

## Standard Paper

# *Lithocalla* (Ascomycota, Lecanorales), a new genus of leprose lichens containing usnic acid

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### Abstract

The identity of *Lecanora ecorticata* has been uncertain due to conflicting reports of the presence of zeorin and the loss of the holotype. Phylogenetic analysis suggests that the species probably belongs in *Ramalinaceae*, where it is the first leprose species reported. Zeorin was found to be absent in recently collected material of *L. ecorticata*, including specimens from the type locality, and the report of zeorin in the lost holotype is considered to be due to contamination. A new genus, *Lithocalla*, is erected to accommodate *L. ecorticata* and a second closely related species, that occurs in the Falkland Islands, is newly combined as *Lithocalla malouina*. A lectotype is selected for *L. ecorticata*. *Lithocalla* is characterized by a leprose thallus containing usnic acid with fatty acids and terpenoids, but no zeorin; ascomata and conidiomata are unknown.

**Key words:** Falkland Islands, Norway, sterile crusts, usnic acid

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### Introduction

The taxonomy of persistently sterile leprose lichens has advanced greatly over the last thirty years. In the 1990s, when most of these species were included in the genus *Lepraria*, more detailed studies of morphology and especially chemistry began to lead to a better understanding of the taxa, and to a sustained increase in the number of accepted species (Laundon 1992; Tønsberg 1992). In the absence of sexual structures, *Lepraria* was regarded as a form-genus of unknown phylogenetic position, and taxonomic advances were mainly made at the species level. Ekman & Tønsberg (2002) showed that most species of *Lepraria* formed a monophyletic group related to the crustose to fruticose genus *Stereocaulon*; however, *Lepraria flavescens* Clauzade & Cl. Roux was shown to belong in *Lecanora*, and *L. lesdainii* (Hue) R. C. Harris and *L. obtusatica* Tønsberg were only distantly related to each other and to the other species in the analysis. Nelsen *et al.* (2008) showed that *Lepraria usnica* Sipman belonged in *Pilocarpaceae* and that *L. coriensis* (Hue) Sipman occurred outside *Lecanorales*. Lendemer & Hodkinson (2013) showed that *Lepraria coriensis*, *L. texta* K. Knudsen *et al.* and some newly described leprose species belonged in the genus *Leprocaulon*, previously comprising fruticose species, and *Leprocaulon* was placed in a newly erected order *Leprocaulales*. Hodkinson & Lendemer (2013) showed that *Lepraria moroziana* Lendemer and *L. obtusatica* belonged in *Arthoniales*.

Despite the transfer of many species to other genera, *Lepraria* is still a large genus, comprising at least 82 accepted species. Although sexual reproductive structures are absent, speciation in this genus has evidently continued. Saag *et al.* (2009) gave a

useful summary of the genus, but at least 24 species have been described since their survey.

Laundon (2003) described a new leprose species from Great Britain (type locality) and Canada as *Lecanora ecorticata*, which was said to contain usnic acid as the major substance, with atranorin, zeorin and fatty acids reported as accessory substances (either present or absent in individual specimens). The placement in *Lecanora* was based on chemistry. Lendemer & Hodkinson (2013) included leprose species containing usnic acid and zeorin in their study and showed that these belonged either in *Lecanora* s. lat. or in *Leprocaulon*. Although *Lecanora ecorticata* had been described as a leprose species with usnic acid and zeorin, its status in regard to the other usnic acid-containing species could not be assessed due to the lack of molecular data for *L. ecorticata*, and the authors excluded the name from western North America.

Although the holotype of *Lecanora ecorticata* was said to contain zeorin, the holotype was lost while on transit during a loan, and three paratypes analyzed by Martin Kukwa (annotations within the herbarium packets) contained up to two terpenoids, but no zeorin. To remove the uncertainty surrounding the identity of the species, the chemistry and phylogenetic relationships of *L. ecorticata* are investigated here.

### Materials and Methods

#### Chemistry

Specimens were investigated by thin-layer chromatography (TLC) in solvent systems A and G, using standard methods (Orange *et al.* 2010).

#### Sequence acquisition

DNA was extracted from recently collected or frozen specimens using the Qiagen DNeasy Plant Mini Kit; the manufacturer's

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instructions were followed except that warm water was used for the final elution. PCR amplification was carried out using Bioneer AccuPower PCR Premix 50 µl reaction tubes. The following regions were amplified:

1. The two internal transcribed spacer regions and the 5.8S region (ITS1-5.8S-ITS2) of the nuclear ribosomal gene, using the primers ITS1F and ITS4.
2. Part of the large ribosomal subunit, using the primers nu-LSU-155-5', LR3, LR5 and LR7.
3. Part of the small subunit of the mitochondrial ribosomal DNA (mtSSU) using the primers mrSSU1 and mrSSU3R.
4. Part of the RNA polymerase II largest subunit (*RPB1*) gene, using the primers RPB1-AF, RPB1-Cr and RPB1-gR1asc.

The PCR thermal cycling parameters for the first three followed Orange *et al.* (2017), and those for *RPB1* followed Gueidan *et al.* (2007).

Sequencing was performed by The Sequencing Service (College of Life Sciences, University of Dundee; [www.dnaseq.co.uk](http://www.dnaseq.co.uk)) using Applied Biosystems BigDye v3.1 chemistry on an Applied Biosystems 3730 automated capillary DNA sequencer.

### Phylogenetic analysis

Alignment of assembled sequences was carried out using BioEdit (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). ClustalW was used to create an initial alignment, which was edited manually and trimmed to remove long free ends. The initial alignments of ITS, LSU and mtSSU were degapped and realigned in PRANK (Löytynoja & Goldman 2010; Löytynoja 2014), using the web interface at <http://wasabiapp.org/software/prank/>. The initial *RPB1* alignment was degapped and realigned in MACSE (Ranwez *et al.* 2011), using the web interface at <http://mbb.univ-montp2.fr/macse>. All parts of the resulting alignments were used for analysis, except for poorly aligned regions of *RPB1*, which were excised.

BLAST searches in GenBank of each gene region were carried out to find closely related sequences. Subsequently, the dataset used for a study of the phylogeny of *Ramalinaceae* (Kistenich *et al.* 2018) was used to investigate the placement of the newly generated sequences.

Phylogenetic relationships and support values were investigated using maximum likelihood (ML) bootstrapping, as implemented in RAxML (Stamatakis 2006; Stamatakis *et al.* 2008), hosted on the CIPRES Science Gateway (Miller *et al.* 2010). After initial analyses of each gene region separately, sequences were concatenated using SequenceMatrix (Vaidya *et al.* 2011).

Analyses with RAxML used rapid bootstrapping with 1000 iterations and the GTRGAMMA substitution model; a search for the best-scoring ML tree was carried out with the bootstrap analysis in a single run. For the concatenated dataset, a partitioned analysis was carried out, using ITS, LSU, mtSSU, and the three codon positions of *RPB1* and of *RPB2* as partitions. *Boreoplaca ultrafrigida* Timdal and *Ropalospora lugubris* (Sommerf.) Poelt were used as outgroup.

The resulting trees were visualized using MEGA v4 (Tamura *et al.* 2007). Support values of  $\geq 70\%$  ML bootstrapping were regarded as significant. Sequences used in the analyses are shown in Table 1.

Pairwise distances between ITS sequences of putative *Lecanora ecorticata* were calculated using MEGA v4.

## Results

### Chemistry

TLC data were obtained from *c.* 11 specimens from Great Britain (of which 5 were sequenced), 2 from Norway, and 10 from the Falkland Islands (of which 5 were sequenced). The following substances were found (Fig. 1):

Usnic acid: constant, major compound.

Fatty acids: two rather well-defined spots  $R_f$  class 4–5 in G (major or minor), appearing as a single spot  $R_f$  class 6 in solvent system A; traces of additional fatty acids.

Unidentified terpenoid A,  $R_f$  class 6 in solvent system G, dirty grey-brown after heating, UV+ dull pink; accompanied by an ill-defined trace of terpenoid UV+ dull purple after heating, immediately above in solvent system G.

Unidentified terpenoid B, faint purplish after heating,  $R_f$  class 6 in G, UV+ violet-purple.

Traces of additional terpenoids. Note that the purplish spot in Fig. 1A at  $R_f$  class 3 is not a terpenoid; similar spots are often present on chromatograms of a wide range of lichen species. Zeorin was absent in all specimens.

Atranorin: traces in five British specimens and one Falkland Island specimen. In solvent system G, atranorin overlaps with usnic acid, and because of this it is indicated by a purple coloration (e.g. Fig. 1A, lane 13).

Stictic acid: present in a single collection from the Falkland Islands.

It was not possible to resolve all the minor terpenoids and fatty acids using TLC, and the substances are best understood when numerous samples are run together on the same plate. However, terpenoid A was restricted to material from Europe, and terpenoid B to material from the Falkland Islands. European material contained two main fatty acids, whereas material from the Falkland Islands lacked these, and instead had a trace of fatty acid at a slightly higher position in G.

### Phylogenetic analysis

ITS sequences were newly prepared for 11 specimens (one sequence extending well into the LSU), mtSSU sequences for 4 specimens and an *RPB1* sequence for one specimen (Table 1).

BLAST searches of the ITS, LSU and mtSSU regions returned mostly members of the family *Ramalinaceae*, or species until recently placed in *Ramalinaceae*, such as *Japewia tornensis* (Nyl.) Tønsberg, as the most closely related sequences. A search using the *RPB1* sequence did not return any closely related sequences. Maximum likelihood analyses of each gene region separately with sequences of *Ramalinaceae* and others of Kistenich *et al.* (2018) suggested in each case that the sequence was nested within *Ramalinaceae*, but with very low support values (data not shown). As support values were so low, a meaningful comparison of the topology of the tree resulting from each analysis was not possible, and a concatenated alignment of the four gene regions was prepared for further analysis. Following an analysis using most of the sequences of Kistenich *et al.* (2018), a more manageable reduced dataset was prepared and analyzed; sequences used are shown in Table 1. *Ramalinaceae* was recovered, but with low support. However, Clades A, C, D, F and G of Kistenich *et al.* (2018) were recovered with good support (only one member of Clade

**Table 1.** Specimens of *Lithocalla* and related species used in the phylogenetic analyses (Fig. 2) with voucher information and GenBank Accession numbers. New sequences are in bold. \* = outgroup.

Species	Voucher	ITS	LSU	mtSSU	RPB1	RPB2
<i>Aciculopsora salmonea</i>	–	MG925948	–	MG925842	MG926137	–
<i>Adelolecia pilati</i>	–	MG925949	AY300826	AY567713	AY756379	MG926227
<i>Arthrosporum popolorum</i>	–	MG925950	MG926039	MG925843	MG926138	MG926228
<i>Austrolecia antarctica</i>	–	MG925951	MG926040	MG925844	–	–
<i>Bacidia arceutina</i>	–	AF282083	MG926041	MG925846	MG926140	MG926230
<i>B. rosella</i>	–	AF282086	AY300829	AY300877	AY756412	AM292755
<i>Bacidina arnoldiana</i>	–	AF282093	MG926048	MG925854	MG926149	MG926238
<i>B. brittoniana</i>	–	MG925954	MG926050	–	MG926151	MG926241
<i>B. phacodes</i>	–	AF282100	MG926049	AY567725	–	MG926240
<i>Badimia dimidiata</i>	–	MG925956	MG926052	AY567774	–	–
<i>Bellicidia incompta</i>	–	AF282092	MG926043	MG925849	MG926144	MG926233
<i>Biatora globulosa</i>	–	AF282073	MG926055	KF662414	MG926155	KF662450
<i>B. ligni-mollis</i>	–	KF650968	–	KF662418	–	–
<i>B. vacciniicola</i>	–	MG925960	MG926060	MG925861	MG926159	MG926245
<i>B. vernalis</i>	–	AF282070	DQ838752	DQ838753	–	–
<i>Bibbya albomarginata</i>	–	MG926024	MG926115	MG925927	MG926212	MG926286
<i>B. ruginosa</i>	–	MG926033	MG926121	MG925937	MG926218	MG926294
<i>B. vermifera</i>	–	AF282109	MG926047	MG925852	MG926148	MG926237
<i>Bilimbia sabuletorum</i>	–	AM292670	AY756346	AY567721	AY756413	AM292761
<i>Boreoplaca ultrafrigida*</i>	–	HM161512	DQ986797	DQ986813	DQ986855	DQ992421
<i>Catillaria contristans</i>	–	MG925962	MG926063	MG925863	MG926162	–
<i>C. erysiboides</i>	–	MG925963	–	AY567732	MG926163	–
<i>C. scotinodes</i>	–	AM292673	MG926064	AM292720	MG926164	AM292763
<i>Catinaria atropurpurea</i>	–	MG925965	MG926065	MG925865	MG926165	MG926246
<i>Catolechia wahlenbergii</i>	–	HQ650649	DQ986794	DQ986811	KJ766824	DQ992424
<i>Cenozosia inanis</i>	–	–	MG926066	MG925866	–	–
<i>Cliostomum corrugatum</i>	–	MG925966	MG926067	AY567722	MG926166	KF662436
<i>Eschatogonia prolifera</i>	–	MG925969	MG926070	MG925870	MG926169	MG926249
<i>Frutidella caesioatra</i>	–	MG925971	–	MG925872	MG926171	–
<i>Glyphopeltis ligustica</i>	–	MG925972	MG926071	MG925873	MG926172	MG926250
<i>Herteliana taylorii</i>	–	–	AY756351	AY756369	AY756385	–
<i>Japewia tornoensis</i>	–	HQ650656	–	HQ660559	–	–
<i>Japewiella tavaresiana</i>	–	MG925975	–	–	–	–
<i>Krogia coralloides</i>	–	MG925977	MG926072	MG925875	MG926173	MG926251
<i>Lecania aipospila</i>	–	MG925978	MG926073	MG925876	MG926174	MG926252
<i>L. erysibe</i>	–	AM292682	MG926074	AM292733	MG926176	AM292769
<i>Lecanora poliophaea</i>	–	MG925981	MG926078	MG925879	MG926178	MG926254
<i>Lecidea albohyalina</i>	–	KF650950	MG926079	KF662398	–	KF662438
<i>Lithocalla ecorticata</i>	Orange 20531 (NMW)	<b>KT962179</b>	<b>KT962179</b>	<b>KT962184</b>	–	–
<i>L. ecorticata</i>	Orange 22747 (NMW)	<b>KT962182</b>	–	–	–	–
<i>L. ecorticata</i>	Orange 22857 (NMW)	<b>KT962183</b>	–	–	–	–
<i>L. ecorticata</i>	Orange 23561 (NMW)	<b>MT857018</b>	–	–	–	–
<i>L. ecorticata</i>	Orange 23566 (NMW)	<b>MT857019</b>	–	–	–	–
<i>L. ecorticata</i>	Orange 23569 (NMW)	<b>MT857020</b>	–	<b>MT857017</b>	<b>MT879700</b>	–
<i>L. ecorticata</i>	Orange 24348 (NMW)	<b>MT857021</b>	–	–	–	–
<i>L. malouina</i>	Orange 20168 (NMW)	<b>KT962178</b>	–	<b>MT857015</b>	–	–
<i>L. malouina</i>	Orange 22461 (NMW)	<b>KT962180</b>	–	–	–	–

(Continued)

Table 1. (Continued)

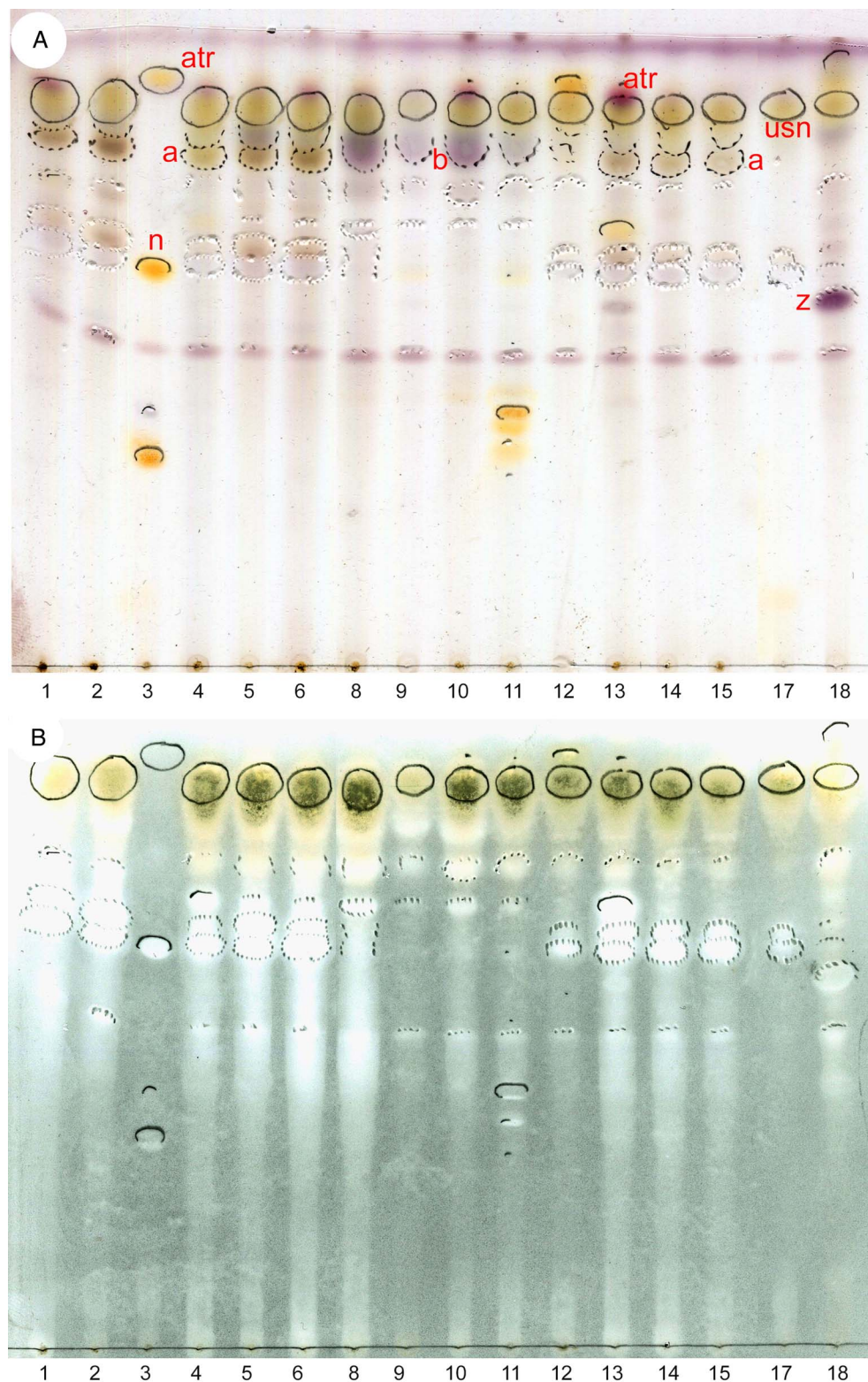
Species	Voucher	ITS	LSU	mtSSU	RPB1	RPB2
<i>Lithocalla malouina</i>	Orange 22571 (NMW)	<b>KT962181</b>	–	–	–	–
<i>L. malouina</i>	Orange 22920 (NMW)	<b>MT857022</b>	–	<b>MT857016</b>	–	–
<i>Lueckingia polyspora</i>	–	MG925984	MG926082	MG925882	–	–
<i>Megalaria grossa</i>	–	AF282074	MG926083	MG925883	MG926181	MG926257
<i>M. versicolor</i>	–	–	AY584651	AY584622	DQ912379	DQ912401
<i>Mycobilimbia pilularis</i>	–	KF650954	–	KF662402	–	KF662442
<i>Niebla melanothrix</i>	–	MG926038	MG926128	MG925945	MG926225	MG926303
<i>Parallopsora brakoeae</i>	–	MG925989	–	MG925891	–	–
<i>P. leucophyllina</i>	–	MG925994	–	MG925897	MG926189	MG926265
<i>Phyllopsora breviuscula</i>	–	MG925990	MG926087	MG925892	MG926185	MG926262
<i>P. gossypina</i>	–	MG925967	MG926068	MG925867	MG926167	MG926247
<i>P. lividocarpa</i>	–	MG925995	–	MG925898	MG926190	–
<i>P. nigrocincta</i>	–	MG925998	–	MG925901	–	–
<i>P. parvifoliella</i>	–	MG925999	MG926092	MG925902	MG926193	MG926267
<i>Physcia aipolia</i>	–	DQ782836	DQ782904	DQ912290	–	–
<i>Porpidinia tumidula</i>	–	MG926008	MG926099	–	MG926200	MG926272
<i>Protoblastenia rupestris</i>	–	MG926010	MG926101	MG925913	MG926202	MG926274
<i>Psora decipiens</i>	–	MG926011	MG926102	AY567772	AY756396	MG926275
<i>Psoroma karstenii</i>	–	–	MG926103	MG925916	–	–
<i>Ramalina fraxinea</i>	–	MG926014	MG926105	MG925918	MG926204	MG926277
<i>Rolfidium bumammum</i>	–	MG926027	MG926117	MG925930	MG926213	MG926288
<i>Ropalospora lugubris*</i>	–	MG926020	–	MG925922	–	–
<i>Schadonia fecunda</i>	–	–	AY756362	AY756376	–	–
<i>Scutula circumspecta</i>	–	–	–	MG925848	MG926143	–
<i>Speerschnidera euploca</i>	–	–	AY300862	AY300912	–	–
<i>Sporacestra pertexta</i>	–	MG926000	MG926093	MG925903	MG926194	MG926268
<i>Squamarina gypsacea</i>	–	HQ650647	–	–	DQ986853	DQ992420
<i>Tasmidella variabilis</i>	–	MG926022	–	MG925924	–	MG926282
<i>Tephromela atra</i>	–	HQ650608	DQ986766	DQ986879	–	DQ992452
<i>Thalloidima candida</i>	–	AF282117	MG926118	MG925932	MG926215	MG926290
<i>T. toniniana</i>	–	MG926036	MG926124	MG925942	MG926221	MG926298
<i>Thamnolecania brialmontii</i>	–	AF282066	MG926112	MG925925	MG926209	MG926283
<i>Toninia squalescens</i>	–	–	–	MG925939	–	–
<i>T. squalida</i>	–	AF282103	MG926123	MG925940	MG926220	MG926297
<i>T. thiopsora</i>	–	MG926035	–	MG925941	–	–
<i>Toniniopsis aromatica</i>	–	AF282126	MG926113	MG925926	MG926210	MG926284
<i>T. bagliettoana</i>	–	–	MG926042	MG925847	MG926142	MG926232
<i>T. subincompta</i>	–	AF282125	MG926046	MG925851	MG926147	MG926236
<i>Tylothallia biformigera</i>	–	AF282077	MG926129	MG925946	MG926226	MG926304
<i>Waynea californica</i>	–	–	MG926130	MG925947	–	MG926305

E was used in the reduced analysis). *Lithocalla* collections form a strongly supported clade, which is basal to Clades F and G, but with poor support. Within *Lithocalla* there are two main strongly supported clades, representing material from Great Britain and the Falkland Islands, respectively (Fig. 2).

Pairwise differences between ITS sequences from 10 putative *Lecanora ecorticata* specimens suggested a relatively high

intraspecific diversity, if it was assumed that all belong to a single species. Average pairwise distances for all 10 sequences were 0.0018 to 0.0351 (mean 0.0209). Average pairwise distances between the British specimens were in the range 0.0018–0.0053 (mean 0.0039); the three Falkland Island sequences were identical. The intraspecific diversity in the British material is comparable to that found in 13 out of 15 members of





**Fig. 1.** Thin-layer chromatography of *Lithocalla* in solvent system G. A, plate after treatment with sulphuric acid and heating. B, the same plate before heating, sprayed with water to reveal fatty acids. Lanes 1, 2, 4–6, 12–17, *Lithocalla ecorticata*; lanes 8–11, *L. malouina*; lane 18, *Leprocaulon calcicola*; lane 3, control with norstictic acid and atranorin. Labels: a = terpenoid A, b = terpenoid B, atr = atranorin, usn = usnic acid, n = norstictic acid, z = zeorin. Collection numbers of samples in lanes: 1, *Orange* 9704; 2, 7572; 4, 8541; 5, 10336; 6, 17628; 8, 20168; 9, 22461 (part); 10, 22461 (part); 11, 22571; 12, 20531; 13, 22747; 14, 8753; 15, 8898; 17, 22857; 18, hb. Earland-Bennett.

*Parmeliaceae* investigated by Del-Prado *et al.* (2010), whereas the considerably higher diversity amongst all 10 sequences was comparable to that of *Parmotrema reticulatum* (Taylor) M. Choisy, which has been shown to comprise a number of lineages representing cryptic species (Del-Prado *et al.* 2011, 2016).

The analysis suggests that *Lecanora ecorticata* is a member of the *Ramalinaceae*, but of uncertain position within the family, and with no closely related genera. However, because of the low support values it is possible that it belongs outside *Ramalinaceae*. Because there are no closely related genera, a new genus is established below for *L. ecorticata*. Based on the limited material

available, the genus is considered to comprise two closely related species, of which the second is described here as new.

## Taxonomy

### *Lithocalla* Orange gen. nov.

Mycobank No.: MB 836548

Thallus crustose, leprose, diffuse, arising from aggregations of separate granules, eventually forming a cracked crust; lower parts of thallus comprising decolourized granules, medulla and hypothallus not developed. Prothallus occasionally seen in shade, sparse. Photobiont a green alga. Ascomata and conidiomata unknown.

Type species: *Lithocalla ecorticata* (J. R. Laundon) Orange.

**Phylogenetic placement.** Ascomycota, Lecanorales, Ramalinaceae.

**Chemistry.** Usnic acid, atranorin (accessory), stictic acid (accessory), fatty acids, terpenoids.

**Etymology.** Derived from the Greek nouns *Lithos* (stone) and *Callos* (beauty), a reference to the beautiful pale yellow colonies dusting rock overhangs. The name has a Latin termination, and should be treated as feminine.

### *Lithocalla ecorticata* (J. R. Laundon) Orange comb. nov.

Mycobank No.: MB 836550

*Lecanora ecorticata* J. R. Laundon, *Nova Hedwigia* **76**, 100–102 (2003).—*Lepraria ecorticata* (J. R. Laundon) Kukwa, *Mycotaxon* **97**, 64 (2006); type: Great Britain, England, Devon (Vice-County (V.C.) 4), Ilfracombe, Torrs Walks, 21/50.47, on shaded vertical rocks, 1 September 1971, J. R. Laundon 2851 (BM—holotype [lost]) [Thin-layer chromatography: atranorin, usnic acid and zeorin, according to the protologue]; Wales, V.C. 49, Caernarvonshire, Beddgelert, Bethania, 2.25 miles south of Snowdon, SH626.509, abundant on underhang of wall running alongside a rough track, 30 April 1983, R. O. Millar (BM – 000763146—lectotype, designated here! MBT 393476) [Thin-layer chromatography: usnic acid, some terpenoids (in traces), 2005, Martin Kukwa].

(Figs 3 & 4)

**Prothallus** absent or occasionally sparsely present in shade. **Thallus** crustose, diffuse, often forming large colonies, arising from aggregations of separate granules to form a leprose crust, pale yellow-green (Munsell 5GY 7–8/2), becoming blue-grey in deep shade; thallus eventually up to 2 mm thick, but then most of the thickness is of dead decolourized granules; thicker thalli may become cracked; granules fine, 60–100 µm diam., ecorticate, without projecting hyphae. **Medulla** and **hypothallus** absent.

**Ascomata** and **conidiomata** unknown.

**Fig. 2.** Phylogenetic relationships of *Lithocalla*, based on a maximum likelihood (ML) analysis of the five-gene alignment. Sequence and voucher information is presented in Table 1. The tree was rooted using *Boreoplaca ultrafrigida* and *Ropalospora lugubris*. The ML bootstrap value is shown adjacent to each branch. Branches in bold indicate a support of ML bootstrap  $\geq 70\%$ . The starting node of Ramalinaceae is indicated by an arrow. The starting nodes of Clades C, D, F and G recovered in an analysis of Ramalinaceae by Kistenich *et al.* (2018) are indicated by a letter.

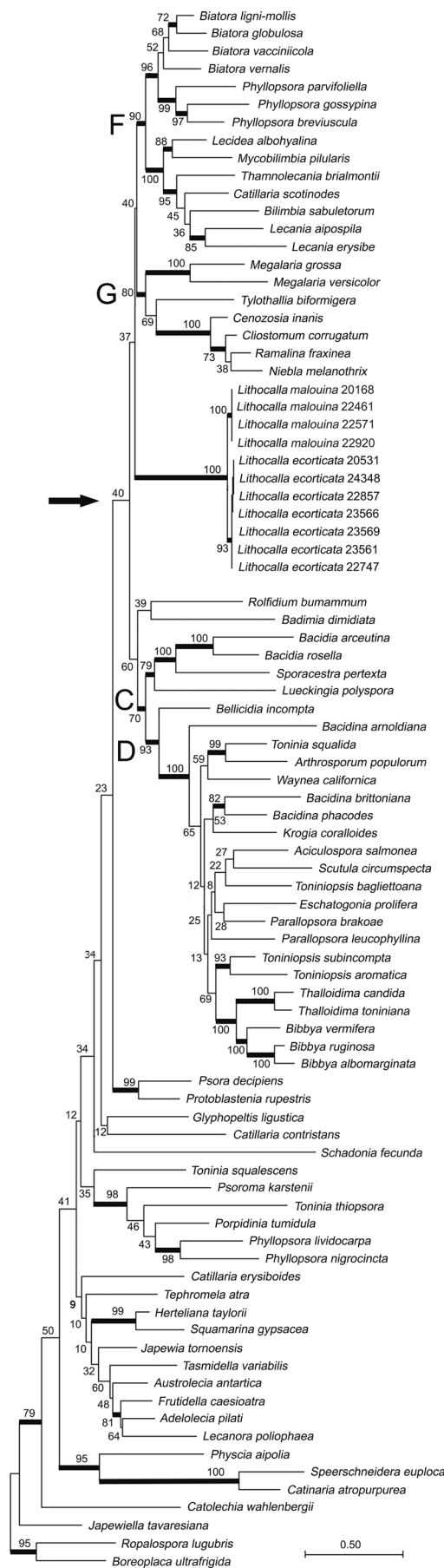






Fig. 3. Rock outcrop supporting *Lithocalla ecorticata* (Orange 23566) at the type locality of the species, Torrs Park, North Devon. In colour online.



Fig. 4. *Lithocalla ecorticata* (Orange 23566) on rain-sheltered surfaces at the type locality of the species. In colour online.

**Chemistry.** Unidentified terpenoid A at  $R_f$  class 6 in solvent system G, dirty grey-brown after heating, UV+ dull pink (minor), additional trace of a terpenoid at  $R_f$  class 6, immediately below usnic acid; two fatty acids,  $R_f$  class 4–5 in G (major or minor), appearing as a single spot  $R_f$  class 6 in solvent system A, traces of additional fatty acids; usnic acid (major); atranorin (accessory, minor). Zeorin is absent. Thallus K–, C–, KC+ pale yellow, PD–.

**Ecology.** On rain-sheltered siliceous rocks, extending further into sheltered cavities than most other British lichens, and typically forming pure stands. It is sometimes conspicuous on drystone walls in woodland, picking out the recesses with a pale yellow colour. *Lepraria incana* (L.) Ach. is often associated with it but does not extend as far into shelter. The species is widespread in Great Britain, though mainly in the north and west. On the basis of confirmed material, it occurs from sea level up to 480 m altitude (North Wales), but probably extends much higher. Confirmed

specimens have been seen from south-west Norway (to 640 m altitude in Aurland). Other records need confirmation (see below).

**Notes.** Laundon (2003) reported atranorin, usnic acid and zeorin in the holotype of *Lecanora ecorticata*. In eight additional specimens he found usnic acid (always present), atranorin (present in two), zeorin (present in two), and an unidentified fatty acid (present in three). Thin-layer chromatography of the three British paratypes in BM (Coppins & Brinklow 12859, Millar, and James s. n.) performed by Martin Kukwa in 2005–2006 (annotations in the herbarium packets) showed usnic acid and up to two terpenoids (but no zeorin); a trace of atranorin was found in one specimen.

The report of zeorin in the holotype of *Lecanora ecorticata*, and the subsequent loss of the holotype while in transit for a loan, have cast doubt on the identity of this species in a world context, although in Great Britain it has been recorded consistently. Laundon cited four paratypes, three from Great Britain and one from Canada. The British paratypes belong to a species which is locally frequent, especially in north and west Britain on base-poor siliceous rocks. The type locality of *Lecanora ecorticata* was visited in October 2016; there was little doubt that only one leprose usnic acid-containing species was present, and that this belonged to the widespread British species (Figs 3 & 4). The rock here is too acidic to support *Lepraria calcicola* Earl.-Benn. *et al.*, the British species that most closely resembles *Lecanora ecorticata*. Two specimens from the type locality were collected and ITS sequences prepared, and these agree with sequences from other parts of Britain. Thus, the lost holotype is considered here to have been conspecific with the British paratypes, and the report of zeorin in the holotype is considered to be due to a contaminant. *Lepraria incana* often grows adjacent to *L. ecorticata*, and could be a source of the contamination, although in this case divaricatic acid should also be visible on the TLC plate. One of the paratypes of *Lecanora ecorticata* (from Beddgelert) is designated here as a lectotype. Orange 23569 is a recent, sequenced, collection from this locality.

The species (as *Lecanora ecorticata* or *Lepraria ecorticata*) has been reported from several European countries but many reports are evidently incorrect, and all need to be re-examined. Published records include France (Roux *et al.* 2020; very rare, record based on Aptroot *et al.* (2007), no data on chemistry), Germany (Boch & Sparrus 2006; chemistry not mentioned but the substratum of granite in the company of *Lepraria incana* sounds correct. John *et al.* 2016; zeorin, divaricatic acid, usnic acid, thus probably a mixture with *Lepraria incana*), Belgium (Diederich *et al.* 2009, a redetermination of the record of *Lecanora leuckertiana* Zedda in Sérusiaux *et al.* (2003); calcareous rock, zeorin), Czech Republic (Bayerová & Kukwa 2004; zeorin), Slovakia (Vondrák *et al.* 2015; chemistry not mentioned, but all on bark), Poland (Kukwa 2006; zeorin, some on bark), Russia (Tarasova *et al.* 2016; zeorin) and Belarus (Tsurukau *et al.* 2016; zeorin, all on bark). All records with zeorin, or on bark, can be assumed to be incorrect.

Outside Europe, *Lithocalla ecorticata* has been reported from India (Bajpai *et al.* 2018; zeorin reported as present or absent), Mongolia (Hauck *et al.* 2013; zeorin reported), Borneo (Paukov *et al.* 2017), Canada (Laundon 2003; chemistry not stated), Chile (Kukwa 2006; chemistry not specifically stated but material from Poland was reported to have zeorin), China (Kukwa 2006)



and Antarctica (Øvstedal & Lewis Smith 2004). Lendemer & Hodkinson (2013) pointed out that *Leprocaulon knudsenii* Lendemer & B.P. Hodk. and other taxa might well have been determined as *L. ecorticata* on the basis of morphology and chemistry, before molecular data were available, and they provisionally excluded *L. ecorticata* from the flora of western North America.

Material of leprose, usnic acid-containing species should be investigated with molecular methods whenever possible, until the taxonomy and distribution are clarified.

**Additional specimens examined.** Paratypes of *Lecanora ecorticata*: **Great Britain: England: V.C. 1**, West Cornwall, Isles of Scilly, Island of Tresco, Great Rock, in sheltered underhang, 16 iv 1968, P.W. James (BM – 000763144) [Thin-layer chromatography: usnic acid, trace of two terpenoids (no zeorin), 26 Jan. 2006, Martin Kukwa]. **Scotland: V.C. 98**, Main Argyll, Cowall, Benmore Forest, Clach Bheinn, ‘The Bishop’, 26/12.89, on mica-schist in deep underhang, forming an extensive effuse cover over the rock, 1988, B. J. Coppins 12859 & R. K. Brinklow (BM – 000763136) [Thin-layer chromatography: usnic acid, perhaps with a trace of atranorin, 2000, J. R. Laundon; usnic acid, two terpenoids (no zeorin), trace of atranorin, 26 Jan. 2006, Martin Kukwa].

Non-paratypes: **Great Britain: Wales: V.C. 43**, Radnorshire, near Rhayader, Wenallt, 22/944.696, 1991, *Orange* 8541 (NMW – C.2004.002.221); **V.C. 44**, Carmarthenshire, near Rhandirmwyn, Dinas, 22/7778.46407, 2015, *Orange* 22857 (NMW – C.2015.005.76); **V.C. 48**, Merioneth, near Corris, Minffordd, Nant Cildydd, 23/7388.1136, 2015, *Orange* 22747 (NMW – C.2015.005.70); Minffordd, Dol-y-cae, Cadair Idris National Nature Reserve, SH7272.1154, 2019, *Orange* 24348 (NMW); **V.C. 49**, Caernarvonshire, NE of Beddgelert, Nantgwynant, SH6269.5072, 2016, *Orange* 23569 (NMW); **V.C. 52**, Anglesey, Cemaes, Porth Padrig, 23/375.943, 1995, *Orange* 10336 (NMW – C.2004.002.226); Amlwch, Parys Mountain, 23/4433.9052, 2011, *Orange* 20531 (NMW – C.2015.005.77). **England: V.C. 4**, North Devon, Ilfracombe, Torrs Park, 21/5053.4721, 2016, *Alan Orange* 23561 (NMW); Ilfracombe, Torrs Park, SS5036.4723, 2016, *Orange* 23566 (NMW); **V.C. 5**, Withypool, River Barle upstream of Landacre Bridge, 21/711.499, 1993, *Orange* 9704 (NMW – C.2004.002.219); **V.C. 34**, West Gloucestershire, Hope Mansel, Beechwood, 32/632.184, 1989, *Orange* 7572 (NMW – C.2003.002.68). **Scotland: V.C. 97**, Westernness, Morvern, Loch Aline, Am Miodar, 17/6845.4473, 2008, *Orange* 17628 (NMW – C.2007.001.333).—**Norway: Hordaland: Kvam**, Torvikbygd, Kolltveit, 32VLM416846, 1991, *Orange* 8753 (NMW – C.2004.002.216). **Sogn og Fjordane: Aurland**, Vassbygd, south side of Vassbygdvatnet, 32 VMN076487, 1991, *Orange* 8898 (NMW – C.2004.002.215); Aurland, Høgås, 32VMN031541, 1991, *Orange* 9033 (NMW – C.2004.002.217).

*Lithocalla malouina* (Øvstedal) Fryday & Orange comb. nov.

Mycobank No.: MB 837877

*Lepraria malouina* Øvstedal, *Lichenologist* 44, 494 (2012); type: Falkland Islands, Weddell Island, summit of Circum Peak, UTM 21F TC 3039 [51.927500°S, 60.923500°W], 650 ft [198 m], 6 January 1968, H. A. Imshaug (42023) & R. C. Harris (MSC-0108539-holotypus).



Fig. 5. *Lithocalla malouina*, Orange 22571. Scale = 1 mm. In colour online.



Fig. 6. *Lithocalla malouina*, habitat. Locality of Orange 22920, on stones below rain-sheltered south-facing overhang; visible on several stones below centre. In colour online.

*Lithocalla malouina* is similar in morphology to *Lithocalla ecorticata* but differs in chemistry and ITS sequence. The ITS region differs from *Lithocalla ecorticata* in 14 transitions, 2 transversions, a 1 bp indel and a 3 bp indel. The thallus contains terpenoid B but lacks the terpenoid A found in *L. ecorticata*. Fatty acids occur in traces but the two main fatty acids present in *L. ecorticata* are absent.

(Figs 5 & 6)

**Chemistry.** Unidentified terpenoid B at  $R_f$  class 6 in G (minor; faint purplish after heating, UV+ violet-purple), traces of fatty acids, usnic acid (major), rarely stictic acid, rarely atranorin (minor). Thallus K–, C–, KC+ pale yellow, PD–.

**Ecology and distribution.** On shaded and rain-sheltered, often south-facing siliceous rock and stones below rock overhangs; Falkland Islands.



**Table 2.** Examples of lichen genera containing leprose species.

Genus	Family	Order	Ascomata known in genus	Usnic acid present in genus	Reference
<i>Andreiomyces</i>	<i>Andreiomycetaceae</i>	<i>Arthoniales</i>	no	isousnic acid	Hodkinson & Lendemer 2013
<i>Botrylepraria</i>	unknown	<i>Verrucariales</i>	no	no	Kukwa & Pérez-Ortega 2010
<i>Lecanora</i>	<i>Lecanoraceae</i>	<i>Lecanorales</i>	yes	yes	Lendemer & Hodkinson 2013
<i>Lepraria</i>	<i>Stereocaulaceae</i>	<i>Lecanorales</i>	no	no	Ekman & Tønsberg 2002
<i>Leprocaulon</i>	<i>Leprocaulaceae</i>	<i>Leprocaulales</i>	no	yes	Lendemer & Hodkinson 2013
<i>Lithocalla</i>	<i>Ramalinaceae</i>	<i>Lecanorales</i>	no	yes	this paper
<i>Septotrapelia</i>	<i>Pilocarpaceae</i>	<i>Lecanorales</i>	yes	yes	Bungartz <i>et al.</i> 2013

**Notes.** Material of *Lithocalla* from the Falkland Islands is morphologically indistinguishable from European material, but there are significant differences in the ITS sequence, small differences in the mtSSU sequence, and consistent small differences in chemistry. Consequently, material from the two regions is regarded here as two separate species. The existence of *Lithocalla* in these two distant regions suggests that the genus may be widespread in temperate regions of the world, although reports to date of *Lithocalla* from Asia, North America and Antarctica need to be confirmed. The status of the two species can be better assessed once molecular data from other parts of the world are available.

*Lithocalla malouina* was described as containing stictic acid and usnic acid, but stictic acid is not constant. Alan Fryday (personal communication) recently confirmed the presence of stictic acid in the holotype, but found it to be absent in three paratypes. Stictic acid was present in only one (*Orange* 22571) out of ten specimens collected by the author between 2011 and 2015.

**Selected specimens examined.** **Falkland Islands:** *West Falkland:* north-west of Port Purvis, near Seal Cave, 51.3548°S, 59.3655°W, on rocks below a high overhang, 2011, *Orange* 20168 (NMW – C.2015.004.126); near Port Howard, Mount Maria, 51.60919°S, 59.55919°W, alt. 275 m, on rock below overhang on SW-facing crags, 2015, *Orange* 22461 (NMW – C.2015.004.127); north-east of Port Stephens, 52.05007°S, 60.68256°W, on stones below overhanging rocks, 2015, *Orange* 22571 (NMW – C.2015.004.128); Saunders Island, headland east of Rookery, 51.31378°S, 60.07867°W, 2015, *Orange* 23012 (FINH). *East Falkland:* Riverside, near Vantan Arroyo, 51.73810°S, 58.28033°W, 2015, *Orange* 22920 (NMW).

## Discussion

*Lithocalla* represents a new lichen lineage, in addition to those already known, that contain persistently sterile or rarely fertile leprose species. Examples of other such lineages are shown in Table 2. All the genera in the table have leprose species that have been combined in *Lepraria* at some point since 1990, but the majority are now excluded following mostly molecular analyses. However, *Botrylepraria* was initially segregated based on morphology and chemistry (Canals *et al.* 1997), and this species is more byssoid than truly leprose. With the transfer of *Lepraria coriensis* and *L. texta* to *Leprocaulon* (Lendemer & Hodkinson 2013), there are only two species still attributed to *Lepraria* that contain usnic acid. One of these, *Lepraria leuckertiana*, is likely to belong in *Lecanora*, the genus in which it was first described. The other, *Lepraria straminea* Vain., was regarded as dubiously belonging in *Lepraria* by Øvstedal & Lewis Smith (2001), and


very probably belongs elsewhere. Thus, despite the great chemical diversity in *Lepraria* (Saag *et al.* 2009), usnic acid appears to be absent from the genus.

There are few lichens with a crustose, truly leprose thallus, which contain usnic acid together with either fatty acids or zeorin but without other major compounds. *Lithocalla* differs from all of these by the absence of zeorin. The minor terpenoids present in *Lithocalla* are unlikely to be confused with zeorin in thin-layer chromatography, and reports of zeorin are considered to be due to the presence of contaminants or to confusion with morphologically similar species. *Leprocaulon knudsenii* is morphologically similar and also occurs on non-calcareous rocks; it contains only usnic acid and zeorin (Lendemer & Hodkinson 2013). *Leprocaulon textum* (K. Knudsen *et al.*) Lendemer & B.P. Hodk. in addition, contains fatty acids and atranorin in at least minor amounts (Knudsen & Elix 2007). *Leprocaulon calcicola* contains only usnic acid and zeorin, but in the field it lacks the yellow tinge of *Lithocalla* and it occurs on mortared walls (Orange *et al.* 2017). *Leprocaulon coriense* (Hue) Lendemer & B.P. Hodk. and *L. beechingii* Lendemer differ in the well-delimited lobes often with raised margins (Elix 2006; Lendemer 2020). *Lecanora leuckertiana* has a cottony medulla (Zedda 2000), whereas a medulla is absent in *Lithocalla*. *Lecanora thysanophora* R.C. Harris has a conspicuous prothallus (Harris *et al.* 2000). In *Septotrapelia usnica* (Sipman) Kalb & Bungartz a medulla is usually present, and the granules can become coarse and pseudocorticate (Elix 2006; Bungartz *et al.* 2013). The combination of usnic acid and zeorin can be found in other sorediate crustose lichens but these have at least some corticate areas, for example *Lecanora compallens* Herk & Aptroot and *L. orosthea* (Ach.) Ach. *Chaenotheca furfuracea* (L.) Tibell and *Psilolechia lucida* (Ach.) M. Choisy have a brighter yellow-green thallus containing pulvinic acid derivatives and rhizocarpic acid, respectively.

Although analyses suggest that *Lithocalla* occupies an isolated position within the family *Ramalinaceae*, data from additional gene regions are needed to confirm its position in the family, and its relationship to other genera. If *Lithocalla* is really a member of the *Ramalinaceae*, then it appears to be the only leprose genus in the family. Of the 32 genera delimited by Kistenich *et al.* (2018), only 12 contained species that produced vegetative propagules, and only *Biatora* and *Mycobilimbia* contained species that were both crustose and produced vegetative propagules.

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