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Thamnolecania yunusii (Ramalinaceae) – A new species of lichenised fungus from Horseshoe Island (Antarctic Peninsula)

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Research Article

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Abstract

The new terricolous lichen species *Thamnolecania yunusii* Halıcı, Güllü, Bölükbaşı & Kahraman, which is characterised by its cream to greyish brown granulose-crustose thallus without vegetative propagules, is described from Horseshoe Island in the South-West Antarctic Peninsula region. All *Thamnolecania* species are known only from the Antarctic. The only species of the genus with a crustose thallus is *T. racovitzae*, but it differs from *T. yunusii* by growing on rocks, having an effuse to subeffigurate thallus that is sometimes isidiate and with shorter and narrower ascospores (*c.* 15 × 3.5 µm *vs.* 15.5–19.5 × 3.5–5.5 µm). The nrITS, mtSSU and RPB1 gene regions of the new species were studied and the phylogenetic position of the species was shown to be in the same clade as *Thamnolecania gerlachei*, *T. brialmontii* and *T. racovitzae*, but occurs on a different branch from these species. As *T. yunusii* is an Antarctic endemic, like the other *Thamnolecania* species, and most of the morphological characters fit well with this genus, we describe this new species under the genus *Thamnolecania*.

Introduction

Horseshoe Island is located in Marguerite Bay within the West Antarctic Peninsula. According to the BAS Database (2022), 29 lichenized fungal species and 15 moss species have been reported from Horseshoe Island. Apparently, these numbers do not represent the biodiversity of Antarctic terrestrial vegetation and more detailed and professional studies should be carried out. In a project aiming to determine the lichen biodiversity of this island with the aid of DNA-barcoding, the first author collected lichens from Horseshoe Island during Turkish Antarctic Expedition VI, 2022. From these collections, *Candelariella ruzgarii* Halıcı, A.M. Kahraman & Güllü (Halıcı, Kahraman Yiğit, Bölükbaşı, & Güllü, 2023a) and *Lendemeriella vaczii* Halıcı, Kahraman Yiğit & Güllü (Halıcı, Bölükbaşı, Güllü, Kahraman Yiğit, & Barták, 2023b) have already been described and published.

Thamnolecania (Vain.) Gyeln. is a genus of lichenized fungi comprising Antarctic endemic species established by Gyelnik (1933). This genus is a well-supported clade including *T. brialmontii* (Vain.) Gyeln., *T. gerlachei* (Vain.) Gyeln. and *T. racovitzae* (Vain.) S.Y. Kondr., Lőkös & Hur (Kondratyuk et al., 2014; Reese Næsborg, Ekman & Tibell, 2007). These three species were first described under the genus *Lecanora* by Vainio (1903) as subgenus *Thamnolecania*. Reese Næsborg et al. (2007) indicated that an alternative classification would be to make a wider circumscription of the genus *Bilimbia* that included *Thamnolecania* and affiliated taxa such as *Lecania croatica* (Zahlbr.) Kotlov and *Lecidea sphaerella* Hedl.

Morphological, anatomical and phylogenetical analyses of the specimens collected on mosses from Horseshoe Island by the first author in 2022 showed that these samples belong to an undescribed species in *Lecania* s. lat. and very closely related to the genus *Thamnolecania*. Therefore, in this paper, we describe this novel taxon under the genus *Thamnolecania* but also suggest that it may be transferred to a novel genus when more data becomes available.

Material & methods

Morphological and anatomical studies

Samples of lichenized fungi were collected from Horseshoe Island (Antarctic Peninsula). Collected specimens are deposited in "Erciyes University Herbarium (ERCH), Kayseri, Turkey)." The specimens were identified by using standard microscope methods. The chemistry was analysed using spot tests, (i.e. 10% KOH (K) and sodium hypochlorite (C)) or thin-layer chromatography (TLC) using solvents A (toluene/1,4-dioxane/glacial acetic acid; 36:9:1) and solvent C (toluene/glacial acetic acid; 20:3; Huneck, Yoshimura, Huneck, & Yoshimura, 1996;

Table 1. nrITS, mtSSU and RPB1sequences used in the analyses. The new sequences are in bold.

Specimen	Locality	ITS	mtSSU	RPB1	References
ERCH HS 0.044 *Thamnolecia yunusii*	Horseshoe Island, Antarctica	**OR159910**	**OR159911**	**OR246912**	This study.
Thamnolecia brialmontii	King George Island, Antarctica	DQ534467			Kim et al. (2006); Reese Næsborg et al. (2007)
	South Sandwich Islands, Antarctica	AM292676	AM292726		
		AM292677			
Thamnolecania gerlachei	King George Island, Antarctica	DQ534468			Kim et al. (2006); Reese Næsborg et al. (2007)
	South Sandwich Islands, Antarctica		AM292737		
			AM292736		
Thamnolecania racovitzae	South Sandwich Islands, Antarctica	AM292687			Reese Næsborg et al. (2007)
Bilimbia lobulata	Norway	MK812573	AM292712		Reese Næsborg et al. (2007)
		MK812712	AM292713		
Bilimbia microcarpa	Canada	AM292669	AM292714		Reese Næsborg et al. (2007)
			AM292715		
Bilimbia sabuletorum	China	OM267695			Reese Næsborg et al. (2007); Ekman, Andersen and Wedin (2008); Miadlikowska et al. (2014); Han, Zhang and Guo (2022); Vondrák et al. (2023)
	Czech Republic		OQ646137		
	Finland			KJ766839	
	Norway			AY756413	
	–		AM292717		
Lecania aipospila	Norway	MK811636	MG925876	MG926174	Reese Næsborg et al. (2007); Kistenich, Timdal, Bendiksby and Ekman (2018); Marthinsen, Rui and Timdal (2019)
		MK812577	AM292723		
Lecania atrynoides	Sweden		AM292724		Reese Næsborg et al. (2007); Ekman et al. (2008)
	UK			AY756416	
Lecania baeomma	Norway		AM292725		Reese Næsborg et al. (2007)
Lecania crytella	Czech Republic	OL457925	OK465534		Vondrák et al. (2022)
		OK332970	OK465584		
Lecania cyrtellina	Austria	MZ409482			Reese Næsborg et al. (2007); Thüs, Kluessendorf and Weber (2021)
	Sweden		AM292729		
			AM292730		
Lecania croatica	Russia	OQ717882			Vondrák et al. (2023)
	Czech Republic	OQ717881			
	Ukraine		OQ682969		
	Austria		OQ682970		
Lecania dubitans	USA	AM504059	AM292732		Reese Næsborg et al. (2007)
			AM292731		
	Sweden	AM504058			
Lecania erysibe	Faroe Islands	AM504061			Reese Næsborg et al. (2007); Kistenich et al. (2018); Marthinsen et al. (2019)
	Norway	MK812191			
	Scotland		MK812191		
	UK			MG926176	
Lecania fructigena	United Kingdom	MZ159732			Miadlikowska et al. (2014); Gaya, Steier, Wright and Woods (2021).
	Finland		KJ766413		
	–			KJ766860	

(*Continued*)

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Table 1. (*Continued*)

Specimen	Locality	ITS	mtSSU	RPB1	References
Lecania fuscella	Sweden	AM292684	MG925877		Reese Næsborg et al. (2007); Kistenich et al. (2018)
		AM292685	AM292735		
Lecania hutchinsiae	Norway	AM504062			Reese Næsborg et al. (2007)
	Scotland	AM292689	AM292739		
Lecania inundata	Scotland		AM292740		Reese Næsborg et al. (2007)
Lecania leprosa	Czech Republic		OK465585		Vondrák et al. (2022)
Lecania naegelii	Czech Republic	OQ717419	OQ682973		Reese Næsborg et al. (2007); Haughland et al. (2021); Vondrák et al. (2023)
	Canada	ON116039			
	Austria		AM292741		
Lecania nylanderiana	Sweden		AM292742		Reese Næsborg et al. (2007); Kistenich et al. (2018)
			MG925878		
Lecania prasinoides	Czech Republic		OQ682974		Vondrák et al. (2023)
Lecania proteiformis	Germany	AM504071			Reese Næsborg et al. (2007)
	Lithuania	AM600968			
Lecania rabenhorstii	Czech Republic	OK332971	OK465586		Reese Næsborg et al. (2007); Vondrák et al. (2022)
	Sweden	AM292693	AM292743		
Lecania sambucina	Czech Republic	OQ717884			Reese Næsborg et al. (2007); Vondrák et al. (2022); Vondrák et al. (2023)
	–		AM292745		
	Sweden	AM292695			
	Russia		OQ682975		
Lecania spadicea	Greece	MG925980		MG926177	Kistenich et al. (2018);
Lecidea sphaerella	Slovakia	AM292701			Reese Næsborg et al. (2007); Printzen (2014); Vondrák et al. (2022); Vondrák et al. (2023)
	–		KF662400		
	Czech Republic	KF650952	OQ646282		
Lecania sylvestris	Slovakia	AM292699			Reese Næsborg et al. (2007)
Lecania turicensis	Spain	AM292748			Reese Næsborg et al. (2007)
Sphaerophorus globosus	Norway	MN483149			Ekman et al. (2008); Spribille et al. (2020)
	Canada		AY256762		
	UK			AY756424	

Orange, James & White, 2001). Measurements were made only from sections in water. Ascospores were measured outside the asci. The average is followed by its standard deviation, and the maximum and minimum values are given in parentheses.

Molecular methods

DNA isolation, PCR and sequencing

Total genomic DNA was extracted using the commercial DNA isolation kit (DNeasy Plant Mini Kit; Qiagen). The isolation was carried out according to the instructions prepared by the manufacturer in the kit. The most universal primers were used in this study. The complete ITS plus 5.8S rDNA was amplified using primers ITS1F and ITS4 (Gardes & Bruns, 1993; White, Bruns, Lee & Taylor, 1990). The mtSSU gene region was amplified by using the primers mtSSU1F and mtSSU3R (Zoller, Scheidegger & Sperisen, 1999). The RPB1 gene region was amplified using the primers RPB1-5F (Denton, McConaughy & Hall, 1998) and fRPB1-11aR (Liu, Whelen & Hall, 1999). Each sample was prepared for a total of 50 µl of standard reaction. Optimum amplification conditions were obtained with 25 µl of 2 × Taq PCR MasterMix in each tube with 1 mM of the primers ITS1F and ITS4, ~100 ng of DNA extracts and it was completed with 50 µl double distilled water. PCR amplifications were conducted with an initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 55 °C or 56 °C and 52 °C (nrITS, mtSSU and RPB1, respectively) for 1 min, 72 °C for 90 s and a final extension at 72 °C for 10 min. Positive amplification of the gene regions was determined using agarose gel electrophoresis. Samples were visualised under UV light. Sequence analyses of lichen samples from which PCR products were obtained were performed by Epigen Biotechnology Laboratory (Ankara, Turkey).

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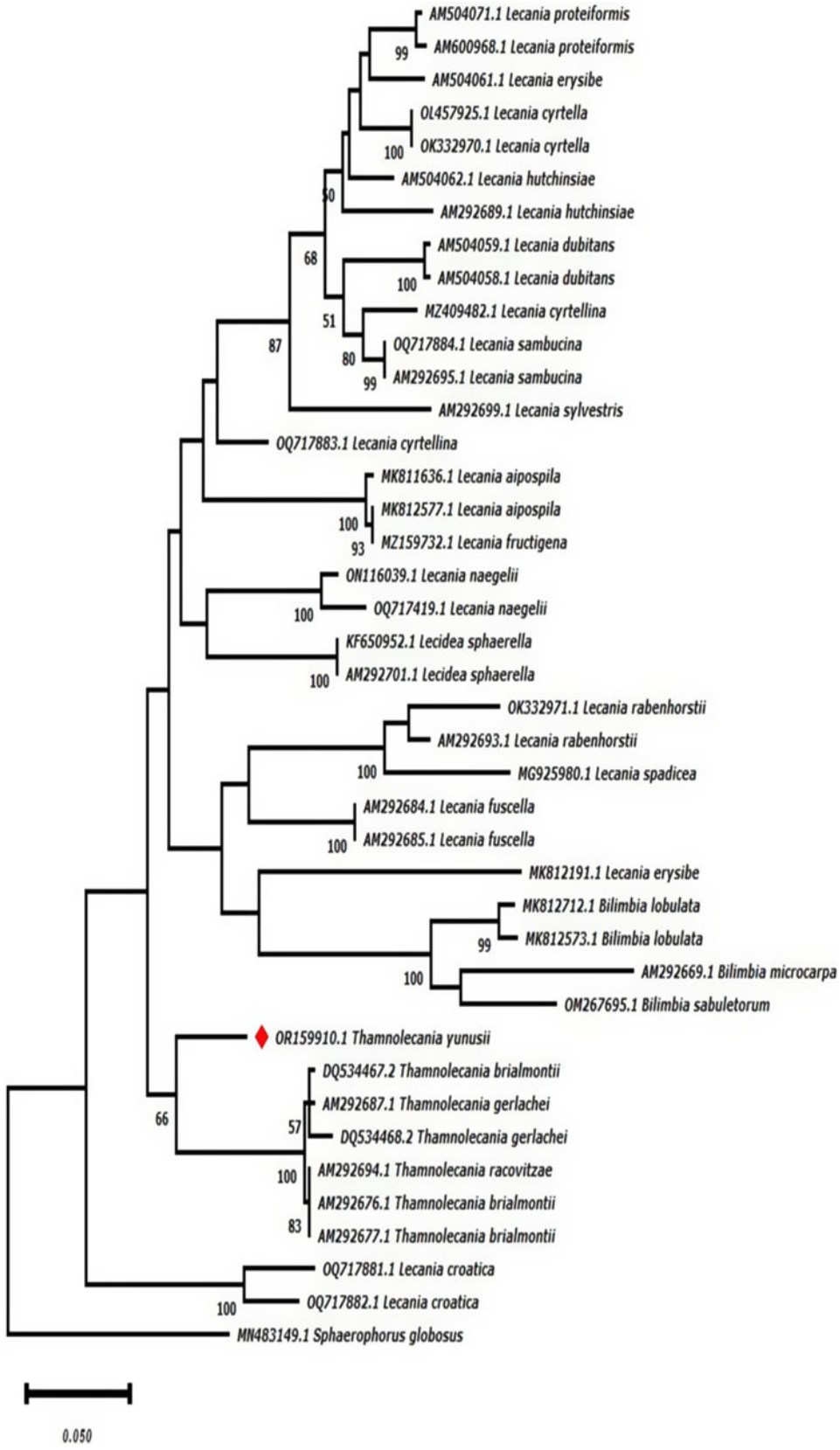

Figure 1. Concatenated maximum likelihood phylogenetic tree (ITS + mtSSU) of *Thamnolecania* and related genera. Posterior probabilities are shown above branches. Numbers at nodes represent the ML bootstrap support (values ≥ 50%). The new species *T. yunusii* is highlighted. *Sphaerophorus globosus* was used as outgroup.

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Additional sequences

The final dataset consisted of newly generated sequences (1 sequence for ITS, 1 sequence for mtSSU and 1 sequence for RPB1) from this study and 39 nrITS, 45 mtSSU and 11 RPB1 sequences obtained from GenBank (Table 1). *Sphaerophorus globosus* was used as the outgroup in nrITS, mtSSU and RPB1 phylogenetic trees.

Sequence alignment and phylogenetic analysis

nrITS, mtSSU and RPB1 sequences of all species were aligned and optimised manually using ClustalW in BioEdit V7.2.6.1 for preparing the phylogenetic trees. In MEGA XI, only parsimony-informative regions were used for analysis. Indeterminate regions were excluded from the alignment (Hall, 1999; Tamura, Stecher & Kumar, 2021). The final alignment comprised 579 (nrITS), 785 (mtSSU) and 619 (RPB1) columns.

One-thousand bootstrap replications were performed by bootstrap analysis for the estimation of confidence levels of the clades. Phylogenetic relationships and support values were investigated using maximum likelihood (ML) bootstrapping, as implemented in MEGA XI. Kimura's two-parameter model was used for the analysis of the ML method. Genbank numbers of used sequences in phylogenetic trees within this study are given in Table 1. Sequence data obtained from ITS and mtSSU gene regions were combined using the Geneious v2023.2.1.

Results

Evaluating phylogenetic analysis results

Three independent phylogenetic trees for *Thamnolecania* and related genera were produced from 68 sequences (39 for nrITS, 15 for mtSSU) from GenBank and three new sequences (1 sequence for nrITS, 1 sequence for mtSSU and 1 sequence for RPB1) from the new species. We obtained the RPB1 gene of the species, but because of the insufficient RPB1 data of *Thamnolecania* genus in GenBank, we did not construct the RBP1 gene phylogenetic tree. All species names are followed by the GenBank accession numbers or voucher information (Table 1). When concatenated maximum likelihood (ITS + mtSSU) tree was examined, *Thamnolecania yunusii*, which we described as a new species, clearly showed branching with other species belonging to the genus *Thamnolecania* with a high bootstrap value (Fig. 1). Within the genus *Thamnolecania*, the new species clearly occupied a separate branch from the rest of the genus. The phylogenetic analysis did not designate any species identical to the new species in the genus *Thamnolecania*. Polymorphism statistics and Blast comparisons of the new species and related species are shown in Table 2.

Table 2. Polymorphism statistics for each marker (nrITS and mtSSU) from datasets corresponding to the genus *Thamnolecania* and Blast comparison of the new species with related species in the GenBank.

Datasets	nrITS	mtSSU
Species included (n)	7	4
Conserved sites	558	705
Variable sites	57	29
Singleton variable sites	55	27
Parsimony inf. sites	2	2
Transitional pairs (si)	9	6
Transversional pairs (sv)	8	8
R (si/sv)	1.1	0.7
Number of sites (bp)	622	757
Blast comparison of the new species	**Related species in GenBank**	**The percentage similarity with BLAST**
	Thamnolecania brialmontii (ITS)	505/569(89%)
Thamnolecania yunusii	*Thamnolecania brialmontii* (mtSSU)	740/774(96%)
	Thamnolecania gerlachei (ITS)	503/570(88%)
	Thamnolecania gerlachei (mtSSU)	703/738(95%)
	Thamnolecania racovitzae (ITS)	500/572 (87%)

Taxonomy

Thamnolecania yunusii Halıcı, Güllü, Bölükbaşı & Kahraman sp. nov. (Fig. 2)

MycoBank No.: MB 848346

Type: Antarctic Peninsula, Horseshoe Island: Sally Cove, Bourgeois Fjord, Marguerite Port, 67°48′30″S 67°17′39″W, alt. 10 m, on mosses, 17 February 2022, leg. M. G. Halıcı *ERCH HS 0.044* (ERCH—holotype).

Diagnosis: Characterised by cream to greyish brown granulose, crustose thallus without vegetative propagules growing on mosses with shorter hymenium (30–50 µm high) compared with the other known members of the genus.

Etymology: Named in honour of Yunus Emre, also known as Derviş Yunus (Yunus the Dervish) (1238–1328), who was a Turkish folk poet and Sufi mystic who greatly influenced Turkish culture. Yunus Emre, who passed away 700 years ago, especially examined the love of humanity and nature in his poems. In order to keep the name of this important heartfelt person alive in nature, we decided to name the new species after him.

Description: Thallus effuse, cream to greyish brown sometimes with a greenish tinge, granulose, up to 0.3 mm thick; granules up to 0.5 mm diam; medulla I-. Photobiont trebouxioid, cells subglobose to globose, 9–13 × 8–12 µm diam.

Apothecia common, mostly aggregated, biatorine, dark brown to almost black, epruinose, mostly flat, sometimes slightly concave, 0.3–0.4 mm diam., with a distinct thalline margin concolourous with the thallus. The thalline margin is frequently apparent. In section: proper exciple c. 25 µm thick, thalline margin c. 40 µm thick, inner section hyaline to becoming brown towards the cortex (K+ purple-brown; Lecania-brown pigment); composed of narrow irregularly radiating hyphae c. 1 µm wide; epihymenium 15–25 µm, brown. Hymenium hyaline, 30–50 µm high, upper parts greenish brown, K+ purple-brown (Lecania-brown pigment); paraphyses simple or sometimes branched at the tips, septate, capitate to moniliform mostly with a brown cap. Hypothecium hyaline, 60–80 µm high, is composed of randomly orientated hyphae. Asci Biatora-type, cylindrical or sometimes clavate, 40–45 × 8–10 µm, ascospores hyaline, 3– septate, randomly arranged in ascus, mostly multiseriate towards the apices, vermiform with

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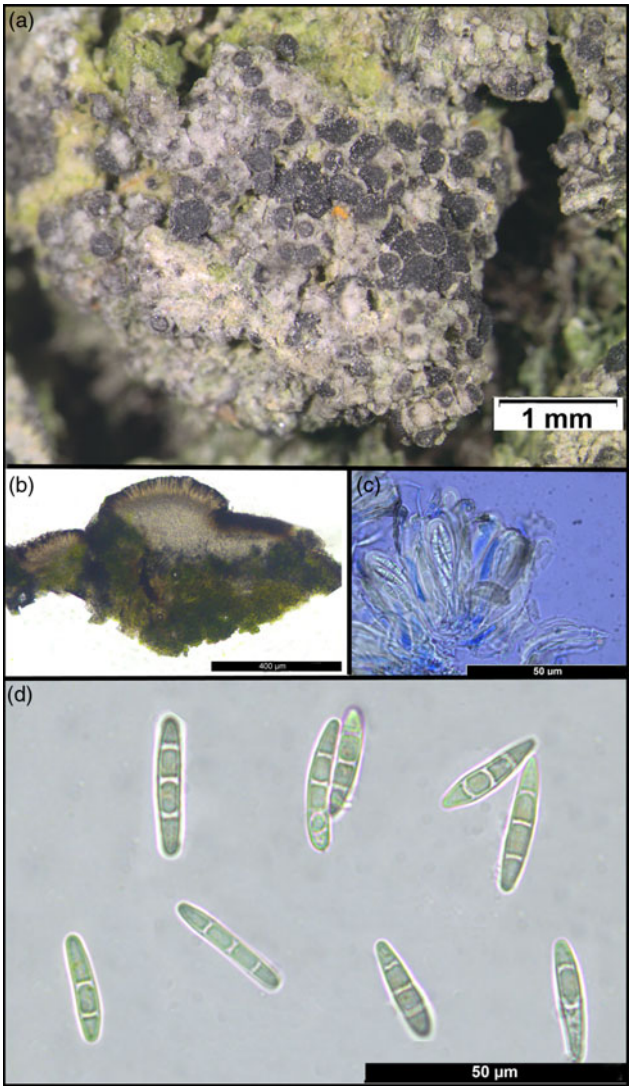

Figure 2. *Thamnolecania yunusii*. (a) Habitus. (b) Apothecial section in water. (c) Asci and paraphyses in methylene blue. (d) 3– septate hyaline ascospores.

rounded or sharp ends, (13.5–)15.5–*17.5*–19.5(–22.5) × (3–)3.5–*4.5*–5.5(–6.5) µm ($n = 30$) and l/w ratio: (2.46–)3.43–*4.27*–5.1–(6.17) µm ($n = 30$). Conidiomata is not observed.

Chemistry: No secondary components were detected in TLC. All spot tests are negative.

Ecology and distribution: The species is so far only known from Horseshoe Island, Antarctica. On Horseshoe Island (Antarctic Peninsula, Antarctica), it grows on mosses with *Parvoplaca athallina* (Darb.) Arup, Søchting & Frödén.

Discussion

Our phylogenetic analyses show that *Thamnolecania yunusii* clearly differs from other species of the genus. The phylogenetic trees obtained as a result of using nrITS and mtSSU gene regions showed that the new species grouped together with *T. gerlachei, T. brialmontii* and *T. racovitzae* species. Although the new species forms a clade with other *Thamnolecania* species, it occurred on a different branch (with higher bootstrap support) from other species. However, since we do not currently have sufficient sequence data to justify erecting a new genus, we include it within the genus *Thamnolecania*. With more detailed studies in the future, it is possible that this new species can be transferred to a novel genus separated from *Thamnolecania* s. str. in *Lecania* s. lat.

The genus *Thamnolecania* is characterised by most species having a fruticose habit, but sometimes also foliose, squamulose or crustose (Øvstedal & Smith, 2001), having three-septate relatively large ascospores up to 22 µm long, a high hymenium (60–100 µm) and relatively large apothecia (up to 3 mm diam.) (Reese Næsborg et al., 2007). Interestingly, all the members of the genus are apparently Antarctic endemics. The new species, *T. yunusii,* is morphologically characterised by its granulose, crustose thallus without vegetative propagules. Although all the other known species of the genus have a higher hymenium (60–100 µm) as indicated above, *T. yunusii* has a lower hymenium (30–50 µm high) differing from the other species. The only other *Thamnolecania* species with a crustose thallus is *T. racovitzae*, but this species differs from *T. yunusii* by growing on rocks, having an effuse to subeffigurate thallus that is sometimes isidiate, and with shorter and narrower ascospores (*c.* 15 × 3.5 µm *vs.* 15.5–19.5 × 3.5–5.5 µm) (Øvstedal & Smith, 2001).

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Competing interests. The authors declare none.

Authorship. Mehmet Gökhan Halıcı collected the specimen and write the manuscript. Mithat Güllü made the phylogenetic analyses. Ekrem Bölükbaşı made the molecular studies. Merve Kahraman Yiğit made the morphological and anatomical analyses.

References

BAS Database (2022). British Antarctic Survey, Database. https://www.bas.ac.uk/data/.

Denton, A. L., McConaughy, B. L., & Hall, B. D. (1998). Usefulness of RNA polymerase II coding sequences for estimation of green plant phylogeny. *Molecular Biology and Evolution*, *15*(8), 1082–1085. doi: 10.1093/oxfordjournals.molbev.a026007

Ekman, S., Andersen, H. L., & Wedin, M. (2008). The limitations of ancestral state reconstruction and the evolution of the ascus in the Lecanorales (lichenized Ascomycota). *Systematic Biology*, *57*(1), 141–156. doi: 10.1080/10635150801910451

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

Gaya, E., Steier, J., Wright, R., & Woods, R. (2021). *Barcoding Fungi from Royal Botanic Garden Kew Fungarium*. Unpublished.

Gyelnik, V. (1933). Lichenes varii novi criticique. *Acta pro Fauna et Flora Universali*, *2*(1), 5–6.

Halıcı, M., Bölükbaşı, E., Güllü, M., Kahraman Yiğit, M., & Barták, M. (2023b). *Lendemeriella vaczii*, a new lichenized fungal species from Antarctic Peninsula-with a key to the genus *Lendemeriella* (in press).

Halıcı, M. G., Kahraman Yiğit, M., Bölükbaşı, E., & Güllü, M. (2023a). New record and new species of lichenized fungal genus *Candelariella* Müll. Arg. in Antarctica. *Polish Polar Research*, *44*(1), 69–83. doi: 10.24425/ppr.2022.140370

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, *41*, 95–98.

Han, L., Zhang, H., & Guo, S. (2022). *Bilimbia sabuletorum [Nucleotide Data]*: The National Center for Biotechnology Information. https://www.ncbi.nlm.nih.gov.

Haughland, D. L., Hood, A., Thauvette, D., Toni, S. A., Cao, M., Birch, J. D., … Shier, C. (2022). Getting to know our biomonitor neighbours: urban lichens and allied fungi of Edmonton, Alberta, Canada. *Opuscula Philolichenum*, *21*, 33–18. doi: 10.5061/dryad.sqv9s4n6d

Huneck, S., Yoshimura, I., Huneck, S., & Yoshimura, I. (1996). *Identification of Lichen Substances*. Berlin, Heidelberg: Springer, pp. 11–123.

Kim, J. H., Ahn, I. Y., Hong, S. G., Andreev, M., Lim, K. M., Oh, M. J., & Hur, J. S. (2006). Lichen flora around the Korean Antarctic scientific station, King George Island, Antarctic. *The Journal of Microbiology*, *44*(5), 480–491.

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. doi: 10.12705/675.1

Kondratyuk, S., Lőkös, L., Kim, J. A. J. A., Kondratiuk, A., Jeong, M. H., Zarei-Darki, B., & Hur, J. S. (2014). *Oxnerella safavidiorum* gen. et spec. nov.(Lecanoromycetidae, Ascomycota) from Iran (Asia) proved by phylogenetic analysis. *Acta Botanica Hungarica*, *56*(3–4), 379–398. doi: 10.1556/abot.56.2014.3-4.13

Liu, Y. J., Whelen, S., & Hall, B. D. (1999). Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. *Molecular Biology and Evolution*, *16*(12), 1799–1808. doi: 10.1093/oxfordjournals.molbev.a026092

Marthinsen, G., Rui, S., & Timdal, E. (2019). OLICH: A reference library of DNA barcodes for Nordic lichens. *Biodiversity Data Journal*, *7*, e36252. doi: 10.3897/BDJ.7.e36252

Miadlikowska, J., Kauff, F., Högnabba, F., Oliver, J. C., Molnár, K., Fraker, E., & Stenroos, S. (2014). A multigene phylogenetic synthesis for the class Lecanoromycetes (Ascomycota): 1307 fungi representing 1139 infrageneric taxa, 317 genera and 66 families. *Molecular Phylogenetics and Evolution*, *79*, 132–168. doi: 10.1016/j.ympev.2014.04.003

Orange, A., James, P. W., & White, F. J. (2001). *Micro-chemical Methods for the Identification of Lichens*. London: British Lichen Society.

Øvstedal, D. O., & Smith, R. (2001). *Lichens of Antarctica and South Georgia: A Guide to Their Identification and Ecology*. Cambridge, UK: Cambridge University Press.

Printzen, C. (2014). A molecular phylogeny of the lichen genus *Biatora* including some morphologically similar species. *The Lichenologist*, *46*(3), 441–453. doi: 10.1017/S0024282913000935

Reese Næsborg, R. R., Ekman, S., & Tibell, L. (2007). Molecular phylogeny of the genus *Lecania* (Ramalinaceae, lichenized Ascomycota). *Mycological Research*, *111*(5), 581–591. doi: 10.1016/j.mycres.2007.03.001

Spribille, T., Fryday, A. M., Pérez-Ortega, S., Svensson, M., Tønsberg, T., Ekman, S., … & Sharman, L. (2020). Lichens and associated fungi from Glacier Bay National Park, Alaska. *The Lichenologist*, *52*(2), 61–181. doi: 10.1017/S0024282920000079

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, *38*(7), 3022–3027.

Thüs, H., Kluessendorf, J., & Weber, L. (2021). *Lecania cyrtellina [Nucleotide Data]*: The National Center for Biotechnology Information. https://www.ncbi.nlm.nih.gov.

Vainio, E. A. (1903). *Résultats Voyage du S.Y.* Belgica: Botanique Lichens.

Vondrák, J., Svoboda, S., Košnar, J., Malíček, J., Šoun, J., Frolov, I., Svensson, M., & Palice, Z. (2023). *Martin7 – A Reference Database of DNA Barcodes for European Epiphytic Lichens*. Unpublished.

Vondrák, J., Svoboda, S., Malíček, J., Palice, Z., Kocourková, J., Knudsen, K., & Hofmeister, J. (2022). From Cinderella to princess: an exceptional hotspot of lichen diversity in a long-inhabited central-European landscape. *Preslia*, *94*(1), 143–181. doi: 10.23855/preslia.2022.143

White, T. J., Bruns, T., Lee, S. J. W. T., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A Guide to Methods and Applications*, *18*(1), 315–322.

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

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