MycoKeys 96: 97–112 (2023)
doi: 10.3897/mycokeys.96.98029
https://mycokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
MycoKeys
Launched to accelerate biodiversity research

Pseudolepraria, a new leprose genus revealed in Ramalinaceae (Ascomycota, Lecanoromycetes, Lecanorales) to accommodate *Lepraria stephaniana*

Martin Kukwa[1], Magdalena Kosecka[1], Agnieszka Jabłońska[1], Adam Flakus[2], Pamela Rodriguez-Flakus[2], Beata Guzow-Krzemińska[1]

1 *Department of Plant Taxonomy and Nature Conservation, Faculty of Biology, University of Gdańsk, Wita Stwosza 59, PL-80-308 Gdańsk, Poland* **2** *W. Szafer Institute of Botany, Polish Academy of Sciences, Lubicz 46, PL-31-512 Kraków, Poland*

Corresponding authors: Martin Kukwa (martin.kukwa@ug.edu.pl);
Beata Guzow-Krzemińska (beata.guzow-krzeminska@ug.edu.pl)

Academic editor: Thorsten Lumbsch | Received 25 November 2022 | Accepted 25 February 2023 | Published 24 March 2023

Citation: Kukwa M, Kosecka M, Jabłońska A, Flakus A, Rodriguez-Flakus P, Guzow-Krzemińska B (2023) *Pseudolepraria*, a new leprose genus revealed in Ramalinaceae (Ascomycota, Lecanoromycetes, Lecanorales) to accommodate *Lepraria stephaniana*. MycoKeys 96: 97–112. https://doi.org/10.3897/mycokeys.96.98029

Abstract

The new genus *Pseudolepraria* Kukwa, Jabłońska, Kosecka & Guzow-Krzemińska is introduced to accommodate *Lepraria stephaniana* Elix, Flakus & Kukwa. Phylogenetic analyses of nucITS, nucLSU, mtSSU and RPB2 markers recovered the new genus in the family Ramalinaceae with strong support. The genus is characterised by its thick, unstratified thallus composed entirely of soredia-like granules, the presence of 4-*O*-methylleprolomin, salazinic acid, zeorin and unknown terpenoid, and its phylogenetic position. The new combination, *P. stephaniana* (Elix, Flakus & Kukwa) Kukwa, Jabłońska, Kosecka & Guzow-Krzemińska, is proposed.

Keywords

Lichenized fungi, morphology, Neotropics, secondary metabolites, sterile lichens, taxonomy

Introduction

During the evolution in some groups of lichenized fungi the ability to reproduce sexually has been apparently lost completely and some phylogenetic lineages are known to develop exclusively asexual lichenized propagules. This includes *Lepraria* Ach.

(Ascomycota, Lecanoromycetes, Lecanorales, Stereocaulaceae), a well-known genus which up to quite recently comprised only crustose lichens with morphologically simple thalli consisting of soredia-like granules laying directly on substrate or on a layer of hypothalline hyphae (e.g., Ekman and Tønsberg 2002; Kukwa 2002; Sipman 2004; Flakus and Kukwa 2009; Flakus et al. 2011a; Lendemer 2011a, b, 2013a; Lendemer and Hodkinson 2013; Guzow-Krzemińska et al. 2019a). However, Lendemer and Hodkinson (2013) found, based on molecular data, that some fruticose species previously referred to *Leprocaulon* Nyl. also represented *Lepraria* s.str. and they were subsequently transferred to the latter genus. In contrast to their simplified morphology, the species produce a vast variety of secondary lichen metabolites, which are an invaluable tool, together with morphological characters that may be sparse, in the recognition of species and their identification (e.g., Laundon 1989, 1992; Tønsberg 1992; Sipman 2004; Kantvilas and Kukwa 2006; Flakus and Kukwa 2007; Saag et al. 2009; Flakus et al. 2011a; Lendemer 2011a, 2013a; Lendemer and Hodkinson 2013; Guzow-Krzemińska et al. 2019a; Kukwa 2019). It is also noteworthy that some species until recently classified as *Lepraria* have been shown to belong to other genera (e.g., *Leprocaulon* and *Septotrapelia* Aptroot & Chaves; Bungartz et al. 2013; Lendemer and Hodkinson 2013) or even new genera were established for some peculiar species, e.g., *Andreiomyces* Hodkinson & Lendemer within Arthoniomycetes (Hodkinson and Lendemer 2013), *Botryolepraria* Canals et al., related to Verrucariaceae in Eurotiomycetes (Kukwa and Pérez-Ortega 2010) and *Lithocalla* Orange in Lecanorales (probably in Ramalinaceae) in Lecanoromycetes (Orange 2020).

Lepraria includes at present c. 75 species (Wijayawardene et al. 2017; Guzow-Krzemińska et al. 2019a; Barcenas-Peña et al. 2021), most of which were described based on chemical (secondary metabolites) and morphological features and some also by molecular markers (e.g., Laundon 1989, 1992; Tønsberg 1992; Lendemer 2011a, 2012, 2013a; Lendemer and Hodkinson 2013; Guzow-Krzemińska et al. 2019a; Barcenas-Peña et al. 2021). One of the species that was placed in *Lepraria* based solely on morphological similarity to other members of the genus was *L. stephaniana* Elix, Flakus & Kukwa (Flakus et al. 2011a). This species is characterised by the thick, unstratified and non-lobed thallus composed of coarse soredia-like granules with soft appearance, and the production of 4-*O*-methylleprolomin, salazinic acid and terpenoids. 4-*O*-methylleprolomin was known only in a single *Pannaria* species before its discovery in *L. stephaniana* (Flakus et al. 2011a). *Lepraria stephaniana* has been known until recently only from the type locality, however during field studies in 2017 in Bolivia we found two new localities of the species (one close to the type locality) (Guzow-Krzemińska et al. 2019b). Sequencing of molecular markers of those two recently collected specimens revealed that *L. stephaniana* is unrelated to other species of *Lepraria* s.str., but instead it appeared to be nested within Ramalinaceae as a previously unsequenced lineage close to *Cliostomum* Fr., *Ramalina* Ach. and allied genera. In this paper we introduce the new genus *Pseudolepraria* for this peculiar lineage within Ramalinaceae.

Materials and methods

Taxon sampling

The studied specimens are deposited in B, BG, KRAM, LPB, NY and UGDA herbaria. Morphology was examined by using Nikon SMZ 800N stereomicroscope. The secondary chemistry of all samples was studied by thin layer chromatography (TLC) following methods by Culberson and Kristinsson (1970) and Orange et al. (2001a).

DNA extraction, PCR amplification and DNA sequencing

DNA was extracted using a modified CTAB method (Guzow-Krzemińska and Węgrzyn 2000). We analysed four fungal markers: nucITS rDNA, mtSSU rDNA, nucLSU rDNA, and RPB2 gene. For this purpose we used the following primers: ITS1F (Gardes and Bruns 1993) and ITS4A (Kroken and Taylor 2001) for nucITS rDNA; mrSSU1 and mrSSU3R (Zoller et al. 1999) for mtSSU rDNA; ITS4A-5' (Kroken and Taylor 2001; Nelsen et al. 2011) and LR5 (Vilgalys and Hester 1990) for nucLSU rDNA; fRPB2-5F and fRPB2-7cR (Liu et al. 1999) for RPB2 gene. Additionally, nucITS rDNA region from green algal partner was amplified using Al1500bf (Helms et al. 2001) and ITS4M primers (Guzow-Krzemińska 2006). PCR was performed in a volume of 25 µl using StartWarm HS-PCR Mix (A&A Biotechnology) following the manufacturer's protocol. 1 µl of genomic DNA was used for amplification. The PCR cycling parameters are available in Suppl. material 1.

The efficiency of the PCR was checked by visualising the reaction products on a 1% agarose gels stained with SimplySafe (Eurx) dye in order to determine DNA fragment lengths. Afterwards, PCR products were purified using Clean-Up Concentrator (A&A Biotechnology). The sequencing was performed in Macrogen Europe (The Netherlands), using amplification primers. The newly obtained sequences were deposited in GenBank database and their accession numbers are listed in Table 1.

Sequence alignment and phylogenetic analysis

The newly generated sequences were compared to the sequences available in the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/) using BLASTn search (Altschul et al. 1990). For the phylogenetic analyses we used representatives of Ramalinaceae and *Boreoplaca ultrafrigida* Timdal and *Ropalospora lugubris* (Sommerf.) Poelt were used as outgroup taxa according to previous studies (Kistenich et al. 2018; Orange 2020; van den Boom and Magain 2020). The independent alignments for each marker were generated in MAFFT using auto option and default parameters (Katoh and Standley 2013). The datasets were then subjected to Guidance2 server (Landan and Graur 2008; Penn et al. 2010; Sela et al. 2015; http://guidance.tau.ac.il/) for further analysis. The MSA algorithm was set to MAFFT and 100 bootstrap replicates were used.

Table 1. Species used in this study with their GenBank accession numbers. New sequences are marked in bold.

Species	nucITS rDNA	nucLSU rDNA	mtSSU	RPB2	Algal nucITS rDNA
Aciculopsora salmonea	MG925948	–	MG925842	–	
Aciculopsora srilankensis	MK400258	–	MK400211	–	
Bacidia arceutina	AF282083	MG926041	MG925846	MG926230	
Bacidia rosella	AF282086	AY300829	AY300877	AM292755	
Bacidina arnoldiana	AF282093	MG926048	MG925854	MG926238	
Bacidina phacodes	AF282100	MG926049	AY567725	MG926240	
Badimia dimidiata	MG925956	MG926052	AY567774	–	
Bellicidia incompta	AF282092	MG926043	MG925849	MG926233	
Biatora globulosa	AF282073	MG926055	KF662414	KF662450	
Biatora vacciniicola	MG925960	MG926060	MG925861	MG926245	
Biatora vernalis	AF282070	DQ838752	DQ838753	–	
Bibbya albomarginata	MG926024	MG926115	MG925927	MG926286	
Bibbya vermifera	AF282109	MG926047	MG925852	MG926237	
Bilimbia sabuletorum	AM292670	AY756346	AY567721	AM292761	
Boreoplaca ultrafrigida	HM161512	DQ986797	DQ986813	DQ992421	
Catillaria scotinodes	AM292673	MG926064	AM292720	AM292763	
Catinaria atropurpurea	MG925965	MG926065	MG925865	MG926246	
Catolechia wahlenbergii	HQ650649	DQ986794	DQ986811	DQ992424	
Cenozosia inanis	–	MG926066	MG925866	–	
Cliomegalaria symmictoides	MW622003	MW621867	MW622006	–	
Cliostomum corrugatum	MG925966	MG926067	AY567722	KF662436	
Cliostomum haematommatis	MK446224	–	MK446223	–	
Eschatogonia prolifera	MG925969	MG926070	MG925870	MG926249	
Kiliasia athallina	MG926023	MG926114	–	MG926284	
Kiliasia sculpturata	MG926034	MG926122	MG925938	MG926295	
Krogia coralloides	MG925977	MG926072	MG925875	MG926251	
Lecania aipospila	MG925978	MG926073	MG925876	MG926252	
Lecania erysibe	AM292682	MG926074	AM292733	AM292769	
Lecania fuscella	AM292684	MG926075	MG925877	–	
Lecidea albohyalina	KF650950	MG926079	KF662398	KF662438	
Lithocalla ecorticata	KT962179	–	KT962184	–	
Lithocalla malouina	KT962178	–	MT857015	–	
Lueckingia polyspora	MG925984	MG926082	MG925882	–	
Megalaria grossa	AF282074	MG926083	MG925883	MG926257	
Megalaria versicolor	–	AY584651	AY584622	DQ912401	
Mycobilimbia pilularis	KF650954	–	KF662402	KF662442	
Mycobilimbia tetramera	–	KJ766600	KJ766439	KJ766957	
Niebla homalea	MG925987	–	MG925888	–	
Namibialina melanothrix	MG926038	MG926128	MG925945	MG926303	
Parallopsora brakoae	MG925989	–	MG925891	–	
Parallopsora leucophyllina	MG925994	–	MG925897	MG926265	
Phyllopsora breviuscula	MG925990	MG926087	MG925892	MG926262	
Phyllopsora gossypina	MG925967	MG926068	MG925867	MG926247	
Phyllopsora parvifoliella	MG925999	MG926092	MG925902	MG926267	
Physcidia wrightii	MN334233	–	MN334227	–	
***Pseudolepraria stephaniana* Kukwa 19740**	**OQ172237**	**OQ172242**	**OQ172251**	**–**	**OQ303855**
***Pseudolepraria stephaniana* Kukwa 19267**	**OQ172236**	**OQ172243**	**OQ172250**	**OQ160272**	**OQ303854**
Ramalina dilacerata	MG926013	MG926104	MG925917	–	
Ramalina fraxinea	MG926014	MG926105	MG925918	MG926277	
Ramalina mannii	MG926019	MG926111	–	MG926280	
Ramalina pollinaria	MG926017	MG926108	AM292752	MG926278	
Ramalina sinensis	MG926018	MG926110	MG925921	–	

Species	nucITS rDNA	nucLSU rDNA	mtSSU	RPB2	Algal nucITS rDNA
Rolfidium bumammum	MG926027	MG926117	MG925930	MG926288	
Ropalospora lugubris	MG926020	–	MG925922	–	
Scutula circumspecta	–	–	MG925848	–	
Sporacestra pertexta	MG926000	MG926093	MG925903	MG926268	
Stirtoniella kelica	MG926021	–	MG925923	–	
Thalloidima candidum	AF282117	MG926118	MG925932	MG926290	
Thalloidima toninianum	MG926036	MG926124	MG925942	MG926298	
Thamnolecania brialmontii	AF282066	MG926112	MG925925	MG926283	
Toninia cinereovirens	AF282104	AY756365	AY567724	AM292781	
Toninia populorum	MG925950	MG926039	MG925843	MG926228	
Toniniopsis aromatica	AF282126	MG926113	MG925926	MG926284	
Toniniopsis subincompta	AF282125	MG926046	MG925851	MG926236	
Tylocliostomum viridifarinosum	NR_174049	–	–	–	
Tylothallia biformigera	AF282077	MG926129	MG925946	MG926304	
Waynea californica	–	MG926130	MG925947	MG926305	
Vermilacinia breviloba	MN811352	MN811548	–	MN757330	

The Guidance confidence scores were calculated and columns with a score < 0.93 were excluded from the alignments. The terminal ends were trimmed. Single-locus matrices consisted of 61 sequences for nucITS, 62 sequences for mtSSU, 51 sequences for nucLSU, and 44 sequences for RPB2. The best ML tree was inferred for each locus using IQ-TREE with 1000 ultrafast bootstrap replicates as implemented in the IQ-TREE web server (Nguyen et al. 2015; Chernomor et al. 2016; Kalyaanamoorthy et al. 2017; Hoang et al. 2018). Congruence was examined by eye and no significant conflict between loci was observed.

For the final analysis, we concatenated four markers which resulted in a dataset of 66 terminals and 3766 positions. The concatenated dataset was subjected to IQ-TREE analysis to find best-fitting nucleotide substitution models (Nguyen et al. 2015; Chernomor et al. 2016; Kalyaanamoorthy et al. 2017; Hoang et al. 2018). The model selection was restricted to models implemented in MrBayes and the following nucleotide substitution models for the four predefined subsets were selected: GTR+F+I+G for mtSSU rDNA, K2P+I+G for nucITS, and SYM+I+G for both nucLSU rDNA and RPB2 markers. The search for maximum likelihood tree was performed in IQ-TREE and followed with 1000 standard bootstrap replicates (Nguyen et al. 2015; Chernomor et al. 2016; Kalyaanamoorthy et al. 2017; Hoang et al. 2018).

Bayesian analysis was carried out using a Markov Chain Monte Carlo (MCMC) method, in MrBayes v. 3.2.6 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) on the CIPRES Web Portal (Miller et al. 2010) using previously selected models (see above). Two parallel MCMC runs were performed, each using four independent chains and ten million generations, sampling every 1000th tree. The resulting log files were analysed using Tracer 1.7.2 (Rambaut et al. 2018). Posterior probabilities (PP) were determined by calculating a majority-rule consensus tree after discarding the initial 25% trees of each chain as the burn-in. The convergence of the chains was confirmed by the convergent diagnostic of the Potential Scale Reduction Factor

(PSRF), which approached 1 and the 'average standard deviation of split frequencies' was < 0.01).

Phylogenetic trees were visualised using FigTree v. 1.4.3 (Rambaut 2009) and modified in Inkscape (https://inkscape.org/). Bootstrap support (BS values ≥ 70) and PP values (values ≥ 0.95) are given near the branches on the phylogenetic tree. The data were deposited at TreeBASE (Submission ID: 30149).

Results and discussion

For this work we successfully sequenced nucITS, mtSSUand nucLSU from two specimens and additionally RPB2 from one specimen of *Lepraria stephaniana* collected in Bolivia (Table 1). BLAST searches of the nucITS, nucLSU, mtSSU and RPB2 markers surprisingly showed the highest similarities to representatives of the family Ramalinaceae, i.e., the genera *Cenozosia* A. Massal., *Cliostomum* and *Ramalina*. Phylogenetic analysis of the concatenated dataset shows that *L. stephaniana* is nested inside Ramalinaceae. The newly sequenced specimens of the species were resolved in a distinct and highly supported clade sister to a clade consisting of *Cliostomum* s.str. represented by the type species *C. corrugatum* (Ach.) Fr., *Cenozosia inanis* (Mont.) A. Massal. and a subclade of several species of *Ramalina*, *Namibialina melanothrix* (Laurer) Spjut & Sérus., *Niebla* (Ach.) Rundel & Bowler and *Vermilacinia breviloba* Spjut & Sérus. (Fig. 1). A new monotypic genus, *Pseudolepraria*, is introduced for this lineage of *Lepraria stephaniana* and is characterised by a thick, unstratified thallus composed of soredia-like granules, and the presence of 4-*O*-methylleprolomin, salazinic acid, zeorin and unknown terpenoid.

Pseudolepraria is the first genus forming leprose and sterile thalli that can be placed with high support within Ramalinaceae. Orange (2020) described the genus *Lithocalla* and placed it with uncertainty in Ramalinaceae. In our phylogeny, *Lithocalla* forms the sister group to Ramalinaceae sensu Kistenich et al. (2018), but this may be an artifact of the taxon sampling. The particular placement of the genus was beyond the scope of this study. *Lithocalla* was introduced for two species, which were originally placed, due to morphological similarities, in *Lepraria*, i.e., *L. ecorticata* (J. R. Laundon) Kukwa and *L. malouina* Øvstedal (Kukwa 2006; Fryday and Øvstedal 2012). Both, *Lithocalla ecorticata* (J. R. Laundon) Orange known from Great Britain and Norway, and *L. malouina* (Øvstedal) Fryday & Orange found in the Falkland Islands (Fryday and Øvstedal 2012; Orange 2020), differ from *Pseudolepraria stephaniana*, in their distribution in colder climates, by the production of usnic and fatty acids, the absence of zeorin and the exclusively saxicolous habitat (Orange 2020).

Species resembling *Pseudolepraria* in the Ramalinaceae have up until recently been included in *Crocynia* (Ach.) A. Massal. This genus was established for lichens with a non-corticate, byssoid, felt-like thallus and historically included several species now placed mostly in *Lepraria* (e.g., Laundon 1989, 1992; Kistenich et al. 2018). According to Lücking et al. (2017) *Crocynia* comprised three species and two of them were included in the phylogeny of Ramalinaceae by Kistenich et al. (2018), where they

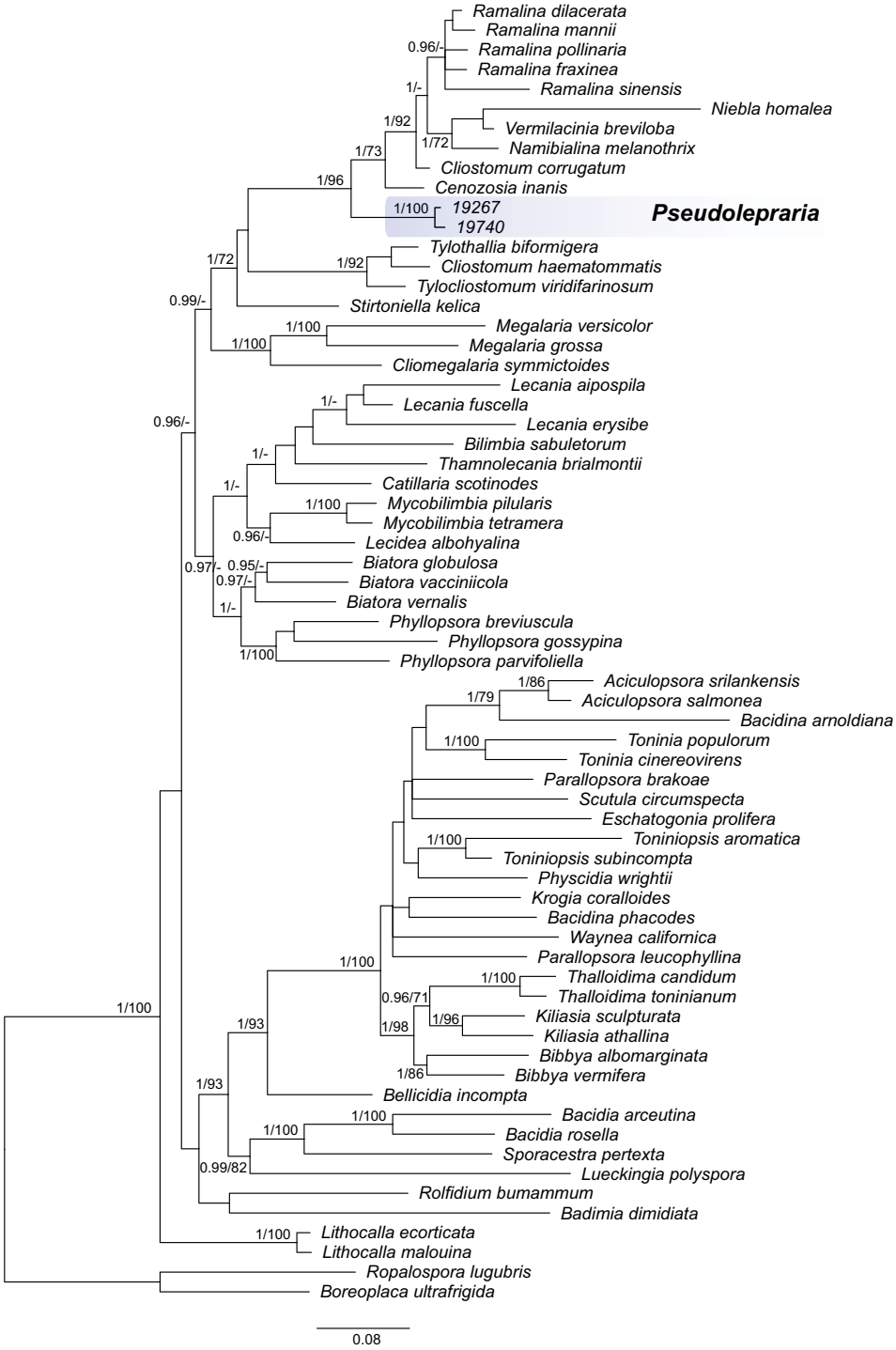

Figure 1. Majority-rule consensus tree resulting from MrBayes analysis of the concatenated mtSSU, nucITS, nucLSU and RPB2 markers with Bayesian PP (values ≥ 0.95) and IQ-TREE bootstrap support values (BS values ≥ 70) given near the branches. *Pseudolepraria* is marked in blue.

formed a clade nested inside *Phyllopsora* Müll. Arg. Consequently, *Crocynia* was synonymised with *Phyllopsora*, also because of morphological similarities (Kistenich et al. 2018). The status of the third species, *C. microphyllina* Aptroot (Lumbsch et al. 2011), and three species discussed by Sipman (2018) remains uncertain. The species historically placed in *Crocynia* differ from *Pseudolepraria* in the byssoid thalli not producing 4-*O*-methylleprolomin and in sometimes producing apothecia (Cáceres 2007; Lumbsch et al. 2011; Aptroot and Cáceres 2014; Sipman 2018).

Pseudolepraria is very similar to *Lepraria* s.str. in sharing the same thallus morphology and, to a certain extent, secondary chemistry (presence of salazinic acid and terpenoids) (e.g., Aptroot 2002; Sipman 2004; Saag et al. 2009; Flakus et al. 2011a; Lendemer 2011a, b). They differ, apart from the phylogenetic position, only in the presence of the very rarely reported 4-*O*-methylleprolomin, a diphenyl ether previously found only in one *Pannaria* species (Flakus et al. 2011a). *Pseudolepraria* differs also in the habitat preferences. It was found only in tropical forests at low elevations (300–470 m a.s.l.), whereas *Lepraria* in tropical South America, including Bolivian ecosystems, are found mostly above 1000 m above sea level (only one locality of *L. finkii* (B. de Lesd.) R.C. Harris found at the elevation of 890 m), in montane forests and open high Andean vegetation (Flakus and Kukwa 2007; Flakus et al. 2011a, b, 2012, 2015; Guzow-Krzemińska et al. 2019a). This is in agreement with the statement presented by Orange et al (2001b), who considered *Lepraria* to be restricted to montane habitats in the tropics.

Poelt (1987) considered the genus *Lepraria* as a 'box of analogous groups of lichens of completely different origin, held together by the same highly specialized thallus type'. Poelt (1987) also stated that the leprarioid thallus type and the loss of generative reproduction developed in evolution through the reduction of the thallus structures as an adaptation for growing in bark crevices and on over-hanging rocks in ecologically specialised group of lichenized fungi, which includes *Lepraria*, but also, as Poelt (1987) mentioned, *Leproplaca* (Nyl.) Nyl. and some species of the genus *Chrysothrix* Mont. (Poelt 1987). However, this is only partly true, as some lichen groups with this type of thallus (e.g., species of *Lepraria neglecta* group) can grow also in other habitats (e.g., Laundon 1992; Lendemer 2013b). Nevertheless, the statement of Poelt (1987) was true and innovative at this time and it was later shown that the leprarioid thallus indeed originated in several unrelated lichen lineages (e.g., Ekman and Tønsberg 2002; Kukwa and Pérez-Ortega 2010; Hodkinson and Lendemer 2013; Malíček et al. 2018; Guzow-Krzemińska et al. 2017; Orange 2020). Furthermore, some leprarioid genera are known exclusively in sterile state, like *Andreiomyces* (Arthoniales, Arthoniomycetes), *Botryolepraria* (Verrucariales, Eurotiomycetes), *Lepraria* and *Lithocalla* (both in Lecanorales, Lecanoromycetes) (Ekman and Tønsberg 2002; Kukwa and Pérez-Ortega 2010; Hodkinson and Lendemer 2013; Orange 2020). *Pseudolepraria* is another addition to this group, however, as only a few collections are available, it may eventually be found with ascomata.

Buschbom and Mueller (2006) suggested that the asexual way of reproduction is advantageous because the symbiosis with the optimal photobiont for a given environment allows the rapid dissemination of both partners. Therefore, it is more important for the mycobiont to keep the relationship with suitable algal species; however this does not mean that the symbiosis in asexually reproducing species cannot be broken.

Kosecka et al. (2021) showed for some *Lepraria* species that the mycobiont can form thalli with different, locally adapted algal strains. We partially sequenced the nucITS region of the photobiont of *Pseudolepraria stephaniana* (Table 1) and found that both thalli associate with the same green algal partner (100% of identity). BLAST hits were closest to *Symbiochloris*, *Dictyochloropsis*, *Massjukichlorella* and *Watanabea* spp., all of which were quite dissimilar to the photobiont sequences of *Pseudolepraria stephaniana*.

Taxonomy

Pseudolepraria **Kukwa, Jabłońska, Kosecka & Guzow-Krzemińska, gen. nov.**
MycoBank No: 847408

Diagnosis. Characterised by thick, unstratified thallus composed of soredia-like granules, the presence of 4-*O*-methylleprolomin, salazinic acid, zeorin, and unknown terpenoid, and the phylogenetic position within Ramalinaceae.

Generic type. *Pseudolepraria stephaniana* (Elix, Flakus & Kukwa) Kukwa, Jabłońska, Kosecka & Guzow-Krzemińska

Description. As this is a monotypic genus the description below constitutes the generic description.

Etymology. The new name refers to the similarity to the genus *Lepraria*, in which this particular species was originally placed.

Pseudolepraria stephaniana **(Elix, Flakus & Kukwa) Kukwa, Jabłońska, Kosecka & Guzow-Krzemińska, comb. nov.**
MycoBank No: 847409
Fig. 2

Lepraria stephaniana Elix, Flakus & Kukwa, in Flakus et al., Lichenologist 43: 64, 2011 (2010). Basionym.

Type. Bolivia. Dept. La Paz: Prov. Iturralde, between Ixiamas and Santa Rosa de Maravillas villages, elev. 305 m, 13°49'16"S, 68°07'18"W, preandean Amazon forest, on bark of tree, 28 July 2008, M. Kukwa 6828 (holotype: UGDA L!; isotypes: B!, BG!, KRAM!, LPB!, NY!).

Description (adopted from Flakus et al. 2011a). Thallus crustose, thick, usually not delimited nor lobed, green-grey to creamy-white, not stratified, but sometimes with a poorly differentiated, pseudo-medullary layer of decaying granules. Hypothallus indistinct. Granules coarse with soft appearance, irregularly rounded, up to 100(–200) μm in diam., composed of very lax hyphae mixed with algal cells, usually with projecting hyphae up to c. 30(–50) μm long. Photobiont green, cells globose, up to 12 μm in diam.

Chemistry. Substances detected: 4-*O*-methylleprolomin (major), salazinic acid (minor), zeorin (minor) and an unknown terpenoid (minor) with Rf class values A6,

Figure 2. Morphology of *Pseudolepraria stephaniana* (type). Scale bar: 0.5 mm.

B6, C6. Thallus reactions: K+ yellow turning brownish to red, P+ yellow, C–, KC– (see also Flakus et al. 2011a).

Distribution and habitat. The species is known only from three localities in Bolivia. It was found on bark of trees in transition Chaqueño-Amazon or preandean Amazon forests at elevation between c. 300 to 470 m a.s.l. (Flakus et al. 2011a; Guzow-Krzemińska et al. 2019b).

Specimens used for DNA extraction (Table 1). Bolivia. Dept. La Paz: Prov. Abel Iturralde, between Santa Rosa de Maravillas and Tumupasa, 13°58'43"S, 67°58'14"W, elev. 300 m, natural Preandean Amazon forest, corticolous, 25 May 2017, M. Kukwa 19740 (LPB, UGDA). Dept. Santa Cruz: Prov. Ichilo, Parque Nacional y Área Natural de Manejo Integrado Amboró, Sendero a la Cascada, near Villa Amboró, 17°44'02"S, 63°35'05"W, elev. 470 m, transition Chaqueño-Amazon forest, in the valley, corticolous, 11 May 2017, M. Kukwa 19267 (LPB, UGDA).

Acknowledgements

We are very grateful to James C. Lendemer (New York Botanical Garden), Tor Tønsberg (University of Bergen) and an anonymous reviewer for invaluable comments,

and the members of Herbario Nacional de Bolivia, Instituto de Ecología, Universidad Mayor de San Andrés, La Paz, for their generous cooperation. This research received funding from the National Science Centre (project no 2015/17/B/NZ8/02441).

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. Journal of Molecular Biology 215(3): 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Aptroot A (2002) New and interesting lichens and lichenicolous fungi in Brazil. Fungal Diversity 9: 15–45.

Aptroot A, Cáceres MES (2014) A key to the corticolous microfoliose, foliose and related crustose lichens from Rondônia, Brazil, with the description of four new species. Lichenologist 46(6): 783–799. https://doi.org/10.1017/S0024282914000358

Barcenas-Peña A, Diaz R, Grewe F, Widhelm T, Lumbsch HT (2021) Contributions to the phylogeny of *Lepraria* (Stereocaulaceae) species from the Southern Hemisphere, including three new species. The Bryologist 124(4): 494–505. https://doi.org/10.1639/0007-2745-124.4.494

Bungartz F, Hillmann G, Kalb K, Elix JA (2013) Leprose and leproid lichens of the Galapagos, with a particular focus on *Lepraria* (Stereocaulaceae) and *Septotrapelia* (Pilocarpaceae). Phytotaxa 150(1): 1–28. https://doi.org/10.11646/phytotaxa.150.1.1

Buschbom J, Mueller GM (2006) Testing "species pair" hypotheses: Evolutionary processes in the lichen-forming species complex *Porpidia flavocoerulescens* and *Porpidia melinodes*. Molecular Biology and Evolution 23(3): 574–586. https://doi.org/10.1093/molbev/msj063

Cáceres MES (2007) Corticolous crustose and microfoliose lichens of northeastern Brazil. Libri Botanici 22: 1–168.

Chernomor O, von Haeseler A, Minh BQ (2016) Terrace aware data structure for phylogenomic inference from supermatrices. Systematic Biology 65(6): 997–1008. https://doi.org/10.1093/sysbio/syw037

Culberson CF, Kristinsson H (1970) A standardized method for the identification of lichen products. Journal of Chromatography A 46: 85–93. https://doi.org/10.1016/S0021-9673(00)83967-9

Ekman S, Tønsberg T (2002) Most species of *Lepraria* and *Leproloma* form a monophyletic group closely related to *Stereocaulon*. Mycological Research 106(11): 1262–1276. https://doi.org/10.1017/S0953756202006718

Flakus A, Kukwa M (2007) New species and records of *Lepraria* (Stereocaulaceae, lichenized Ascomycota) from South America. Lichenologist 39(5): 463–474. https://doi.org/10.1017/S0024282907007116

Flakus A, Kukwa M (2009) *Lepraria glaucosorediata* sp. nov. (Stereocaulaceae, lichenized Ascomycota) and other interesting records of *Lepraria*. Mycotaxon 108(1): 353–364. https://doi.org/10.5248/108.353

Flakus A, Elix JA, Rodriguez P, Kukwa M (2011a) New species and records of *Lepraria* (Stereocaulaceae, lichenized Ascomycota) from South America. Lichenologist 43(1): 57–66. https://doi.org/10.1017/S0024282910000502

Flakus A, Oset M, Jabłońska A, Rodriguez Saavedra P, Kukwa M (2011b) Contribution to the knowledge of the lichen biota of Bolivia. 3. Polish Botanical Journal 56(2): 159–183.

Flakus A, Etayo J, Schiefelbein U, Ahti T, Jabłońska A, Oset M, Bach K, Rodriguez Saavedra P, Kukwa M (2012) Contribution to the knowledge of the lichen biota of Bolivia. 4. Polish Botanical Journal 7(2): 427–461.

Flakus A, Sipman HJM, Rodriguez Flakus P, Jabłońska A, Oset M, Meneses QRI, Kukwa M (2015) Contribution to the knowledge of the lichen biota of Bolivia. 7. Polish Botanical Journal 60(1): 81–98. https://doi.org/10.1515/pbj-2015-0001

Fryday AM, Øvstedal DO (2012) New species, combinations and records of lichenized fungi from the Falkland Islands (Islas Malvinas). Lichenologist 44(4): 483–500. https://doi.org/10.1017/S0024282912000163

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Molecular Ecology 2(2): 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Guzow-Krzemińska B (2006) Photobiont flexibility in the lichen *Protoparmeliopsis muralis* as revealed by ITS rDNA analyses. Lichenologist 38(5): 469–476. https://doi.org/10.1017/S0024282906005068

Guzow-Krzemińska B, Węgrzyn G (2000) Potential use of restriction analysis of PCR-amplified DNA fragments in taxonomy of lichens. Mycotaxon 76: 305–313.

Guzow-Krzemińska B, Malíček J, Tønsberg T, Oset M, Łubek A, Kukwa M (2017) *Lecanora stanislai*, a new, sterile, usnic acid containing lichen species from Eurasia and North America. Phytotaxa 329(3): 201–211. https://doi.org/10.11646/phytotaxa.329.3.1

Guzow-Krzemińska B, Jabłońska A, Flakus A, Rodriguez-Flakus P, Kosecka M, Kukwa M (2019a) Phylogenetic placement of *Lepraria cryptovouauxii* sp. nov. (Lecanorales, Lecanoromycetes, Ascomycota) with notes on other *Lepraria* species from South America. MycoKeys 53: 1–22. https://doi.org/10.3897/mycokeys.53.33508

Guzow-Krzemińska B, Flakus A, Kosecka M, Jabłońska A, Rodriguez-Flakus P, Kukwa M (2019b) New species and records of lichens from Bolivia. Phytotaxa 397(4): 257–279. https://doi.org/10.11646/phytotaxa.397.4.1

Helms G, Friedl T, Rambold G, Mayrhofer H (2001) Identification of photobionts from the lichen family Physciaceae using algal-specific ITS rDNA sequencing. Lichenologist 33(1): 73–86. https://doi.org/10.1006/lich.2000.0298

Hoang DT, Chernomor O, Von Haeseler A, Minh BQ, Vinh LS (2018) UFBoot2: Improving the ultrafast bootstrap approximation. Molecular Biology and Evolution 35(2): 518–522. https://doi.org/10.1093/molbev/msx281

Hodkinson BP, Lendemer JC (2013) Next-generation sequencing reveals sterile crustose lichen phylogeny. Mycosphere 4(6): 1028–1039. https://doi.org/10.5943/mycosphere/4/6/1

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17(8): 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods 14(6): 587–589. https://doi.org/10.1038/nmeth.4285

Kantvilas G, Kukwa M (2006) A new species of *Lepraria* (lichenized Ascomycetes) from Tasmania's wet forests. Muelleria 23: 3–6. https://doi.org/10.5962/p.291578

Katoh K, Standley DM (2013) MAFFT: multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010

Kistenich S, Timdal E, Bendiksby M, Ekman S (2018) Molecular systematics and character evolution in the lichen family Ramalinaceae (Ascomycota: Lecanorales). Taxon 67(5): 871–904. https://doi.org/10.12705/675.1

Kosecka M, Guzow-Krzemińska B, Černajová I, Škaloud P, Jabłońska A, Kukwa M (2021) New lineages of photobionts in Bolivian lichens expand our knowledge on habitat preferences and distribution of *Asterochloris* algae. Scientific Reports 11(1): e8701. https://doi.org/10.1038/s41598-021-88110-0

Kroken S, Taylor JW (2001) A gene genealogical approach to recognize phylogenetic species boundaries in the lichenized fungus *Letharia*. Mycologia 93(1): 38–53. https://doi.org/10.1080/00275514.2001.12061278

Kukwa M (2002) Taxonomic notes on the lichen genera *Lepraria* and *Leproloma*. Annales Botanici Fennici 39: 225–226.

Kukwa M (2006) Notes on taxonomy and distribution of the lichen species *Lepraria ecorticata* comb. nov. Mycotaxon 97: 63–66.

Kukwa M (2019) *Lepraria juanfernandezii*, a new lichen species from the Southern Hemisphere. Plant and Fungal Systematics 64(2): 233–235. https://doi.org/10.2478/pfs-2019-0019

Kukwa M, Pérez-Ortega S (2010) A second species of *Botryolepraria* from the Neotropics and the phylogenetic placement of the genus within Ascomycota. Mycological Progress 9(3): 345–351. https://doi.org/10.1007/s11557-009-0642-0

Landan G, Graur D (2008) Local reliability measures from sets of co-optimal multiple sequence alignments. Pacific Symposium on Biocomputing 13: 15–24.

Laundon JR (1989) The species of *Leproloma*-the name for the *Lepraria membranacea* group. Lichenologist 21(1): 1–22. https://doi.org/10.1017/S0024282989000034

Laundon JR (1992) *Lepraria* in the British Isles. Lichenologist 24(4): 315–350. https://doi.org/10.1017/S002428299200046X

Lendemer JC (2011a) A taxonomic revision of the North American species of *Lepraria* s.l. that produce divaricatic acid, with notes on the type species of the genus *L. incana*. Mycologia 103(6): 1216–1229. https://doi.org/10.3852/11-032

Lendemer JC (2011b) A standardized morphological terminology and descriptive scheme for *Lepraria* (Stereocaulaceae). Lichenologist 43(5): 379–399. https://doi.org/10.1017/S0024282911000326

Lendemer JC (2012) Perspectives on chemotaxonomy: Molecular data confirm the existence of two morphologically distinct species within a chemically defined *Lepraria caesiella* (Stereocaulaceae). Castanea 77(1): 89–105. https://doi.org/10.2179/11-042

Lendemer JC (2013a) A monograph of the crustose members of the genus *Lepraria* Ach. s. str. (Stereocaulaceae, Lichenized Ascomycetes) in North America north of Mexico. Opuscula Philolichenum 12(1): 27–141.

Lendemer JC (2013b) Shifting paradigms in the taxonomy of lichenized fungi: Molecular phylogenetic evidence corroborates morphology but not chemistry in the *Lepraria neglecta* group. Memoirs of the New York Botanical Garden 108: 127–153.

Lendemer JC, Hodkinson BP (2013) A radical shift in the taxonomy of *Lepraria* s.l.: Molecular and morphological studies shed new light on the evolution of asexuality and lichen growth form diversification. Mycologia 105(4): 994–1018. https://doi.org/10.3852/12-338

Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: Evidence from an RNA Polymerase II Subunit. Molecular Biology and Evolution 16(12): 1799–1808. https://doi.org/10.1093/oxfordjournals.molbev.a026092

Lücking R, Hodkinson BP, Leavitt SD (2017 [2016]) The 2016 classification of lichenized fungi in the Ascomycota and Basidiomycota – Approaching one thousand genera. The Bryologist 119(4): 361–416. https://doi.org/10.1639/0007-2745-119.4.361

Lumbsch HT, Ahti T, Altermann S, Amo De Paz G, Aptroot A, Arup U, Bárcenas Peña A, Bawingan PA, Benatti MN, Betancourt L, Björk CR, Boonpragob K, Brand M, Bungartz F, Cáceres MES, Candan M, Chaves JL, Clerc P, Common R, Coppins BJ, Crespo A, Dal-Forno M, Divakar PK, Duya MV, Elix JA, Elvebakk A, Fankhauser JD, Farkas E, Itatí Ferraro L, Fischer E, Galloway DJ, Gaya E, Giralt M, Goward T, Grube M, Hafellner J, Hernández M JE, Herrera Campos MA, Kalb K, Kärnefelt I, Kantvilas G, Killmann D, Kirika P, Knudsen K, Komposch H, Kondratyuk S, Lawrey JD, Mangold A, Marcelli MP, McCune B, Messuti MI, Michlig A, Miranda González R, Moncada B, Naikatini A, Nelsen MP, Øvstedal DO, Palice Z, Papong K, Parnmen S, Pérez-Ortega S, Printzen C, Rico VJ, Rivas Plata E, Robayo J, Rosabal D, Ruprecht U, Salazar Allen N, Sancho L, Santos De Jesus L, Santos Vieira T, Schultz M, Seaward MRD, Sérusiaux E, Schmitt I, Sipman HJM, Sohrabi M, Søchting U, Søgaard MZ, Sparrius LB, Spielmann A, Spribille T, Sutjaritturakan J, Thammathaworn A, Thell A, Thor G, Thüs H, Timdal E, Truong C, Türk R, Umaña Tenorio L, Upreti, DK van den Boom P, Vivas Rebuelta M, Wedin M, Will-Wolf S, Wirth V, Wirtz N, Yahr R, Yeshitela K, Ziemmeck F, Wheeler T, Lücking R (2011) One hundred new species of lichenized fungi: a signature of undiscovered global diversity. Phytotaxa 18: 1–127. https://doi.org/10.11646/phytotaxa.18.1.1

Malíček J, Palice Z, Vondrák J, Łubek A, Kukwa M (2018) *Bacidia albogranulosa* (Ramalinaceae, lichenized Ascomycota), a new sorediate lichen from European old-growth forests. MycoKeys 44: 51–62. https://doi.org/10.3897/mycokeys.44.30199

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. 2010 Gateway Computing Environments Workshop (GCE). 14 Nov. 2010. New Orleans Convention Center, New Orleans, 8 pp. https://doi.org/10.1109/GCE.2010.5676129

Nelsen MP, Lücking R, Mbatchou JS, Andrew CJ, Spielmann AA, Lumbsch TH (2011) New insights into relationships of lichen-forming Dothideomycetes. Fungal Diversity 51(1): 155–162. https://doi.org/10.1007/s13225-011-0144-7

Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300

Orange A (2020) *Lithocalla* (Ascomycota, Lecanorales), a new genus of leprose lichens containing usnic acid. Lichenologist 52(6): 425–435. https://doi.org/10.1017/S0024282920000419

Orange A, James PW, White FJ (2001a) Microchemical Methods for the Identification of Lichens. British Lichen Society, London, 101 pp.

Orange A, Wolseley P, Karunaratne V, Bombuwala K (2001b) Two leprarioid lichens new to Sri Lanka. Bibliotheca Lichenologica 78: 327–333.

Penn O, Privman E, Ashkenazy H, Landan G, Graur D, Pupko T (2010) GUIDANCE: a web server for assessing alignment confidence scores. Nucleic Acids Research 38: W23–W28. https://doi.org/10.1093/nar/gkq443

Poelt J (1987) On the reduction of morphological structures in lichens. Bibliotheca Lichenologica 25: 35–45.

Rambaut A (2009) FigTree ver. 1.4.3. http://tree.bio.ed.ac.uk/software/figtree [Accessed 4 Oct. 2016]

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67(5): 901–904. https://doi.org/10.1093/sysbio/syy032

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19(12): 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Saag L, Saag A, Randlane T (2009) World survey of the genus *Lepraria* (Stereocaulaceae, lichenized Ascomycota). Lichenologist 41(1): 25–60. https://doi.org/10.1017/S0024282909007993

Sela I, Ashkenazy H, Katoh K, Pupko T (2015) GUIDANCE2: accurate detection of unreliable alignment regions accounting for the uncertainty of multiple parameters. Nucleic Acids Research 43: W7–W14. https://doi.org/10.1093/nar/gkv318

Sipman HJM (2004) Survey of *Lepraria* species with lobed thallus margins in the tropics. Herzogia 17: 23–35.

Sipman HJM (2018) Three new lichen species and 48 new records from Vanuatu. Australasian Lichenology 82: 106–129.

Tønsberg T (1992) The sorediate and isidiate, corticolous, crustose lichens in Norway. Sommerfeltia 14(1): 1–331. https://doi.org/10.2478/som-1992-0002

van den Boom P, Magain N (2020) Three new lichen species from Macaronesia belonging in Ramalinaceae, with the description of a new genus. Plant and Fungal Systematics 65(1): 167–175. https://doi.org/10.35535/pfsyst-2020-0011

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172(8): 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Wijayawardene NN, Hyde KD, Rajeshkumar KC, Hawksworth DL, Madrid H, Kirk PM, Braun U, Singh RV, Crous PW, Kukwa M, Lücking R, Kurtzman CP, Yurkov A, Haelewaters D, Aptroot A, Lumbsch HT, Timdal E, Ertz D, Etayo J, Phillips AJL, Groenewald JZ, Papizadeh M, Selbmann L, Dayarathne MC, Weerakoon G, Jones EBG, Suetrong S, Tian Q, Castañeda-Ruiz RF, Bahkali AH, Pang K-L, Tanaka K, Qin DD, Sakayaroj J, Hujslová M, Lombard L, Shenoy BD, Suija A, Maharachchikumbura SSN, Thambugala KM, Wanasinghe DN, Sharma BO, Gaikwad S, Pandit G, Zucconi L, Onofri S, Egidi E, Raja HA, Kodsueb R, Cáceres MES, Pérez-Ortega S, Fiuza PO, Monteiro SJ, Vasilyeva LN, Shivas RG, Prieto M, Wedin M, Olariaga I, Lateef AA, Agrawal Y, Fazeli SAS, Amoozegar MA, Zhao GZ, Pfliegler WP, Sharma G, Oset M, Abdel-Wahab MA, Takamatsu S, Bensch K, de Silva NI, De Kesel A, Karunarathna A, Boonmee S, Pfister DH, Lu Y-Z, Luo Z-L, Boonyuen N, Daranagama DA, Senanayake IC, Jayasiri SC, Samarakoon MC, Zeng X-Y,

Doilom M, Quijada L, Rampadarath S, Heredia G, Dissanayake AJ, Jayawardana RS, Perera RH, Tang LZ, Phukhamsakda C, Hernández-Restrepo M, Ma X, Tibpromma S, Gusmao LFP, Weerahewa D, Karunarathna SC (2017) Notes for genera: Ascomycota. Fungal Diversity 86(1): 1–594. https://doi.org/10.1007/s13225-017-0386-0

Zoller S, Scheidegger C, Sperisen C (1999) PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes. Lichenologist 31(5): 511–516. https://doi.org/10.1006/lich.1999.0220

Supplementary material 1

The PCR parameters

Authors: Beata Guzow-Krzemińska, Magdalena Kosecka

Data type: PCR parameters (word document)

Explanation note: The PCR parameters are presented for each marker.

Link: https://doi.org/10.3897/mycokeys.96.98029.suppl1