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A comprehensive study of *Macrochaete oelandica* sp. nov., the second known cyanobiont of lichen *Placynthium nigrum* in Europe

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Abstract: Cyanobacteria, a diverse group of photosynthetic bacteria, act as primary symbionts for carbohydrate production in about 10% of lichenized fungi, referred to as cyanolichens. This study investigates the effect of different living conditions on the morphology of one cyanobacterium, comparing the cyanobacterium in the lichen symbiosis with the same species in culture. The lichen *Placynthium nigrum* served as a suitable model. We examined the cyanobacterial 16S rRNA, fungal mtSSU rDNA, and MCM7 sequences using ML analyses, along with analysis of 16S–23S ITS sequences to explore DNA helix structures. Based on the results, we propose here a new cyanobacterial species, *Macrochaete oelandica.* The morphological analyses revealed that a genetically identical cyanobacterium obtained from the same lichen thallus exhibits different morphological characteristics depending on their living conditions: incompletely developed features due to the limitations when living in symbiosis within the lichen thallus versus fully developed morphological features when cultured.

Key words: Cyanobacteria, cyanolichens, evolution, helices, integrative taxonomy, phylogeny, symbiosis

INTRODUCTION

With an origin dating back approximately 2.5 billion years, cyanobacteria are the most morphologically diverse group among prokaryotes and the only bacteria capable of oxygenic photosynthesis (HENTSCHKE & GAMA JUNIOR 2022). They utilize CO_2 as their primary carbon source (GARCIA–PICHEL 2009) and produce carbohydrates, primarily glycogen, which can constitute up to 60% of their biomass (FRIGAARD 2018).

Cyanobacteria, as primary producers of biomass and oxygen, play a key role in almost all ecosystems, including extreme habitats. They can grow as single cells or form large macroscopic colonies, alone or in symbiotic associations with other organisms (HINDÁK 2008). Cyanobacteria live as the primary photosynthetic partner in many lichenized fungi. This symbiosis significantly alters the nutrient requirements of the symbionts, and expands their ecological adaptability (RIKKINEN 2017).

The understanding of microbial symbiotic interactions continues to be a growing field across multiple scientific disciplines (FARRAR 1976; HAWKSWORTH & GRUBE 2020; GRIMM et al. 2021). Lichens serve as a prime example of such relationships, consisting of a symbiotic interaction between a fungus (mycobiont), photosynthetic microorganisms (photobionts) such as prokaryotic cyanobacteria (cyanobionts) or eukaryotic green algae (phycobionts), and other organisms (HAWKSWORTH & GRUBE 2020).

The structure of the lichen formation, the lichen thallus, is of utmost importance in the formation and maintenance of the symbiosis, having a direct influence on the morphology of both major lichen symbionts. For instance, distinct thallus morphologies can be produced when a single mycobiont interacts with different photobionts, such as cyanobacteria or green algae (JAMES & HENSSEN 1976; ARMALEO & CLERC 1991; ERTZ et al. 2018). Recent research has shown that even genetically different strains of the same photobiont genus can result

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ABBREVIATIONS AND EXPLANATIONS
MCM7 – mini–chromosome maintenance complex component 7, DNA replication licensing factor, protein–coding gene;
ML – maximum likelihood;
mtSSU – small subunit of the mitochondrial rDNA;
PD – petri dish;
Prothallus – an arrangement of fungal hyphae only on the margin of the lichen without the photobiont;
Thallus – an arrangement of fungal hyphae with photosynthetic symbionts in the lichen; also a vegetative structure in cyanobacteria.

in different lichen thallus morphologies (Steinová et al. 2019, 2022). Furthermore, distinct morphotypes of the same photobiont are correlated with differences in lichen thallus morphology (Košuthová et al. 2020). Investigations of the impact of lichen thallus structure on photobiont morphology have provided valuable insights. The mycobiont constructs a hyphal framework surrounding the photobiont cells and other symbiotic microorganisms, forming a network of biotic associations that enhances the fitness of the entire phenotype. Photobionts primarily contribute by providing energy through photosynthesis, even though this symbiotic lifestyle can lead to a stressful environment that negatively affects the morphological structure and development of photobionts (Nash 2008). Earlier research (Degelius 1954, 1974) demonstrated varying morphological expressions in photobionts that were clustered in packets versus those appearing filamentous, growth forms that depend on whether they live in symbiosis within lichens or grow solitary in unialgal cultures.

While there has been a significant focus on molecular studies concerning the mycobiont in lichens, research on photobionts, particularly cyanobionts in cyanolichens, has been limited (Hoffman & Lendemer 2018). Recent progress in DNA sequencing and cultivation techniques has enhanced our understanding of photobiont communities (Dal Grande et al. 2018; Villanueva et al. 2018; Muggia et al. 2020; Kosecka et al. 2021). Nonetheless, studies on lichen cyanobionts are rare and often based solely on molecular data without isolation and cultivation (Otálora et al. 2010; Magain et al. 2017; Lavoie et al. 2020; Dal Forno et al. 2021). The16S rRNA used in the past often resulted in low taxonomic resolution (Paulsrud et al. 2000; O'Brien et al. 2005; Strunecký et al. 2023), and it remains to be determined whether this marker will provide greater resolution for unicellular cyanobacterial photobionts.

Accurate taxonomic evaluation of cyanobionts requires a polyphasic approach that integrates phylogeny, morphology and ecology, which necessitates studying the photobiont in isolation (Dvořák et al. 2015). The slow growth and difficulties of getting photobionts in culture have, however, hindered the identification of general patterns in cyanobiont diversity (Jung et al. 2021). To reliably identify cyanobionts, it is important to correlate in vitro traits with those exhibited in the symbiotic stage within the lichen thallus, which has been studied for green algal symbionts (Garrido–Benavent et al. 2022), but never in detail for cyanobionts.

The cyanobiont in the lichen *Placynthium* (Huds.) Gray (Peltigerales, Ascomycota) was previously considered belonging to the genera *Dichothrix* Zanardini ex Bornet et Flahault or *Scytonema* C. Agardh ex Bornet et Flahault (Jørgensen 2012). However, the study by Berrendero Gómez et al. (2016) revealed that the cyanobiont of *P. nigrum* is in fact one of three species in the newly described genus *Macrochaete*. This genus belongs to the order Nostocales and the family Rivulariaceae (Strunecký et al. 2023).

One of the main morphological characteristics of *Macrochaete* is its ability to produce semi–hyaline hairs at the end of filaments, which distinguishes it from the representatives of the genus *Calothrix* C.Agardh ex Bornet et Flahault. To date, three *Macrochaete* species have been described, each living under different environmental conditions. These include the free living arctic–alpine *M. psychrophylla* Berrendero, Johansen et Kaštovský, the tropical *M. santannae* Berrendero, Johansen et Kaštovský, and a lichen cyanobiont *M. lichenoides* Berrendero, Johansen et Kaštovský, which was collected from Lanzarote in the Canary Islands (Spain) living in *P. nigrum* (Berrendero Gómez et al. 2016).

In this study, we use an integrative taxonomy to describe the new species of *Macrochaete* living as a cyanobiont of *P. nigrum* formally as *Macrochaete oelandica* Hindáková et A. Košuthová, sp. nov. We demonstrate how different morphological characteristics depend on lifestyle by using unialgal cultures.

MATERIAL AND METHODS

Material. *Placynthium nigrum*, along with its associated cyanobiont, was collected by Alica Košuthová and Martin Westberg (collection number 1300) during an excursion conducted in 2017 on Öland, the second largest Swedish island in the Baltic Sea. This lichen was collected from calcareous outcrops situated in the open Alvar region of Dröstorp Nature Reserve. Subsequently, this specimen has been preserved in the herbarium of the Swedish Museum of Natural History (S) under the accession number S–F453718.

Isolation and cultivation of the cyanobiont from the lichen thallus of *Placynthium nigrum*. In order to isolate the cyanobiont, lobes of freshly collected lichen thallus were rinsed under running water and allowed to air–dry briefly. Subsequently, using a binocular microscope, these lobes were removed using a sterile blade and placed on a microscope slide in a few drops of water, where gentle pressure with a coverslip facilitated the release of the cyanobiont. The obtained material was transferred on solidified BG–11 agar on a PD, sealed with Parafilm and labeled. The cultivation was conducted within a cultivation room maintained at a constant temperature of 21 °C and an illumination level of 20 $\mathrm{mmol.m^{-2}.s^{-1}}$ for the duration of one year. This was carried out at the Institute of Botany, Plant Science and Biodiversity Centre, Slovak Academy of Sciences (PSBC SAS), situated in Bratislava, Slovakia. Throughout the cultivation period, short filaments were gradually formed, and subsequently inoculated on new PDs. Periodic supplementation of the PD with a modified liquid medium based on CHU No. 10 (CHU 1942) supported the development of semi–hyaline hair, a characteristic of the taxon under investigation, as previously described by Berrendero et al. (2008).

The unialgal culture Hindakova_AL_227, which represents the solo cyanobiont, was used for molecular analyses under the extraction code AL469 (Fig. 1). The term "solo cyanobiont" refers to a cyanobiont isolated from a lichen and growing separately (solo) as an unialgal culture, was accepted by Roth & Goodenough (2021). We have consistently utilized the term "solo" throughout our text. The culture Hindakova_AL_227 was maintained within PD containing BG–11 agar and is stored in the phycological cultivation facility at the Institute of Botany, PSBC SAS. The dried part of the cultivated photobiont designated as Hindakova_AL_227 serves as the holotype. Together with the formaldehyde–fixed material and the living cultivated photobiont, it is deposited at the Institute of Botany, PSBC SAS.

Morphological studies. The examination of morphological characteristics was carried out using the Zeiss Stemi 305 stereomicroscope and Leitz Diaplan light microscope, with specimens prepared as a hand–cut sections. Measurements of the cyanobiont in symbiosis were conducted in water. For more precise measurements, calibrated digital photographs were taken employing NIS–Elements (Nikon, Japan) with a precision of 0.1 µm. In order to document the maximum of the morphological features of the solo cyanobiont the obtained isolates were regularly observed over a 12–months period. Macroscopic and microscopic images were obtained from natural material or samples immersed in water, unless stated otherwise, employing NIS–Elements (Nikon, Japan) and Zeiss AxioCam ICc3.

Molecular data production. Total DNA was extracted from fresh material using the DNeasy Plant Mini Kit (Qiagen, Germany) following the manufacturer's instruction and modifications described by Košuthová et al. (2020). We PCR amplified ≈0.6 kb of the small subunit of the fungal mitochondrial rDNA (mtSSU) and ≈1.3 kb of both cyanobacterial 16S rRNA and16S–23S ITS. The primer combination used for mtSSU were mrSSU1 and mrSSU3R (Zoller et al. 1999). For partial 16S rRNA and the whole 16S–23S ITS sequence, we used the primer combinations CYA106F and cyan781Rab (Nubel et al. 1997) and P2 (known also as CYA359 forward, Nubel et al. 1997) and P1 (Wilmotte et al. 1993), respectively. Symmetric PCR amplifications were performed using Illustra^{TM} Hot Start PCR beads (GE Healthcare, USA), according to the manufacturer's instructions. The PCR cycling conditions used for the fungal mtSSU were performed as in Košuthová et al. (2016). The PCR cycling conditions for the cyanobacterial 16S rRNA and 16S–23S ITS were: 94 °C for 5 min. linked to 35 cycles (94 °C for 1 min., 60 °C for 1 min., and 72 °C for 1 min.), with a final extension of 72 °C for 8 min. After examination by gel electrophoresis, amplification products were purified using ExoSAP–IT (USB Corp., USA). Sequencing of both strands was performed by MACROGENE (Macrogen Europe B. V., Amsterdam, Netherland, http://dna.macrogen–europe.com) using two additional primers for the cyanobacterial 16S–23S ITS region, P5 and P8 (Wilmotte et al. 1993). All newly obtained sequences in this study were submitted to GenBank under accession numbers summarized in Fig. 1 and S1.

Sequence editing, alignment and phylogenetic analyses. The generated sequences were assembled and edited using Geneious version R8 (http://www.geneious.com, Kearse et al. 2012). All edited sequences underwent initial identity verification through BLAST searches (Zhang et al. 2000). The alignment of these sequences was automatically performed using the Muscle algorithm (Edgar 2004) in AliView 1.09 (Larsson 2014). Introns and ambiguously aligned regions were adjusted manually following Lutzoni et al. (2000). Three separate alignments were created. The first simulated the proposed monophyly of the cyanobacterial family Rivulariaceae, aiding in determining the position of the newly described cyanobacterial species. The second focused on analysing helical structures. The third was assembled to check for the phylogenetic position and identity of the lichen (fungal) sample. A total of 79 cyanobacterial nucleotide sequences were employed for phylogenetic analyses to demonstrate the position of the newly described cyanobacterial species. This dataset encompassed 1,284 bp of the 16S rRNA gene, incorporating representatives from other genera within the Nostocales, in conjunction with additional data sourced from GenBank (utilized in Berrendero Gómezet al. 2016; Safar et al. 2019), using *Scytonematopsis contorta* Vaccarino et Johansen as an outgroup. *Macrochaete oelandica* was represented by four new sequences derived from two distinct DNA extractions; one obtained from the lichen with DNA extraction voucher AL555 and the other obtained from the unialgal culture with DNA extraction voucher AL469 (GenBank accession numbers for 16S rRNA and 16S–23S ITS are summarized in GenBank accession numbers under the Taxonomy section).

For the fungal dataset, we included 48 fungal nucleotide sequences of mtSSU and MCM7 in a concatenated multiple sequence alignment with 25 terminals. In total, this dataset comprised 1309 nucleotide positions (616 bp for mtSSU and 693 bp for MCM7) and included sequences previously used by Košuthová et al. (2016, S1). It included the lichen sample *P. nigrum* (DNA voucher AL237) from which the morphological and culture studies were conducted, and for which only mtSSU data (GenBank accession number OR149206) was obtained. Sequences used in this study are summarized in Fig. 1 and S1. Single ambiguous base pairs within otherwise highly conserved regions within the alignments were adjusted or removed manually, allowing smaller final blocks and gap positions within the final blocks. Phylogenetic relationships and confidence were inferred from a single cyanobacterial 16S rRNA dataset and one combined fungal (mtSSU and MCM7) dataset using Maximum Likelihood (ML). For the ML analysis, the same settings were used as in the individual gene analyses utilizing RAxML. Likelihood and ML bootstrapping were executed through RAxML 8 (Stamatakis 2014) implementing a general time reversible (GTR) model of nucleotide substitution with gamma distributed rate heterogeneity (GTRGAMMA). One thousand bootstrap (BS) replicates were completed using the parametric BS algorithm of RAxML–HPC v.8 on XSEDE using the CIPRES Web Portal (Miller et al. 2010).

The D1–D1' and Box B helices in 16S–23S ITS were identified using CIMS online (Labrada et al. 2023). Their secondary structures were estimated via RNA folding form online at mFold server 3.4 (Zuker 2003) with default settings. Similarity matrices were estimated in MEGA X (Kumar et al. 2018) program for 16S rRNA and 16S–23S ITS sequences separately. We used p–distance with uniform rates and complete deletion.

The description of the new species adhered to the guidelines and regulations outlined in the International Code of Nomenclature for algae, fungi, and plants (Turland et al. 2018) and was based on the monophyletic species concept sensu Johansen & Casamatta (2005).

RESULTS AND DISCUSSION

Phylogenetic analyses and similarity matrices

Our cyanobacterial phylogenetic analysis resulted in a well–supported topology that places our two accessions of cyanobacterium from lichen and culture, respectively, as a clade sister to, yet distinct from, a clade of

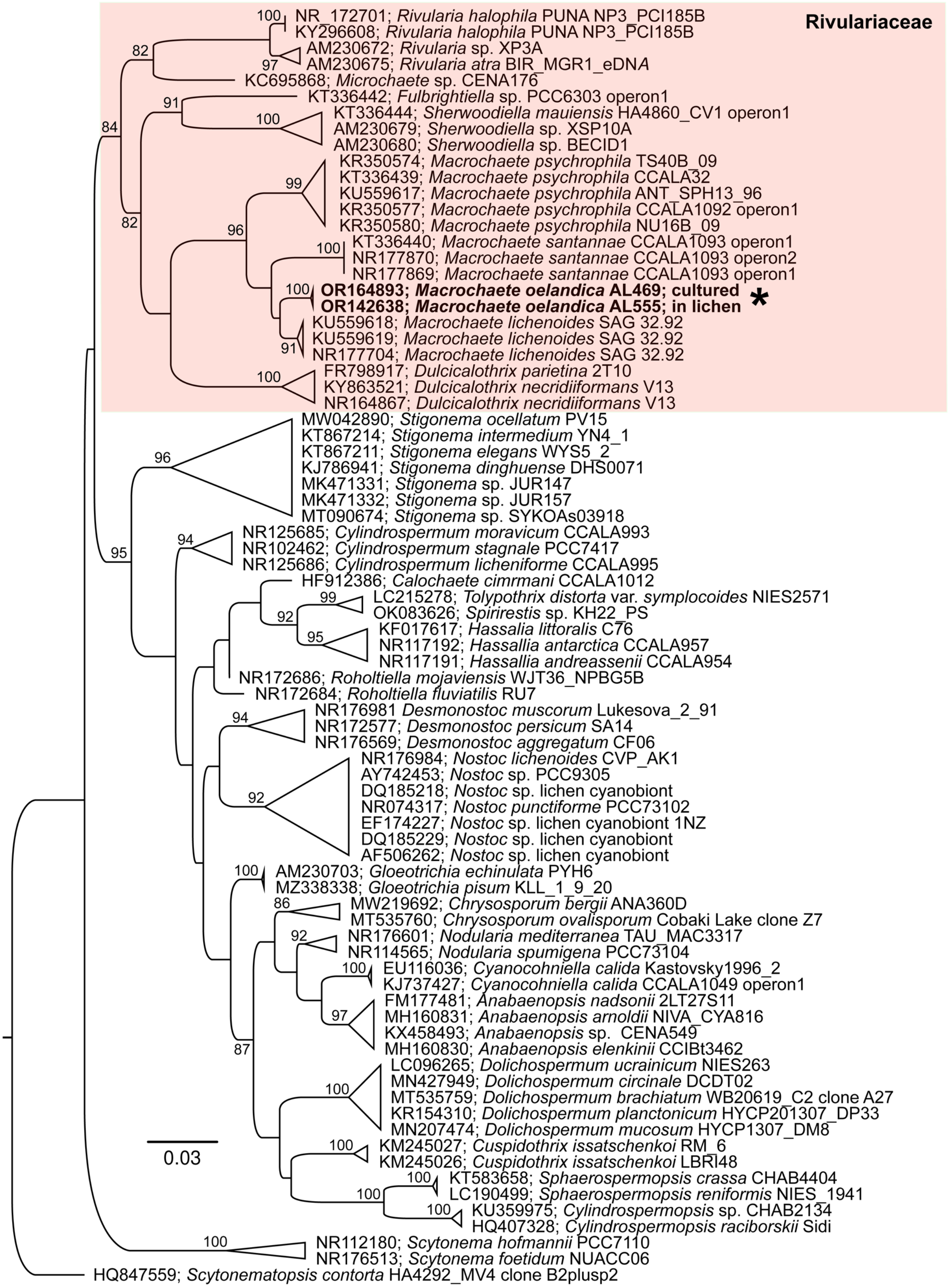


Fig. 1. The most likely tree from the RAxML phylogenetic analyses based on 16S rRNA matrix indicating phylogenetic complexity within the members of the family Rivulariaceae (marked pink) inferred by Maximum Likelihood with bootstrap values representing ML > 80%. The length of the 16S rRNA gene alignment was 1284 bp long and it comprises total of 79 nucleotide sequences. The new species of *Macrochaete* is indicated by bold and asterisk. The scale bars indicate 0.03 of substitution per side.

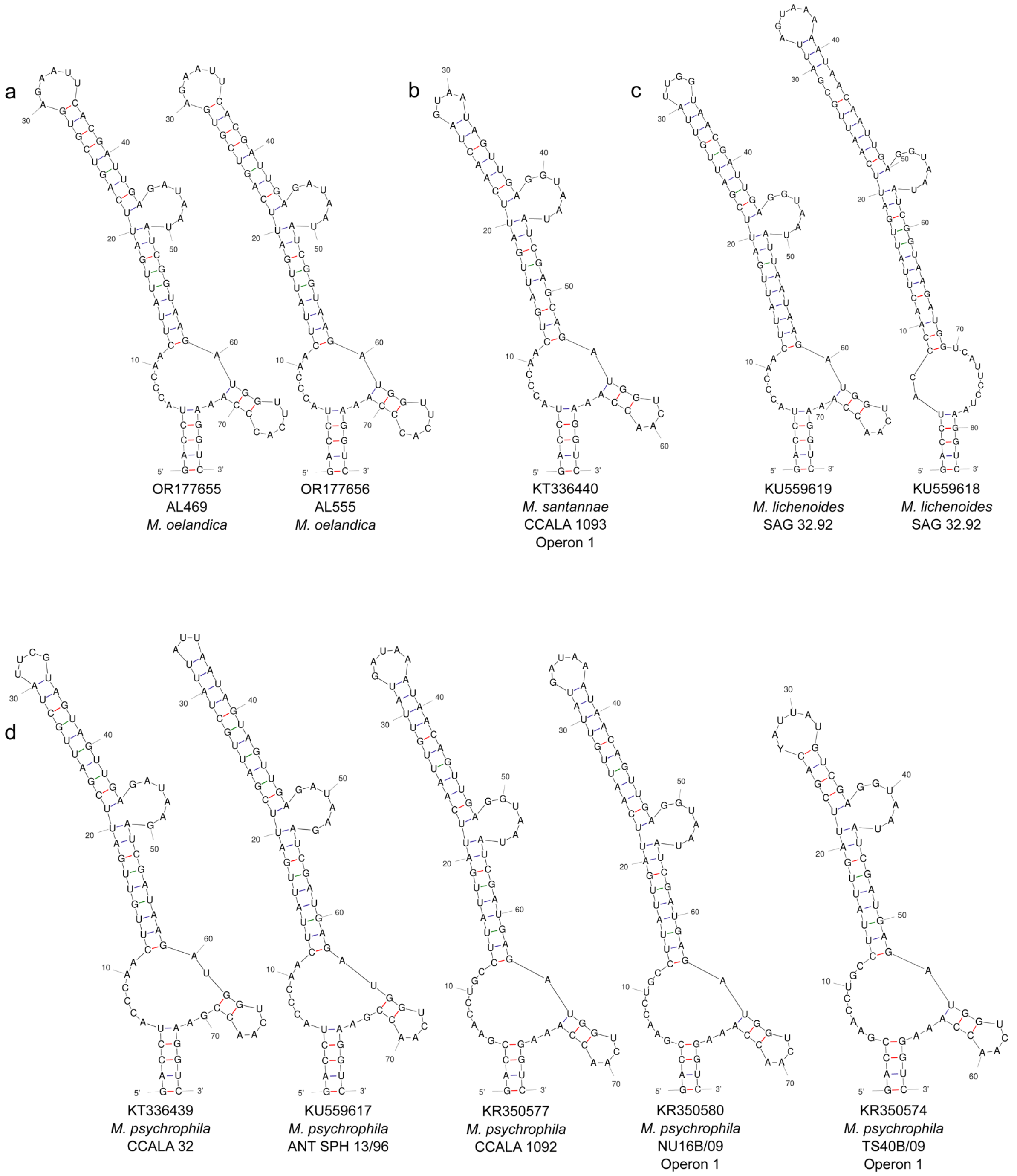
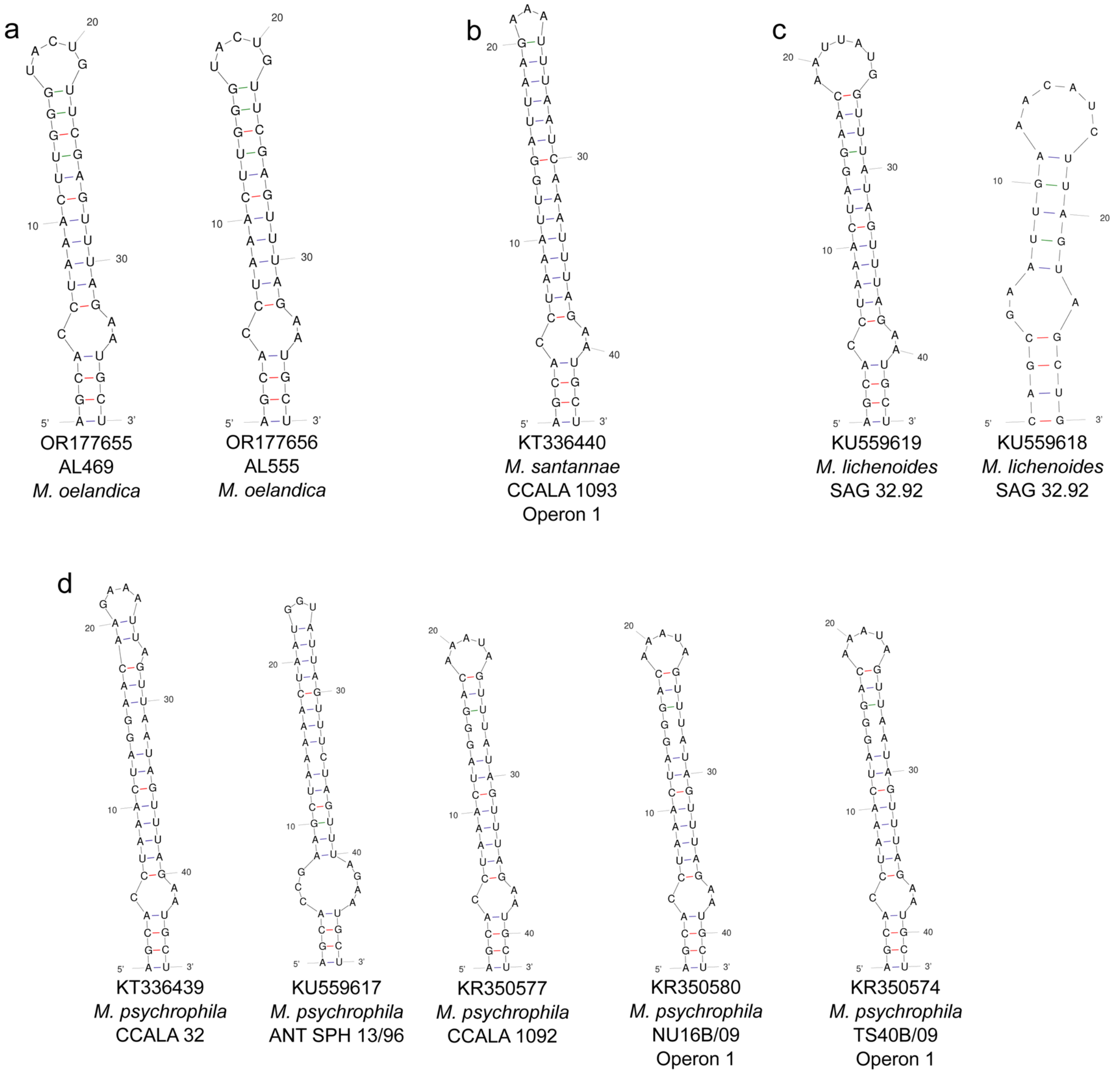


Fig. 2. Secondary structures of D1–D1' helices in 16S–23S ITS within the genus *Macrochaete*: (a) *M. oelandica*, (b) *M. santannae*, (c) *M. lichenoides*, and (d) *M. psychrophila*.

three accessions of *M. lichenoides* (Fig. 1). The overall topology of the tree is consistent with the results of Berrendero Gómez et al. (2016). Saraf et al. (2019) demonstrated that the family Calotrichaceae is distinct from Rivulariaceae including the genera *Macrochaete*, *Dulcicalothrix*, and *Calotrix* sensu lato. The genus *Calothrix* s. l. was split in two genera, *Fulbrightiella* N. Kumar, P. Singh, et J.R. Johansen and *Sherwoodiella* J.R. Johansen et P. Singh (Kumar et al. 2022). Representatives of the genus *Calothrix* s. l. are presented in Fig. 1. The most recently, Strunecký et al. (2023) analyzed both families Rivulariaceae and Calotrichaceae merging them under the name Rivulariaceae for now. We agree with this conclusion and accept the current placement of the genus *Macrochaete* in the family Rivulariaceae (Strunecký et al. 2023, Molinari Novoa in Guiry, M.D. & Guiry, G.M. 2023).

Our findings regarding the taxonomy of *Macrochaete* has confirmed that *M. oelandica* belongs in this monophyletic genus, supported by a ML value of 96%. Currently *Macrochaete* includes four distinct species, *M. lichenoides,*



OR177655
AL469
M. oelandica

OR177656
AL555
M. oelandica

KT336440
M. santannae
CCALA 1093
Operon 1

KU559619
M. lichenoides
SAG 32.92

KU559618
M. lichenoides
SAG 32.92

KT336439
M. psychrophila
CCALA 32

KU559617
M. psychrophila
ANT SPH 13/96

KR350577
M. psychrophila
CCALA 1092

KR350580
M. psychrophila
NU16B/09
Operon 1

KR350574
M. psychrophila
TS40B/09
Operon 1

Fig. 3. Secondary structures of Box B helices in 16S–23S ITS within the genus *Macrochaete*: (a) *M. oelandica*, (b) *M. santannae*, (c) *M. lichenoides*, and (d) *M. psychrophila*.

M. oelandica, M. psychrophila, and *M. santannae*. The corresponding phylogenetic tree where *Macrochaete oelandica* is highlighted in bold and with an asterisk, is presented in Fig.1. Furthermore, we estimated similarity matrices of the species within *Macrochaete*. The *M. oelandica* sequences are 100% similar in 16S rRNA and 98.66% similar in 16S–23S ITS. The 16S rRNA most similar sequence to *M. oelandica* was *M. lichenoides* with 99.54% in 16S rRNA and 83.97% in 16S–23S ITS. On the other hand, *M. psychrophila* sequences were the most distant from *M. oelandica* with ~96% similarity in 16S rRNA and ~77–80% in ITS (Table S3). High 16S rRNA similarity suggest that the *M. oelandica* and *M. lichenoides* are recently diverged. If we apply 98.7% (Stackebrandt & Ebers 2006) threshold, we can assume that sequences of *M. oelandica* and *M. lichenoides* belong to the same species. However, 16S rRNA similarity has been shown to be too conservative to recognize inter species differences and other markers need to be applied (Stanojković et al. 2022; Dvořák et al. 2023). In any case, 16S–23S ITS dissimilarity was well below the similarity threshold advocated by Pietrasiak et al. (2019) supporting that *M. oelandica* is indeed a new species.

Through fungal phylogenetic analysis, we successfully determine the identity of the collected lichen, which is essential information for assigning the cyanobiont to the mycobiont. The resulting topology (Fig. S1) closely resembled the findings presented in the study by Košuthová et al. (2016). Particularly, our sample AL237 serving as the foundation for this research, was nested within the *Placynthium nigrum* species clade. This placement is consistent with the morphological characteristics of the sample, which exhibits a dark brown to blackish lichen thallus covered by granular to coralloid isidia, accompanied by the distinctive presence of a blue–black prothallus

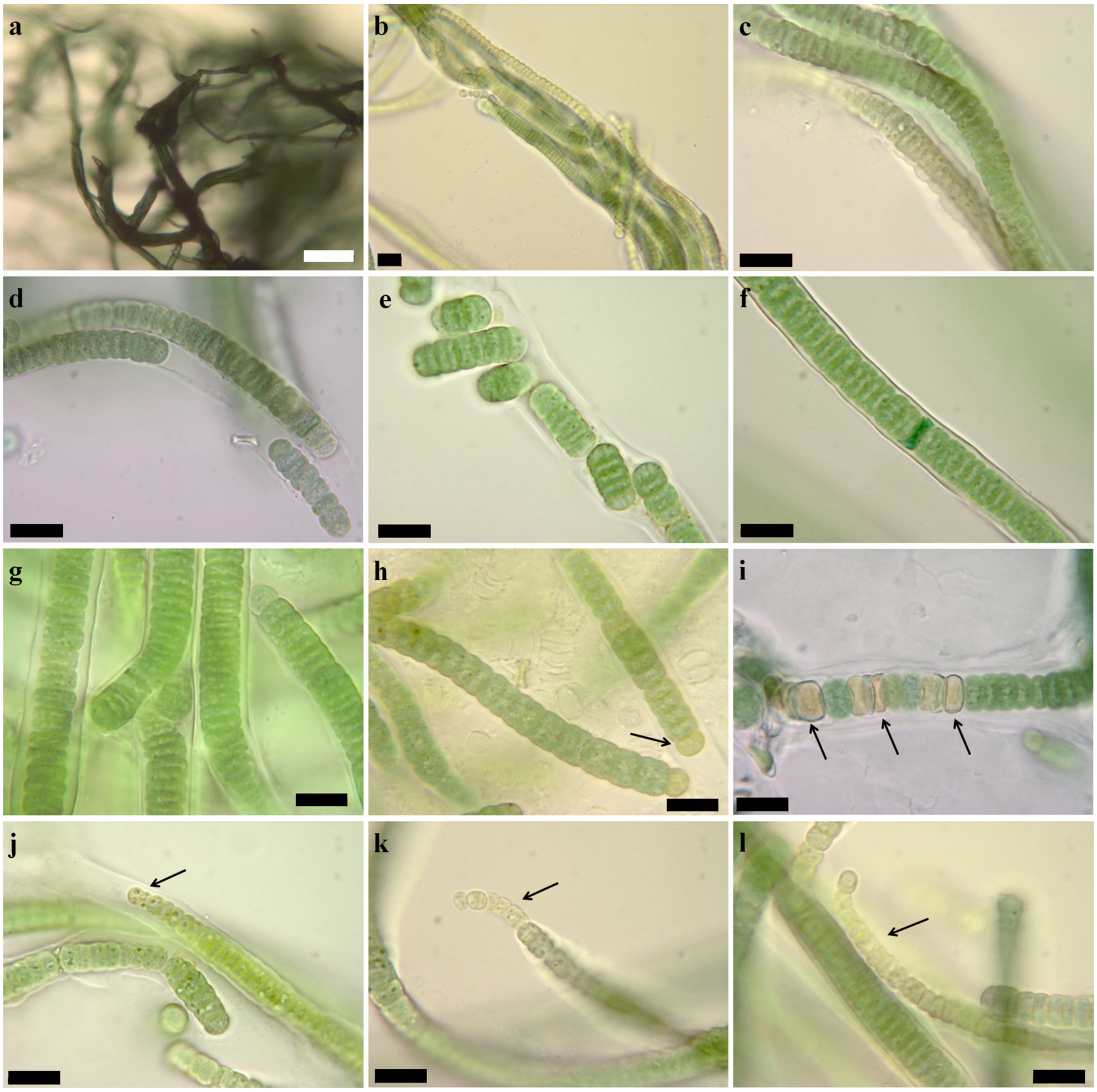


Fig. 4. *Macrochaete oelandica* sp. nov. as solo cyanobiont in culture (strain Hindáková_AL_227): (4a) filaments joined into fascicles of varying thickness; (4b, c) detail on filaments forming fascicles; (4d, e) production of hormogonia; (4f) filament with necridic cell; (4g) false branching of filament; (4h) filaments with one basal heterocyte and (4i) more intercalary heterocytes (arrows); (4j–l) filaments with semi–hyaline terminal hairs (arrow). Scale bars 100 µm (4a) and 10 µm (4b–l).

(Fig. S2) verifying its identity as *Placynthium nigrum*.

16S–23S ITS secondary structure

The D1–D1' and Box B helices of the two *Macrochaete oelandica* strains were found to be identical and no tRNA was identified in the species (Figs 2, 3). However, the D1–D1' of *M. oelandica* possessed a unilateral sub–terminal 6 nt bulge, which has been identified as a specific feature for the genus *Macrochaete* (Berrendero Gómez et al. 2016). While the D1–D1' of *M. oelandica* had a similar secondary structure to other *Macrochaete* species, the species possessed some unique features. For instance, the terminal hairpin loop had a sequence of 5'–AGAAUUC–3', which is unique among the *Macrochaete* species. Additionally, a difference was identified at the bilateral terminal bulge where the 3' part had a unique sequence of 5'–UUCAC–3'. Similarly, the Box B helices of *M. oelandica* had a similar structure to other *Macrochaete* species, composed of a terminal hairpin loop and basal bilateral bulge, but exhibited a unique sequence of the terminal loop 5'–UACUG–3'. These unique features provide further evidence for a delimitation of *M. oelandica* as a new species.

Ecological and morphological evaluation

In the study by Berrendero Gómezet al. (2016), *Macrochaete* was described as closely resembling *Calothrix,* yet distinguished by the production of a pair of heteromorphic basal heterocytes and end cells in the form of long hyaline hairs in low–phosphorus environments.

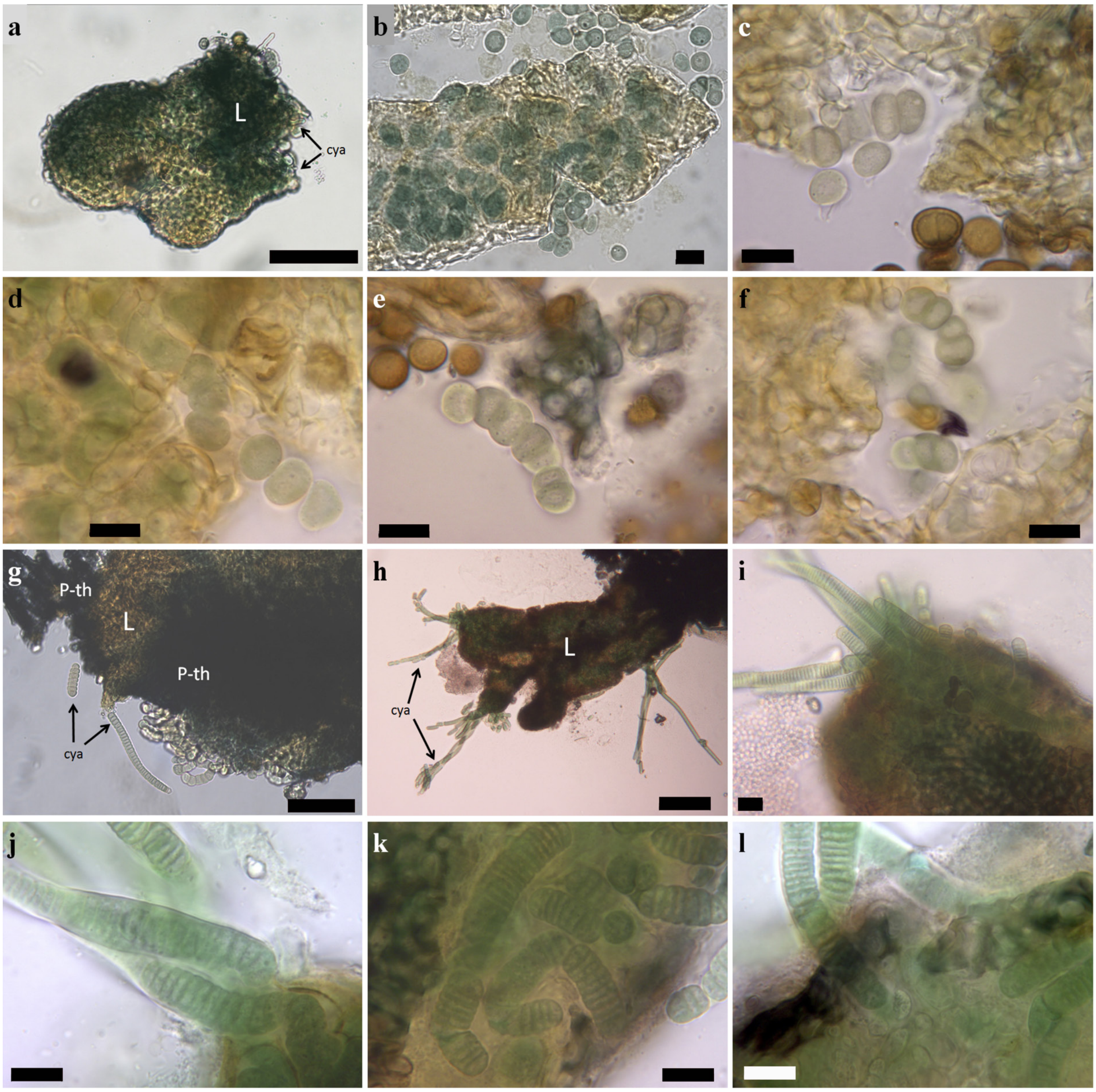


Fig. 5. *Macrochaete oelandica* sp. nov. as cyanobiont in the lichen *Placynthium nigrum*: (5a–f) single or clustered cells releasing from the mycobiont thallus, or (5g–l) forming short or longer filaments of up to 35 cells when released from the mycobiont prothallus; (P–th) mycobiont prothallus; (L) lobe of the lichen, (cya) cyanobiont, marked with arrows. Scale bars 50 µm (5a, g), 100 µm (5h) and 10 µm (5b–f, i–l).

The name "*Macrochaete*" originates from the Greek words "macro" meaning "large," and "chaete" meaning "hair," referring to this genus' characteristic trait. Among the known species, *M. psychrophila*, the type species, is one of the two free–living species observed in soil crusts in cold environments such as the Alps, Antarctica, and the Himalayas. Its ability to tolerate sub–freezing temperatures for most of the year sets it apart from the other species. The second known free–living species, *M. santannae* has a distribution in the tropics makes it ecologically distinct from *M. psychrophila*. Additionally, this tropical species possesses longer terminal hairs (up to 80 cells) compared to cold–adapted species (up to 50 cells). The third species, *M. lichenoides*, lives as a lichen symbiont in *Placynthium nigrum*. Interestingly, this cyanobacterium lacks one of the key diagnostic characters of the genus, the long terminal hairs (Berrendero Gómez et al. 2016). Despite attempts to stimulate hair formation using the CHU No. 10 low phosphorus medium, cultivated strains of *M. lichenoides* did not exhibit this characteristic feature. A possible explanation may be due to the lichen habitat's limited requirement for phosphatase activity associated with hair development, therefore the hair formation is simply unnecessary (Berrendero Gómez et al. 2016). Liba et al. (2006) and Sigurbjornsdottir et al. (2015) observed the same phenomena in association with the fungus or associated bacteria. The fourth species identified within this genus is described here as *M. oelandica*, which is also a lichen symbiont. The characteristic hair formation of *Macrochaete* is not present throughout the entire life cycle of *M. oelandica*, it develops only during the mature stage (Fig. 4j–l). This is in contrast to *M. lichenoides*, where this feature is entirely absent.

In the investigations conducted by Vančurová et al. (2018, 2021), Steinová et al. (2019), and Škvorová et al. (2022), instances of "photobiont switching" have been observed and explained by environmental and geographic factors. Notably *Placynthium nigrum* demonstrates the capability to establish symbiotic relationships with various cyanobacteria across different geographical gradients. Both *Macrochaete lichenoides* and *M. oelandica* are known from this lichen species, with *M. lichenoides* being prevalent in tropical to temperate zones, including the Canary Islands in Spain, and *M. oelandica* being found in semi–continental oceanic climates in nemoral to nemo–boreal zones, specifically collected from the island of Öland in the Baltic Sea in Sweden. Despite Öland's sunny climate and high risk of UV irradiation, *M. oelandica* does not possess a colored sheath (lack of scytonemin) to protect against radiation, as known in e.g. the filamentous epilithic cyanobacteria *Scytonema mirabile* (Dillwyn) Bornet or *Stigonema mirabile* Beck (Hindák 2008). However, as highlighted by Hindáková et al. (2017), the lichen thallus surface is densely colonized with various epiphytic coccal cyanobacteria, surrounded by colored mucilage that may serve as a protection against UV–B radiation. These findings highlight the adaptability of lichens to diverse environmental conditions and their potential for forming multiple symbiotic relationships with cyanobacteria.

Taxonomy

Taxonomic classification of the cyanobiont: phylum Cyanobacteria, class Cyanophyceae, order Nostocales, family Rivulariaceae, genus Macrochaete (Strunecký et al. 2023, Molinari–Novoa in Guiry M.D. & Guiry G.M. 2023).

Macrochaete oelandica Hindáková et A. Košuthová, sp. nov. (Fig. 4a–l)

Description of the solo cyanobiont in culture: Thallus filamentous, heteropolar, but not clearly differentiated throughout the life cycle. Filaments solitary or in groups, able to form macroscopic colonies (Fig. 4a–c), with false branching (Fig. 4g). Sheath initially thin (Fig. 4d), later thick (Fig. 4f, g), firm, wide, also protruding, almost not layered, open at the end, always colorless (Fig. 4c, j). Trichomes short to long (up to 300 µm), straight or twisted and intertwined, constricted at the cross walls. The cells grey–green to dark green, shorter than wider, dimensions: i) at the base of filaments 5.3 – 5.9 µm wide, 2.8 – 3.2 µm high, ii) in the middle part of filaments 4.7 – 9.1 µm wide and 1.3 – 4.5 µm high, iii) at the end of filaments as more–less rounded cells: 3.4 – 7.9 µm wide and 3 – 6.1 µm high. At the end of filaments semi–hyaline hairs (Fig. 4j–l) composed from 5 to 7 irregularly more–less spherical cells, 3.2 – 4.8 µm wide and 2.3 – 4.6 µm high, constricted at the cell walls. Heterocyte one basal (Fig. 4h), 4.9 – 8.4 µm × 3 – 7.4 µm in size, rarely as two basal or in an intercalary position (Fig. 4i). Reproduction by hormogonia of varying numbers of cells, usually three to nine (Fig. 4d, e). Necrotic cells (Fig. 4f) rarely present in the filaments, the production of hormogonia is not associated with them. Akinetes not observed.

Holotype: SAV! AH2024–001. Dried cultivated cyanobiont, deposited at the Institute of Botany, PSBC SAS. Holotype specimen illustrated in Fig. 4.
Reference strain: Hindakova_AL_227, isolated by Alica Hindáková.
Strain information: The live culture is stored in the phycological culture collection at the Institute of Botany, PSBC SAS, Bratislava, Slovakia.
Type locality: Sweden. Öland, Gårdby parish, Dröstorp Nature Reserve, Tornrör, 1200 m SW of Skarpa Alby; Lat/long: 56,585620 N; 16,581270 E; Open area with calcareous outcrops; date: 17 June 2017; Leg. Alica Košuthová and Martin Westberg (coll. number 1300), det. Alica Košuthová. Lichen specimen is illustrated in S2.
Materials analyzed: The strain Hindakova_AL_227 served as material for DNA analyses of cultures cyanobacterium 16S rRNA and 16S–23S ITS (DNA accession number: AL469 – cultured). The lichen *Placynthium nigrum* (S–F453718) was utilized for the analysis of the cyanobacterium of 16S rRNA and 16S–23S ITS (DNA accession number AL555).
GenBank accession numbers: 16S rRNA: OR164893 (DNA accession number AL469 – cultured) and OR142638 (DNA accession number AL555 – in lichen); 16S–23S ITS: OR17765 (DNA accession number AL469 – cultured) and OR177656 (DNA accession number AL555 – in lichen).
Etymology: "oelandica" referring to the place of occurrence of the studied lichen – Öland.
Ecologyand distribution: lichen cyanobiont. Known only from Öland.

Observations

In this study, we examined the morphology of *Macrochaete oelandica* under two different living conditions. First, we studied the cyanobiont in fresh material of the lichen *Placynthium nigrum.* Here, the cells were variously compressed in encapsulated clusters in the lichen thallus, forming short (2–6 cell) to longer filaments (up to 10 cells, Fig. 5a–f), and even longer when released through the prothallus (up to 35 cells; Fig. 5g–l). The released cells were oval–shaped, irregular in size and shape within one filament (4.4 – 10.9 µm × 4.9 – 8.4 µm; Fig. 5b–f), pale grey–green in color, with a thin sheath surrounding the cells closely. Chromatoplasma and centroplasma were clearly differentiated, but neither heterocytes, nor semi–hyaline hairs were observed (Fig. 5i–l).

Next, we examined *M. oelandica* as solo organism in unialgal culture. Initially, cut pieces of lichen thalli were placed on BG–11 agar, where the cyanobiont could be released from among the fungal hyphae (Figs 4g–h, 5). After several weeks, the thallus of the cyanobacterium was filamentous and heteropolar (Fig. 4h), but not clearly differentiated throughout the life cycle. Sheaths were either thin, closely surrounding the cells (Fig. 4d), or thicker and protruding, colorless, homogenous, and almost not layered (Fig. 4c, f, g, j). The filaments were solitary, straight, of different lengths, more or less equally wide along the entire length, and constricted at the cross walls. At the end, they had a rounded cell (Fig. 4d, g), not elongated into cellular hair. The cells were significantly shorter than wider (4.9 – 9.1 µm wide, 1.5 – 2.6 µm high), with chromatoplasma and centroplasma clearly differentiated. Semi–hyaline hairs composed of up to 7 cells have been observed at the end of old filaments (Fig. 4j–l). Hormogonia (3–9 cells) were produced in different parts of the filaments (Fig. 4d, e).Heterocytes were observed during the mature stage (Fig. 4h, i).

Notes: *Macrochaete oelandica* has the ability to produce semi–hyaline hair at the end of the filament, which is one of the main morphological characteristic of the genus *Macrochaete* (Berrendero Gómez et al. 2016). The terminal hairs of the new species, (up to 7 cells) are short in comparison both with *M. santannae,* which has longer terminal hairs up to 80 cells, and with *M. psychrophila* up to 50 cells. In *M. lichenoides,* terminal hairs were not observed at all. *Macrochaete oelandica* also differs by producing colorless sheaths (Fig. 4c–j) compared with other *Macrochaete* species where yellow brown color occurs at least at the lower part of the sheath (Berrendero Goméz et al. 2016). Although necrotic cells are present in *M. oelandica* filaments (Fig. 4f), the production of hormogonia is not associated with them. Unlike the original diagnosis of the genus *Macrochaete*, *M. oelandica* often forms distinct macroscopic colonies (Fig. 4a, b) due to false branching of filaments (Fig. 4g). Based on its first and so far only known occurrence on Öland, we assume that *Macrochaete oelandica* inhabits semi–continental oceanic climate characterized by significant temperature fluctuations between summer and winter.

CONCLUSION

Previous studies (Degelius 1954, 1974) demonstrated that the cell shape of photobionts varies depending on whether they live in symbiosis in lichens or solo in culture. In our research, we investigated the cyanobiont identity by comparing DNA sequences under these two different living conditions, and concluded that they belong to the same species, *Macrochaete oelandica* (Fig. 1). This alignment allowed for the examination and comparison of their morphological characteristics. In the lichen thalli, the cyanobiont had the shape of oval cells or filaments of different lengths, variously coiled in a limited space (Fig. 5d–f), similar in appearance to that of the cyanobacteria of the genus *Nostoc* (Košuthová et al. 2021, 2022). After placing the cyanobiont on BG–11agar medium for several weeks, we observed changes in the formation of filaments, which became more heteropolar (Fig. 5i–l). In the mature stage, heterocytes were produced (Fig. 4h–i). The observed features of photobionts, clustered in packets or filamentous, between the same cyanobacterium when existing in symbiosis versus solo in culture underscore its phenotypic plasticity to diverse environmental conditions. The environmental factors not only dictate the phenotypes produced, but also serve as the arena where distinct morphologies experience different growth, survival, and selection (West–Eberhard 1989). Competitive interaction, temperature fluctuations, pH levels, and environmental stress are considered as influential forces shaping phenotypic plasticity (Neustupa & Šťastný 2006; Neustupa 2008; Hašler et al. 2011). In conclusion, *Macrochaete oelandica* stands as a model to interpret the complex morphology of a cyanobiont using an integrative taxonomical approach, which involved collecting genetical, morphological, and ecological data from two distinct living conditions: as a symbiont in the lichen *Placynthium nigrum* and solo as unialgal culture. We found that *M. oelandica* is morphologically distinct from other species (see the text above), with a unique ITS secondary structure. Our approach can be considered as a pilot for lichenized fungi in general, but in particular for studies of cyanolichens.

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REFERENCES

Armaleo, D. & Clerc, P. (1991): Lichen chimeras: DNA analysis suggests that one fungus forms two morphotypes. – Exp. Mycol.15: 1–10.

Berrendero, E.; Perona, E. & Mateo, P. (2008): Genetic and morphological characterization of *Rivularia* and *Calothrix* (Nostocales, Cyanobacteria) from running water. – Int. J. Syst. Evol. Microbiol. 58: 447–60.

Berrendero Gómez, E.; Johansen, J. R.; Kaštovský, J.; Bohunická, M. & Čapková, K. (2016): *Macrochaete* gen.nov. (Nostocales, Cyanobacteria), a taxon morphologically and molecularly distinct from *Calothrix*. – J. Phycol. 52: 638–655.

Dal Forno, M.; Lawrey, J. D.; Sikaroodi, M.; Gillevet, P. M.; Schuettpelz, E. & Lücking, R. (2020): Extensive photobiont sharing in a rapidly radiating cyanolichen clade. – Mol. Ecol. 30: 1755–1776.

Dal Grande, F.; Rolshausen, G.; Divakar, P. K.; Crespo, A.; Otte, J.; Schleuning, M. & Schmitt, I. (2018): Environment and host identity structure communities of green algal symbionts in lichens. – New Phytol. 217: 277–289.

Degelius, G. (1954): The lichen genus *Collema* in Europe. – Symb. Bot. Upsal. 13: 1–499.

Degelius, G. (1974): The lichen genus *Collema* with special reference to the extra–European species. – Symb. Bot. Upsal. 20: 1–215.

Dvořák, P.; Jahodářová, E.: Stanojković, A.; Skoupý, S. & Casamatta, D.A. (2023): Population genomics meets the taxonomy of cyanobacteria. – Algal Res. 72: 103128.

Dvořák, P.; Poulíčková, A.; Hašler, P.; Belli, M.; Casamatta, D. A. & Papini, A. (2015): Species concepts and speciation factors in cyanobacteria, with connection to the problems of diversity and classification. – Biodivers. Conserv. 24: 739–757.

Edgar, R. C. (2004): MUSCLE: multiple sequence alignment with high accuracy and high throughput. – Nucleic Acids Res. 32: 1792–1797.

Ertz, D.; Guzow–Krzemińska, B.; Thor, G.; Łubek, A. & Kukwa, M. (2018): Photobiont switching causes changes in the reproduction strategy and phenotypic dimorphism in the Arthoniomycetes. – Sci. Rep. 8: 4952.

Farrar, J. F. (1976): Ecological physiology of the lichen *Hypogymnia physodes*, ii. effects of wetting and drying cycles and the concept of 'physiological buffering'. – New Phytol. 77: 105–113.

Frigaard, N–U. (2018): Chapter 2 – Sugar and Sugar Alcohol Production in Genetically Modified Cyanobacteria, in Genetically Modified Cyanobacteria. – In: Holban, A. M. & Grumezescu, A. M. (eds): Handbook of Food Bioengineering, Genetically Engineered Foods. – p. 31–47, Academic Press, United Kingdom.

Garcia–Pichel, F. (2009): Cyanobacteria (Third Edition). – Encyclopedia of Microbiology 18: 107–124.

Garrido–Benavent, I.; Chiva, S.; Bordenave, C. D.; Molins, A. & Barreno, E. (2022): *Trebouxia maresiae* sp. nov. (Trebouxiophyceae, Chlorophyta), a new lichenized species of microalga found in coastal environments. – Crypt. Algol. 43: 135–145.

Grimm, M.; Grube, M.; Schiefelbein, U.; Zühlke, D.; Bernhardt, J. & Riedel, K. (2021): The lichens' microbiota, still a mystery? – Front. Microbiol. 12: 714.

Hašler, P., Dvořák, P., Ondřej, V., Kitner, M., Hloušková, P. & Poulíčková, A. (2011): The importance of the polyphasic approach in a comparative study of *Nodularia* (Nostocales, Cyanobacteria). – Preslia 83: 167–182.

Hawksworth, D. L. & Grube, M. (2020): Lichens redefined as complex ecosystems. – New Phytol. 227: 1281–1283.

Hentschke, G. S. & Gama Junior, W. A. (2022): Trends in Cyanobacteria: a contribution of systematics and biodiversity studies. – In: Lopes, G.; Silva, M. & Vasconcelos, V. (eds): The Pharmacological Potential of Cyanobacteria. – pp. 1–22, Academic Press, Elsevier, United Kingdom.

Hindák, F. (2008): Colour atlas of cyanophytes. – 253 pp., VEDA, Bratislava.

Hindáková, A.; Wedin, M.; Westberg, M. & Košuthová A. (2017): Shades of olive in the cyanolichens *Placynthium*. – Fritschiana 85: 29–30.

Hoffman, J. R. & Lendemer, J. C. (2018): A meta–analysis of trends in the application of Sanger and next–generation sequencing data in lichenology. – Bryologist 121: 133–147.

James, P. W. & Henssen, A. (1976): The morphological and taxonomic significance of cephalodia. –In: Brown, D. H.; Hawksworth, D. L. & Bailey, R. H. (eds) Lichenology: Progress and problems. – pp. 27–77, Academic, London.

Johansen, J. R. & Casamatta, D. A. (2005): Recognizing cyanobacterial diversity through adoption of a new species paradigm. – Algological Studies 117: 71–93.

Jørgensen, P. M. (2007): Placynthiaceae. – Nordic Lichen Flora 3: 134–142.

Jung, P.; Brust, K.; Schultz, M.; Büdel, B.; Donner, A. & Lakatos, M. (2021): Opening the Gap: Rare Lichens With Rare Cyanobionts – Unexpected Cyanobiont Diversity in Cyanobacterial Lichens of the Order Lichinales. – Front. Microbiol. 12: 728378.

Kearse, M.; Sturrock, S. & Meintjes, P. (2012): The Geneious 6.0.3 read mapper. – Auckland, New Zealand: Biomatters, Ltd.

Kosecka, M.; Guzow–Krzemińska, B. & Černajová, I.(2021): New lineages of photobionts in Bolivian lichens expand our knowledge on habitat preferences and distribution of *Asterochloris* algae. – Sci. Rep. 11: 8701.

Košuthová, A.; Fernández–Brime, S.; Westberg, M. & Wedin, M. (2016): *Collolechia* revisited and a re–assessment of ascus characteristics in *Placynthiaceae* (Peltigerales, Ascomycota). – Lichenologist 48: 3–12.

Košuthová, A.; Bergsten, J., Westberg, M. & Wedin, M. (2020): Species delimitation in the cyanolichen genus *Rostania*. – BMC Evol. Biol. 20: 115.

Košuthová, A.; Westberg, M. & Wedin, M. (2022): A revision of the *Rostania occultata* (Collemataceae) complex in Fennoscandia. – Lichenologist 54: 13–24.

Kumar, N.; Saraf, A.; Pal, S.; Mishra, D.; Singh, P. & Johansen, J. (2022): Circumscription of *Fulbrightiella* gen. nov. and *Sherwoodiella* gen. nov., two novel genera in the *Calotrichaceae* (Nostocales, Cyanobacteria). – J. Phycol. 59: 204–220.

Kumar, S.; Stecher, G. Li. M.; Knyaz, C. & Tamura, K. (2018): MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. – Mol. Biol. Evol. 35: 1547–1549.

Labrada, N. A.; McGovern, C. A.; Thomas, A. L.; Hurley, A. C.; Mooney, M. R. & Casamatta, D. A. (2023): The CIMS (Cyanobacterial ITS motif slicer) for molecular systematics. – Alg. Res. 72: 103–128.

Larsson, A. (2014): AliView: a fast and lightweight alignment viewer and editor for large datasets. – Bioinformatics 30: 3276–3278.

Lavoie, C.; Renaudin, M.; McMullin, R. T.; Gagnon, J.; Roy, C.; Beaulieu, M.–E.; Bellenger, J. P. & Villarreal, J. C. (2020): Extremely low genetic diversity of *Stigonema* associated with *Stereocaulon* in eastern Canada. – Bryologist 2: 188–203.

Liba, C. M.; Ferrara, F. I. S.; Manfio, G. P.;

Fantinatti–Garboggini, F.; Albuquerque, R. C.; Pavan, C.; Ramos, P. L.; Moreira–Filho, C. A. & Barbosa, H. R. (2006): Nitrogen–fixing chemoorganotrophic bacteria isolated from cyanobacteria–deprived lichens and their ability to solubilize phosphate and to release amino acids and phytohormones. – J. Appl. Microbiol. 101: 1076–86.

Lutzoni, F.; Wagner, P.; Reeb, V. & Zoller, S. (2000): Integrating ambiguously aligned regions of DNA sequences in phylogenetic analyses without violating positional homology. – Syst. Biol. 49: 628–651.

Magain, J.; Miadlikowska, B.; Goffinet, E.; Sérusiaux, F. & Lutzoni, F. (2017): Macroevolution of Specificity in Cyanolichens of the Genus *Peltigera* Section *Polydactylon* (Lecanoromycetes, Ascomycota). – Syst. Biol. 66: 74–99.

Molinari–Novoa, E.A. – In: Guiry, M. D. & Guiry, G. M. (26 December 2023): *AlgaeBase*. – World–wide electronic publication. National University of Ireland, Galway. http://www.algaebase.org; searched on 24 January 2024.

Muggia, L.; Nelsen, M. P.; Kirika, P. M.; Barreno, E.; Beck, A.; Lindgren, H.: Lumbsch, T. & Leavitt, S. D. (2020): Formally described species woefully underrepresent phylogenetic diversity in the common lichen photobiont genus *Trebouxia* (Trebouxiophyceae, Chlorophyta): An impetus for developing an integrated taxonomy. – Mol. Phylogen. Evol. 149: 106821.

Nash, T. H. (2008): Lichen Biology 2nd ed. – 502 pp., Cambridge University, Cambridge.

Neustupa, J. & Šťastný J. (2006): The geometric morphometric study of Central European species of the genus Micrasterias (Zygnematophyceae, Viridiplantae). – Preslia 78: 253–263.

Neustupa, J.; Šťastný, J. & Hodač, L. (2008): Temperature–related phenotypic plasticity in the green microalga *Micrasterias rotata*. – Aquat. Microb. Ecol. 51: 77–86.

Nübel, U.; Garcia–Pichel, F. & Muyzer, G. (1997): PCR primers to amplify 16S rRNA genes from cyanobacteria. – Appl. Environ. Microbial. 63: 3327–3332.

O'Brien, H. E.; Miadlikowska, J. & Lutzoni, F. (2005): Assessing host specialization in symbiotic cyanobacteria associated with four closely related species of the lichen fungus *Peltigera*. – Eur. J. Phycol. 40: 363–378.

Otálora, M. A. G.; Aragón, G.: Molina, M. C.; Martínez, I. & Lutzoni, F. (2010): Disentangling the *Collema–Leptogium* complex through a molecular phylogenetic study of the Collemataceae (Peltigerales, lichen–forming Ascomycota). – Mycologia 102: 279–290.

Paulsrud, P.; Rikkinen, J. & Lindblad, P. (2020): Spatial patterns of photobiont diversity in some *Nostoc*–containing lichens. – New Phytol. 146: 291–299.

Pietrasiak, N.; Osorio–Santos, K.; Shalygin, S.; Martin, M. P. & Johansen, J. R. (2019): When Is A Lineage A Species? A Case Study In *Myxacorys* gen. nov. (Synechococcales: Cyanobacteria) With The Description of Two New Species From The Americas. – J. Phycol. 55: 976–996.

Rikkinen, J. (2017): Symbiotic cyanobacteria in lichens. – In: Grube, M.; Seckelbach, J. & Muggia, L. (eds): Algae and cyanobacteria in symbiosis. – pp. 147–167, World Scientific, Publishing Europe, London.

Roth, R. & Goodenough, U. (2021): Lichen 1. Solo fungal and algal partners. – Algal Res. 58: 02356.

Saraf, A.; Suradkar, A.; Dawda, H. G.; Gaysina, L. A.; Gabidullin, Y.; Kumat, A.; Behere, I.; Kotulkar, M.; Batule, P. & Singh, P. (2019): Phylogenetic complexities of the members of Rivulariaceae with the re–creation of the family Calotrichaceae and description of *Dulcicalothrix necridiiformans* gen nov., sp nov., and reclassification of *Calothrix desertica*. – FEMS Microbiol. Lett. 366: fnz219.

Sigurbjörnsdóttir, M. A.; Andrésson, O. S. & Vilhelmsson, O. (2015): Analysis of the *Peltigera membranacea* metagenome indicates that lichen–associated bacteria are involved in phosphate solubilization. – Microbiology 161: 989–96.

Stackebrandt, E. & Ebers, J. (2006): Taxonomic parameters revisited: Tarnished gold standards. – Microbiol. today. 33: 152–155.

Stamatakis, A. (2014): RAxML version 8: a tool for phylogenetic analysis and post–analysis of large phylogenies. – Bioinformatics 30: 1312–1313.

Stanojković, A.; Skoupý, S.; Škaloud, P. & Dvořák, P. (2022): High genomic differentiation and limited gene flow indicate recent cryptic speciation within the genus *Laspinema* (cyanobacteria). – Front. Microbiol. 13: 977454.

Steinová, J.; Škaloud, P.; Yahr, R.; Bestová, H. & Muggia, L. (2019): Reproductive and dispersal strategies shape the diversity of mycobiont photobiont association in *Cladonia* lichens. – Mol. Phylogen. Evol. 134: 226–237.

Steinová, J.; Holien, H.; Košuthová, A. & Škaloud, P. (2022): An Exception to the Rule? Could Photobiont Identity Be a Better Predictor of Lichen Phenotype than Mycobiont Identity? – *J. Fungi.* 8: 275.

Strunecký, O.; Pavlovna Ivanova, A. & Mareš, J. (2023): An updated classification of cyanobacterial orders and families based on phylogenomic and polyphasic analysis. – J. Phycol. 59: 12–51.

Škvorová, Z.; Cernajová, I.; Steinová, J.; Peksa, O.; Moya, P. & Škaloud, P. (2022): Promiscuity in Lichens Follows Clear Rules: Partner Switching in *Cladonia* Is Regulated by Climatic Factors and Soil Chemistry. – Front. Microbiol. 12: 781585.

Turland, N. J.; Wiersema, J. H.; Barrie, F. R.; Greuter, W.; Hawksworth, D. L.; Herendeen, P. S.; Knapp, S.; Kusber, W–H.; Li, D–Z.; Marhold, K.; May, T. W.; McNeill, J.; Monro, A. M.; Prado, J.; Price, M. J. & Smith, G. F. (2018): International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017.Regnum Vegetabile 159. – Koeltz Botanical Books, Glashütten. DOI https://doi.org/10.12705/Code.2018.

Villanueva, C. D.; Hašler, P.; Dvořák, P.; Poulíčková, A. & Casamatta, D. A. (2018): *Brasilonema lichenoides* sp. nov. and *Chroococcidiopsis lichenoides* sp. nov. (Cyanobacteria): two novel cyanobacterial constituents isolated from a tripartite lichen of headstones. – J. Phycol. 54: 224–233.

Vančurová, L.; Muggia, L.; Peksa, O.; Řídká, T. & Škaloud, P. (2018): The complexity of symbiotic interactions influences the ecological amplitude of the host: A case study in *Stereocaulon* (lichenized Ascomycota). – Mol. Ecol. 27: 3016–3033.

Vančurová, L.; Malíček, J.; Steinová, J. & Škaloud, P. (2021): Choosing the Right Life Partner: Ecological Drivers of Lichen Symbiosis. – Front. Microbiol. 12: 769304.

West–Eberhard, M. (1989): Phenotypic plasticity and the origins of diversity. – Ann. Rev. Ecol. Syst. 20: 249–278.

Wilmotte, A.; van de Peer, Y.; Goris, A.; Chapelle, S.; De Baere, R.; Nelissen, B.; Neefs, J.–M.; Hennebert, G. L. & De Wachter, R. (1993):Evolutionary relationships among higher fungi inferred from small ribosomal subunit RNA sequence analysis. – System. Appl. Microbiol. 16: 436–444.

Zhang, Z.; Schwartz, S.; Wagner, L. & Miller, W. (2000): A greedy algorithm for aligning DNA sequences. – J. Comput. Biol. 7: 203–214.

Zoller, S.; Scheidegger, Ch. & Sperisen, Ch. (1999): PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen–forming ascomycetes. – Lichenologist 31: 511–516.

Zuker, M. (2003): Mfold web server for nucleic acid folding and hybridization prediction. – Nucleic Acids Res. 31: 3406–3415.

Supplementary material

The following supplementary material is available for this article:

Supplementary Fig. S1. The most likely phylogenetic tree from a RAxML analysis based on 1309 aligned characters.

Supplementary Fig. S2. The lichen *Placynthium nigrum* – picture of holotype S–F453718.

Supplementary Table S3. Similarity matrices comparing the genetic divergence within the Macrochaete genus based on 16S rRNA and 16S–23S ITS gene sequences respectively..

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

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