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# A new leprose *Leprocaulon* (Ascomycota, *Leprocaulales*) from Great Britain

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**Abstract:** *Leprocaulon calcicola* is described as new from walls in SE England; it is leprose, pale to mid blue-grey, and contains zeorin and usnic acid. It differs from *L. knudsenii* from North America in its habitat on mortared walls rather than non-calcareous rock and in its ITS sequence. '*Lecanora*' *ecorticata* differs in the yellower colour, and the presence of unidentified fatty acids and traces of unknown terpenoids (but not zeorin) by thin-layer chromatography. Leprose lichens with usnic acid are still poorly known and sequencing must be used to support morphological and chemical studies.

**Key words:** churches, lichen, taxonomy, usnic acid, walls

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## Introduction

Knowledge of persistently sterile leprose lichens has advanced greatly in the past 25 years. Laundon (1992) and Tønsberg (1992) introduced a modern and workable species-concept in the genus *Lepraria*, based on a combination of morphology and chemistry, although the phylogenetic relationships of the species were still unknown. Ekman & Tønsberg (2002) used DNA studies to establish that most of the European species of *Lepraria* belonged in *Stereocaulaceae*, but three of the species studied belonged elsewhere. Saag *et al.* (2009) produced an overview of the world species of *Lepraria*, which then comprised 57 species, and the description of new species has continued. Regional reviews include Lendemer (2013*a*) for North America and Elix (2009) for Australia. Molecular studies are continuing to provide valuable insights at the species (Lendemer 2013*b*) and generic levels (Hodkinson & Lendemer 2013).

The treatment of sterile leprose species containing usnic acid has been inconsistent. Laundon (2003) considered that these did not belong in *Lepraria*; consequently, he transferred *Crocynia coriensis* Hue to *Lecanora* and described a new leprose species containing usnic acid as *Lecanora ecorticata* J. R. Laundon. Both were later transferred to *Lepraria* by Sipman (2004) and Kukwa (2006) respectively. Sipman (2003) described another usnic acid-containing species as *Lepraria usnica* Sipman. Using mtSSU and nrLSU data, Nelsen *et al.* (2008) showed that *Lepraria coriensis* and *L. usnica* did not belong in *Lepraria* s. str., but in an unknown family and in *Pilocarpaceae*, respectively. Lendemer & Hodkinson (2013) showed that in addition to the core group of *Lepraria* s. str. in *Stereocaulaceae*, *Lepraria* s. lat. was distributed between a further five clades within *Lecanoromycetes*. Leprose species containing usnic acid occurred in four of the clades: 1) a new family *Leprocaulaceae* for the genus *Leprocaulon*, containing fruticose and leprose species, and including *Lepraria coriensis*; 2) *Pilocarpaceae*, including *Lepraria usnica* in a new genus *Nelsenium* (later synonymized

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with *Septotrapelia* by Bungartz *et al.* 2013); 3) '*Lecanora*-1' and 4) '*Lecanora*-2', both in *Lecanoraceae*. Recent work suggests that *Lecanora ecorticata* belongs in a fifth clade (A. Orange, unpublished data).

In 2007 the second author recognized an unfamiliar '*Lepraria*' on the Roman walls of Colchester. Based on morphology and chemistry, a provisional identification of *Lepraria usnica* was made and some of the material placed in a freezer. However, in 2015 the material was removed from the freezer and sequenced, and was found to belong in *Leprocaulon*. Further specimens were collected and sequenced. ITS sequences suggest that the material is distinct from the leprose *Leprocaulon* species treated by Lendemer & Hodkinson, and it is described here as new.

## Materials and Methods

DNA was extracted from recently collected or frozen specimens using the Qiagen DNeasy Plant Mini Kit; the manufacturer's instructions were followed except that warm water was used for the final elution. PCR amplification was carried out using Bioneer AccuPower PCR Premix in 50 µl tubes. The two internal transcribed spacer regions and the 5.8S region (ITS1-5.8S-ITS2) of the nuclear ribosomal genes were amplified, using the primers ITS1F and ITS4. The PCR thermal cycling parameters were: initial denaturation for 5 min at 94 °C, followed by 5 cycles of 30 s at 94 °C, 30 s at 55 °C, and 1 min at 72 °C, then 30 cycles of 30 s at 94 °C, 30 s at 52 °C and 1 min at 72 °C. PCR products were visualized on agarose gels stained with ethidium bromide, and purified using the Sigma GenElute PCR Clean-Up Kit. Sequencing was performed by The Sequencing Service (College of Life Sciences, University of Dundee, www.dnaseq.co.uk) using Applied Biosystems Big-Dye Ver 3.1 chemistry on an Applied Biosystems model 3730 automated capillary DNA sequencer. Reference numbers for DNA extractions performed in this study are given in square brackets.

Alignment of assembled sequences was carried out using BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html); the alignment was edited manually. Ambiguously aligned regions were excluded from the analysis.

Phylogenetic relationships and support values were investigated using a Bayesian approach. The models of evolution for the three regions ITS1, 5.8S and ITS2 (HKY, K80 and GTR + I respectively) were selected using the Akaike Information Criterion (AIC) in MrModeltest 2.2 (Nylander 2004). Gaps were treated as missing data. Using MrBayes, two partitioned analyses of two parallel runs were carried out for 500 000 generations, with trees sampled every 100 generations. Stationarity was considered to have been reached when the average standard deviation of split frequencies dropped to <0·01. A burn-in sample of 1250 trees was discarded from each run, respectively. Support values of ≥95% Bayesian posterior probabilities were regarded as significant. Sequences used in the analysis are shown in Table 1.

Thin-layer chromatography (TLC) was carried out using Solvent System G (Orange *et al.* 2010).

## Results

The aligned ITS1-5.8S-ITS2 region comprised 501 sites, of which 49 were deleted as ambiguous. The unrooted 50% majority-rule consensus tree from the Bayesian analysis of the ITS region (Fig. 1) supports the distinctness of the new species from other species of *Leprocaulon.*

## The Species

### Leprocaulon calcicola Earl.-Benn., Orange, Hitch & M. Powell sp. nov.

MycoBank No.: MB 818724

Thallus leprose, finely granular, without medulla, sterile, containing zeorin and usnic acid.

Type: Great Britain, England, East Suffolk, V.C. 25, Wickham Market, Chapel Lane, 62/302.557, on mortar and dead moss between flints of garden wall by minor road, 6 May 2016, *C. J. B. Hitch* [E2117] (NMW - C.2016.005.110—holotype; GenBank Accession no: KX674679).

(Fig. 2)

*Prothallus* not observed. *Thallus* crustose, leprose, diffuse, 100–500 µm thick, pale to mid blue-grey in herbarium, brighter and greener in the field; granules fine, 60–120 µm diam., ecorticate, without projecting hyphae; lower part of thallus of decolourized granules, true medulla absent. *Photobiont* a green alga, cells spherical, 8–15 µm diam., sometimes divided into autospores.

*Ascomata* and *conidiomata* unknown.

*Chemistry*. Zeorin, usnic acid; acetone extract on filter paper K−, C−, KC+ yellow, PD−.

*Habitat and distribution*. On vertical or near-vertical surfaces of old mortared

TABLE 1. Leprocaulon *species used for the phylogenetic analysis in this study together with location, voucher information and GenBank number. New sequences are in bold. Author reference numbers for DNA extractions in square brackets.*

| Species | Country | Voucher | GenBank Acc. No. |
|---|---|---|---|
| *L. adhaerens* | USA | *Lendemer* 13546 (NY) | KC184096 |
| *L. adhaerens* | USA | *Lendemer* 12341 (NY) | KC184097 |
| *L. adhaerens* | USA | *Buck* 53439 (NY) | KC184098 |
| *L. adhaerens* | USA | *Lendemer* 9760 (NY) | KC184099 |
| *L. adhaerens* | USA | *Lendemer* 13546 (NY) | KC184100 |
| *L. adhaerens* | USA | *Morse* 16380 (NY) | KC184101 |
| *L. americanum* | USA | *Lendemer* 11445 (NY) | KC184111 |
| *L. americanum* | USA | *Knudsen* 9605 (NY) | KC184112 |
| *L. calcicola* | England | *Earland-Bennett* [E1908] (hb. Earland-Bennett) | **KX674674** |
| *L. calcicola* | England | *Earland-Bennett & Skinner* [E2077] (hb. Earland-Bennett) | **KX674675** |
| *L. calcicola* | England | *Earland-Bennett & Skinner* [E2079] (hb. Earland-Bennett) | **KX674676** |
| *L. calcicola* | England | *Earland-Bennett & Skinner* [E2080] (hb. Earland-Bennett) | **KX674677** |
| *L. calcicola* | England | *Earland-Bennett & Skinner* [E2081] (hb. Earland-Bennett) | **KX674678** |
| *L. calcicola* | England | *Hitch* [E2117] (NMW) | **KX674679** |
| *L. calcicola* | England | *Hitch* & *Powell* [E2173] (NMW) | **KX674680** |
| *L. knudsenii* | USA | *Knudsen* 9256 (NY) | KC184114 |
| *L. knudsenii* | USA | *Lendemer* 11476 (NY) | KC184115 |
| *L. knudsenii* | USA | *Lendemer* 19641 (NY) | KC184116 |
| *L. microscopicum* | Norway | *Ekman* 3685 (BG) | AF517921 |
| *L. microscopicum* | Germany | *Buck* 55927 (NY) | KC184109 |
| *L. microscopicum* | Germany | *Buck* 55960 (NY) | KC184110 |
| *L. santamonicae* | USA | *Knudsen* 12058 (NY) | KC184105 |
| *L. santamonicae* | USA | *Kocourková s.n.* (NY) | KC184106 |
| *L. santamonicae* | USA | *Lendemer* 11383 (NY) | KC184107 |
| *L. santamonicae* | USA | *Lendemer* 19660 (NY) | KC184108 |
| *L. terricola* | USA | *Knuds*en 2658.2 (SBBG) | KC184102 |
| *L. terricola* | USA | *Knudsen* 9608 (NY) | KC184103 |
| *L. terricola* | USA | *Knudsen* 2658.2 (UCR) | KC184104 |
| *L. textum* | USA | *Lendemer* 11500 (NY) | KC184113 |

walls, on shaded or sunny surfaces; often growing directly on mortar, but also overgrowing mosses (both healthy and decaying) and flints. So far known from a small number of localities in SE England.

*Notes*. Analysis of ITS sequences (Fig. 1) places material from SE England in the genus *Leprocaulon*, in a subclade with *L. americanum* Lendemer & Hodkinson, *L. knudsenii* Lendemer & Hodkinson and *L. textum* (K. Knudsen *et al.*) Lendemer & Hodkinson. All members of the subclade contain zeorin and usnic acid, whereas other members of the genus contain either this combination (*L. microscopicum* (Vill.) Gams ex D. Hawksw.), or zeorin and pannarin, or argopsin (Lendemer & Hodkinson 2013). *Leprocaulon americanum* is a fruticose species, but *L. knudsenii* and *L. textum* are leprose. *Leprocaulon textum* is said to also contain atranorin (major or minor compound) and angardianic and/or roccellic acid (minor) (Knudsen & Elix 2007). The new species is most similar in morphology and chemistry to *L. knudsenii*, which also has a finely granular thallus with no medulla, and contains only zeorin and usnic acid. The granules in *L. knudsenii* are reported as (30)–35–*45*–55–(81) µm ($\bar{x} \pm 1$SD, with maximum and minimum values in parentheses), which are slightly finer than in *L. calcicola*. Based on the available sequences, the new species is well-separated from *L. knudsenii* in the phylogenetic tree. In addition, *L. knudsenii* is so far reported only from non-calcareous rocks in the mountain ranges of California.

Amongst British species, the fine granules and lack of medulla give *Leprocaulon calcicola* a resemblance to *Lepraria incana* (L.) Ach.

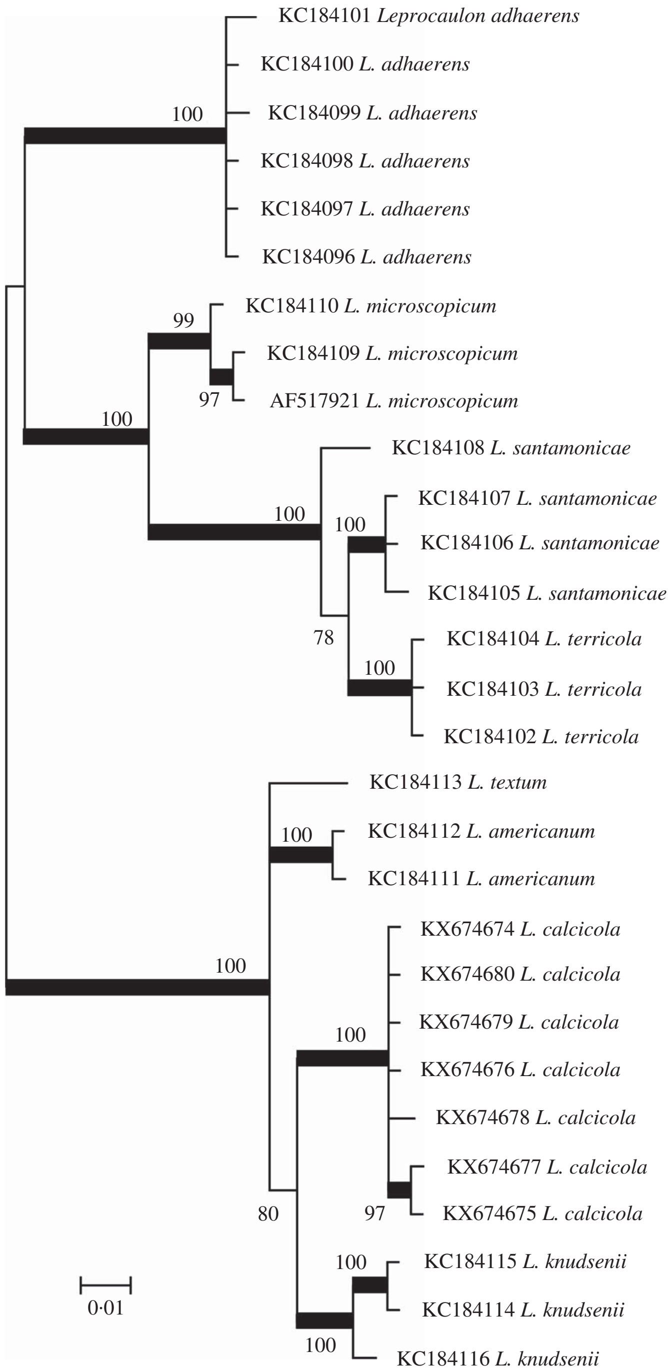


FIG. 1. Phylogenetic relationships of *Leprocaulon* species, based on a partitioned Bayesian analysis of the ITS region. The tree is mid-point rooted. Branches in bold indicate a support of PP ≥ 95%.

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FIG. 2. *Leprocaulon calcicola*, holotype. Scale = 500 µm. In colour online.

and *Lecanora ecorticata*, although both of these are most abundant on acidic substrata. *Lecanora ecorticata* has a distinct pale yellow tinge in the field which is absent in *L. calcicola*, despite the presence of usnic acid. A spot test (carried out on an acetone extract spotted onto filter paper) should distinguish *L. calcicola* from leprose British species except for *L. ecorticata*; the K−, KC+ yellow reaction indicates usnic acid. Thin-layer chromatography will distinguish the new species from *L. ecorticata*, which contains usnic acid, unidentified fatty acids, traces of unknown terpenoids and occasionally a trace of atranorin, but no zeorin. Reports of zeorin in some specimens of *L. ecorticata* (Laundon 2003) are considered here to be due to contamination from associated lichens. Amongst extra-British species, confusion would be possible with *Septotrapelia usnica* (Sipman) Kalb & Bungartz (syn. *Lepraria usnica*, *Nelsenium usnicum*), *Lecanora leuckertiana* Zedda (Zedda 2000), and the leprose species related to *Lecanora* which were investigated by Lendemer & Hodkinson (2013). It is possible that some of these will prove different in subtle morphological features or chemistry. For example, Elix (2006) reported contortin as a minor but constant compound in material of *S. usnica* from Australia. A key to leprose species with usnic acid is not attempted here as knowledge of these lichens is still poor and a key is likely to be misleading. For instance, Lendemer & Hodkinson did not give descriptions of the three unidentified leprose species which they found to belong in *Lecanora*, and they noted that the name *Lecanora ecorticata* may have been misapplied to more than one taxon. Until regional floras are well known, leprose lichens containing usnic acid should be sequenced in case further morphologically similar species occur.

The new species is only the second species in the genus *Leprocaulon* reported from Europe, the other being *L. microscopicum*. The valid name for the latter species is in dispute (Hawksworth *et al.* 2013; Lendemer & Hodkinson 2013; Lendemer 2014).

*Additional specimens examined.* **Great Britain:** *England*: **V.C. 19,** North Essex, Colchester, Priory Street, 62/000.250, on mossy mortar and undersides of septarian nodules and tile (Roman) of Roman wall, E-facing, 23 x 2007, *P. Earland-Bennett* [E1908] (hb. P. Earland-Bennett); Colchester, Priory Street, 51/999.250, on mossy mortar of

Roman wall, facing east, 23 x 2007, *P. Earland-Bennett* s. n. (hb. P. Earland-Bennett); Colchester, Priory Street, 52/999.250, on mossy mortar of Roman wall, east-facing, 23 iii 2016, *P. M. Earland-Bennett* & *J. F. Skinner* [E2080] (hb. P. Earland-Bennett); Colchester, Vineyard Gate, 52/997.250, in mossy mortar crevices (in full sunlight) of Roman wall (facing south), 23 iii 2016, *P. M. Earland-Bennett* & *J. F. Skinner* [E2077] (hb. P. Earland-Bennett); Colchester, Balkerne Hill, 52/992.252, on crumbly mortar of Roman wall, 23 iii 2016, *P. M. Earland-Bennett* & *J. F. Skinner* [E2079] (hb. P. Earland-Bennett); Colchester, 52/999.250, on mossy mortar and undersides of septarian nodules and tile of west side of bastion of Roman wall, 23 iii 2016, *P. M. Earland-Bennett* & *J. F. Skinner* [E2081] (hb. P. Earland-Bennett). **V.C. 25,** East Suffolk, Wickham Market, 62/302.557, on shaded mortar of flint wall (facing north), 27 x 2007, *P. Earland-Bennett* s. n. (hb. P. Earland-Bennett). **V.C. 26,** West Suffolk, Hessett Church, 52/93.61, on string course shaded by buttress on east-facing cutback of south wall, 27 v 2016, *C. J. B. Hitch* & *M. Powell* [E2173] (NMW - C.2016.005.111).

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