Lendemeriella vaczii, a new lichenized fungal species from Antarctic Peninsula-with a key to the genus *Lendemeriella*

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Abstract
Lichens are most dominant elements of Antarctic terrestrial vegetation, however, they are still not well known. In this paper, *Lendemerialla vaczii* is described as a new lichen species to science from the James Ross Island and Horseshoe Island, Antarctic Peninsula, based on morphology and phylogenetic analysis. The new species is characterized by brownish cream or buff-colored areolate thallus lacking vegetative propagules, black and lecideine apothecia and very thin (up to 1 µm) septa in ascospores. Phylogenetic analysis of nrITS sequence data shows that new species clusters in the genus *Lendemeriella* with a high bootstrap support. The new species is compared with other *Lendemeriella* species and other related crustose *Teloschistaceae* species without anthraquinones and a comprehensive description is provided. An identification key to 10 species of *Lendemeriella* is also provided.

Key words: Antarctica, biodiversity, *Caloplacoidae*, James Ross Island, *Teloschistaceae*

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Introduction

Lendemeriella is a recently described genus including nine species (*L. aureopruinosa*, *L. borealis*, *L. dakotensis*, *L. exsecuta*, *L. lucifuga*, *L. nivalis*, *L. reptans*, *L. sorocarpa*, *L. tornoensis*) mainly with an Arctic-alpine, boreal-montane or Mediterranean distribution in the northern hemisphere (Kondratyuk et al. 2020, Frolov et al. 2021). The members of the genus are rarely reported from southern hemisphere. *L. exsecuta* has been reported from South Shetland and South Orkney Islands (Øvstedal and Smith 2001, Index Fungorum Partnership[1]) and *L. tornoensis* from New Zealand (Galloway 2004). The genus is phylogenetically positioned in the subfamily

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Caloplacoidae and 4 species of this genus (*L. exsecuta*, *L. nivalis*, *L. sorocarpa* and *L. tornoensis*) are considered members of *Caloplaca exsecuta* group by Vondrák et al. (2019). In this paper, we describe *Lendemeriella vaczii* as a new species from the James Ross Island and the Horseshoe Island, Antarctica. The two islands differ in their geomorphology and vegetation cover; for more information, *see* Láska et al. (2011), Yildirim (2019), Davies et al. (2013).

Material and Methods

Sample of lichenized fungi was collected from the James Ross Island and the Horseshoe Island (Antarctica) by the first author and deposited in the Erciyes University Herbarium Kayseri, Türkiye (ERCH). Lichen specimen was examined by standard microscopic techniques. Hand-cut sections were studied in water, potassium hydroxide (K) and Lugol's solution (I). Measurements of biometrical characteristics were made in water. The measurements were followed by the calculations of means, standard deviation (SD) of $\bar{x}_2$. "n" was the total number of measurements for all samples of that species.

Isolation, DNA extraction, amplification, and sequencing

Hand-cut sections of five apothecia from collected specimen were prepared for DNA isolation and DNA was extracted with a commercial DNA extraction kit (DNeasy Plant Mini Kit; Qiagen) in line with the manufacturer's instructions. PCR amplifications for the internal transcribed spacer region (*ITS1-5.8S-ITS2* rDNA) RNA genes were performed with total 50 µl standard reaction volume for each sample. Optimum amplification conditions were obtained with 25 µl 2 × Taq PCR MasterMix in each tube with 19 µl of distilled water, 2 µl of DNA extracts and 2 µl of the primers *ITS1F* and *ITS4* (Gardes and Bruns 1993, White et al. 1990). The thermal cycling conditions included an initial denaturation step of 95°C for 5 min., followed by 35 cycles of 95°C for 45 sec. (denaturation), 54°C for 45 sec. (annealing), and 72°C for 60 sec. (extension) followed by a final extension period of 72°C for 10 min. Sequence analyzes of the lichen samples obtained from the PCR products were performed by the BM Labosis laboratory (Ankara, Turkey).

Phylogenetic analyses

All *ITS* sequences (Table 1) were aligned and the alignments were performed using a ClustalW and subsequently optimized by hand in BioEdit V7.2.6.1 (Hall 1999). Ambiguous regions were delimited and excluded from the alignment (Hall 1999). Only parsimony-informative regions were finally analyzed in MEGA XI (Tamura et al. 2021).

BLAST searches in GenBank of each gene region were carried out to find closely related sequences. A total of 43 ITS rDNA sequences were analyzed. Bootstrap analysis for the estimation of confidence levels of the clades was performed on 1000 bootstrap replications. Phylogenetic relationships and support values were investigated using maximum likelihood (ML) bootstrapping, as implemented in MEGA XI. Kimura two-parameter model was used for the analysis of the ML method. *Blastenia ammiospila* (Wahlenb.) Arup, Søchting & Frödén was used as outgroup.

Species	Genbank Number	Locality
JR 0.409 *Lendemeriella vaczii*	OQ812127	James Ross Island,
HS 0.146 *Lendemeriella vaczii*	OQ812125	Horseshoe Island, Antarctica
Pisutiella phaeothamnos	MG954114	Turkey
	JN813419	Greece
Pisutiella congrediens	MG954115	Turkey
Pisutiella conversa	MG954112	Russia
	MG954113	Russia
Kuettlingeria diphyodes	MN103120	China
	MN103121	China
	KR912046	Russia
	KJ816761	Russia
Lendemeriella reptans	MH104934	USA
	JQ686192	USA
Lendemeriella lucifuga	MG954216	Czech Republic
	MG954217	Czech Republic
Lendemeriella sorocarpa	MG954132	Russia
	MG773658	-
Lendemeriella exsecuta	MG954225	Russia
	MG954227	USA
Lendemeriella borealis	MW227317	Russia
	MG954129	Russia
Lendemeriella tornoensis	MG954220	USA
	MG954221	USA
Lendemeriella nivalis	MG954222	USA
Lendemeriella aureopruniosa	MN814228	Russia
	MN814229	Russia
Pyrenodesmia alociza	KJ021239	Bulgaria
	MT967436	Sweden
Pyrenodesmia erodens	MH104927	Türkiye
Pyrenodesmia concreticola	KC884542	Russia
Pyrenodesmia chalybea	MN986922	-
	MN989247	Greece
Pyrenodesmia bicolor	MH104922	Russia
Pyrenodesmia peliophylla	MH104930	USA
Pyrenodesmia aractina	MH104919	Czech Republic
Pyrenodesmia variabilis	MZ244103	USA
	MZ244104	USA
Pyrenodesmia molariformis	KC416145	Ukraine
Pyrenodesmia duplicata	HQ611272	Finland
Pyrenodesmia atroflava	MH104921	Greece
Pyrenodesmia neotaurica	MN305807	Ukraine
Pyrenodesmia teicholyta	MH104935	Ukraine
Pyrenodesmia erythrocarpa	MN305806	Ukraine
Huneckia wrightii	*MT967388*	*Ecuador, Galapagos*
Huneckia crocina	*MT967384*	*Argentina*
Huneckia rheinigera	*MT967387*	*Australia*
Huneckia pollini	*MT967386*	*Spain*
Blastenia ammiospila	*MF114841*	-

Table 1. Genbank Numbers of used specimens in the analyses.

Results

Phylogenetic Analysis

In the BLASTn search, the ITS sequence of *Lendemeriella vaczii* (the final alignment contained 522 bp after trimming) was most similar to *Lendemeriella borealis* (Vain.) S.Y. Kondr. (89.06%, 53 mismatches and no gaps). Altogether, 262 nucleotides were found to be conserved sites (C), and 252 nucleotides were found to be variable sites (V) in ITS gene region. The dataset used for a study of the phylogeny of the subfamily Caloplacoideae of the Teloschistaceae (the genera *Huneckia*, *Pisutiella* and *Pyrenodesmia*) in the ML phylogenetic analysis was used to investigate the placement of the newly generated sequence (Fig. 1). When the ML phylogenetic tree was examined, the genera in the subfamily Caloplacoideae of the Teloschistaceae clearly showed a separate branching. The ITS ML phylogenetic tree clearly showed that the new species differed from other species in the genus *Lendemeriella* and other closely related genera (Fig. 1).

Taxonomy

***Lendemeriella vaczii* Halıcı, Kahraman Yiğit & Güllü sp. nov.** (Fig. 2)
Mycobank No: MB 848346
***Diagnosis*:** Differs from the other members of the genus by having black and lecideine apothecia, smaller ascospores ((11–)12.5–*14*–16.5(–17) × (5–)6–*7.5*–8.5(–10) µm) with very thin or undeveloped isthmus.
***Type*:** Antarctic Peninsula, James Ross Island: SE margin of the Berry Hill Mesa, 63° 48′ 42.0″ S, 57° 50′ 5.4″ W, alt. 345 m., on basaltic rocks, 17 January 2017, leg. M. G. Halıcı & M. Barták (ERCH JR. 0.409–holotype). Thanks to shallow depressions in the mesa surface formed in between the individual volcanic stones, the availability of water from melting snow accumulated in the depressions was relatively high during austral summer season. Another water source was condensation of water vapour from cloud bases touching the mesa plateau frequently. Therefore, the mesa plateau was relatively rich in lichen flora with dominating species *Usnea sphacelata* and *Umbilicaria decussata*, and the other ones found at the collection site (*see* Ecology and Distribution).
***Etymology*:** Named in honour of Assistant Prof. Mgr. Peter Váczi, Ph.D. (Masaryk University, Brno, Czech Republic), who researched Antarctic terrestrial vegetation and helped the first author in the field excursions in the James Ross Island. Last few decades, he has been engaged into the studies of ecophysiology of lichens from Antarctics, the James Ross Island in particular. He is a member of the team at the Masaryk University, Brno, that focuses on stress physiology of Antarctic lichens.
Description: *Thallus* epilithic, inside the crevices as small patches up to 1.1 mm, areolate, brownish cream or buff-colored, thin to well developed. Areoles angular, brownish cream or buff-colored, flat to slightly convex, up to 0.15 mm wide and 0.3 mm thick. Cortex up to 60 µm, brownish hyaline. *Algal layer* 150–180 µm thick; algae green, chlorococcoid, subglobose to globose, 11–13 × 14–16 µm. Medulla up to 120 µm thick, full of oil droplets, pseudoparenchymatous, the isodiametric cells are 3.5–4.5 × 3.5–4.5 µm. *Apothecia* 0.1–0.2 mm diam., sessile, flat, rounded to angular, lecideine, disc black, epruinose, thalline exciple absent, true exciple carbonized, up to 25 µm thick. Epihymenium brownish green with cinereorufa green pigment (Meyer and Printzen 2000), 25 µm thick. Hymenium hyaline, 65 µm thick, without extracellular oil droplets or crystals.

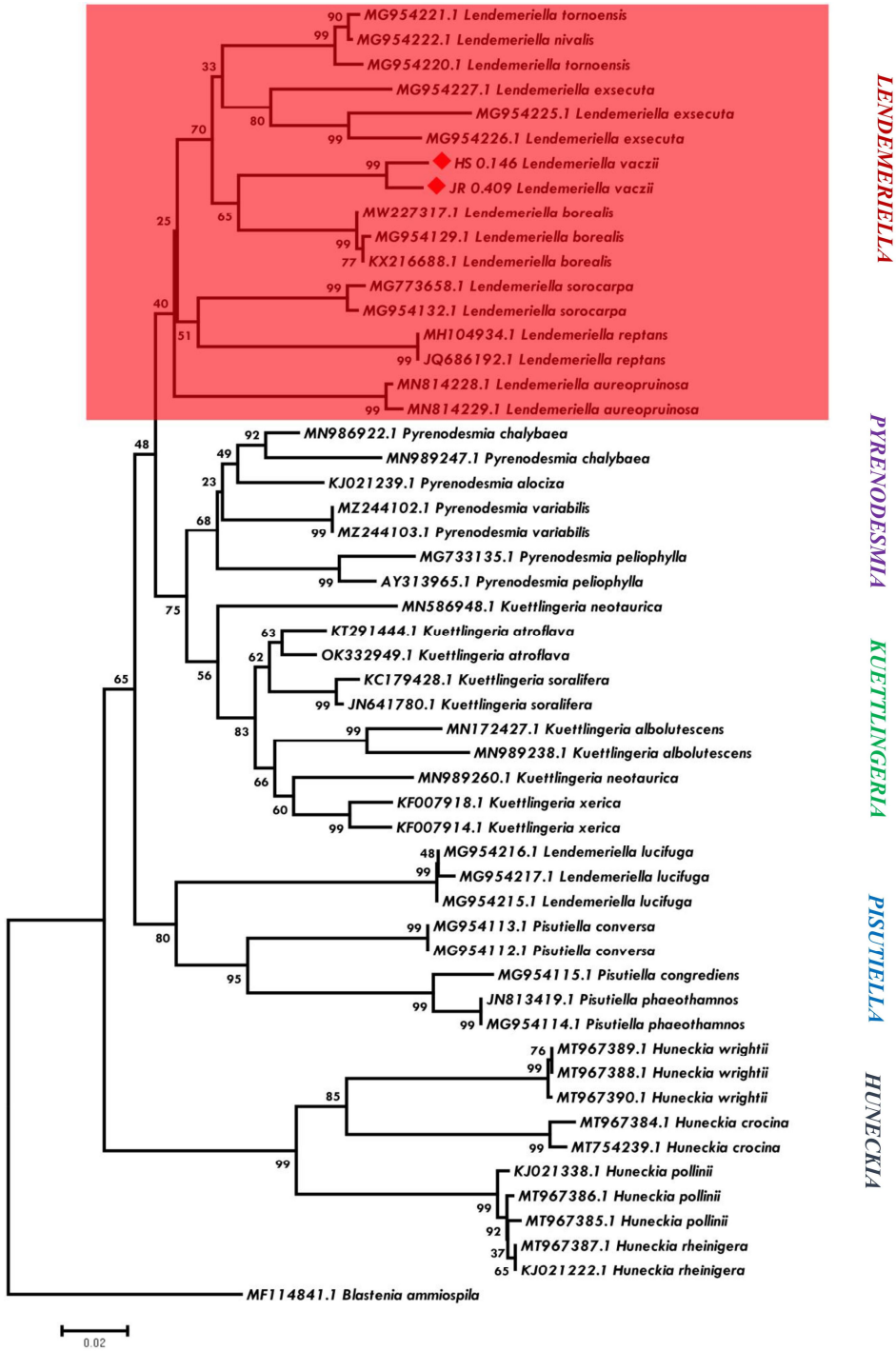

Fig. 1. ITS ML phylogeny of *L. vaczii* and related genera. The new species *L. vaczii* is presented with JR 0.409 and HS 0.146 code.

Hypothecium golden brown or light brown, 60 µm thick with extracellular oil droplets. Paraphyses 2 µm thick at the lower parts, clavate or slighlty enlarged at the tips, tips are pigmented and 3 µm thick, not branched or sometimes branched, septate. Asci clavate, 50–60 × 12–15 µm, 8-spored (n=10). Ascospores oblong-ellipsoid, hyaline, (11–)12.5–*14*–16.5(–17) × (5–)6–*7.5*–8.5(–10) µm, isthmus very thin or undeveloped, up to 1 µm when present, l/w ratio: (1.45)1.63–*1.94*–2.25(2.8) (n=30). Pycnidia not observed.
Chemistry: Epihymenium and upper exciple K-, N+ red.
Ecology and Distribution: *L. vaczii* grows on basaltic rocks at relatively high altitudes, in the James Ross Island, Ulu Peninsula and Horseshoe Island (Antarctic Peninsula) with lichens such as *Amandinea latemarginata*, *Gondwania sublobulata*, *Lecanora flavocrassa*, *Lecidella stigmatae*, *Myriolecis semipallida*, *Ochrolechia parella*, *Usnea subantarctica*.
Specimen examined: Antarctic Peninsula, Horseshoe Island: SE of the Sally Cove, 67° 48′ 58″ S, 67° 18′ 9″ W, alt. 70 m., on basaltic rocks, 17 February 2022, leg. M. G. Halıcı (ERCH HS. 0.146).

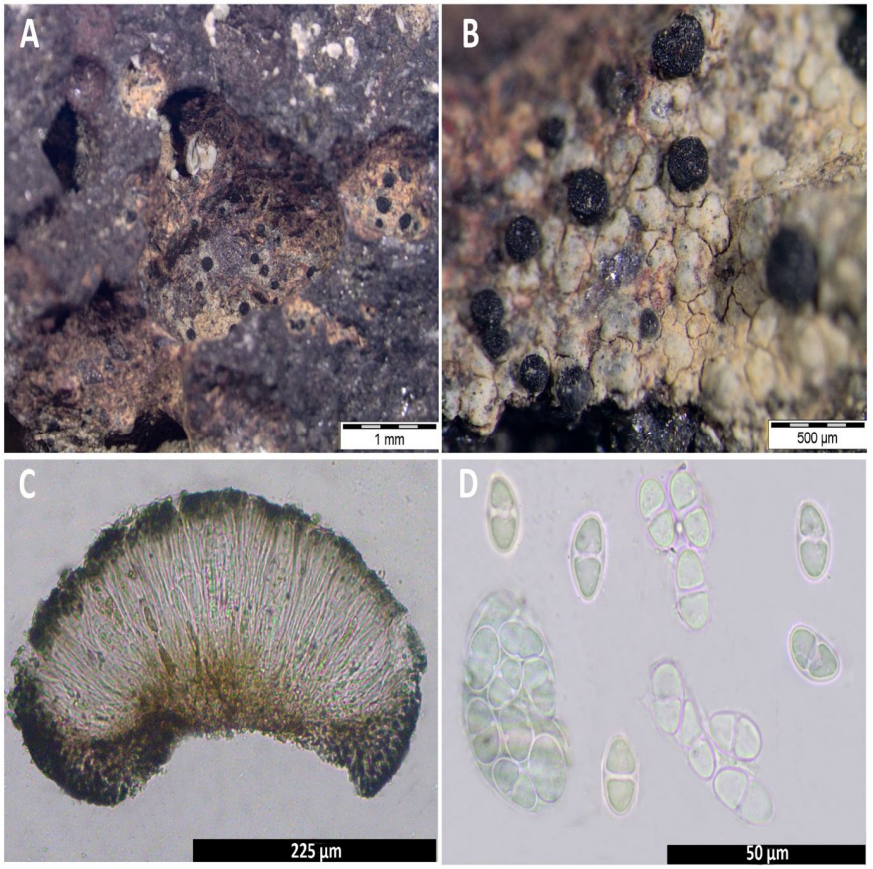

Fig. 2. *Lendemeriella vaczii.* A. – Habitus. B. – Areolles and apothecia in closer view. C. – Apothecial anatomy. D. – Ascus and ascospores.

Discussion

The circumscription of lichen-forming fungal species has traditionally been guided by morphological, chemical and ecological features. However, because lichens generally display few taxonomically useful characteristics, of which many are widely variable, the homology of character states within and among groups is difficult to assess. Therefore, molecular data have gained importance in lichen systematics and now have a significant impact on the classification and taxonomy of lichenized ascomycetes (Divakar and Crespo 2015). In most cases, our phylogenetic analyses support the traditional species delimitation based on morphological and chemical traits. Phylogenetic analysis results clearly suggest that the new species belongs to the genus *Lendemeriella* and is clearly differentiated from other species of the genus (Fig. 1).

The new species differs from the other known *Lendemeriella* species not only phylogenetically, but also morphologically and anatomically (*see* the below-described differences). The other epilithic species of the genus are *L. aureopruinosa*, *L. exsecuta* and *L. reptans*. From these species, *L. exsecuta* is also known from Antarctic Peninsula (Øvstedal and Smith 2001) and has mostly biatorine to lecideine apothecia with a blackish disc as *L. vaczii*, but differs from the new species by having ascospores with wider septa (3–5 µm *vs.* 1–1.5 µm) (Søchting et al. 2008). *Lendemeriella aureopruinosa* is a recently described saxicolous taxon from Yakutia, Russia (Frolov et al. 2021), with a thin grey thallus and small apothecia (0.3-0.6 mm in diameter), with a dark orange disc usually bearing epipsamma and often with a grey true exciple containing the pigment Cinereorufa-green. However, this taxon also differs from *L. vaczii* by having ascospores with wider septa [(3.0–)3.5–4.0–4.3(–7.0) µm *vs.* 1–1.5 µm)] and also typical bright aureate pruina on young apothecia (Frolov et al. 2021). The last epilithic species of the genus *L. reptans* has a sorediate thallus (Hodkinson and Lendemer 2012). The other species of the genus having aseptate ascospores or very thin septate ascospores are *L. nivalis* and *L. tornoensis* but these species grow on mosses and have much longer ascospores (*see* the key below).

C. exsecuta, *C. sorocarpa*, *C. tornoensis* and *C. nivalis*, which are in the "*Caloplaca exsecuta*" group (Vondrák et al. 2019) are classified under the genus *Lendemeriella* by Kondratyuk et al. (2020). "*Caloplaca conversa*" group which is characterized with black apothecia and Sedifolia-grey pigment in all parts of the thallus and at the apothecial margin are classified under the genus *Pisutiella* by Kondratyuk et al. (2020). The new species *L. vaczii* does not have Sedifolia-grey pigment in the thallus cortex nor in the apothecial margin but has Cinereorufa-green pigment. The other crustose Teloschistacae member with brown-black apothecial disk, which was reported as *Caloplaca* aff. *diphyodes* from the James Ross Island, is classified under the genus *Kuettlingeria* and this genus is also characterized by presence of Sedifolia-grey pigment in the thallus cortex and in the apothecial margin (Frolov et al. 2021).

An identification key to the species of Lendemeriella

1. Sorediate.....**2**
1. Non-sorediate.....**4**
2. On bark or twigs.....**3**
2. On rocks.....
... ***L. reptans*** **(Lendemer & B.P. Hodk.) S.Y. Kondr.** (Hodkinson and Lendemer 2012).
3. Apothecia present, thallus endophloedic or as thin gray film.....
..... ***L. sorocarpa*** **(Vain.) S.Y. Kondr.** (Wetmore 2004).
3. Apothecia absent, thallus areolate.....
..... ***L. lucifuga*** **(G. Thor) S.Y. Kondr.** (Kubiak and Zalewska 2009).
4. On rocks.....**5**
4. On wood bark or mosses.....**7**
5. Ascospore septa > 3 µm.....**6**
5. Ascospore septa < 3 µm…..... ***L. vaczii*** **Halıcı, Kahraman Yiğit, Güllü sp. nov.**
6. Ascospore septa 3–5 µm, occurs in zonal tundra and the alpine belt of high mountains and rarely in the upper part of the forest bell, 7-chloroemodin present.....
..... ***L. exsecuta*** **(Nyl.) S.Y. Kondr.** (Søchting et al. 2008).
6. Ascospore septa 3–7 µm, grows in the forest belt in the mountains and the rarely in the alpine belt, 7-chrloroemodin absent.....
..... ***L. aureopruinosa*** **I.V. Frolov, Vondrák, Arup, Konoreva, S. Chesnokov, Yakovczenko & Davydov** (Frolov et al. 2021).
7. On bark.....**8**
7. On mosses.....**9**
8. Thallus light brown, areolate, apothecial disc dull orange to orange, has circumboreal distribution..... ***L. borealis*** **(Vain.) S.Y. Kondr.** (Wetmore 2007).
8. Thallus gray brown, subsquamulose to areolate, apothecial disc brown, known only from North America..... ***L. dakotensis*** **(Wetmore) S.Y. Kondr.** (Wetmore 1994).
9. Ascospores 27–32 × 4–5.5 µm, aseptate or with an indication of a septum
..... ***L. nivalis*** **(Körb.) S.Y. Kondr.** (Søchting et al. 2008).
9. Ascospores 16–19 × 5–8 µm and septa 1–2 µm.....
L. tornoensis **(H. Magn.) S.Y. Kondr.** (Søchting et al. 2008).

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