TÜBİTAK

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2022) 46: 500-506
© TÜBİTAK
doi:10.55730/1300-008X.2725

Shackletonia backorii (Teloschistaceae)-A new species of lichenised fungus from James Ross Island (Antarctic Peninsula)

Mehmet Gökhan HALICI[1,*], Mithat GÜLLÜ[1], Ekrem BÖLÜKBAŞI[2], Merve KAHRAMAN YİĞİT[1]
[1]Erciyes University, Department of Biology, Faculty of Science, Kayseri, Turkey
[2]Suluova Vocational School, Department of Environmental Protection Technologies, Amasya University, Amasya, Turkey

Received: 28.05.2022 • **Accepted/Published Online:** 03.08.2022 • **Final Version:** 19.09.2022

Abstract: The new lichen species *Shackletonia backorii* Halıcı, Güllü & Kahraman is described from James Ross Island, which is located in the North-East Antarctic Peninsula region. The new species is morphologically most similar to *S. insignis* (Søchting & Øvstedal) Søchting, Frödén & Arup, which was described from Livingstone Island (North-West Antarctic Peninsula region). These two species have a similar bluish grey to blackish thallus with a microfruticose and coralloid structure and red brown or almost black zeorine apothecia, but *S. backorii* has rhomboid ascospores and grows on soil whereas *S. insignis* has ellipsoid ascospores and occurs in sheltered crevices of rocks. The nrITS and mtSSU gene regions of the new species are studied and the phylogenetic position of the species is in the genus *Shackletonia* within the subfamily *Xanthorioideae* and it is clearly separated from the other species of the genus.

Key words: Antarctica, biodiversity, lichenized fungi, *Teloschistaceae*.

1. Introduction

According to our previous findings, James Ross Island which is in the North-East Antarctic Peninsula region has a rich and special lichen biodiversity when compared with the other parts of the continent because three lichen species were described as new to science and nine lichen species were reported as new to Antarctica from this island (Halıcı et al., 2017, 2018, 2020, 2021a, 2021b, 2022; Kahraman and Halıcı, 2021). The reason for this rich and special lichen biodiversity is probably because of the island's special location in the transition zone between maritime and continental climate types (Bednarek-Ochyra et al., 2000). Another reason for the rich lichen biodiversity is the large deglaciated areas (170 km^2) present on James Ross Island.

The genus *Shackletonia* Søchting, Frödén & Arup was described by Arup et al. (2013), belonging to subfamily *Xanthorioideae* and is currently represented by four species: *S. buelliae* (Olech & Søchting) Søchting, Frödén & Arup, *S. hertelii* (Søchting, Øvstedal & Sancho) Søchting, Frödén & Arup, *S. insignis* (Søchting & Øvstedal) Søchting, Frödén & Arup and *S. siphonospora* (Olech & Søchting) Søchting, Frödén & Arup. All four species have an Antarctic distribution with *S. hertelii* also being reported from southern Patagonian and *S. siphonospora* from the Falkland Islands. Two species previously classified under the genus *Shackletonia* (*S. cryodesertorum* Garrido-Ben., Søchting & Pérez-Ort. and *S. sauronii* (Søchting & Øvstedal) Søchting, Frödén & Arup were transferred to the newly described genus *Erichansenia* by Kondratyuk et al. (2020). These authors suggested that *Erichansenia* differs from *Shackletonia* in having better developed epilithic thallus, in having mostly completely black apothecia with a dark greenish blue outer exciple, in having *Lecidea* green pigment in the cortex of thallus and exciple. Besides the genus, *Erichansenia* has distribution in both northern and southern hemispheres but *Shackletonia* with only southern distribution. These two genera phylogenetically are positioned in the outermost position to the other genera of the *Xanthorioideae* (Arup et al., 2013; Kondratyuk et al., 2020).

Morphological, anatomical, and phylogenetical analyses of two specimens collected on soil from relatively high altitudes of James Ross Island by the first author in 2017, showed that they belong to an undescribed species of *Shackletonia* and we describe it here as *S. backorii*, the fifth species of the genus *Shackletonia*.

2. Material & methods

2.1. Morphological and anatomical studies

Samples of lichens were collected from James Ross Island (Antarctic Peninsula) by the first author and are deposited in ERCH. Macroscopic observations were made with an

* Correspondence: mghalici@gmail.com

Olympus S2X7 dissecting microscope equipped with an OLYMPUS SC30 image capture system. Handmade sections of ascomata were examined in a Leica DM2500 light microscope and microphotographs were taken with a Flexacam C1 digital camera. Microscopic measurements were made on material mounted in water. Chemicals used either for tissue dissociation and examination, or for testing possible colour reactions of ascomatal elements were: 10 % KOH (K), sodium hypochlorite (C), and 10 % nitric acid (N). Ascospores were measured outside the asci. The average is followed by its standard deviation, and the maximum and minimum values are given in parentheses. The thickness of the spore septum was measured at the outer wall.

2.2. Molecular methods

2.2.1. DNA isolation, PCR, and sequencing

Total DNA was isolated from the specimens by means of the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. Hand-cut sections of about five apothecia per collected specimen were prepared for DNA isolation. PCR amplification for the internal transcribed spacer region (ITS1-5.8S-ITS2 rDNA) and the mitochondrial small subunit gene regions was achieved using 2 × Taq PCR MasterMix in 20-µL tubes with 16 µL of distilled water, 2 µL of DNA extracts, and 2 µL of primers ITS5 and ITS4 (Gardes and Bruns, 1993; White et al., 1990) or mrSSU1 and mrSSU3R (Zoller et al., 1999). The following PCR temperature profiles were employed: 5 min at 94 °C, then 30 cycles of 1 min at 94 °C, 1 min at 52 or 54 °C (nrITS, mtSSU, respectively), and 3 min at 72 °C, with a final extension of 8 min at 72 °C. The PCR reactions were visualized on 1% agarose gel. Sequence analyzes of the lichen samples obtained from the PCR products were performed by the BM Labosis Laboratory (Ankara, Turkey).

2.2.2. Additional sequences

The final dataset consisted of newly generated sequences (2 sequences for ITS and 1 sequence for mtSSU) from this study and 23 ITS and 15 mtSSU sequences obtained from GenBank (Table 1). *Teuvoahtiana rugulosa* was used as the outgroup in both ITS and mtSSU phylogenetic trees.

2.2.3. Sequence alignment and phylogenetic analysis

All ITS and mtSSU sequences were aligned and edited manually using ClustalW in Bioedit V7.2.6.1 (Hall, 1999). All ambiguous characters were removed and only unambiguous characters were analyzed. The final alignment comprised 579 (nrITS) and 785 (mtSSU) columns. Phylogenetic trees with bootstrap values were obtained in MEGA XI using the Maximum Likelihood method with a rapid bootstrap with 1000 bootstrap replications (Tamura et al., 2021).

The final dataset consisted of newly generated sequences (2 sequences for ITS and 1 sequence for mtSSU) from this study and 23 ITS and 15 mtSSU sequences obtained from GenBank (Table 1). Sequences were subjected to BLAST searches to confirm their identities. Only high-quality sequences were used for phylogenetic analyses. Sequences were manually edited using BioEdit V7.2.6.1 (Hall, 1999) and FinchTV 1.4.0 (Geospiza Inc., Seattle, WA, USA). All new sequences were deposited in GenBank. *Teuvoahtiana rugulosa* was used as an outgroup in both ITS and mtSSU trees. Apart from *Shackletonia*, two high-diverse genera of subfamily *Xanthorioideae* with a considerable amount of molecular data, *Erichansenia* and *Xanthomendoza* were also analysed for comparison. The polymorphism statistics of the three genera compared are shown in Table 2.

3. Results

3.1. Evaluating phylogenetic analysis results

Two independent phylogenetic trees for the genus *Shackletonia* were produced from 41 sequences (23 for ITS, and 15 for mtSSU) from GenBank and three new sequences (sequences for ITS and 1 sequence for mtSSU) from the new species (Table 2). All species names are followed by the GenBank accession numbers or voucher information (Table 2). When the ITS and mtSSU phylogenetic tree were examined compared with the related genera in the *Xanthorioideae* family, the genus *Shackletonia* in which our new species is located, clearly formed a separate clade with a 100% bootstrap value from the other genera (Figure 1 and Figure 2). Within the genus *Shackletonia*, the new species clearly branched out from other species. The phylogenetic analyzes did not designate any species identical to the new species in the genus *Shackletonia*.

Figure 1. ITS ML phylogeny of *S. backorii* and related species. The new species *S. backorii* is presented in JR code.

3.2. Taxonomy

Shackletonia backorii Halıcı, Güllü & Kahraman sp. nov. (Figure 3)

MycoBank No.: MB 844771

Type: Antarctic Peninsula, James Ross Island: Berry Hill Mesa, 63°48'S, 57°50'W, alt. 345 m, on soil, 11 January 2017, leg. M. G. Halıcı *ERCH JR 0.328* (ERCH—holotype).

Diagnosis: Similar to *Shackletonia insignis* with crustose to microfruticose thallus but differs by terricolous habit and rhomboid ascospores.

Etymology: Named in honour of Prof. Dr. Martin Bačkor (Pavol Jozef Šafárik University in Košice, Faculty of Science), who helped the first author in the field excursions in James Ross Island.

Description: Thallus lichenized, not parasitic, crustose to microfruticose, ash grey to dark grey, some parts of the thallus almost black in patches, up to 0.5 mm thick. Thallus is composed of vertical lobes or more or less coralloid squamules. Thallus cortex differentiated, thin, with dark pigments. Medulla dense or medium dense

Table 1. ITS and mtSSU sequences used in the analyses. The new sequences are in bold.

	ITS	**Locality**	**mtSSU**	**Locality**
JR 0.328 *Shackletonia backorii*	**ON950087**	James Ross Island, Antarctica	**ON950086**	James Ross Island, Antarctica
JR 0.341 *Shackletonia backorii*	**ON950083**	James Ross Island, Antarctica	-	-
Shackletonia buelliae	KC179117	South Shetland Islands, Antarctica	KC179578	South Shetland Islands, Antarctica
Shackletonia hertelii	KC179118	Chile	KC179579	Chile
Shackletonia insignis	KC179119	South Shetland Islands, Antarctica	-	-
Shackletonia siphonospora	KC179121	South Shetland Islands, Antarctica	-	-
Erichansenia epithallina	MH155282	USA, California,	-	-
Erichansenia epithallina	MH155283	Hot Creek	-	-
Erichansenia sauronii	KC179120	Russia, Caucasus	-	-
Erichansenia cryodesertorum	JX036057	Antarctica, South Shetland Islands	KU599932	McMurdo Dry Valleys, Antarctica
Erichansenia cryodesertorum	JX036132	McMurdo Dry Valleys, Antarctica	-	-
Erichansenia cryodesertorum	JX036144	McMurdo Dry Valleys, Antarctica	-	-
Xanthomendoza mendozae	JQ301688	McMurdo Dry Valleys, Antarctica	JQ301523	Bolivia, Potosí, Salar de Uyuni, Isla Inca Huasi
Xanthomendoza mendozae	EU681350	Bolivia, Potosí, Salar de Uyuni, Isla Inca Huasi	EU680939	Sweden
Xanthomendoza borealis	KP314378	Sweden	KT291526	East Antarctica, Hallett Peninsula
Xanthomendoza borealis	KT291473	Svalbard (High Arctic)	KC179617	Greenland
Xanthomendoza fulva	AM408423	East Antarctica, Hallett Peninsula, Cape Hallett, Ross Sea coast of Victoria Land	-	-
Xanthomendoza fulva	AM408426	Switzerland	-	-
Xanthomendoza hasseana	AM292816	Switzerland	KC179619	USA, Arizona
Xanthomendoza hasseana	AM697877	California, Santa Barbara, USA	-	-
Xanthomendoza oregana	AM697876	California, Santa Barbara, USA	KT291527	California, Santa Barbara, USA
Xanthomendoza oregana	KJ396108	California, Santa Barbara, USA	-	-
Xanthomendoza poeltii	JQ301689	Norway	JQ301525	Sweden, Halland
Xanthomendoza poeltii	EU681361	Sweden, Halland	KC179622	Sweden
Xanthomendoza trachyphylla	-	Sweden	KC179623	USA, North Dakota
Xanthomendoza trachyphylla	-	-	KT291513	Pakistan (Northern Areas), Northwestern Himalaya
Teuvoahtiana rugulosa	KY614443	Chile	KY614517	Chile

Table 2. Polymorphism statistics for each marker (nrITS and mtSSU) from datasets corresponding to the genera *Shackletonia*, *Erichansenia* and *Xanthomendoza* belonging to the subfamily *Xanthorioideae*.

Datasets	**nrITS**	**mtSSU**
Species included (n) Conserved sites Variable sites	25 277 296	16 683 90
Singleton variable sites	66	36
Parsimony inf. sites	229	50
Transitional pairs (si)	46	11
Transversional pairs (sv)	42	11
R (si/sv)	1.1	1.0
Number of sites (bp)	579	785

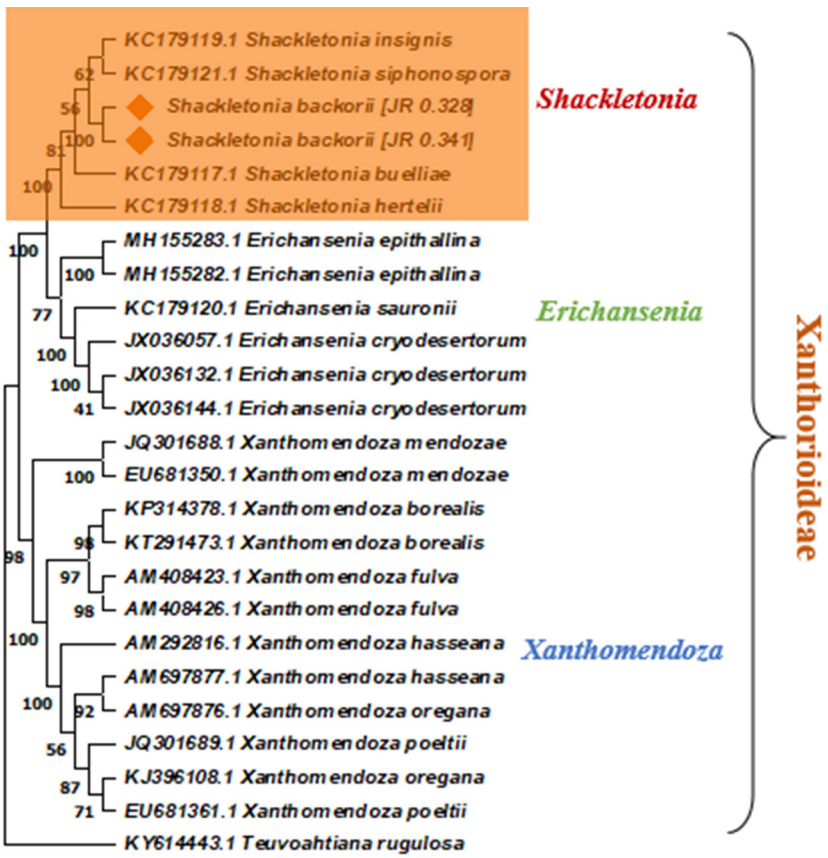

Figure 1. ITS ML phylogeny of *S. backorii* and related species. The new species *S. backorii* is presented in JR code.

with interwoven hyphae, pseudoparenchymatous, with oil droplets. Algal layer of the thallus 65–95 µm thick, algae green, chlorococcoid.

Apothecia zeorine, numerous, sessile to stipitate, up to 0.3 mm wide. Apothecial disc flat or somewhat concave, roundish to angular, red brown or almost blackish, the young ones have a lighter colour with a greenish tinge. Thalline margin distinct, prominent, concolorous with the thallus, smooth in young apothecia but slightly curved in the older ones. Proper margin thin but easily

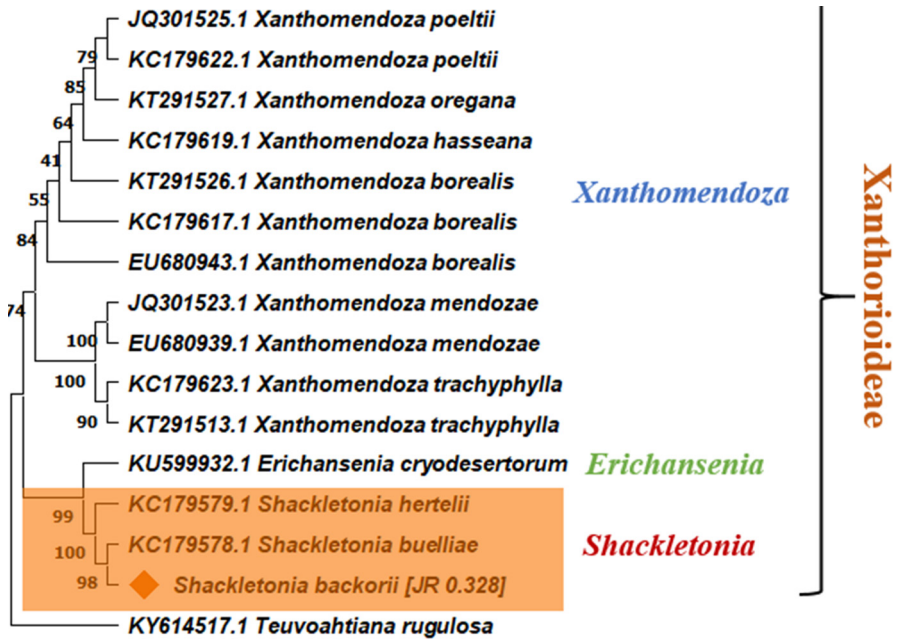

Figure 2. mtSSU ML phylogeny of *S. backorii* and related species. The new species *S. bachorii* is presented in JR code.

seen with x10 magnification, lighter than the thalline margin in colour. Cortex of excipulum consisting of strongly glutinized prosoplectenchymatous tissue. Proper excipulum fan-shaped. Epithecium golden brown or light brownish, 15–20 µm, K + pink violet. Hymenium hyaline, 45–70 µm. Paraphyses branched from tips or the medium parts, septate, with oil droplets, 1.5–4 µm thick, apices not enlarged or slightly enlarged. Hypothecium hyaline with a yellowish tinge, rich in oil droplets, 70–95 µm. Asci 8-spored, 40–45 × 10–15 µm. Ascospores hyaline, polaribilocular, lemon-shaped to broadly rhomboid, (10–)11.5–12.5–13.5(–14) × (5–)6–7.5–9(–11) µm, septa (1.5)2–3–4(5) µm, l/w: (1.18)1.34–1.67–1.99(2.27); s/l: (0.14–)0.15–0.24–0.32(–0.38). Pycnidia not observed.

Chemistry: Thallus K-, C-, KC-, PD-, apothecial disc K+ violet.

Ecology: *Shackletonia backorii* is a terricolous lichen recorded between 200–350 m altitude on James Ross Island (Antarctic Peninsula). This species prefers exposed habitats and was found together with lichens such as *Megaspora verrucosa* (Ach.) Hafellner & V. Wirth and *Lecidella* spp.

Additional specimen examined: Antarctic Peninsula, James Ross Island: Southern east part of Johnson Mesa, 63°49′46.2″S, 57°54′21.6″W, alt. 292 m, on soil, 14 January 2017, leg. M. G. Halıcı, *ERCH JR 0.341*.

4. Discussion

According to our phylogenetical analyses, the new species clearly belongs to the genus *Shackletonia* and is clearly separate from other species of the genus. Morphologically, *S. backorii* is most similar to *S. insignis*, which was described from Livingstone Island (North-West Antarctic Peninsula region). These two species have similar microfruticose and coralloid structures of the bluish grey to blackish thallus and red brown or almost black zeorine apothecia, but *S. backorii* has rhomboid ascospores and grows on soil whereas *S. insignis* occurs in sheltered crevices of rocks and has ellipsoid ascospores (Søchting et al., 2004). *Erichansenia cryodesertorum* (Garrido-Ben., Søchting & Pérez-Ort.) S.Y. Kondr., Kärnefelt & A. Thell, an epilithic species described from McMurdo Dry Valleys of Antarctica in the genus *Shackletonia* (Garrido-Benavent et al., 2016) but subsequently transferred to *Erichansenia* (Kondratyuk et al., 2020), sometimes develops microfruticose morphs similar to these two species. This is a common feature of Antarctic epilithic lichens and, according to Garrido-Benavent et al. (2016), is possibly related to the higher water retention capacity and light harvesting efficiency of this morphology. *Erichansenia cryodesertorum* clearly differs from *S. backorii* by its completely black, lecideine apothecia. Previously, the only known terricolous species of the genera *Shackletonia* and *Erichansenia* was *S. siphonospora*, but this species differs from *S. backorii* by having simple ascospores and almost invisible thallus which is a dark greyish crust and is known from King George Island, Livingstone Island (Olech and Søchting, 1993) and the Falkland Islands (Fryday et al., 2019). The other members of these two genera *S. buelliae* and *E. sauronii* (Søchting & Øvstedal) S.Y. Kondr., Kärnefelt & A. Thell are known to be lichenicolous

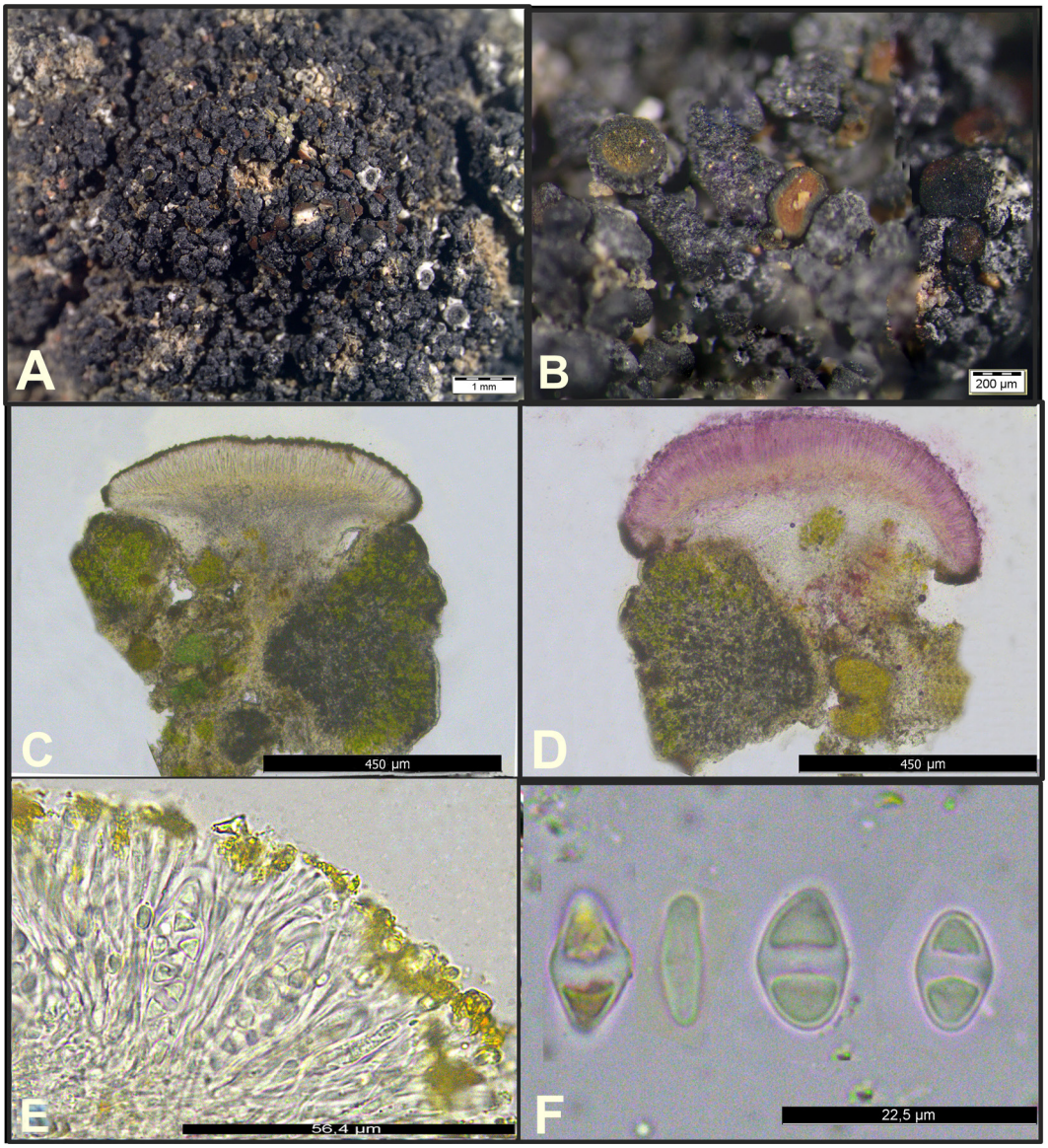

Figure 3. *Shackletonia backorii*. **A.** Habitus (The white lichen between the squamules of *S. backorii* is *Megaspora verrucosa*). **B.** Apothecia and squamules in closer view. **C.** Apothecial section in water. **D.** Apothecial section in K. **E.** Asci and paraphyses in water. **F.** Lemon-shaped to rhomboid ascospores.

(Olech and Søchting, 1993; Søchting et al., 2004). *Caloplaca psoromatis* Olech & Søchting is another Antarctic species known to be lichenicolous on apothecia of *Pannaria hookeri* (Borrer) Nyl. and *Psoroma* spp. which probably belongs in the genus *Shackletonia* but unfortunately, this species has no molecular data and has not yet been transferred to this genus (Øvstedal and Lewis Smith, 2004; Garrido-Benavent et al., 2016). This species has more or less rhomboid ascospores, as in *S. backorii*. Three species: *E. cryodesertorum*, *S. buelliae*, and *S. siphonospora* have typically Lecidea green pigment in the exciple (Garrido-Benavent et al., 2016) but *S. backorii* does not.

An identification key to the species of *Shackletonia*:

1. Ascospores polaribilocular 2
1. Ascospores simple ..... *S. siphonospora* (Olech & Søchting) Søchting, Frödén & Arup
2. Thallus ± microfruticose 3
2. Thallus crustose or not visible 4
3. Terricolous, ascospores rhomboid ..... *S. backorii* Halıcı, Güllü & Kahraman
3. Growing on rock crevices, ascospores ellipsoid ..... *S. insignis* (Søchting & Øvstedal) Søchting, Frödén & Arup
4. Thallus not visible, on *Buellia* spp. ..... *S. buelliae* (Olech & Søchting) Søchting, Frödén & Arup

4. Thallus crustose, dark greyish, saxicolous on maritime rocks or sometimes lichenicolous ..... *S. hertelii* (Søchting, Øvstedal & Sancho) Søchting, Frödén & Arup

Acknowledgements
The first author thanks Erciyes University for their financial support to conduct the fieldwork on James Ross Island, Antarctica, İTÜ Polrec for their support, and J.G. Mendel station for infrastructure and facilities. This project was supported by TÜBİTAK 118Z587 coded Project and TÜBA (Turkish Academy of Science). Dr. Alan FRYDAY (Michigan State University, USA) is thanked for the scientific contribution and for checking the language of the manuscript. Dilara BİŞGİN is thanked for taking the anatomical sections.

References

Arup U, Søchting U, Frödén P (2013). A new taxonomy of the family Teloschistaceae. Nordic Journal of Botany 31 (1): 016-083. doi:10.1111 /j.1756-1051.2013.00062.x

Bednarek-Ochyra H, Váňa J, Ochyra R, Lewis-Smith RI (2000). The liverwort flora of Antarctica. W. Szafer Institute of Botany, Polish Academy of Sciences, Cracow, Poland.

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. doi:10.1111/j.1365-294X.1993.tb00005.x

Fryday AM, Orange A, Ahti T, Øvstedal DO, Crabtree DE (2019). An annotated checklist of lichen-forming and lichenicolous fungi reported from the Falkland Islands (Islas Malvinas). Glalia: revista electrónica del Grupo Latinoamericano de Liquenólogos 8 (1): 1-100.

Garrido-Benavent I, Søchting U, de los Ríos Murillo A, Pérez-Ortega S (2016). *Shackletonia cryodesertorum* (Teloschistaceae, Ascomycota), a new species from the McMurdo Dry Valleys (Antarctica) with notes on the biogeography of the genus *Shackletonia*. Mycological Progress 15(7): 743-754. Doi:10.1007/s11557-016-1204-x

Halıcı MG, Güllü M, Parnikoza I (2017). *Sagediopsis bayozturkii* sp. nov. on the lichen *Acarospora macrocyclos* from Antarctica with a key to the known species of the genus (Ascomycota, Adelococcaceae). Polar Record 53 (3): 271-275. doi:10.1017 / S0032247417000043

Halıcı MG, Bartak M, Güllü M (2018). Identification of some lichenised fungi from James Ross Island (Antarctic Peninsula) using nrITS markers. New Zealand Journal of Botany 56 (3): 276-290. doi: 10.1080/0028825X.2018.1478861

Halıcı MG, Osmanoğlu OM, Kahraman M (2020). A new record of lichenized fungus species for Antarctica: *Peltigera castanea* Goward, Goffinet & Miądl. Czech Polar Reports 10 (1): 50-58. doi:10.5817/CPR2020-1-5

Halıcı MG, Kahraman M, Kistenich S, Timdal E (2021a). *Toniniopsis bartakii*-A new species of lichenised fungus from James Ross Island (Antarctic Peninsula). Turkish Journal of Botany, 45 (3):216-223. doi:10.3906/bot-2101-24

Halıcı MG, Kahraman M, Osmanoğlu O, Bartak M (2021b). New records of lichenized fungi for Antarctica. Polish Polar Research 42 (3): 203-219. doi: 10.24425/ppr.2021.137145

Halıcı MG, Kahraman M, Scur MC, Kitaura MJ (2022). *Leptogium Pirireisii*, a new species of lichenized Ascomycota (Collemataceae) from James Ross Island in Antarctica. New Zealand Journal of Botany 60 (1): 68-76. doi:10.1080/00288 25X.2021.1939735

Hall TA (1999). BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/ NT. Nucleic Acids Symposium Series 41: 95-98.

Kahraman M, Halıcı MG (2021). *Buellia epigaea* (Pers.) Tuck, a new record of lichenized fungus species for Antarctica. Czech Polar Reports 11 (1): 9-15. doi:10.5817/CPR2021-1-2

Kondratyuk SY, Lőkös L, Farkas E, Kärnefelt I, Thell A et al. (2020). Three new genera of the Teloschistaceae proved by three gene phylogeny. Acta Botanica Hungarica 62 (1-2): 109-136. doi:10.1556/034.62.2020.1-2.7

Olech M, Søchting U (1993). Four new species of *Caloplaca* from Antarctica. The Lichenologist 25 (3): 261-269.

Øvstedal DO, Smith RI (2004). Additions and corrections to the lichens of Antarctica and South Georgia. Cryptogamie, Mycologie 25 (4): 323-331.

Søchting U, Øvstedal DO, Sancho LG (2004). The lichens of Hurd Peninsula, Livingston Island, South Shetlands, Antarctica. Bibliotheca Lichenologica 88: 607-658.

Tamura K, Stecher G, Kumar S (2021). MEGA11: molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution 38 (7): 3022-3027. doi:10.1093/molbev/msab120

White TJ, Bruns T, Lee SJWT, 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