

Diversity of *Lecidea* (Lecideaceae, Ascomycota) species revealed by molecular data and morphological characters

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Abstract: The diversity of lichens, especially crustose species, in continental Antarctica is still poorly known. To overcome difficulties with the morphology based species delimitations in these groups, we employed molecular data (nuclear ITS and mitochondrial SSU rDNA sequences) to test species boundaries within the genus *Lecidea*. Sampling was done along a north–south transect at five different areas in the Ross Sea region (Cape Hallett, Botany Bay to Mount Suess, Taylor Valley, Darwin Area and Mount Kyffin). A total of 153 specimens were collected from 13 localities. Phylogenetic analyses also include specimens from other regions in Antarctica and non-Antarctic areas. Maximum parsimony, maximum likelihood and Bayesian analyses agreed in placing the samples from continental Antarctica into four major groups. Based on this phylogenetic estimate, we restudied the micromorphology and secondary chemistry of these four clades to evaluate the use of these characters as phylogenetic discriminators. These clades are identified as the following species *Lecidea cancriformis*, *L. andersonii* as well as the new species *L. polypycnidophora* Ruprecht & Türk sp. nov. and another previously unnamed clade of uncertain status, referred to as *Lecidea* sp. (*L.* UCR1).

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Introduction

The diversity of lichens in Antarctica is poorly known. This is especially true for crustose lichens that are often reduced to minute patches surrounding ascomata under the harsh climatic conditions typical of this ecosystem. The number of available collections is limited, which restricts the ability to assess variability within species. Lecideoid lichens are abundant and rich in species in Antarctica (Hertel 2007) and, because of the poor understanding of morphological and chemical variation, their taxonomy is currently in urgent need of revision. Most of the lecideoid taxa are confined to the sub-Antarctic and maritime Antarctic regions with only a few species known to occur in the more extreme environmental conditions of continental Antarctica. Lecideoid lichens often act as pioneers on rock and pebbles (Hertel 1987). At the more southern and very dry parts of continental Antarctica lecideoid lichens are among the few occurring lichen groups (Stevens *et al.* unpublished, Marshall *et al.* unpublished).

Saxicolous lecideoid lichens in Antarctica include species of the genera *Carbonea*, *Lecanora*, *Lecidea*, and *Lecidella*. Despite the ecological importance of these lichens in polar habitats, the taxonomy is only poorly known and the circumscription of taxa differs between authors. Only a few lecideoid lichens are recorded from continental Antarctica (Pickard & Seppelt 1984, Hale 1987,

Hertel 1987, 1988, Jacobsen & Kappen 1988, Seppelt *et al.* 1988, 1995, Andreev 1990, Kappen *et al.* 1990, Inoue 1991, 1995, Melick *et al.* 1994, Upreti 1996, Broady & Weinstein 1998, Øvstedal & Lewis Smith 2001, Castello 2003).

The diversity of species in Antarctic lichens has been a matter of debate for a long time. While some authors claimed about 97% of the Antarctic lichen species to be endemic (Dodge 1973), others considered *c.* 20% as being more realistic (Hertel 1988, Seppelt *et al.* 1998). In most recent studies, many of the previously described endemic species, have been reinterpreted as variations due to extreme climatic conditions. One prominent example is the Antarctic endemic *Lecidea cancriformis* (Hertel 2007). Different morphotypes of this lichen have been interpreted as different species but recent morphological studies have suggested that they should be synonymised with *L. cancriformis* (Castello 2003). These morphology-based studies (Castello 2003, Hertel 2007) accept two *Lecidea* species (*L. andersonii*, *L. cancriformis*) in continental Antarctica. However, molecular data are largely absent for Antarctic lichens, with only a few exceptions (Dyer & Murtagh 2001, Ohmura & Kanda 2004, Ott *et al.* 2004, Seymour *et al.* 2007, Wirtz *et al.* 2006, 2008, Lee *et al.* 2008). The evaluation of morphological characters and identification of crustose lecideoid lichens is hampered in polar regions, due to common occurrence of chasmolithic

Table I. Investigation sites with the number of the collected *Lecidea* specimens and the total number of collected specimens from Ross Sea Region. The dates of the expeditions at the Ross Sea Region are in parenthesis. Specimens for collection sites 6–9 were provided as herbarium vouchers from R. Seppelt and M. Andreev for a broader taxon sampling across the entire continental Antarctica.

Collection sites	<i>Lecidea</i> specimens	Total number of specimens collected
1. Cape Hallett (2004)	1	20
2 a. Botany Bay–Granite Harbour–Discovery Bluff	28	70
b. Mount Suess–Sperm Bluff (2000, 2008)	5	14
3. Taylor Valley 2003	11	21
4. Darwin Area: a. Brown Hills (2004)	46	67
b. Lake Wellman (2007)	7	18
c. Diamond Hill - Bartrum Basin (2009)	53	115
5. Mount Kyffin (2003)	2	58
6. Wilkes Land (R. Seppelt)	10	-
7. Mac. Robertson Land (M. Andreev)	2	-
8. Princess Elizabeth Land (M. Andreev)	2	-
9. Dronning Maud Land (M. Andreev)	3	-

growth and ascomata lacking ascospores or with only sparsely developed ascospores (Hertel 2009).

To overcome difficulties of assessing species diversity in Antarctic lecideoid lichens, we employed molecular data (nuclear ITS and mitochondrial SSU ribosomal DNA sequences) to test species boundaries. We used a phylogenetic species concept based on a two-gene dataset in this study to circumscribe species in these lichens (Grube & Kroken 2000). Based on our phylogenetic estimate we re-evaluated morphological and chemical characters to identify characters that can be used to identify these distinct lineages. Here we have focused on samples belonging to the genus *Lecidea* s. str. Collections were made from five different regions in continental Antarctica along a north–south coastal transect within the Ross Sea region (72–84°S). 153 specimens of the genus *Lecidea* were found in all habitats (Tables I & II, Figs 1 & 2). To complete the sampling we included samples from other regions of continental Antarctica (Tables I & II, Fig. 1), Arctic regions (Svalbard/Norway, Greenland), Austria, Sweden and the USA (Table II).

Material and methods

Investigation sites

The collection sites are located on the western coast of the Ross Sea (Table I, Figs 1 & 2). The Transantarctic Mountains are to the west and strongly affect the local climate. In particular, föhn-like winds contribute to the unusual dryness of the whole of southern Victoria Land and the McMurdo Dry Valleys in particular (Green *et al.* 1992). The saxicolous vegetation is extremely sparse. Lichens are only found where special microclimatic conditions occur and where they benefit from protection from abrasion of wind blown particles, from meltwater from melting snow and ice and by increased warmth (Green *et al.* 2007).

1. Cape Hallett, northern Victoria Land (72°19'S, 170°16'E), 0–300 m alt., January 2004

The area is a low lying gravel spit with a steep slope to the south rising from sea level to about 300 m alt.; the scree on the slope protects the vegetation from the strong winds. The area is ice free during the late spring and summer, the melting water forms numerous small channels through the flatter vegetated areas and precipitation is low (Pannowitz *et al.* 2005). The level area is composed of beach gravels and cliff talus and a high nutrient input is guaranteed by a rich birdlife in the area (Green *et al.* 2000).

2. a. Granite Harbour–Botany Bay–Discovery Bluff, Victoria Land, Ross Dependency (77°00'S, 162°27'–163°35'E), 0–435 m alt., January 2000, 2008
During the summer season the area is snow free and protected from high winds. Several small streamlets of melting water flow from the snowfields of the adjacent ridges (Pannowitz *et al.* 2005) and the area has an exceptional moss and lichen vegetation (Taylor 1913).
b. Mount Suess (77°02'S, 161°34'–161°43'E), 730–755 m alt. Sperm Bluff (77°03'–77°05'S, 161°43'E) 522 m alt., 2008

These two inland mountains are within a glacial ice sheet (south of Mackay Glacier). The region is snow free in summer and a well sheltered area with a pond at the bottom is located below the top of Mount Suess. Mount Suess consists of a complex of granodiorite, gneiss, dolerite and sandstone (Gunn & Warren 1962). Sperm Bluff is composed of big granite boulders, ephemeral streams appear not to be present.

3. Taylor Valley, Victoria Land (77°34'–77°41'S, 162°15'–163°05'E), 0–1143 m alt., January 2003
The collecting sites are located close to the Canada Glacier (40 m alt.), Mount Falconer north from Lake Fryxell (718 m alt.) and Aasgard Range at the Rhone Glacier (1143 m alt.). Taylor Valley is a classic dry valley and is a part of the McMurdo Dry Valleys, which

Table II. Specimen information - location, collector, date, herbarium number and Genbank-accession numbers of mtSSU, ITS.

No.	Name of species	Sample	Herbarium	ITS	mtSSU
1	<i>Farnoldia jurana</i> ssp. <i>jurana</i> (Schaer.) Hertel	AUSTRIA, Salzburg: Pongau 22 Jul 06, leg: R. Türk & U. Ruprecht	R. Türk 39660	EU263920	GU074511
2	<i>Lecidea andersonii</i> Filson	ANTARCTICA, Dronning Maud Land: Schirmacher Oasis 20 Mar 03, leg & det: M. Andreev	LE A0515601	GU074445	GU074469
3	<i>L. andersonii</i>	ANTARCTICA, Dronning Maud Land: Schirmacher Oasis 21 Mar 03, leg & det: M. Andreev	LE A0515901	GU074446	GU074470
4	<i>L. andersonii</i>	ANTARCTICA, Victoria Land: Botany Bay 16 Jan 08, leg & det: U. Ruprecht	R.Türk 43008	GU074450	GU074463
5	<i>L. andersonii</i>	ANTARCTICA, Victoria Land: Botany Bay 16 Jan 08, leg & det: U. Ruprecht	R.Türk 43011	GU074452	GU074464
6	<i>L. andersonii</i>	ANTARCTICA, Victoria Land: Granite Harbour 15 Jan 08, leg & det: U. Ruprecht	R. Türk 43006	GU074453	GU074462
7	<i>L. andersonii</i>	ANTARCTICA, Victoria Land: Granite Harbour 17 Jan 08, leg & det: U. Ruprecht	R.Türk 43022	GU074454	GU074465
8	<i>L. andersonii</i>	ANTARCTICA, Victoria Land: Mount Suess 8 Jan 08, leg & det: U. Ruprecht	R. Türk 42995	GU074447	GU074458
9	<i>L. andersonii</i>	ANTARCTICA, Victoria Land: Mount Suess 8 Jan 08, leg & det: U. Ruprecht	R. Türk 42996	GU074448	GU074459
10	<i>L. andersonii</i>	ANTARCTICA, Victoria Land: Sperm Bluff 12 Jan 08, leg & det: U. Ruprecht	R. Türk 43002	GU074449	GU074460
11	<i>L. andersonii</i>	ANTARCTICA, Victoria Land: Sperm Bluff 12 Jan 08, leg & det: U. Ruprecht	R. Türk 43003	GU074451	GU074461
12	<i>L. andersonii</i>	ANTARCTICA, Windmill Islands: Bailey Peninsula 4 Jan 89, leg & det: R. Seppelt	ADT18513	GU074443	GU074468
13	<i>L. andersonii</i>	ANTARCTICA, Windmill Islands: Bailey Peninsula 25 Dez 85, leg: R. Seppelt, det: U. Ruprecht	ADT24109	GU074444	GU074467
14	<i>L. andersonii</i>	NORWAY, Svalbard: Ny Ålesund 18 Aug 08, leg: A. Tribsch, det: H. Hertel	R. Türk 43425	GU074442	GU074466
15	<i>L. atrobrunnea</i> (Ramond) Schaer.	ANTARCTICA, Antarctic Peninsula: Danco Coast 9 Jan 06, leg & det: M. Andreev	LE A060124	GU074457	GU074505
16	<i>L. atrobrunnea</i>	ANTARCTICA, Antarctic Peninsula: Willhelm Archipelago 12 Jan 06, leg & det: M. Andreev	LE A060414	-	GU074504
17	<i>L. atrobrunnea</i>	ANTARCTICA, South Shetland Island: Livingston Island 3 Feb 02, leg & det: R. Türk	R.Türk 36121	GU074455	GU074509
18	<i>L. atrobrunnea</i>	N. GREENLAND: near Nikolaj Nielsen Kyst, 22 Jul 99, leg & det: E.S. Hansen	Lund, Lich. Groenl. Exsiccati 770	EU259897	GU074510
19	<i>L. atrobrunnea</i> ssp. <i>saxosa</i> Hertel & Leuckert (Ramond) Schaer.	USA, California: Peninsula Range, Tahquitz Peak 4 Oct 04, leg & det: K. Knudsen	U.C. Riverside UCR-174803	GU074456	GU074507
20	<i>L. atrobrunnea</i> ssp. <i>saxosa</i>	USA, California: Peninsula Range, Tahquitz Peak 4 Oct 04, leg & det: K. Knudsen	U.C. Riverside UCR-176128	EU259898	GU074508
21	<i>L. atrobrunnea</i> ssp. <i>stictica</i> Hertel & Leuckert (Ramond) Schaer.	AUSTRIA, Kärnten: Hohe Tauern 2 Jul 03, leg & det: R. Türk	R. Türk 34435	EU259899	GU074506
22	<i>L. auriculata</i> Th. Fr	NORWAY, Svalbard: Ny Ålesund 18 Aug 08, leg: A. Tribsch, det: H. Hertel	R. Türk 43426	GU074428	GU074499
23	<i>L. auriculata</i>	SWEDEN, Jämtland: Åre par., Handöl 28 Aug 05, det: Ulf Arup	Lund L05184	GU074427	GU074500

Table II. Continued

No.	Name of species	Sample	Herbarium	ITS	mtSSU
24	<i>L. cancriformis</i> Dodge & Baker	ANTARCTICA, Northern Victoria Land: Cape Hallett 28 Nov 04, leg: R. Seppelt, det: U. Ruprecht	ADT25151	GU074429	GU074477
25	<i>L. cancriformis</i>	ANTARCTICA, Princess Elizabeth Land: Larssemann Hills 2 Jan 05, leg & det: M. Andreev	LE A057501	GU074440	GU074479
26	<i>L. cancriformis</i>	ANTARCTICA, Ross Dependency: Bartrum Basin 16 Jan 09, leg: R. Türk, det: U. Ruprecht	R. Türk 44712	GU074438	GU074487
27	<i>L. cancriformis</i>	ANTARCTICA, Ross Dependency: Darwin Glacier 26 Jan 09, leg: R. Türk, det: U. Ruprecht	R. Türk 44634	GU074434	GU074486
28	<i>L. cancriformis</i>	ANTARCTICA, Ross Dependency: Darwin Glacier-Brown Hills 11 Dec 04, leg: R. Türk, det: U. Ruprecht	R. Türk 35604	EU257671	GU074480
29	<i>L. cancriformis</i>	ANTARCTICA, Ross Dependency: Lake Wellman 4 Dec 07, leg: R. Türk, det: U. Ruprecht	R. Türk 42988	GU074435	GU074481
30	<i>L. cancriformis</i>	ANTARCTICA, Ross Dependency: Lake Wellman 6 Dec 07, leg: R. Türk, det: U. Ruprecht	R. Türk 42992	GU074436	GU074482
31	<i>L. cancriformis</i>	ANTARCTICA, Ross Dependency: Mount Kyffin 8 Jan 03, leg & det: L. Sancho	MAF-Sancho 2	GU074439	GU074489
32	<i>L. cancriformis</i>	ANTARCTICA, Ross Dependency: Smith Valley 15 Dec 07, leg: M. Stevens, det: U. Ruprecht	R. Türk 44727	GU074437	GU074488
33	<i>L. cancriformis</i>	ANTARCTICA, Victoria Land: Discovery Bluff 19 Jan 08, leg & det: U. Ruprecht	R. Türk 43020	GU074431	GU074484
34	<i>L. cancriformis</i>	ANTARCTICA, Victoria Land: Discovery Bluff 19 Jan 08, leg & det: U. Ruprecht	R. Türk 43023	GU074432	GU074485
35	<i>L. cancriformis</i>	ANTARCTICA, Victoria Land: Granite Harbour 22 Jan 08, leg & det: U. Ruprecht	R. Türk 43028	GU074430	-
36	<i>L. cancriformis</i>	ANTARCTICA, Victoria Land: Mount Suess 09 Jan 08, leg & det: U. Ruprecht	R. Türk 43000	GU074433	GU074483
37	<i>L. cancriformis</i>	ANTARCTICA, Victoria Land: Taylor Valley 23 Jan 03, leg: R. Türk, det: U. Ruprecht	R. Türk 33712	EU257677	GU074478
38	<i>L. confluens</i> (Weber) Ach.	AUSTRIA, Salzburg: Lungau 12 Jul 06, leg & det: R. Türk	R. Türk 39641	EU263921	GU074492
39	<i>L. fuscoatra</i> (L.) Ach.	AUSTRIA, Oberösterreich: Eferding 8 Aug 06, leg & det: F. Berger	R. Türk 40136	EU263922	GU074490
40	<i>L. fuscoatrina</i> Hertel & Leuckert	USA, California: Peninsula Range, Tahquitz Peak 13 May 05, leg & det: K. Knudsen	U.C. Riverside UCR (646Kerry)	EU259900	-
41	<i>L. laboriosa</i> Müll.Arg.	USA, California: Peninsula Range, Tahquitz Peak U.C. Riverside 21 Aug 06, leg & det: K. Knudsen	EU259902 43266UCR1	GU074503	
42	<i>L. lapicida</i> var. <i>lapicida</i> (Ach.) Ach.	AUSTRIA, Osttirol: Hohe Tauern, 21 Jul 07, leg & det: R. Türk	R. Türk 42745	GU074425	GU074493
43	<i>L. lapicida</i> var. <i>pantherina</i> Ach.	AUSTRIA 1997 Hafellner & Hafellner AUSTRIA, Tirol: Ötztal 8 Jun 07, leg & det: R. Türk	GZU Hafellner 42375 R. Türk 42143	AF332118 -	- GU074494
44	<i>L. obhuridata</i> (L.) Nyl.	AUSTRIA, Osttirol: Hohe Tauern 21 Jul 07, leg & det: R. Türk	R. Türk 42730	GU074423	GU074495
45	<i>L. plana</i> (J.Lahm in Körb) Nyl	SWEDEN, Halland: Veinge-Tjärby 26 Aug 03, det: U. Arup	Herbarium Lund L03170	EU259903	GU074497

Table II. Continued

No.	Name of species	Sample	Herbarium	ITS	mtSSU
46	<i>L. plana</i>	SWEDEN, Halland: Veinge-Tjärby 19 Sep 03, det: U. Arup	Herbarium Lund L03316	EU259904	GU074498
47	<i>L. polypycnidophora</i> Ruprecht & Türk sp. nov.	ANTARCTICA, Mac. Robertson Land: Prince Charles Mountains 28 Jan 05, leg: M. Andreev, det: U. Ruprecht	SZU A0513002	GU074441	GU074474
48	<i>L. polypycnidophora</i>	ANTARCTICA, Victoria Land: Taylor Valley 3 Feb 2003, leg: R. Türk, det: U. Ruprecht	SZU Türk 33632	EU257681	GU074471
49	<i>L. polypycnidophora</i>	ANTARCTICA, Victoria Land: Taylor Valley 3 Feb 2003, leg: R. Türk, det: U. Ruprecht	R. Türk 33640	EU257682	GU074473
50	<i>L. polypycnidophora</i>	ANTARCTICA, Victoria Land: Taylor Valley 25 Jan 2003, leg: R. Türk, det: U. Ruprecht	R. Türk 33648	EU257680	GU074472
51	<i>L. silacea</i> (Hoffm.) Ach.	AUSTRIA, Tirol: Ötztal 8 Jun 07, leg & det: R. Türk	R. Türk 42156	GU074424	GU074496
52	<i>Lecidea</i> sp.	NORWAY, Svalbard: Ny Ålesund 18 Aug 08, leg: A. Tribsch	R. Türk 43427	GU074426	GU074502
53	<i>Lecidea</i> sp.	USA, South Dakota: Brule County 8 Aug 03, leg: J.C. Lendemer, det: R.C. Harris	Lichens of South Dakota #1165	EU263929	GU074501
54	<i>L. tessellata</i> Flörke	AUSTRIA, Steiermark: Eisenerzer Alpen 2 Oct 99, leg & det: J. Hafellner	GZU Hafellner 49249	EU263926	GU074491
55	<i>Lecidea</i> UCR1	ANTARCTICA, Victoria Land: Taylor Valley 3 Feb 03, leg: R. Türk, det: U. Ruprecht	R. Türk 33619	EU263927	GU074475
56	<i>Lecidea</i> UCR1	ANTARCTICA, Victoria Land: Taylor Valley 22 Jan 03, leg: R. Türk, det: U. Ruprecht	R. Türk 33704	EU263928	GU074476
57	<i>Porpidia macrocarpa</i> (DC.) Hertel & A. J. Schwab	AUSTRIA, Salzburg: Lungau, 14 July 2005, leg & det: R. Türk	R. Türk 39740	EU263923	GU074512

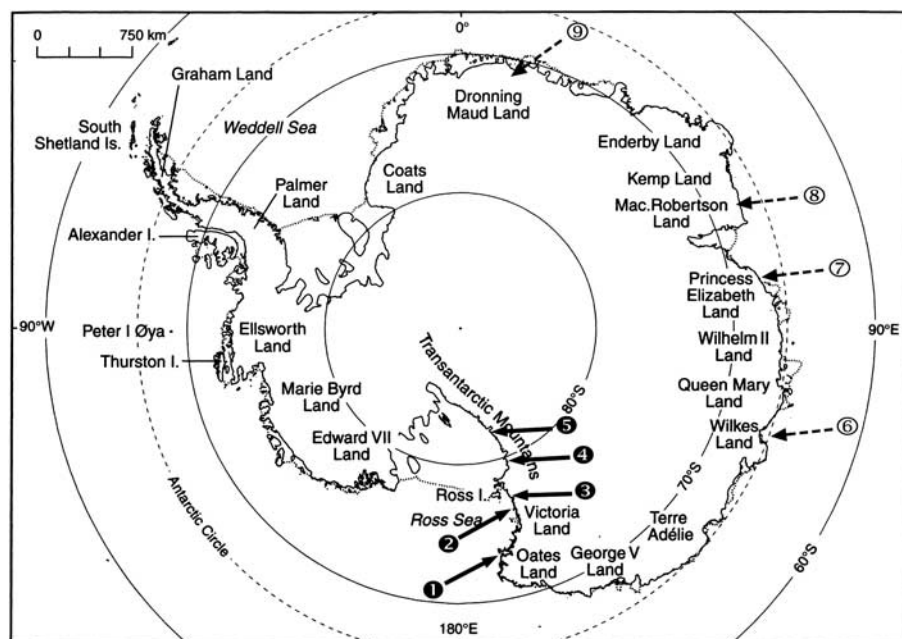


Fig. 1. Investigation areas, general map from Øvstedal & Lewis Smith (2001). 1–5 = Ross Sea area from 72°–84°S, the samples were collected by the authors and collaborators, 6 = Wilkes Land (R. Seppelt), 7 = Princess Elizabeth Land (M. Andreev), 8 = MacRobertson Land (M. Andreev), and 9 = Dronning Maud Land (M. Andreev).

are the largest ice free areas in continental Antarctica (Doran *et al.* 2002). The region is a cold desert with arid soils and exposed bedrocks. Possible sources of water in this region are perennially ice covered lakes, snow (falling and blowing), clouds and fog, ephemeral streams and alpine glaciers (Doran *et al.* 2002).

4. a. Brown Hills (79°46'–79°50'S 158°33'–195°25'E), 400–450 m alt., December 2004

The Brown Hills are located in the north of Darwin Glacier. The Carlyon granodiorite makes up most of the Brown Hills and includes a variably foliated, biotite-hornblende granodiorite and granite (Simpson & Cooper 2002). This site appears to be a particularly dry part of the continental Transantarctic Range.

- b. Lake Wellman (79°54'S, 156°32'–156°53'E) 935–1371 m alt., November 2007

The surroundings of Lake Wellman are characterized by a very dry climate, caused by a high evaporation rate due to low average air humidity and/or continuous winds originating from the cold glacier regions. The bedrock surrounding this area is sandstone from the Beacon Group and dolerite from the Ferrar dolerite sills.

- c. Diamond Hill (79°51'–79°52'S, 159°16'–159°21'E), 371–550 m alt., January 2009

Diamond Hill is located at the eastern edge of the Transantarctic Mountains, close to the Ross Ice Shelf and north from the Darwin Glacier. Climate conditions are characterized by higher air humidity and precipitation that support a higher diversity and abundance of lichens.

- d. Bartrum Basin (79°45'S, 158°30'–158°38'E) 370–430 m alt., January 2009

Located in the north-west of the Brown Hills, Bartrum Basin is apparently very dry. The dominant rock types are dolerite and granite.

5. Mount Kyffin and surroundings, Queen Maud Mountains (83°44'–83°45'S, 172°15'–172°46'E), 450–820 m alt., January 2003

The investigated area is located at the southern edge of Beardsmore Glacier. The mountains are formed by Goldie formation greywacke (Gunn & Walcott 1962) and schist. The collecting sites at the ridges were snow free. The site is characterized by low precipitation rates and severe winters.

Morphological analyses

Light microscopic investigations were carried out with a LEITZ Laborlux S (Leitz, Vienna). Cross sections of apothecia (12 µm thick) were prepared using a Leitz Kryomat 1703 freezing microtome. The sections were stained with Lactophenol blue solution, KOH (10%) and Lugol's iodine solution 50%. Thallus morphology, anatomical features of asci and the ascospores were documented with photographs, using a digital camera (Samsung-Digimax V50).

Chemical analyses

Samples were extracted for four hours in methanol and secondary compounds were characterized by high-performance liquid chromatography (HPLC) using benzoic acid and (E)-1-(9-Anthryl)-2-phenylethene as internal standards. HPLC analyses were performed according to Stocker-Wörgötter & Elix (2002) using a Hitachi /Merck Spectra System with a Beckman 5C18 column, 250 by 4.6 mm, with spectrometric

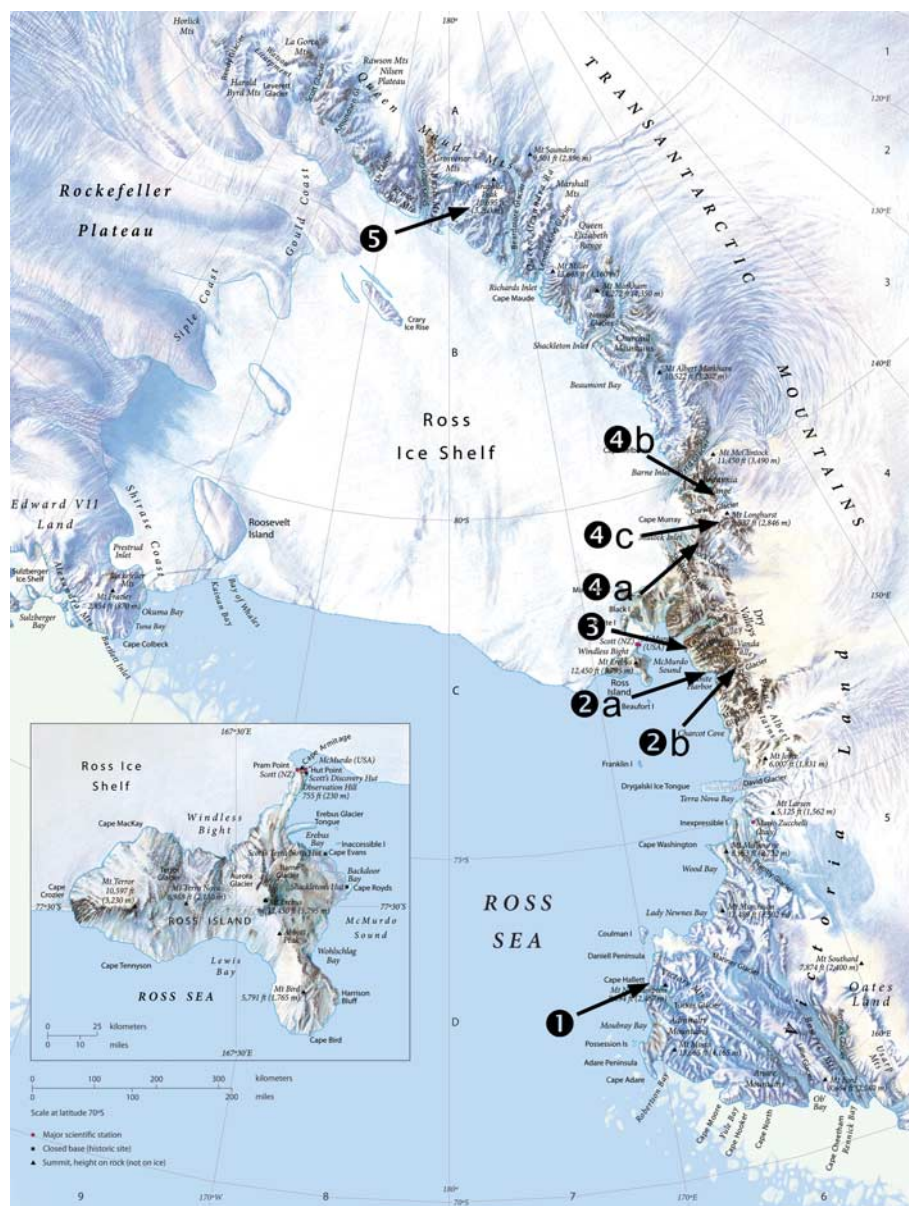


Fig. 2. More detailed view on the snow free regions of the Ross Sea coast at the end of summer (McGonigal & Woodworth 2001). 1 = Cape Hallett, 2a = Granite Harbour–Botany Bay–Discovery Bluff, 2b = Mount Suess–Sperm Bluff, 3 = Taylor Valley, 4a = Brown Hills, 4b = Lake Wellman, 4c = Diamond Hill–Bartrum Basin.

detectors operating at 254 nm with a flow rate of 1 ml min^{-1} . Two solvent systems, A and B with a flow rate of 1 ml min^{-1} were used as follows: The run started with 100% A (1% aqueous ortho-phosphoric acid and methanol in the ratio 7:3) and was raised to 58% B (pure methanol) over 15 min, then to 100% B over a further 15 min, followed by isocratic elution in 100% B for further 10 min.

Standardized TLC with atranorin and norstictic acid as reference substances and solvents A, B' and C was used to identify the chemical profile of the lichens (Culberson & Johnson 1982 and references therein).

DNA-amplification, purification, sequencing

Total DNA was extracted from thallus or apothecia by using the DNeasy Plant Mini Kit (Qiagen) according to the

manufacturer's instructions. The PCR mix contained 0.5 units of recombinant Taq polymerase (Fermentas), 0.2 nM of each of the four dNTPs, $0.3 \mu\text{M}$ of each primer and 1–10 ng genomic DNA. The PCR products were purified using Qiaquick PCR purification kit (Qiagen) and resolved in sterile distilled water. The purified and dried PCR-products were sent to Macrogen (Seoul, Korea) for sequencing using the PCR primers. The obtained sequences were aligned with homologous sequences from the NCBI-Database. In cases where the PCR did not produce bands in a first reaction, we took $1 \mu\text{l}$ of this reaction for a nested PCR with internal primers. Reactions that produced multiple bands were taken for TA cloning. Plasmids were isolated using the Wizard Plus SV Miniprep System (Promega) and inserts were sequenced.

The internal transcribed spacer region (ITS) of the mycobionts' nuclear ribosomal DNA and mitochondrial

Table III. Sequences, temperature and primer map of the used ITS-primers.

Primers	Internal transcribed spacer region rRNA (ITS)		
	Sequence 5' to 3'	Temp.	
ITS1	TCCGTAGGTGAACCTGCGG	56°	White <i>et al.</i> 1990
ITS1 F	CTTGGTCATTTAGAGGAAGTAA	56°	Gardes & Bruns 1993
ITS1 univ	GAACCTGCGGAAGGATCATTA	56°	This study
ITS Land sS	AACCATTGAAGTTGAGGG	56°	This study
RC LR0Rmod1 rev	TCCGCTTATTGATATGCTTAAGTT	56°	This study
ITS4	TCCTCCGCTTATTGATATGC	56°	White <i>et al.</i> 1990
LR21	ACTTCAAGCGTTTCCCTTT	53°	http://www.biology.duke.edu/fungi/mycolab/

small subunit (mtSSU) were amplified and sequenced with the primers described in Tables III & IV. To reduce the costs for sequencing and to accelerate the identification of the large number of *L. andersonii* samples - often more than 20 years old, we designed 'ITS-Land sS' (Table III), a reverse primer to directly and specifically amplify the ITS region of *L. andersonii*, best used with ITS-1F. To be certain that this primer amplified only *L. andersonii*, we tested it on already confirmed *L. andersonii* specimens and on other *Lecidea* species throughout the phylogeny and sequenced the products.

Phylogenetic analysis

Mitochondrial SSU and nuclear ITS sequences were assembled with vector NTI Advance 9 (Invitrogen, Vienna, Austria) and then manually corrected. The alignment of sequences (including out-group taxon) was done using MacClade (Maddison & Maddison 2003).

The alignment was unambiguous and included only a few gaps. For comparison, lecideoid species from other

regions of Antarctica and other parts of the world were sequenced and included in the data matrix together with one sequence downloaded from Genbank as shown in Table II. To test for potential conflict, parsimony bootstrap analyses were performed on each individual dataset, and 75% bootstrap consensus trees were examined for conflict.

To choose a nucleotide substitution model we used Modeltest 3.7 (Posada & Crandall 1998); both tests (hierarchical likelihood ratio test and Akaike information criterion) selected the Tamura-Nei Model (TrNef) with equal base frequencies including estimation of invariant sites and assuming a discrete gamma distribution. A maximum likelihood analysis (ML) was performed using the program Garli 0.96 (http://www.nescent.org/wg_garli/Main_Page) with the estimated TrNef (0.10020) + I + G model and default settings. Nonparametric bootstrap was used to assess robustness of clades, running 2000 pseudo replicates.

Maximum parsimony analyses (MP) were performed using the program PAUP* (Swofford 2003). Heuristic searches with 1000 random taxon addition replicates were conducted with TBR branch swapping and MulTrees option in operation,

Table IV. Sequences, temperature and primer map of the used mtSSU-primers.

	Mitochondrial small subunit rDNA (mtSSU)		
	Sequence 5' to 3'	Temp.	
mrSSU1	AGCAGTGAGGGATATTGGTC	55°	Zoller <i>et al.</i> 1999
mtSSU for2	AATIGCCTTRCGGCKGAAC	54°	This study
mtSSU uni for	GTGCCAGCAGYCGCGGTAATWC	57.5°	This study
mtSSU uni rev	CWTTAASTKWAGACAGCKTGC	57.5°	This study
mtSSU rev2	AAAATAAAGTAAGGGYTTTCGTTAATT	54°	This study
CU6	TGTGGCACGTCTATAGCCCA	55°	http://plantbio.berkeley.edu/~bruns/tour/primers.html

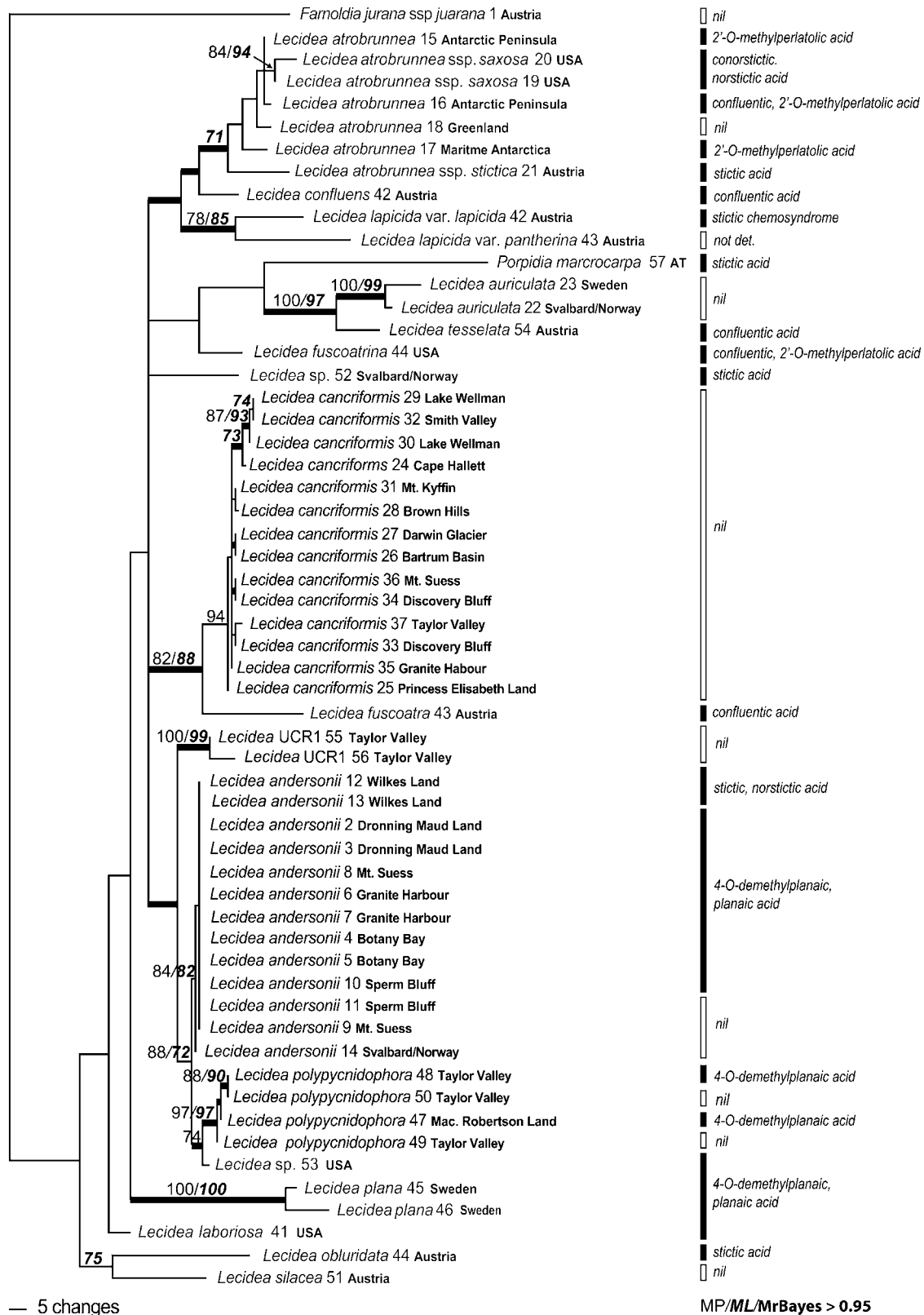


Fig. 3. Phylogeny of Antarctic *Lecidea* spp. This Bayesian tree is based on a combined dataset of ITS and mtSSU with > 0.95 support and directly mapped bootstrap values with > 70 support of maximum parsimony - and maximum likelihood (Garli 0.96) analysis. Secondary metabolites of the specimens are plotted to the right of the tree. The four main clades are indicated in black.

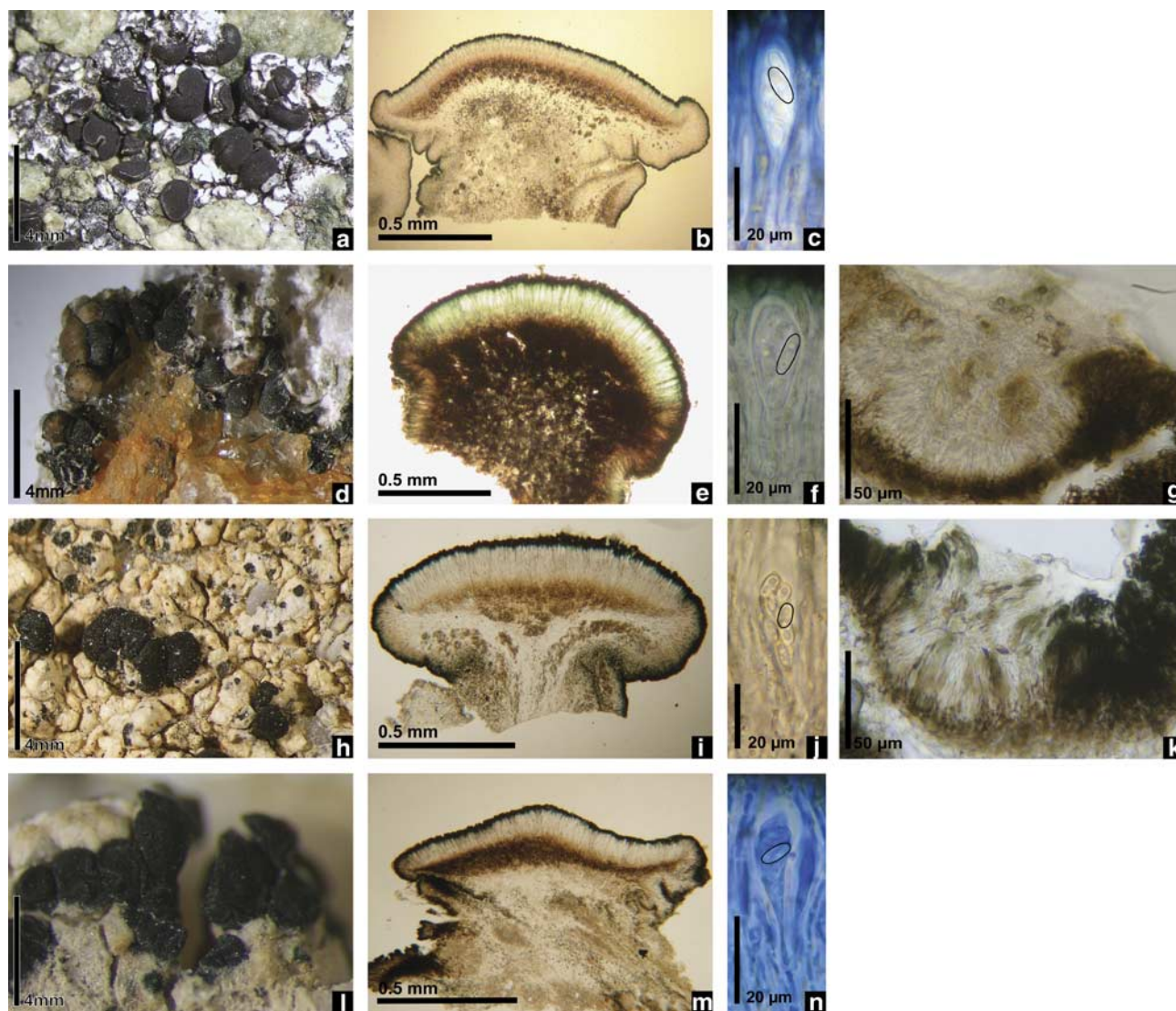


Fig. 4. Overview of the most important morphological attributes of the investigated *Lecidea* specimens. *Lecidea andersonii*: **a.** Sparse thallus with apothecia, **b.** cross-section of apothecium, **c.** ascus with narrowly ellipsoid ascospores. *Lecidea cancriformis*: **d.** brownish thallus with apothecia and a dense group pycnidia bottom left, **e.** cross-section of apothecium with characteristically dark brown hypothecium, **f.** ascus with narrow oblong ascospores, **g.** cross-section of pycnidium with bacilliform conidia. *Lecidea polypycnidophora* sp. nov.: **h.** thallus with apothecia and abundant pycnidia, **i.** cross-section of apothecium, **j.** ascus with ellipsoid ascospores, **k.** cross-section of pycnidium with bacilliform pycnospores. *Lecidea* UCR1: **l.** chasmolithic thallus with aggregated apothecia, **m.** cross-section of apothecium, **n.** ascus with narrowly ellipsoid ascospores.

equally weighted characters and gaps treated as missing data. Bootstrapping was performed based on 2000 replicates with random sequence additions. Homoplasy levels were assessed by calculating consistency index (CI), retention index (RI), and rescaled consistency (RC) index from each parsimony search.

The B/MCMC analyses were conducted using the MrBayes 3.1. program (Huelsenbeck & Ronquist 2001). A run with 10 000 000 generations starting with a random tree and employing four simultaneous chains was executed. Every 100th tree was saved into a file. The first 1 000 000

generations (i.e. the first 10 000 trees) were deleted as the “burn in” of the chain. We plotted the log-likelihood scores of sample points against generation time using TRACER 1.0 (<http://evolve.zoo.ox.ac.uk/software.html?id=tracer>) to check whether stationarity was achieved after the first 1 000 000 generations by checking whether the log-likelihood values of the sample points reached a stable equilibrium value (Huelsenbeck & Ronquist 2001). Additionally, we used AWTY (Nylander *et al.* 2007) to compare splits frequencies in the different runs and to plot cumulative split frequencies to ensure that stationarity was reached. Of the remaining

180 000 trees (90 000 from each of the parallel runs) a majority rule consensus tree with average branch lengths was calculated using the sumt option of MrBayes. Posterior probabilities were obtained for each clade. Only clades that received bootstrap support equal, or above, 70% under parsimony, and likelihood and posterior probabilities equal, or above, 0.95 were considered as strongly supported.

Results

Phylogenetic analyses

In total four different *Lecidea* species could be identified in continental Antarctica and are confirmed by morphological, chemotaxonomic and molecular data including two novel *Lecidea* species. As well as our own material freshly collected at various sites in areas of continental Antarctica we could also use comparison material of *Lecidea*, most of which included originally described species treated in this study. The new sequences were aligned with one ITS sequence downloaded from Genbank (Table II).

After excluding excess sequences the final data matrix for the molecular phylogeny comprised 57 OTUs. The length of the combined alignment (mitochondrial SSU and nuclear ITS rDNA) was 877 unambiguously aligned nucleotide position characters. The MP 75% bootstrap support method for testing datasets for incongruence indicated no strongly supported conflict (data not shown) and hence a combined analysis was performed.

164 positions in the combined alignment were parsimony-informative. MP analyses yielded 298 most parsimonious trees 683 steps long (CI = 0.59, RI = 0.74, RC = 0.44). Tree topologies among maximum parsimony, maximum likelihood and Bayesian analyses were identical in all well-supported clades and so we only present here the Bayesian tree with support values of the other analyses (Fig. 3).

The phylogenetic reconstruction indicates that the samples from continental Antarctica cluster in four well supported clades. Specimens identified as *L. cancriformis* form a monophyletic group that has an unsupported relationship to *L. fuscoatra*. The specimens of *L. andersonii* form one monophyletic group, which contains three chemotypes (stictic/norstictic acid, planaic/4-o-demethylplanaic acid, no secondary metabolites) and a sample from Svalbard (Norway). In addition to these two species, two currently unnamed clades were found and are described below. These are *Lecidea* UCR1 and *L. polypycnidophora*. The two samples of *Lecidea* sp. (*L.* UCR1) form a strongly supported monophyletic group and are sister to the two clades including specimens of *L. andersonii*, *L. polypycnidophora* and an unidentified *Lecidea* sp. from South Dakota (USA). *Lecidea polypycnidophora* with the sequence of the lichen sample LOSD1165 (South Dakota, USA) at the base, form a sister group to *L. andersonii*. The results of this phylogenetic analysis indicates that

four distinct lineages of *Lecidea* spp. occur in continental Antarctica.

The backbone of the phylogeny is not supported and so this analysis does not allow further conclusions regarding the relationships of the species examined. However, the additional samples from Antarctica form strongly supported clades for each of the currently described species. *Lecidea atrobrunnea*, *L. lapicida*, *L. plana*, *L. tessellata* and *L. auriculata* each form clearly defined and well supported monophyletic groups. The currently accepted varieties - including different chemotypes - in two of the species (*L. atrobrunnea*, *L. lapicida*), however, are more distantly related and form different clades in our analyses as can be seen in the chemotypes plotted on the phylogenetic tree in Fig. 3. The species of the genus *Porpidia* are nested within *Lecidea* as shown in previous studies (Buschbom & Barker 2006).

Morphological and chemical analyses

Based on the sequence data from 170 *Lecidea* samples (Table I), we re-examined the morphology and secondary chemistry to identify phenotypic characters that circumscribe the monophyletic clades found in the analysis. We got a complete dataset of molecular, chemical and morphological analyses from a total of 107 specimens. 57 accessions were chosen to build up the final phylogeny after excluding additional accessions of the same species from identical habitats.

The localities of the studied specimens and herbarium information are given in Tables I & II. The most important diagnostic characters of the four *Lecidea* spp. in continental Antarctica are shown in Fig. 4. Descriptions of the species and discussion on variation and differences to similar species are given below.

Lecidea andersonii Filson 1974

Thallus: often poorly developed, rimose to areolate, up to 2 cm in diam., angular and usually adpressed along rock crevices; surface: whitish to grey and brownish; apothecia: frequent, scattered in groups, dense and partially grouped, sessile, constricted at the base, round to irregular, up to 2 mm in diam., disc black, flat to slightly convex, mostly epruinose, rarely with a thin whitish pruina, margin distinct, sometimes becoming flexuose; proper exciple: 80–150 µm high, hyaline, outer margin dark green to brownish green; hypothecium: brownish, 70–100 µm high; hymenium: hyaline, 50–80 µm high; paraphyses: simple, coherent, with swollen apices; epihymenium: dark green, 10–15 µm high; asci: amyloid, *Lecidea*-type, 8-spored; ascospores: narrowly ellipsoid (8-) 9–12 x 3–4, length-width index 2,1–2,3; spot tests: thallus K-, C-, I- and medulla: K-, C-, I+ ; secondary metabolites: chemotype I: 4-O-demethylplanaic and planaic acids; chemotype II: stictic and norstictic acids; chemotype III: nil.

Substrate: on siliceous rocks (granite).

Distribution: bipolar; known from maritime and continental Antarctica, Svalbard/Norway and Iceland. In continental Antarctica, *L. andersonii* occurs only in milder regions with coastal aspects. No specimens were found south of 77° (see Figs 1 & 3 and Tables I & II)

Notes: Our morphological circumscription of *Lecidea andersonii* agrees with the current concept (Filson 1974, Øvstedal & Lewis Smith 2001, Castello 2003, Hertel 2007, 2009). Inoue (1991) placed the species in the *L. auriculata* group where it is characterized by wide ascospores (Hertel 2007). Our phylogenetic analysis does not support this classification, but suggest a close relationship to *Lecidea tessellata* (Fig. 3). The results of our studies on the secondary chemistry of *L. andersonii* differ from that given in the literature. Previously, stictic acid (Hertel 2007) or confluent and glomelliferic acids were reported for *L. andersonii* (Inoue 1991, Øvstedal & Lewis Smith 2001). We could not detect confluent and glomelliferic acids in any *L. andersonii* specimen (Fig. 3). Instead we found three chemotypes (see above) the most common possesses 4-*O*-demethylplanic acid and planic acid. We also studied two specimens from Wilkes Land (type locality) which were confirmed as belonging to *L. andersonii* using molecular data, but contained stictic and norstictic acids. This chemistry agrees with the literature (Hertel 2007). Noteworthy, the long side chain depsides 4-*O*-demethylplanic and planic acids are not biosynthetically related to the beta-orcinol depsidones of the stictic acid chemosyndrome. We included also a sample from Svalbard/Norway (No. 14) in our calculations which could clearly be identified as *L. andersonii* by sequence and morphology. This specimen from Norway lacks secondary metabolites and hence represents a third chemotype together with two Antarctic specimens within this clade (Fig. 3).

Lecidea andersonii was long regarded as an Antarctic endemic (Øvstedal & Lewis Smith 2001, Castello 2003) until Hertel (2007) placed *Lecidea pseudopromiscens* Hertel & Rambold from Iceland into synonymy with *L. andersonii*. Our material from Svalbard/Norway confirms a bipolar distribution for this species.

Similar species in Antarctica include planic and/or 4-*O*-demethylplanic acid or lack secondary metabolites. *L. andersonii* is characterized by an I+ medulla, narrowly ellipsoid ascospores, mostly distinct margin and a sparsely developed thallus.

Lecidea cancriformis C.W. Dodge & G.E. Baker,
Ann. Mo. Bot. Gard. 25: 539 (1938)

Thallus: well developed, delimited, rimose to areolated and often sub-bullate, up to 3 cm in diam., often along crevices; surface: whitish (when eroded) to grey and brown; pycnidia: when present, grouped, with a black ostium, containing

bacilliform conidia, up to 150 µm in diam. Apothecia: scattered in groups, sessile, constricted at the base, up to 1.4 mm in diam., disc black, flat to moderately convex, round to irregular, margin thin, if present; proper exciple: up to 100 µm thick, pale, outer margin dark green to brownish green; hypothecium: brown to black, 70–100 µm high; hymenium: hyaline to pale green, 40–90 µm high; paraphyses: simple, septate, coherent, apices slightly swollen, 4–5 µm thick; epihymenium: dark green, 10–20 µm high; asci: amyloid, *Lecidea*-type, 8-spored; ascospores: narrowly oblong, (8-) 9–12 x (2-) 3–4 µm; Spot tests: thallus and medulla: K-, C-, I-; secondary metabolites: nil.

Substrate: on siliceous rock (granite).

Distribution: endemic to continental Antarctica (Øvstedal & Lewis Smith 2001, Castello 2003, Hertel 2007).

Notes: This species has a whitish (necrotic), grey or brown thallus surface (Øvstedal & Lewis Smith 2001, Hertel 2007), including variable forms with endolithic growth to thick, brownish glossy thalli. Material seen by Castello (2003) included only brown to dark brown or black thalli with a smooth and shiny surface. The taxon is readily distinguished by a negative iodine reaction of the medulla and the absence of orcinol depsides. The size of the hymenium (40–90 µm high) is in our study sometimes higher than previously described. Inoue (1991) indicated a height of 35–50 µm, Øvstedal & Lewis Smith (2001) of 60–65 µm, Castello (2003) of 60–80 µm and Hertel (2009) of 38–55 µm, which shows a high variability. Hertel (2007) mentioned that numerous specimens contain norstictic acid but this was not found in our samples. Under the circumstance of extreme environmental factors (thallus eroded) the species is similar to *L. polypycnidophora* and *L. UCR1*, but readily distinguished by oblong ascospores, a dark brown hypothecium, an I- medulla and infrequent sub-bullate, brown areoles.

Lecidea cancriformis is a widespread species and occurs in every locality sampled in the Ross Sea region. It is especially dominant in dry parts, where it is accompanied by *Acarospora gwynii* and *Lecanora fuscobrunnea* (Stevens *et al.* unpublished, Marshall *et al.* unpublished)

Lecidea polypycnidophora Ruprecht & Türk sp. nov.

Thallus crustosus, areolatus, rotundus, albidus. Medulla I+ violacea. Pycnidia abundantes. Apothecia aggregata, ad basin constricta, nigra, plana usque ad convexa. Hypothecium 30–40 µm altum. Hymenium hyalinum, 50–70 µm altum. Paraphyses simplices, septatae, apices turgidos. Epihymenium virido-nigrum, 15–20 µm altum. Asci ad typum *Lecidea* pertinens. Ascospores hyalinae, non-septatae, ellipsoideae, 6–8 x (3-) 4–5 µm.

Type: Antarctica, a) Holotypus: Victoria Land: Taylor Valley, 25 m alt, 77°36'48.6"S, 163°04'18.4"E, leg. Roman Türk 33632 (SZU), b) Paratypus: Mac. Robertson Land: Prince

Charles Mountains, Field Base Radok, 160 m alt, 70°49'S, 68°05'E, leg. Mikhail Andreev 0513002 (SZU).

Thallus: well developed, areolated, rounded to angular and partly granular, up to 1.5 mm thick, along crevices; surface: whitish to cream-coloured; pycnidia: either in dense groups at fissures or evenly distributed over the thallus, ostiolum grey to black, conidia bacilliform, up to 300 µm in diam.; Apothecia: scattered in small groups, sessile, constricted at the base, round to irregular, up to 1.5 mm in diam., disc black, flat to convex, margin indistinct to distinct; proper exciple: broad, 150–250 µm thick, brownish, outer margin dark green to brownish green; hypothecium: mid-brown, 30–40 µm high; hymenium: hyaline, 50–70 µm high; paraphyses: simple, septate, coherent, apices slightly swollen, 5–6 x 4 µm; epihymenium: dark green, 15–20 µm high; asci: amyloid, *Lecidea*-type, 8-spored; ascospores: ellipsoid, 6–8 x (3–) 4–5 µm, length-width index: 1.4–1.9; spot tests: thallus K-, C-, I- and medulla: K-, C-, I+ faintly violet; secondary metabolites: 4-*O*-demethylplanic acid or nil.

Substrate: on siliceous rocks (sandstone, granite).

Distribution: so far endemic to Continental Antarctica.

Notes: This new species has a well developed thallus with abundant pycnidia, a faintly violet I+ medulla and ellipsoid ascospores. It is similar to *L. andersonii* and *Lecidea* UCR1 but differs in having broader ascospores (length-width index 1.4–1.9 in comparison to 2.1–2.3 and ~2), a well developed, visible thallus and pycnidia. Another similar species is *L. soyaensis* M. Inoue (Inoue 1991). However, this species has a sparsely developed thallus which is largely restricted to the surroundings of the apothecia, a violet-brown hypothecium, and a I+ intensively violet medulla. It further differs chemically in containing the stictic acid chemosyndrome (Hertel 2007) and is probably a synonym of *L. andersonii*.

Additional specimens examined: Antarctica, Victoria Land: Taylor Valley: Türk 33640 (No. 49), 33641, 33648 (No. 50), 33658, 33703.

“*Incertae sedis*” *Lecidea* sp.: *Lecidea* UCR1: Türk 33619 (No.54), Türk 33704 (N. 55)

Thallus: well developed, rimose to areolate, up to 1.5 mm high, along crevices; surface: whitish to grey and pale brown; apothecia: frequent, densely aggregated, sessile, constricted at the base, up to 1.2 mm in diam., disc black matt, flat, round to irregular, inconspicuous margin; proper exciple: broad, up to 200 µm thick, hyaline, outer margin dark green; hypothecium: brown, seldom hyaline, 40–50 µm high; hymenium: hyaline, often with brownish granules, 40–60 µm high; paraphyses: simple, septate, coherent, apices slightly swollen, 4 x 5–6 µm thick; epihymenium: dark green to dark brown, 10–15 µm high; asci: amyloid, *Lecidea*-type, 8-spored; ascospores: narrowly ellipsoid, (7–) 8–9 (–10) x (3–) 4 (–5) µm, length-width index:

~2; spot tests: thallus and medulla: K-, C-, cortex I+, medulla I- (Türk 33704), rarely I+ (Türk 33619); secondary metabolites: nil.

Substrate: on siliceous rock (granite).

Distribution: so far only known from two collections in Taylor Valley (continental Antarctica).

Notes: although morphological and molecular evidence suggest that these two samples belong to a distinct lineage, we refrain from formally describing this as a new species, because of some variability in the sequenced markers and in the lichen chemistry (iodine reaction of the medulla), until further samples become available.

These chasmolithic growing specimens have a well-developed, whitish thallus, densely grouped, sessile, black apothecia with an inconspicuous margin, a broad exciple and narrowly ellipsoid ascospores. They differ from *L. andersonii* by a mostly I- medulla, a well developed thallus and apothecia without a distinct margin. The ascospores are smaller than those of *L. cancriformis*. Further, the hypothecium is lighter in comparison to the dark brown in *L. cancriformis*. In comparison to the new species *L. polypycnidophora* there are no pycnidia, the thallus is completely covered with apothecia, and the ascospores are more oblong.

Discussion

Our analyses demonstrate that the diversity of *Lecidea* spp. in continental Antarctica is higher than previously thought. Geographical data evaluation also shows a decreasing diversity of *Lecidea* species the more continental and drier the habitats are (see Table II and Investigation Sites). It clearly indicates that the species concept in *Lecidea* needs revision and that molecular data are helpful in interpreting subtle morphological differences that have been previously regarded as intraspecific variability. Our combined approach of molecular analyses and morphological and chemical re-examination on the background of the phylogenetic estimate has led to the description of two new species here.

One of these new species, *Lecidea polypycnidophora*, forms a distinct well supported clade as a sister group to *L. andersonii*. *Lecidea polypycnidophora* can clearly be distinguished from its sister groups by its abundant pycnidia, the shape of the spores and a well developed thallus. The other strongly supported clade is formed by two accessions of a *Lecidea* species of uncertain status (*Lecidea* UCR1). We refrain here from formally describing the lichens in this clade as a single novel species because a very important character for identification (iodine reaction of the medulla) brings up two different results. Therefore we cannot say if this is just some variation within a single species or if there are two sub-species in this clade. Additionally there is at least some sequence variation between the two accessions within the *L. UCR1*

clade but still it clearly forms a distinct strongly supported group.

These results agree with similar studies in other groups of lichenized fungi indicating that numerous distinct lineages are hidden under a single lichen-fungus name (Grube & Kroken 2000, Kroken & Taylor 2001, Molina *et al.* 2004, Arguello *et al.* 2007, Lücking *et al.* 2008, Wirtz *et al.* 2008). In these studies, morphological or chemical differences have been previously interpreted as intraspecific variability. Re-examination of morphology with the background of a molecular phylogenetic estimate then revealed, often subtle, morphological and/or chemical characters, supporting the distinction of these clades at species level. There is a growing body of evidence that the approach to current species recognition, which is largely based on morphology and chemistry, underestimates the number of species. Hence, there is a strong possibility that large numbers of undescribed fungal species will not only be found in poorly studied areas, such as tropical forests or in underexplored habitats, for example growing in insects, plants or lichens (Hawksworth & Rossman 1997, Sipman & Aptroot 2001, Arnold & Lutzoni 2007), but also hidden under known species names.

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