10900, Thailand*
[8]*Seto Inland Sea Carbon–neutral Research Center, Hiroshima University (S–CNC), Hiroshima 739–0046, Japan*

Abstract: Subaerophytic cyanobacterial diversity is poorly known in Malaysian rainforests generally and at Penang Hill specifically. A new subaerophytic species of cyanobacteria, *Desmonostoc cockrellii* sp. nov., was isolated from rocky surfaces along trails in The Habitat Penang Hill, Malaysia. Polyphasic analysis identified unique morphological structures such as larger dimensions of akinetes and bubble formations within colonies grown on agarized medium. Toxin–encoding genes for microcystin, nodularin, cylindrospermopsin, anatoxin–a and saxitoxin were not detected. The 16S rRNA gene phylogeny inferred by both maximum likelihood and Bayesian inference placed our four isolates within the *Desmonostoc* clade, distinct from previously characterised *Desmonostoc* species. The length and nucleotide sequences of the secondary structures of Box–B and V2 helices further differentiated them from known members of the genus. Fatty acid profiling showed that saturated fatty acids (53.7–77.5% dwt.) dominated over monosaturated fatty acids (8.6–22% dwt.) and polyunsaturated fatty acids (13.9–24.3% dwt.). The most abundant fatty acids were butyric acid (4:0), palmitic acid (16:0), oleic acid (18:1), γ–linolenic acid (18:3) and docosahexaenoic acid (22:6). Clear differences were apparent between the different isolates of *D. cockrellii* sp. nov. in the presence or absence and percentage composition of these fatty acids.

Keywords: Nostocales, polyphasic characterization, subaerophytic, heterocytous, tropical cyanobacteria, fatty acid

Introduction

The taxonomy of heterocytous cyanobacteria, such as members of the genus *Nostoc* Vaucher ex Bornet et Flahault, is notoriously problematic (Singh et al. 2020). Previous phylogenetic studies have reported *Nostoc* to be polyphyletic (Řeháková et al. 2007; Komárek et al. 2014). Recent polyphasic studies have led to the establishment of *Nostoc* sensu stricto and the erection of fifteen *Nostoc*–like genera including *Desmonostoc* Hrouzek et Ventura (Mishra et al. 2021; Tawong et al. 2022). This approach highlights the importance of assessment of a range of data to differentiate taxa lacking diacritical morphological characters. Molecular evidence forms the primary criterion while morphological, ultrastructural and ecological characters provide secondary support (Singh et al. 2020).

The genus *Desmonostoc* was erected based on 16S rRNA gene sequence analysis (Hrouzek et al. 2013). To date, 14 species of *Desmonostoc* have been described, excluding *D. vinosum* Miscoe et Johansen which does not belong to the *Desmonostoc* phylogenetic clade (Mishra et al. 2021; Pecundo et al. 2023). Discriminating the genus from *Nostoc* and other *Nostoc*–like groups morphologically remains challenging without assimilating phylogenetic data, although the large production of mucilaginous sheath throughout the life cycle, diffluent to parallel arrangement of trichomes (Alvarenga et al. 2018; Pecundo et al. 2021; Almeida et al. 2023), and the apoheterocytic differentiation of akinetes in long chains, with the exception of large, solitary akinetes in *D. magnisporum* Saraf et al. (Saraf et al. 2018) and *D.*

persicum Kabirnataj et al. (Kabirnataj et al. 2020), and the absence of akinetes in *D. aggregatum* Pecundo et al. (Pecundo et al. 2021) and *D. caucasicum* Maltseva, Kulikovskiy et Maltsev (Maltseva et al. 2022), have been presumed to be important morphological traits of members of the genus (Hrouzek et al. 2013). Furthermore, it is ecologically diverse, with reports from freshwater (Singh et al. 2016; Saraf et al. 2018; Nowruzi et al. 2023), saline waters (Alvarenga et al. 2018), terrestrial environments (Hrouzek et al. 2013; Miscoe et al. 2016; Cai et al. 2018; Kabirnataj et al. 2020; Maltseva et al. 2022) and as plant symbionts (Pecundo et al. 2021, 2023). Species have been recorded from Europe and North and South America (Almeida et al. 2023), as well as in Asia including India (Singh et al. 2016; Saraf et al. 2018), Iran (Kabirnataj et al. 2020; Nowruzi et al. 2023), and China (Cai et al. 2018; Pecundo et al. 2021, 2023). Subaerophytic representatives include *D. muscorum* (Bornet et Flahault) Hrouzek et Ventura (Hrouzek et al. 2013), *D. geniculatum* Miscoe, Pietrasiak et Johansen (Miscoe et al. 2016), *D. danxiaense* F. Cai et R. Li (Cai et al. 2018), *D. persicum* (Kabirnataj et al. 2020), and *D. caucasicum* (Maltseva et al. 2022).

Cyanobacteria are known to produce various bioactive products (e.g., fatty acids, carbohydrates, amino acids, carotenoids and phenolic compounds) (Nowruzi et al. 2023). Fatty acid composition has been used to differentiate closely related species and genera of cyanobacteria when grown under controlled conditions (Gugger et al. 2002; Li & Watanabe 2004; Temina et al. 2007; Shukla et al. 2012). In heterocytous cyanobacteria, the dominant fatty acids are C16:0 palmitic acid, C18:0 stearic acid, C16:1 palmitoleic acid and C22:0 behenic acid (Anahas & Muralitharan 2018). Cyanobacteria accumulate saturated fatty acids (SFAs) (50–85% dwt.), followed by monounsaturated fatty acids (MUFAs) (6.6–23.5% dwt.) and polyunsaturated fatty acids (PUFAs) (1.77–16.4% dwt.) (Anahas & Muralitharan 2018). However, inclusion of analysis of the composition of fatty acids in polyphasic studies is still lacking, particularly in strains of *Nostoc* sensu lato (Alvarenga et al. 2018; Maltseva et al. 2022).

Subaerial cyanobacteria are found on air–exposed surfaces of substrata such as soil, rocks, tree bark, leaves and animals (Neustupa & Škaloud 2010). In Peninsular Malaysia, there has been one study focusing on the diversity of the group (Merican et al. 2021). This preliminary assessment of their occurrence in the tropical rainforest of The Habitat Penang Hill recovered six morphospecies of epiphytic cyanobacteria, but genetic approaches were not applied to species identification. An improved knowledge of cyanobacterial diversity at The Habitat Penang Hill would provide support for the importance of the site's status as a UNESCO Biosphere Reserve and promote its conservation. Here, we describe a new *Desmonostoc* species isolated from there using a polyphasic approach that incorporates morphology, 16S rRNA gene phylogeny, putative secondary structures of 16S–23S rRNA internal transcribed spacer (ITS) region and fatty acid profiling.

Materials and Methods

Sample collection, strain cultivation and examination. Four heterocytous cyanobacterial isolates PHNL1, PHNL2, PHNL3 and PHNL4 were isolated from samples collected from the surfaces of boulders along trails in The Habitat Penang Hill (latitude 05°25'32.715"N; longitude 100°16'7.34"E) during a floristic survey conducted in April 2020 (Merican et al. 2021). One litre of full–strength BG–11_0 medium without sodium nitrate was supplemented with 1 % agar and 100 $\mu g.ml^{-1}$ cycloheximide (CHX). This was made by mixing 500 ml of double–strength stock solution with 250 ml of 4 % agar in sterile distilled water (Rippka et al. 1979) which was then added to 250 ml of filter–sterilised cycloheximide solution (400 $\mu g.ml^{-1}$ in sterile distilled water). Also, a 1 l full strength BG–11_0 liquid medium was prepared as above but omitting the agar from the mixture. Nine hundred millilitres of autoclaved stock solution were supplemented with 100 ml syringe–filtered CHX to suppress growth of undesired eukaryotic organisms (Bolch & Blackburn 1996).

Clonal cultures of each of the four isolates were obtained by removing single colonies from the original enrichment culture and transferring them onto new culture media in Petri dishes. The isolates were also grown in 100 ml BG–11_0 liquid medium contained in 250 ml Erlenmeyer flasks. The flasks were agitated three times a day by hand. All cultures were incubated at 25 °C under a 12:12 h light:dark cycle with illumination from a white fluorescent lamp (±27.03 $mmol.m^{-2}.s^{-1}$). Visible colonies developed after 2 to 3 weeks of incubation.

For light microscopy, the isolates were mounted in water on slides for microscopic observation using an Olympus BX–53F (Olympus America Inc., Center Valley, PA, USA) bright field microscope at 100–2000× magnification. Morphological characteristics were recorded, including trichome width, colour and shape, vegetative cell length and width, shape and dimensions of heterocytes and akinetes, apical cell shape and presence or absence of a sheath. Morphometric parameters based on 30 randomly chosen replicates were measured using ToupView v4.11 software. Illustrations were made with the aid of a camera lucida, and photomicrographs were taken to aid identification. Species descriptions of Komárek (2013) were followed. Repeat observations were carried out for a period of 6 months to document morphological changes and life–cycle features.

For transmission electron microscopy, colonies of all four isolates taken from liquid cultures at 3 weeks were harvested into 1.5 ml microcentrifuge tubes, centrifuged at 10,000 rpm for 2 min, and fixed for 24 h in 1.5 ml McDowell–Trump fixative solution prepared with 0.1 M phosphate buffer at pH 7.2 (McDowell & Trump 1976) and then post–fixed with 1% osmium tetroxide. Following fixation, specimens were dehydrated in a graded ethanol in water series (50%, 75%, 95% and 100%) and infiltrated in Spurr's low viscosity epoxy resin with acetone in a 1:1 mixture and left in a rotator overnight (Spurr 1969). Sections of 0.07–0.09 µm thickness were made using an ultramicrotome (PowerTome XL, USA) with a glass knife. Staining used 4% uranyl acetate followed by lead citrate to improve contrast (Reynolds 1963). Copper grids were used to mount sections for observation using transmission electron microscopy (Zeiss EFTEM Libra 20, Germany).

DNA extraction, PCR and sequencing. DNA was extracted from cultures using the G–spin™ Genomic DNA extraction kit for bacteria (iNtRON Biotechnology Inc., Seongnam, Korea) following the manufacturer's protocols. The quality of extracted DNA was determined using a Thermo Scientific™ NanoDrop™ Quawell UV Spectrophotometer Q3000 for ±1.5 $\mu g.ml^{-1}$ between 12 and 24 h. To obtain an approximately 1000 bp fragment size of the 16S rRNA gene, the sequences were amplified using the polymerase chain reaction (PCR) and primer pairs 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). Primers ITSCYA236F (5' – CTG GTT CRA GTC CAG GAT – 3') and ITSCYA225R (5' – TGC AGT TKT CAA GGT TCT– 3') were used to amplify the ITS region (Valerio et al. 2009). The PCR reactions were carried out in a 25 µl final volume consisting of 12.5 µl of MyTaq™ Red Mix (Meridian Bioscience, Cincinnati, OH, USA), 1 µl, each, of forward and reverse primers, 2 µL of DNA template, and 8.5 µl of milli–Q. A negative control was prepared using a reaction tube consisting of all the reaction ingredients excluding the DNA template. PCR reactions were performed in 200 µl reaction tubes using the BioRad T100 thermal cycler or the Major Science Major Cycler thermal cycler. The following thermal cycling parameters were used: 95 °C for 3 min; 94 °C for 1 min, 58 °C for 1 min (30 s at 55 °C for ITS), 72 °C for 2 min (30 s for ITS), repeated for 30 cycles; a final extension of 72 °C for 7 min and a storage step at 4 °C. Once the reaction was complete, 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.

The extracted DNA was also screened by PCR for the presence of genes coding for microcystin (MCY), nodularin

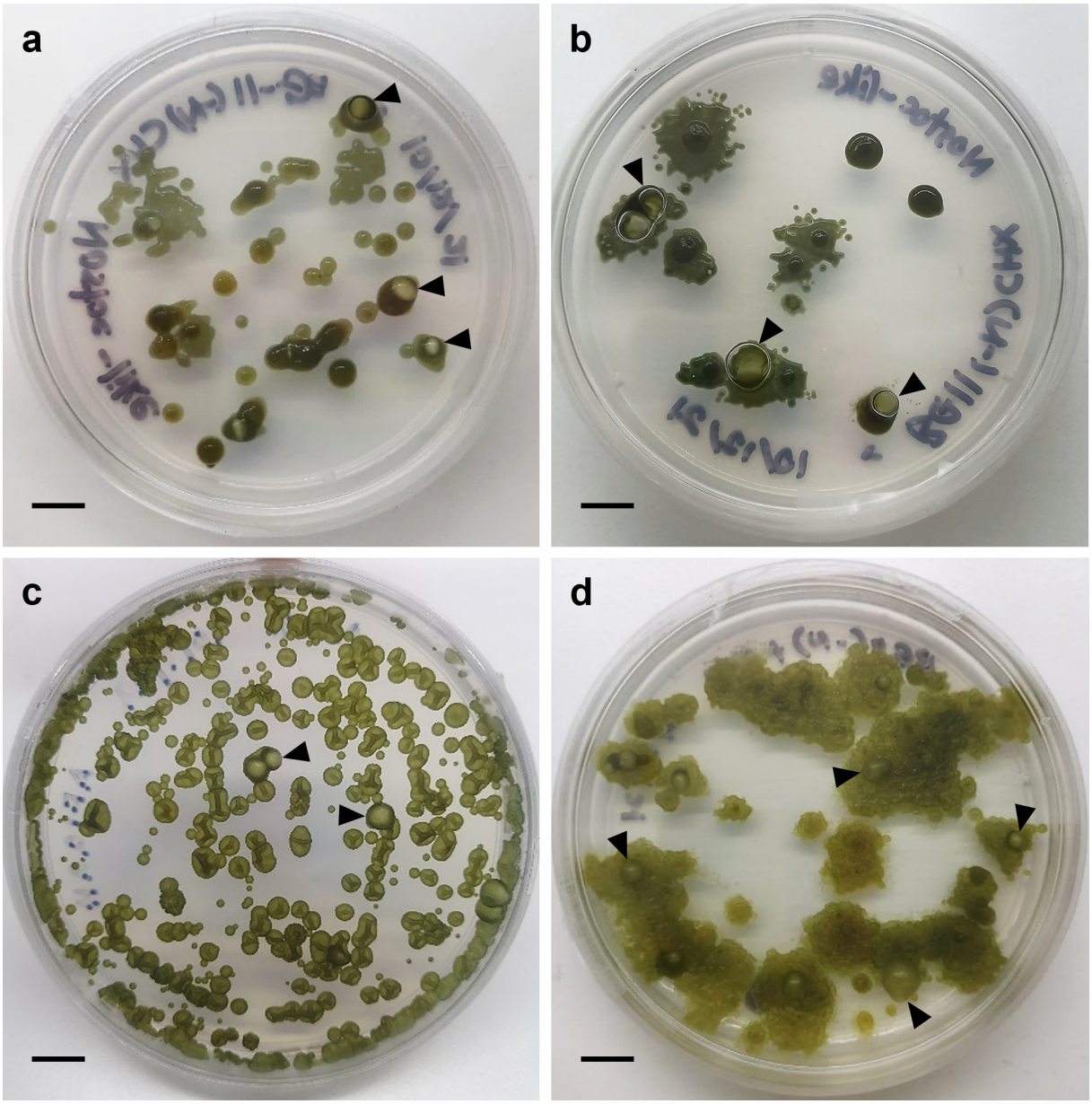

Fig. 1. Colony morphology of *Desmonostoc cockrellii* sp. nov. after 1–4 weeks incubation on nitrogen–free agarized medium, with visible gas bubble formations (arrowheads): (a) isolate PHNL1; (b) isolate PHNL2; (c) isolate PHNL3; (d) isolate PHNL4. Scale bars 1 cm.

(NOD), cylindrospermopsin (CYN), anatoxin (ATX), and saxitoxin (STX) production. Amplification of toxin genes was as follows: mcyE gene (MCY producers) using the primer set mcyE–F2 and mcyE–R4 (RANTALA et al. 2006); ndaF gene (NOD producers) using the primers HEPF/HEPR (JUNGBLUT & NEILAN 2006); the polyketide synthase (PKS) gene and sulfotransferase gene cyrJ (CYN producers) using the primers M4/M5 (SCHEMBRI et al. 2001) and cynsulfF/cylnamR (MIHALI et al. 2008), respectively; the anaF and anaC genes (ATX producers) using the primers atxoaf/atxar (BALLOT et al. 2010) and anxgen–F/anxgen–R (RANTALA–YLINEN et al. 2011), respectively; and the sxt gene (STX producers) using the primer pair sxtaf and sxtar (AL–TEBRINEH et al. 2010). DNA templates from strains which were previously confirmed to be microcystin (*Microcystis aeruginosa* FSS–164), nodularin (*Iningainema pulvinus* ES0614), cylindrospermopsin (*Raphidiopsis raciborski* FSS–127), anatoxin (*Anabaena* UHC0054) or saxitoxin (*Dolichospermum circinale* FSS–124) producers were used as positive controls.

Phylogenetic analysis. The chromatograms were examined, edited, and manually trimmed using the Geneious 11.0 software package (http://www.geneious.com, (KEARSE et al. 2012). Sequence alignments were made using the MUSCLE algorithm in the Geneious 11.0 software using sequences of our isolates, along with published sequences of *Nostoc sensu stricto* and closely related *Nostoc*–like genera retrieved from GenBank. The dataset for phylogenetic analyses consisted of 130 operational taxonomic units (OTUs) and included one outgroup taxon, *Chroococcidiopsis thermalis* PCC7203 (AB039005). Phylogenetic trees were constructed using Maximum Likelihood (ML) and Bayesian Inference (BI) methods. The best–fit model of DNA substitution was determined using the program Kakusan 4 prior to analysis (TANABE 2007). ML analysis was performed using W–IQ–TREE (http://iqtree.cibiv.univie.ac.at) (TRIFINOPOULOS et al. 2016). The analysis used the general time–reversible invariant–sites (GTRI) nucleotide substitution model with the default parameters. Bootstrap proportions were calculated (1000 replicates) to assess the robustness of each node. BI analyses were performed with the program MrBayes v3.2.6 (RONQUIST & HUELSENBECK 2003) using the GTR + I + G model. Two independent analyses, each consisting of four Markov chains, were run simultaneously for 1,500,000 generations, sampling every 200 generations. A burn in of 25% of saved trees was removed, and the remaining trees were used to calculate the Bayesian posterior probability values. ML and BI trees were edited using the program FigTree v1.4.4 (RAMBAUT 2018).

The uncorrected p–distance based on 16S rRNA gene and ITS sequences was assessed using MEGA version 11 (TAMURA et al. 2021). Percentage similarity for the 16S rRNA gene was calculated using the formula: % = 100 × (1–p). In addition, the secondary structures of Box–B and V2 helices found in the 16S–23S rRNA ITS region were predicted using RNAstructure version 6.0 (REUTER & MATHEWS 2010). Alignment of the ITS region was conducted using Geneious Prime software with operons containing tRNA genes.

Fatty acid composition analysis. Dried biomass collected during the stationary phase after 21 days in liquid medium was used for lipid extraction (modified from BLIGH & DYER 1959). A mixture of 1:2 (v/v) chloroform and methanol was added to the dry biomass and sonicated for 20 min. This was left in a fume hood to sediment overnight. Then, a mixture of 1:1 (v/v) distilled water and chloroform was added to give a final methanol: chloroform: water ratio of 1:1:0.5 (v/v) before sonicating for another 10 min. This was centrifuged (1200 ×g) for 2 min to generate a separate lipid phase. Samples of lipids were sent to MYCO2 Sdn Bhd, Penang, Malaysia, for lipid analysis following the method described by the American Oil Chemists Society AOAC 996.06 (HORWITZ & LATIMER 2005). Fatty acid composition was expressed as relative percent weight (% dwt.) of total FAMEs.

RESULTS

Morphological characteristics

The four isolates obtained from The Habitat Penang Hill shared similar growth morphologies. On agar plates, gas bubbles were frequently formed within colonies. Minute colonies grew scattered around larger colonies. These later developed into larger, initially hemispherical, then amorphous colonies, with smooth, lobate, or undulate margins, and convex, umbonate, or pulvinate elevations (Fig. 1). In fresh, nitrogen–free liquid medium, the cultures began as loose agglomerations, eventually forming several spherical masses up to 3 mm in diameter, occasionally accompanied by thin curled–up sheets. Patchy, irregular films were observed adhering to the bottom of the flasks.

Other common features among the isolates were flexuous or entangled filaments, olive–green to blue–green vegetative and specialized cells (akinetes and heterocytes) and the development of akinetes in long chains between heterocytes. Overall, the cell shapes were quite consistent among isolates, but variations in cell dimensions were evident (Table S1). The vegetative cells were wider in isolate PHNL2 and longer in PHNL1 and PHNL2 (but shorter than PHNL1). Intercalary heterocytes of PHNL1 were longer than those of other isolates and PHNL2 had larger terminal heterocytes (longer and wider). PHNL3 and PHNL4 shared almost identical vegetative cell dimensions. PHNL4 also had shorter but slightly wider akinetes than the other isolates.

Desmonostoc cockrellii B.C.G. Lim, Y.L. Choi et F. Merican sp. nov. (Figs. 1–3)

Description: Thallus macroscopic, olive–green or blue–green to yellowish–green. Gas–filled bubbles (3.4–10.0 mm in diameter) frequent within colonies of 1– to 4–week–old cultures (Fig. 1). Filaments 4.6–7.5 µm wide. Sheaths individual or communal, colourless, thin, firm or diffluent. Trichome olive–green to blue–green, uniseriate, isopolar, always constricted at cross–walls, flexuous, kinked, sometimes entangled or coiled, densely or loosely aggregated into macrocolonies, up to 300–celled, aggregated in communal sheath (Fig. 2a), later with sheath compartmentalization (Fig. 2b). Vegetative cells barrel–shaped, ± isodiametric, longer or shorter than wide, 3–7 µm × 3.5–6.6 µm. Apical cells blunt and round. Intercalary heterocytes spherical, ellipsoidal, 4.2–9.6 (10.3) µm × 4–7.1 µm, up to three in series.

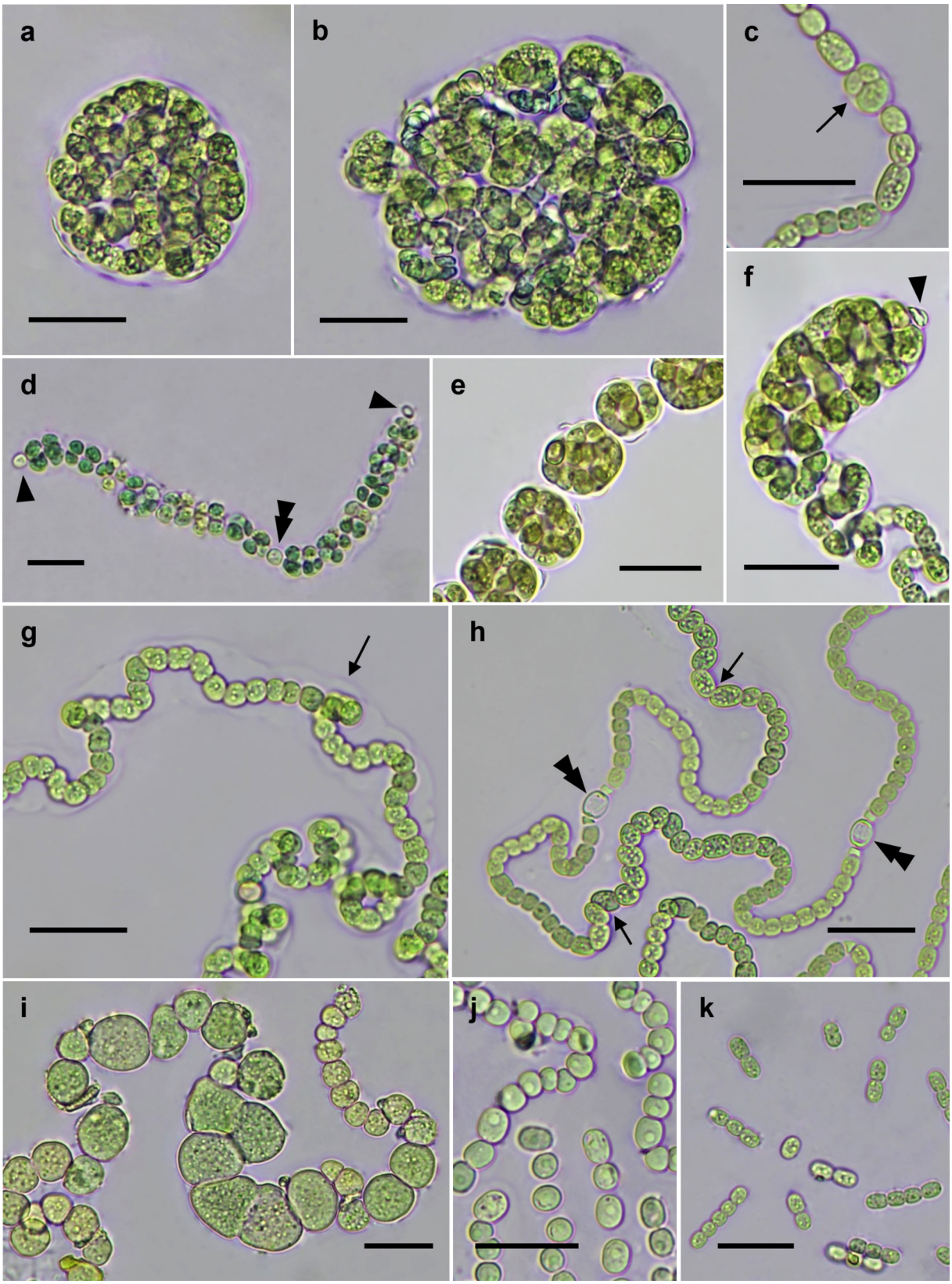

Fig. 2. Morphology of *Desmonostoc cockrellii* sp. nov.: (a) spherical microcolony of densely entangled filaments; (b) a large colony with compartmentalised sheath; (c–e) akinete germination (arrow) and its subsequent divisions (arrowheads and double arrowhead pointing to terminal and intercalary heterocyte, respectively); (f) the release of a trichome from its colony (arrowhead pointing to terminal heterocyte); (g) kinked trichome with diffluent sheath (arrow); (h) apoheterocytic akinete development with acute bending of trichome by akinete pairs as indicated by arrows (double arrowheads pointing to intercalary heterocytes); (i) distinct, large akinetes in one year–old cultures; (j) akinetes with central or peripheral granule; (k) motile hormogonia. Scale bars 20 μm.

Terminal heterocyte spherical, oval, cap–shaped in small colonies, 3.7–7.6 (8.1) µm × 3.3–7.1 µm. Germination of akinete into two vegetative cells (Fig. 2c), growing into cylindrical, elongated grape–like clusters (Fig. 2d), subsequently forming rounded ensheathed colonies with densely packed trichomes (Fig. 2e), each developing a larger colony with a densely entangled trichome (Fig. 2f), later untangling into kinked trichome within diffluent sheath (Fig. 2g). Akinetes arise apoheterocytically in series of 10 to 40 cells, often with one to two pairs of akinetes causing conspicuous or subtle acute bending of the trichome (Fig. 2h), eventually cells in the entire trichome successively differentiate into akinetes. In cultures of less than 3 months, akinetes olive–green to blue–green, ellipsoidal, rarely spherical, sometimes elongated, heavily granulated, smooth, wall colourless, slightly or distinctively larger than vegetative cells, (3) 5–9 (10) µm × 4.5–7.7 µm; akinetes large, spherical, or irregular–shaped in 3– to 6–month–old cultures (Fig. 2i), 7–23 µm × 6.5–19 µm when in chains, 6–12 µm in diameter when solitary, sometimes with a small– to medium–sized granule located peripherally or centrally (Fig. 2j). Reproduction by akinetes as described above, or by fragmentation of trichomes at necridic cells to form hormogonia. Hormogonia short, of 2–10 cells (Fig. 2k), usually with creeping motion, tapering of the apex rarely observed. Thylakoids with formations of hemispherical and spherical loops resembling fascicles, in an undulating arrangement (Fig. 3a, b), or irregularly distributed (Fig. 3c, d).

Holotype: PHNL1 (dried cultured material, preserved

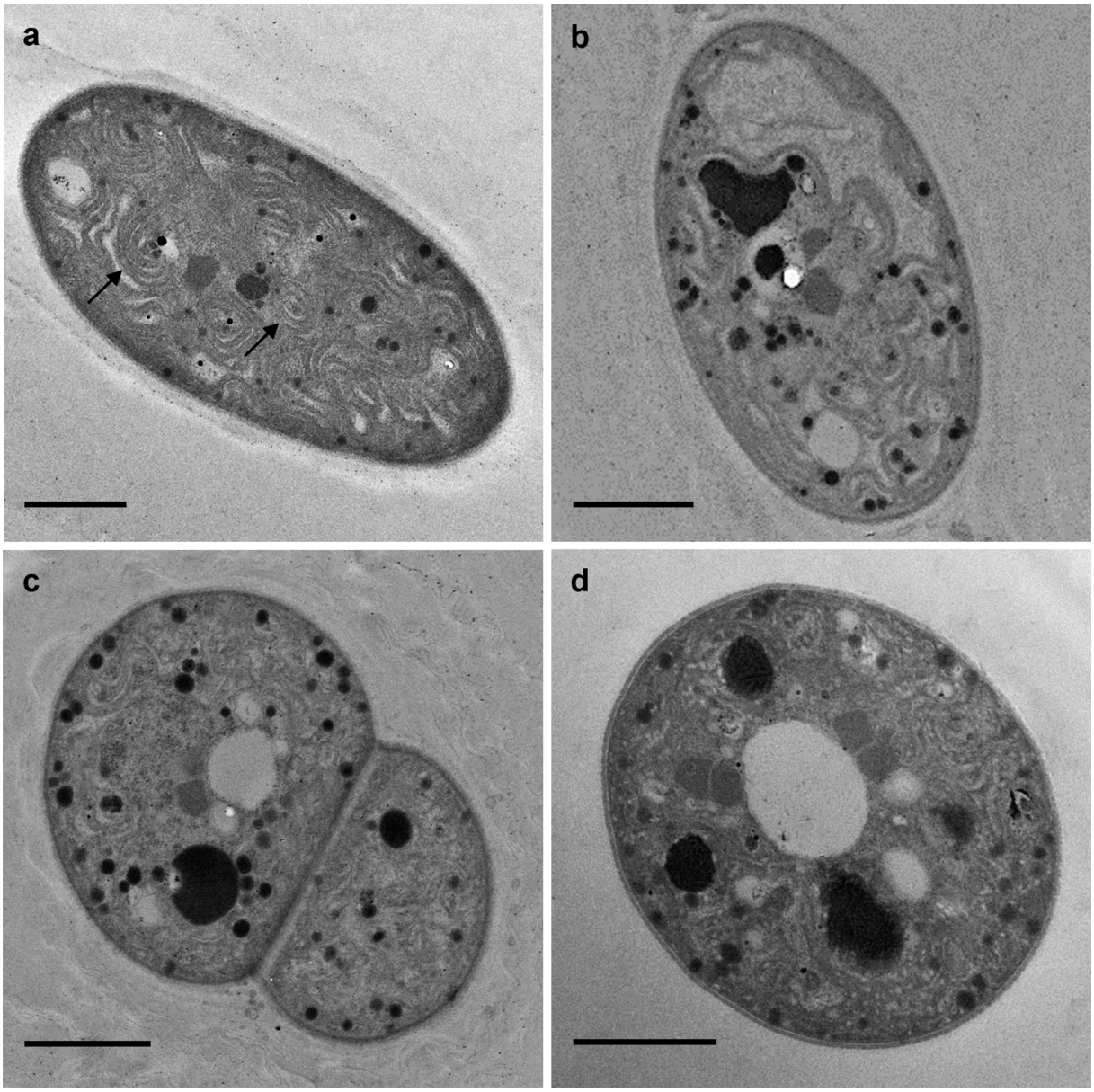

Fig. 3. Ultrastructure of *Desmonostoc cockrellii* sp. nov.: (a, b) undulating, coiling, wavy fascicles of thylakoid membranes in vegetative cells; (c, d) akinetes with thylakoid membranes irregularly distributed throughout the cell volume. Scale bars 1 µm.

in a metabolically inactive state), collected on 20 April 2020 by F. Merican, is deposited in the herbarium of the School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia. Herbarium number: USMP 12364.
Type locality: 05°25'32.715"N, 100°16'7.34"E. Isolated from algal assemblages collected from boulders along the trails in The Habitat Penang Hill, UNESCO Biosphere Reserve, Penang, Malaysia.
Habitat: Subaerophytic and terrestrial.
Etymology: The species is named after Reza Cockrell, co–founder and Director of The Habitat Foundation.
Reference strain: PHNL1 culture maintained in Universiti Sains Malaysia, Penang, Malaysia.
Materials analyzed: isolates PHNL1, PHNL2, PHNL3 and PHNL4.
GenBank accession numbers: PQ466560–PQ466563 (16S rRNA) and PQ466568–PQ466571 (ITS).

Diagnosis: This species is morphologically distinguishable from other *Desmonostoc* species due to its formation of gas bubbles within colonies, larger size of akinetes and the acute bending of its trichome caused by akinete pairs during apoheterocytic development. Phylogenetic analysis using the 16S rRNA gene revealed that this species formed a distinct cluster in the clade of *Desmonostoc* sensu stricto, distant from other *Desmonostoc* taxa. The significant differences in length and structure of the secondary structures of the ITS region also supported the establishment of this new species.

Phylogenetic relationships with other strains
The phylogenetic trees inferred from partial 16S rRNA gene sequences using ML and BI approaches yielded similar topologies, and only the ML tree is presented (Fig. 4). Isolates PHNL1, PHNL2, PHNL3 and PHNL4 were placed within the *Desmonostoc* sensu stricto clade (Fig. 4). They were genetically identical and formed an independent lineage within the clade, with 100% bootstrap support and posterior probability of 1.00 (Fig. 4). The percentage similarity among the 16S rRNA gene sequences was also analysed (Table S2). The p–distance based on 16S rRNA gene sequences showed that the four isolates shared 100% similarity with each other and 96.7–98.6% similarity with other *Desmonostoc* species (excluding *D. vinosum*) in the phylogenetic tree.

The domains Box–B and V2 were identified from the 16S–23S rRNA ITS region and their secondary structures were also predicted. The alignment of the ITS sequences showed that the lengths of Box–B and V2 helices were conserved among the four isolates but varied considerably from those of other *Desmonostoc* species. Overall, the Box–B helices varied considerably in length, ranging from 28 to 54 nt (Table S3). The Box–B helix of our isolates (30 nt long) presented a basal clamp consisting of a 5–bp helix (5' –CAGCA–UGCUG– 3'), followed by a 3:3 base basal bilateral bulge, a 5–bp stem (5' –UGACA–UGUCA– 3') and, finally, a terminal loop containing four bases (GUGG) (Fig. 5). Although similar to that of *D. caucasicum* MZ–C154, close inspection revealed that the Box–B helix of *D. caucasicum* MZ–C154 was 28 nt long, in addition to having a 4–bp stem following the basal bilateral bulge, and a terminal loop with slightly different base sequence (GAGA). The V2 helices of the *Desmonostoc* species included in this analysis showed dissimilarities in terms of both secondary structure (Fig. 6) and length, ranging from 23 to 81 nt (Table S3). The V2 helix of our isolates comprised a 10–bp helix basal clamp, followed by a 2:2 base basal bilateral bulge and then by a 2:3 base bilateral bulge, the latter connected by a 11–bp stem with a side loop with a single adenine base on the 5′ side, followed by a 3:3 base subterminal bilateral bulge and a terminal loop containing four bases (AUAA). The data from this polyphasic analysis also support the erection of a new *Desmonostoc* species.

Molecular screening of toxin–encoding genes
Genes encoding nodularin, microcystin, saxitoxin, anatoxin or cylindrospermopsin were not detected.

Fatty acid profiling
D. cockrellii sp. nov. produced 14 different fatty acids, ranging in length from 4 to 22 carbon atoms. However, there were differences amongst the four isolates based on their presence, absence and percentage composition (Table S4). SFAs (53.7–77.5% dwt.) predominated over MUFAs (8.6–22% dwt.) and PUFAs (13.9–24.3% dwt.) in all four isolates. The most abundant fatty acids were 4:0 butyric acid (6.7–22.2% dwt.), 16:0 palmitic acid (11.3–32.1% dwt.), 18:1n9c oleic acid (8.6–22.0% dwt.), 18:3n6 γ–linolenic acid (2.3–18.5% dwt.) and 22:6n3 *cis*–4,7,10,13,16,19–docosahexanoic acid (4.5–13.1% dwt.). The saturated fatty acids 8:0 caprylic acid, 11:0 undecanoic acid, 14:0 myristic acid, 18:0 stearic acid, 20:0 arachidic acid, 22:0 behenic acid, 23:0 tricosanoic acid, and 24:0 lignoceric acid, together with the monounsaturated fatty acids 14:1 myristoleic acid, 15:1 *cis*–10–pentadecanoic acid, 16:1 palmitoleic acid, and 24:1 nervonic acid were not detected in any of the isolates.

Discussion

The 16S rRNA gene phylogenetic tree placed the isolates within the *Desmonostoc* sensu stricto clade as a distinct and strongly supported cluster (Fig. 4). As recommended by Mishra et al. (2021), we integrated representative sequences of true *Nostoc* members and all closely related *Nostoc*–like genera currently known in order to avoid premature conclusions of classification. *Desmonostoc cockrellii* sp. nov. is clearly distinct from members of the genus *Nostoc* and is phylogenetically distant from all other *Nostoc*–like genera. It also showed 16S rRNA gene sequence similarity (96.7–98.6%) with other *Desmonostoc* species below the accepted threshold

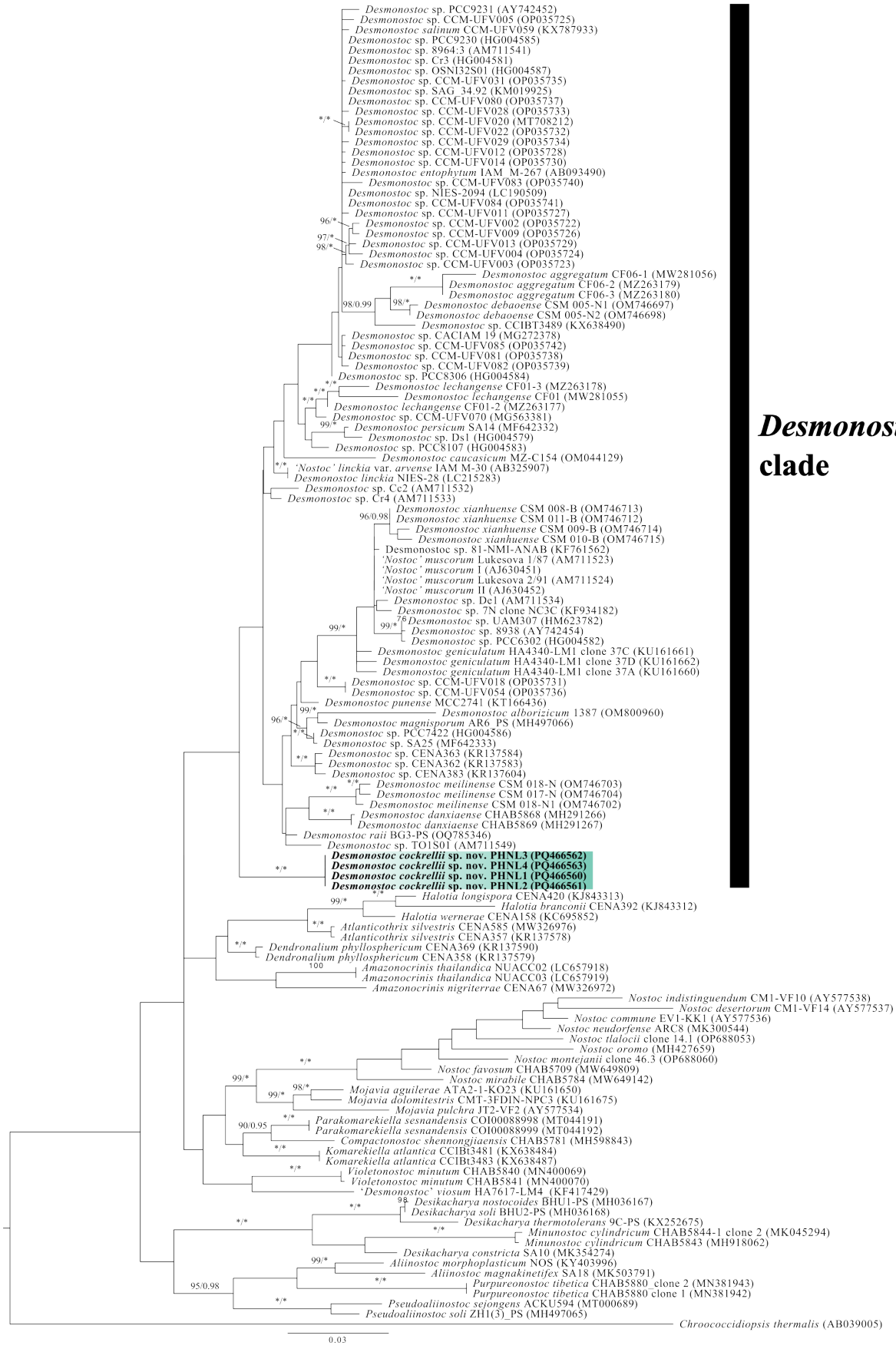

Fig. 4. Maximum likelihood phylogenetic tree based on partial 16S rRNA gene sequences (1536 base pairs). Maximum likelihood bootstrap support values (≥90%) (left) and Bayesian inference posterior probabilities (≥0.90) (right) are shown on the branches. Asterisks indicate 100% bootstrap support and/or 1.00 posterior probabilities.

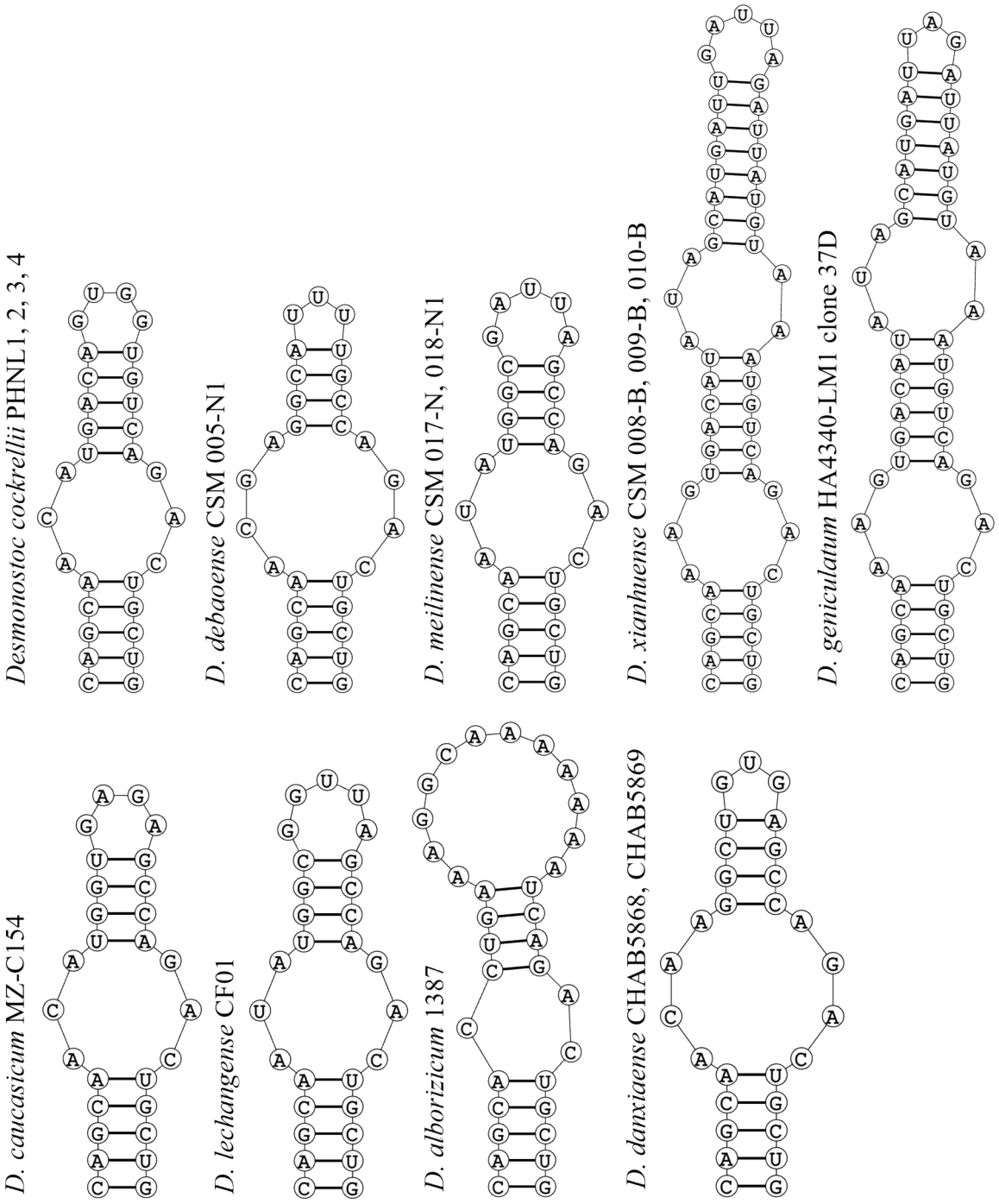

Fig. 5. Secondary structures of Box–B helices of different *Desmonostoc* species.

value of 98.7–99.0% used for discriminating between species of cyanobacteria (Stackebrandt & Jonas 2006; Yarza et al. 2014). Our phylogenetic analyses, therefore, support the erection of a new species of *Desmonostoc*. The 16S–23S rRNA ITS secondary structure is an effective and reliable genetic marker used in cyanobacterial taxonomy to differentiate species within a genus (Iteman et al. 2000; Boyer et al. 2001; Maltseva et al. 2022). The erection of *D. cockrellii* sp. nov. is further supported by the percent dissimilarity values of the ITS sequence alignment (Table S5) and distinct folding patterns of the Box–B and V2 helices. Generally, strains with <4% dissimilarity (p–distance <0.04) are considered to represent the same species, while dissimilarity of >7% (p–distance >0.07) supports separation of cryptic species within a genus (Bohunická et al. 2015; Pecundo et al. 2021). Strikingly, the isolates studied here presented a range of 12.6–41.8% dissimilarity from those *Desmonostoc* species with ITS sequences available from GenBank (Table S5).

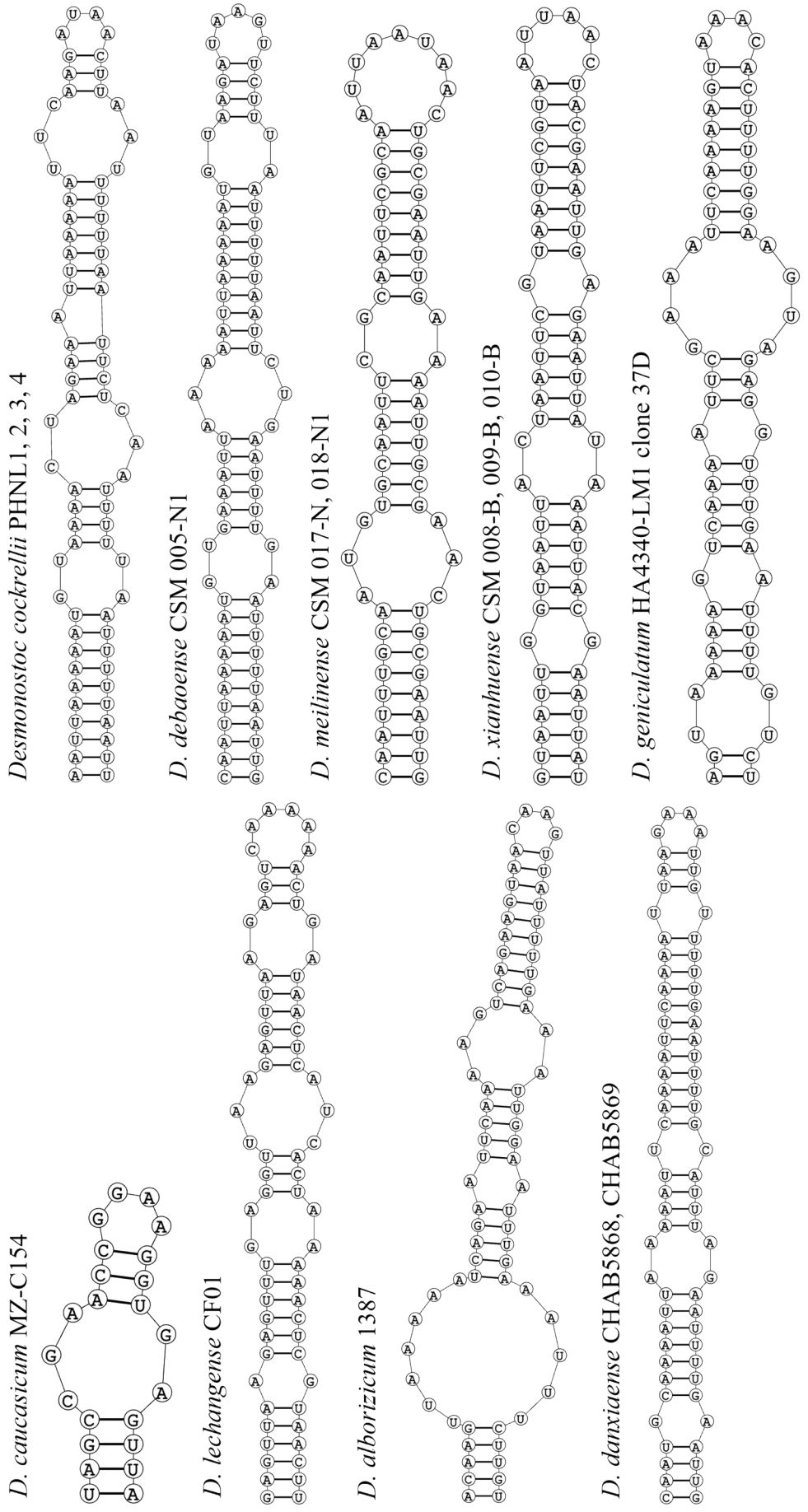

Fig. 6. Secondary structures of V2 helices of different *Desmonostoc* species.

Morphologically, the four isolates had typical *Desmonostoc* characteristics of agglomeration of filaments forming macrocolonies, diffluent mucilage enveloping long chains of akinetes and long filaments with barrel–shaped and mostly isodiametric vegetative cells. The production of akinetes up to three times larger than those of other *Desmonostoc* species was a distinctive feature of *D. cockrellii* sp. nov. (Table S6). The acute bending of its trichome (Fig. 2h) also resembled the morphology of that among the hormogonia of *Mojavia pulchra* Řeháková et J.R. Johansen (Řeháková et al. 2007). The distinction between these two species was that this feature was brought about by pairs of akinetes during the apoheterocytic development of *D. cockrellii* sp. nov., whereas widening of intercalary cells caused the acute bending of the hormogonia of the *M. pulchra*. A prominent feature of *D. cockrellii* sp. nov. was the formation of gas bubbles within colonies on agarized medium, a trait shared by *Nostoc indistinguendum* CM1–VF10 Řeháková et J.R. Johansen (Řeháková et al. 2007). Bubbles formed within a week in *D. cockrellii* sp. nov. whilst those in *N. indistinguendum* took three months. Although both are subaerophytic, *D. cockrellii* sp. nov. differs ecologically from *N. indistinguendum* as the latter was described from the sandy soil of Mojave desert (Řeháková et al. 2007). Colony morphology of *D. cockrellii* sp. nov. is unknown in the field as they were not directly observed during sample collection. While morphological comparisons of natural populations and laboratory–grown cultures should ideally form part of the species identification process (Hentschke & Sant'Anna 2015; Singh et al. 2016), rare representatives of an algal consortia are often masked by other dominant groups.

The fatty acid profiles varied across the four isolates of *D. cockrellii* sp. nov., while clearly differing from those of other heterocytous cyanobacteria (Shukla et al. 2012; Anahas & Muralitharan 2015, 2018; Alvarenga et al. 2018; Maltseva et al. 2022). They exhibited high levels of SFAs (53.7–77.5%) and low levels of PUFAs (13.9–24.3%), similar to those of *D. muscorum* MBDU105 (Table S4). The presence of 16:0 palmitic acid in the new species is consistent with heterocytous strains isolated from saline water (Alvarenga et al. 2018), soil (Maltseva et al. 2022), freshwater habitats and plant hosts (Shukla et al. 2012; Anahas & Muralitharan 2015, 2018). Although the palmitic acid content varied between isolates (11.3–24.8% dwt.), it was still within the range of that in most of the reported strains (13.0–38.5% dwt.) (Anahas & Muralitharan 2015, 2018; Alvarenga et al. 2018; Maltseva et al. 2022), with the exception of those reported by Shukla et al. (2012), i.e. *Anabaena* spp., *Nostoc* spp. (<9% dwt.). This may indicate the influence of cultivation conditions and nutrient availability, as previously reported for fatty acid composition in cyanobacteria (Yadav et al. 2021). The greater 4:0 butyric acid content in isolates PHNL3 (20.7% dwt.) and PHNL4 (22.2% dwt.) was considerably higher than that of *D. muscorum* MBDU105 (0.75% dwt.) and other closely related heterocytous cyanobacteria (<6.75% dwt.) (Anahas & Muralitharan 2015, 2018). The enrichment of 13:0 tridecanoic acid in isolate PHNL1 (28.2% dwt.) was notable compared to that of our other isolates (0–7.7% dwt.) and those of *D. muscorum* MBDU105 (0.57% dwt.) (Anahas & Muralitharan 2018) and *Anabaena variabilis* (4.08–5.64% dwt.) (El Shafay et al. 2021). The quantitative differences of fatty acid content in different strains within the same species has been addressed in earlier reports (Mühling et al. 2005; Stamenković et al. 2020; Maltsev & Maltseva 2021) and posited to derive from genetic drift and mutations (Mühling et al. 2005). Furthermore, the biochemical profile of microalgae can be influenced by the environmental conditions of their natural habitats, as demonstrated by the build–up of photoprotective compounds such as hexadecatrienoic acid and α–linolenic acid in desmid strains inhabiting shallow freshwater (Stamenković et al. 2020). Thus, the genetic foundation of the fatty acid composition may potentially be impacted by the varying degrees of shading and exposure to different light intensities along the trails from which the new species was sampled (Mühling et al. 2005; Stamenković et al. 2020).

The presence or absence of specific fatty acids have been demonstrated to provide useful chemotaxonomic markers for classifying cyanobacterial strains at the genus or species level (Gugger et al. 2002; Mühling et al. 2005; Shukla et al. 2012). Gugger et al. (2002) demonstrated that several fatty acids played a significant role in generic differentiation, such as the presence of large amounts of 16:0 palmitic acid in *Calothrix marchica* PCC7714 and the presence of 18:0 iso–stearic acid and 18:3ω6,9,12c linolenic acid in *Microcystis* strains. The presence of 16:1ω5c and 16:1ω7c palmitoleic acids, and the absence of 16:1ω9c palmitoleic acid and 16:0 anteisohexadecanoic acid, in *Nostoc* sp. 152 allowed the discrimination of this strain from other Nostocales strains belonging to the genera *Aphanizomenon*, *Anabaena*, *Cylindrospermum* and *Calothrix* (Gugger et al. 2002). The presence of 14:0 myristic acid in hepatotoxic strains of *Anabaena* and *Nostoc* was also considered as a taxonomic biomarker, separating them from non–toxic strains without that particular fatty acid (Gugger et al. 2002). In addition, the lack of ɣ–linolenic acid in *Spirulina* strains allowed Mühling et al. (2005) to distinguish them from *Arthrospira* strains containing ɣ–linolenic acid. Some fatty acids like 14:0 myristic acid, 18:0 stearic acid and 16:1 palmitoleic acid were not found in *D. cockrellii* sp. nov. despite being commonly reported for *D. muscorum* MBDU105 (Anahas & Muralitharan 2018) and strains of *Calothrix* (Anahas & Muralitharan 2015), *Nostoc* and *Anabaena* (Gugger et al. 2002; Shukla et al. 2012; Anahas & Muralitharan 2015, 2018). Other fatty acids, namely 12:0 lauric acid, 15:0 pentadecanoic acid, 17:1 cis–10–heptadecanoic acid and 18:2n6c linoleic acid, were detected only in small amounts in some isolates, as also noted in *D. muscorum* MBDU105 and other heterocytous cyanobacterial strains

(*Anabaena* spp., *Nostoc* spp., *Dolichospermum spiroides* MBDU 607 and *Tolypothrix tenuis* MBDU 609) (Anahas & Muralitharan 2018). The content of 20:3n6 cis–8,11,14–eicosatrienoic acid in isolate PHNL2 was 11% dwt., in contrast to other heterocytous strains which contained negligible quantities (<2.41% dwt.) (Anahas & Muralitharan 2018). From the data obtained in the current study, we suggest that 14:0 myristic acid, 18:0 stearic acid, 16:1 palmitoleic acid, 18:2n6c linoleic acid and 20:3n6 cis–8,11,14–eicosatrienoic acid could offer potential as chemotaxonomic markers, but it would be helpful to explore this potential further using a broader diversity of isolates in future polyphasic taxonomic studies, because only a limited number of *Desmonostoc* and other *Nostoc*–like specimens has been subjected to fatty acid analyses (Alvarenga et al. 2018; Anahas & Muralitharan 2018; Maltseva et al. 2022).

Limited information is available on the diversity of nostocacean cyanobacteria in Malaysia's subaerophytic environments. The discovery of *Desmonostoc cockrellii* sp. nov. represents the first novel *Desmonostoc* species described from The Habitat Penang Hill, and expands knowledge of the tropical biogeography of this genus. We suggest that future targeted studies and the application of polyphasic taxonomy will increase knowledge of the diversity of this group.

Acknowledgements

We gratefully acknowledge the support of The Habitat Penang Hill for funding and facilitating the tropical sample collection during the BioBlitz event. We also thank Prof. Wan Maznah Wan Omar, Dr. Sébastien Lavoué and Dr. Farah Alia Nordin for sharing the culturing, PCR and herbarium facilities at the School of Biological Sciences, USM.

Disclosure statement

No potential conflicts of interest are reported by the authors.

Funding

Faradina Merican is supported by The Habitat Foundation research grant. Peter Convey is supported by NERC core funding to the BAS 'Biodiversity, Evolution and Adaptation' Team.

References

Al–Tebrineh, J.; Mihali, T.K.; Pomati, F. & Neilan, B.A. (2010): Detection of saxitoxin–producing cyanobacteria and *Anabaena circinalis* in environmental water blooms by quantitative PCR. – Applied and Environmental Microbiology 76: 7836–7842. DOI: 10.1128%2FAEM.00174–10.

Almeida, A.V.M.; Vaz, M.G.M.V.; Castro, N.V.; Genuário, D.B.; Oder, J.C.; Souza, P.A.M.; Martins, S.B.; Machado, M.; Nunes–Nesi, A. & Araújo, W.L. (2023): How diverse a genus can be: An integrated multi–layered analysis into *Desmonostoc* (Nostocaceae, Cyanobacteriota). – Systematic and Applied Microbiology 46: Article 126422. DOI: 10.1016/j.syapm.2023.126422.

Alvarenga, L.V.; Vaz, M.G.M.V.; Genuário, D.B.; Esteves–Ferreira, A.A.; Almeida, A.V.M.; Castro, N.V.; Lizieri, C.; Souza, J.J.L.; Schaefer, C.E.G. & Nunes–Nesi, A. (2018): Extending the ecological distribution of *Desmonostoc* genus: proposal of *Desmonostoc salinum* sp. nov., a novel cyanobacteria from a saline–alkaline lake. – International Journal of Systematic and Evolutionary Microbiology 68: 2770–2782. DOI: 10.1099/ijsem.0.002878.

Anahas, A.M.P. & Muralitharan, G. (2015): Isolation and screening of heterocystous cyanobacterial strains for biodiesel production by evaluating the fuel properties from fatty acid methyl ester (FAME) profiles. – Bioresource Technology 184: 9–17. DOI: 10.1016/j.biortech.2014.11.003.

Anahas, A.M.P. & Muralitharan, G. (2018): Characterization of heterocystous cyanobacterial strains for biodiesel production based on fatty acid content analysis and hydrocarbon production. – Energy Conversion and Management 157: 423–437. DOI: 10.1016/j.enconman.2017.12.012.

Ballot, A.; Fastner, J.; Lentz, M. & Wiedner, C. (2010): First report of anatoxin–*a*–producing cyanobacterium *Aphanizomenon issatschenkoi* in northeastern Germany. – Toxicon 56: 964–971. DOI: 10.1016/j.toxicon.2010.06.021.

Bligh, E.G. & Dyer, W.J. (1959): A rapid method of total lipid extraction and purification. – Canadian Journal of Biochemistry and Physiology 37: 911–917. DOI: 10.1139/o59–099.

Bohunická, M.; Pietrasiak, N.; Johansen, J.R.; Gómez, E.B.; Hauer, T.; Gaysina, L.A. & Lukešová, A. (2015): *Roholtiella*, gen. nov. (Nostocales, Cyanobacteria)—a tapering and branching cyanobacteria of the family Nostocaceae. – Phytotaxa 197: 84–103. DOI: 10.11646/phytotaxa.197.2.2.

Bolch, C.J.S. & Blackburn, S.I. (1996): Isolation and purification of Australian isolates of the toxic cyanobacterium *Microcystis aeruginosa* Kutz. – Journal of Applied Phycology 8: 5–13. DOI: 10.1007/BF02186215.t.

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. – Molecular Biology and Evolution 18: 1057–1069. DOI: 10.1093/oxfordjournals.molbev.a003877.

Cai, F.; Yang, Y.; Wen, Q. & Li, R. (2018): *Desmonostoc danxiaense* sp. nov. (Nostocales, Cyanobacteria) from Danxia mountain in China based on polyphasic approach. – Phytotaxa 367: 233–244. DOI: 10.11646/phytotaxa.367.3.3.

El Shafay, S.M.; Gaber, A.; Alsanie, W.F. & Elshobary, M.E. (2021): Influence of nutrient manipulation on grodwth and biochemical constituent in *Anabaena variabilis* and *Nostoc muscorum* to enhance biodiesel production. – Sustainability 13: 9081. DOI: 10.3390/su13169081.

Gugger, M.; Lyra, C.; Suominen, I.; Tsitko, I.; Humbert, J.; Salkinoja–Salonen, M.S. & Sivonen, K. (2002): Cellular fatty acids as chemotaxonomic markers of the genera *Anabaena*, *Aphanizomenon*, *Microcystis*, *Nostoc* and *Planktothrix* (cyanobacteria). – International Journal of Systematic and Evolutionary Microbiology 52: 1007–1015. DOI: 10.1099/ijs.0.01917–0.

Hentschke, G. S. & Sant'Anna, C. L. (2015): Current trends and prospects for cyanobacterial taxonomy – are only cultured populations enough? – Algological Studies 147: 3–6. DOI: 10.1127/algol_stud/2014/0185.

Horwitz, W. & Latimer, G.W. (2005): Official methods of analysis of AOAC International 15th edition. – 771 pp., Gaithersburg, USA.

Hrouzek, P.; Lukešová, A.; Mareš, J. & Ventura, S. (2013): Description of the cyanobacterial genus *Desmonostoc* gen. nov. including *D. muscorum* comb. nov. as a distinct, phylogenetically coherent taxon related to the genus *Nostoc*. – Fottea 13: 201–213. DOI: 10.5507/fot.2013.016.

Iteman, I.; Rippka, R.; Tandeau de Marsac, N. & Herdman, M. (2000): Comparison of conserved structural and

regulatory domains within divergent 16S rRNA–23S rRNA spacer sequences of cyanobacteria. – Microbiology 146: 1275–1286. DOI: 10.1099/00221287–146–6–1275.

Jungblut, A.D. & Neilan, B.A. (2006): Molecular identification and evolution of the cyclic peptide hepatotoxins, microcystin and nodularin, synthetase genes in three orders of cyanobacteria. – Archives of Microbiology 185: 107–114. DOI: 10.1007/s00203–005–0073–5.

Kabirnataj, S.; Nematzadeh, G.A.; Talebi, A.F.; Saraf, A.; Suradkar, A.; Tabatabaei, M. & Singh, P. (2020): Description of novel species of *Aliinostoc*, *Desikacharya* and *Desmonostoc* using a polyphasic approach. – International Journal of Systematic and Evolutionary Microbiology 70: 3413–3426. DOI: 10.1099/ijsem.0.004188.

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. DOI: 10.1093%2Fbioinformatics%2Fbts199.

Komárek, J. (2013): Cyanoprokaryota. 3. Teil/3rd part: Heterocytous Genera. – In: Büdel, B.; Gärtner, G.; Krienitz, L. & Schagerl, M. (eds): Süßwasserflora von Mitteleuropa, Bd. 19/3. – 1131 pp., Springer Spektrum Berlin, Heidelberg.

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.

Li, R. & Watanabe, M.M. (2004): Fatty acid composition of planktonic species of *Anabaena* (Cyanobacteria) with coiled trichomes exhibited a significant taxonomic value. – Current Microbiology 49: 376–380. DOI: 10.1007/s00284–004–4380–x.

Maltsev, Y. & Maltseva, K. (2021): Fatty acids of microalgae: diversity and applications. – Reviews in Environmental Science and Bio/Technology 20: 515–547. DOI: 10.1007/s11157–021–09571–3.

Maltsev, Y.; Maltseva, K.; Kulikovskiy, M. & Maltseva, S. (2021): Influence of light conditions on microalgae grodwth and content of lipids, carotenoids, and fatty acid composition. – Biology 10: 1060. DOI: 10.3390/biology10101060.

Maltseva, S.; Bachura, Y.; Erst, T.; Kulikovskiy, M. & Maltsev, Y. (2022): Description of *Desmonostoc caucasicum* sp. nov. (Cyanobacteria) using an integrative taxonomic approach. – Phycologia 61: 514–527. DOI: 10.1080/00318884.2022.2086428.

Maltseva, S.; Kezlya, E. & Krivova, Z. (2022): Phylogeny and fatty acid profiles of *Aliinostoc vietnamicum* sp. nov. (Cyanobacteria) from the soils of Vietnam. – Journal of Phycology 58: 789–803. DOI: 10.1111/jpy.13283.

McDowell, E.M. & Trump, B.F. (1976): Histologic fixatives suitable for diagnostic light and electron microscopy. – Archives of Pathology & Laboratory Medicine 100: 405–414.

Merican, F.; Senee, G.; Ismail, D.E. & Convey, P. (2021): First records of subaerial cyanobacteria and chlorophytes from Penang Hill. – In: Ruppert, N.; Lowman, M. & Nor, S.A.M. (eds): The Biodiversity of Penang Hill: Findings of the ‚Penang Hill BioBlitz 2017'. – pp. 76–91. The Habitat Foundation, Penang.

Mihali, T.K.; Kellmann, R.; Muenchhoff, J.; Barrow, K.D. & Neilan, B.A. (2008): Characterization of the gene cluster responsible for cylindrospermopsin biosynthesis. – Applied and Environmental Microbiology 74: 716–722. DOI: 10.1128/aem.01988–07.

Miscoe, L.H.; Johansen, J.R.; Kociolek, J.P.; Lowe, R.L.; Vaccarino, M.A.; Pietrasiak, N. & Sherwood, A.R. (2016): The diatom flora and cyanobacteria from caves on Kauai, Hawaii. II. Novel cyanobacteria from caves on Kauai, Hawaii. – In: Kies, L. & Schnetter, R. (eds): Bibliotheca Phycologica 123. – pp. 75–152, J. Cramer in Borntraeger Science Publishers, Stuttgart.

Mishra, D.; Saraf, A.; Kumar, N.; Pal, S. & Singh, P. (2021): Issues in cyanobacterial taxonomy: comprehensive case study of unbranched, false branched and true branched heterocytous cyanobacteria. – FEMS Microbiology Letters 368: Article fnab005. DOI: 10.1093/femsle/fnab005.

Mühling, M.; Belay, A. & Whitton, B.A. (2005): Variation in fatty acid composition of *Arthrospira* (*Spirulina*) strains. – Journal of Applied Phycology 17: 137–146. DOI: 10.1007/s10811–005–7213–9.

Neustupa, J. & Škaloud, P. (2010): Diversity of subaerial algae and cyanobacteria growing on bark and wood in the lowland tropical forests of Singapore. – Plant Ecology and Evolution 143: 51–62. DOI: 10.5091/plecevo.2010.417.

Nowruzi, B.; Becerra–Absalón, I. & Metcalf, J.S. (2023): A novel microcystin–producing cyanobacterial species from the genus *Desmonostoc*, *Desmonostoc alborizicum* sp. nov., isolated from a water supply system of Iran. – Current Microbiology 80: Article 49. DOI: 10.1007/s00284–022–03144–5.

Pecundo, M.H.; Cai, F.; Chang, A.C.G.; Ren, H.; Li, N.; Li, R. & Chen, T. (2021): Polyphasic approach identifies two new species of *Desmonostoc* (Nostocales, Cyanobacteria) in the coralloid roots of *Cycas fairylakea* (Cycadales). – Phycologia 60: 653–668. DOI: 10.1080/00318884.2021.1987697.

Pecundo, M.H.; Chen, T.; dela Cruz, T.E.E.; Griffith, M.P.; Hu, Z.; Chen, H. & Li, N. (2023): Discovery of Cyanobacteria associated with cycads and description of three novel species in *Desmonostoc* (Nostocaceae). – Diversity 15: 1132. DOI: 10.3390/d15111132.

Rambaut, A. (2018): FigTree v1.4.4. http://tree.bio.ed.ac.uk/software/figtree/.

Rantala–Ylinen, A.; Kana, S.; Wang, H.; Rouhiainen, L.; Wahlsten, M.; Rizzi, E.; Berg, K.; Gugger, M. & Sivonen, K. (2011): Anatoxin–*a* synthetase gene cluster of the cyanobacterium *Anabaena* sp. strain 37 and molecular methods to detect potential producers. – Applied and Environmental Microbiology 77: 7271–7278. DOI: 10.1128%2FAEM.06022–11.

Rantala, A.; Rajaniemi–Wacklin, P.; Lyra, C.; Lepisto, L.; Rintala, J.; Mankiewicz–Boczek, J. & Sivonen, K. (2006): Detection of microcystin–producing cyanobacteria in Finnish lakes with genus–specific microcystin synthetase gene E (*mcyE*) PCR and associations with environmental factors. – Applied and Environmental Microbiology 72: 6101–6110. DOI: 10.1128/aem.01058–06.

Řeháková, K.; Johansen, J.R.; Casamatta, D.A.; Xuesong, L. & Vincent, J. (2007): Morphological and molecular characterization of selected desert soil cyanobacteria: three species new to science including *Mojavia pulchra* gen. et sp. nov. – Phycologia 46: 481–502. DOI: 10.2216/06–92.1.

Reuter, J.S. & Mathews, D.H. (2010): RNAstructure: software for RNA secondary structure prediction and analysis. – BMC Bioinformatics 11: Article 129. DOI: 10.1186/1471–2105–11–129.

Reynolds, E.S. (1963): The use of lead citrate at high pH as

an electron–opaque stain in electron microscopy. – The Journal of Cell Biology 17: 208–212. DOI: 10.1083/jcb.17.1.208.

Rippka, R.; Deruelles, J.; Waterbury, J.B.; Herdman, M. & Stanier, R.Y. (1979): Generic assignments, strain histories and properties of pure cultures of cyanobacteria. – Journal of General Microbiology 111: 1–61. DOI: 10.1099/00221287–111–1–1.

Ronquist, F. & Huelsenbeck, J.P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. – Bioinformatics 19: 1572–1574. DOI: 10.1093/bioinformatics/btg180.

Saraf, A.; Dawda, H.G.; Suradkar, A.; Behere, I.; Kotulkar, M.; Shaikh, Z.M.; Kumat, A.; Batule, P.; Mishra, D. & Singh, P. (2018): Description of two new species of *Aliinostoc* and one new species of *Desmonostoc* from India based on the polyphasic approach and reclassification of *Nostoc punensis* to *Desmonostoc punense* comb. nov. – FEMS Microbiology Letters 365: Article fny272. DOI: 10.1093/femsle/fny272.

Schembri, M.A.; Neilan, B.A. & Saint, C.P. (2001): Identification of genes implicated in toxin production in the cyanobacterium *Cylindrospermosis raciborskii*. – Environmental Toxicology: An International Journal 16: 413–421. DOI: 10.1002/tox.1051.

Shukla, E.; Singh, S.S.; Singh, P. & Mishra, A.K. (2012): Chemotaxonomy of heterocystous cyanobacteria using FAME profiling as species markers. – Protoplasma 249: 651–661. DOI: 10.1007/s00709–011–0303–4.

Singh, P.; Shaikh, Z.M.; Gaysina, L.A.; Suradkar, A. & Samanta, U. (2016): New species of *Nostoc* (cyanobacteria) isolated from Pune, India, using morphological, ecological and molecular attributes. – Plant Systematics and Evolution 302: 1381–1394. DOI: 10.1007/s00606–016–1337–z.

Singh, P.; Snokhousova, J.; Saraf A.; Suradkar, A. & Elster J. (2020): Phylogenetic evaluation of the genus *Nostoc* and description of *Nostoc neudorfense* sp. nov., from the Czech Republic. – International Journal of Systematic and Evolutionary Microbiology 70: 2740–2749. DOI: 10.1099/ijsem.0.004102.

Spurr, A.R. (1969): A low–viscosity epoxy resin embedding medium for electron microscopy. – Journal of Ultrastructure Research 26: 31–43. DOI: 10.1016/S0022–5320(69)90033–1.

Stackebrandt, E. & Jonas, E. (2006): Taxonomic parameters revisited: tarnished gold standards. – Microbiology Today 33: 152–155.

Stamenković, M.; Steinwall, E.; Nilsson, A.K. & Wulff, A. (2020): Fatty acids as chemotaxonomic and ecophysiological traits in green microalgae (desmids, Zygnematophyceae, Streptophyta): a discriminant analysis approach. – Phytochemistry 170: Article 112200. DOI: 10.1016/j.phytochem.2019.112200.

Tamura, K.; Stecher, G. & Kumar, S. (2021): MEGA11: Molecular Evolutionary Genetics Analysis Version 11. – Molecular Biology and Evolution 38: 3022–3027. DOI: 10.1093/molbev/msab120.

Tanabe, A.S. (2007): KAKUSAN: a computer program to automate the selection of a nucleotide substitution model and the configuration of a mixed model on multilocus data. – Molecular Ecology Notes 7: 962–964. DOI: 10.1111/j.1471–8286.2007.01807.x.

Tawong, W.; Pongcharoen, P.; Pongpadung, P.; Ponza, S. & Saijuntha, W. (2022): *Amazonocrinis thailandica* sp. nov. (Nostocales, Cyanobacteria), a novel species of the previously monotypic *Amazonocrinis* genus from Thailand. – Algae 37: 1–14. DOI: 10.4490/algae.2022.37.3.10.

Temina, M.; Rezankova, H. & Rezanka, T. (2007): Diversity of the fatty acids of the *Nostoc* species and theri statistical analysis. – Microbiological Research 162: 308–321. DOI: 10.1016/j.micres.2006.01.010.

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 Research 44: W232–W235. DOI: 10.1093/nar/gkw256.

Valerio, E.; Chambel, L.; Paulino, S.; Faria, N.; Pereira, P. & Tenreiro, R. (2009): Molecular identification, typing and traceability of cyanobacteria from freshwater reservoirs. – Microbiology 155: 642–656. DOI: 10.1099/mic.0.022848–0.

Yadav, G.; Sekar, M.; Kim, S.H.; Geo, V.E.; Bhatia, S.K.; Sabir, J.S.M.; Chi, N.T.L.; Brindhadevi, K. & Pugazhendhi, A. (2021): Lipid content, biomass density, fatty acid as selection markers for evaluating the suitability of four fast growing cyanobacterial strains for biodiesel production. – Bioresource Technology 325: Article 124654. DOI: 10.1016/j.biortech.2020.124654.

Yarza, P.; Yilmaz, P.; Pruesse, E.; Glockner, F. O.; Ludwig, W.; Schleifer, K. H.; Whitman, W. B.; Euzeby, J.; Amann, R. & Rossello–Mora, R. (2014): Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. – Natural Reviews Microbiology 12: 635–645. DOI: 10.1038/nrmicro3330.

Supplementary material

The following supplementary material is available for this article:

Table S1. Morphological characteristics of the *Desmonostoc cockrellii* sp. nov. isolates.
Table S2. The 16S rRNA gene percent similarity of *Desmonostoc* strains and closely related taxa.
Table S3. Lengthwise comparison of the ITS domains of the 16S–23S rRNA ITS regions for *Desmonostoc* species with tRNA–containing operons.
Table S4. Fatty acid composition profile (dwt. %) of *Desmonostoc cockrellii* sp. nov. and other related strains.
Table S5. Uncorrected p–distance comparison of *Desmonostoc* species based on the tRNA–containing operons of the 16S–23S rRNA ITS region.
Table S6. Morphological comparison of *Desmonostoc cockrellii* sp. nov. with previously described *Desmonostoc* species. The source, locality and habitat conditions (if available) are also provided.

This material is available as part of the online article (http://fottea.czechphycology.cz/contents)

Received July 16, 2024
Revised November 22, 2024
Accepted December 3, 2024
Prepublished online January 24, 2025