

Geographically disjunct phylogenetic lineages in *Leptogium hibernicum* reveal *Leptogium krogiae* sp. nov. from East Africa

Torbjörg BJELLAND, Mika BENDIKSBY and Andreas FRISCH

Abstract: Phylogenetic analyses of nuclear (*Mcm7*, nrITS, nrLSU) and mitochondrial (mrSSU) DNA sequences revealed geographically disjunct lineages in *Leptogium hibernicum*. One of these lineages is described here as *Leptogium krogiae*, which differs from *L. hibernicum* in the thinner thallus (190–210 µm vs. 390–480 µm), shallowly striate lobes instead of deeply furrowed lobes, isidia that are seldom distinctly flattened, and apothecia with a subhymenial euparaplectenchymatous layer. *Leptogium krogiae* is a widespread epiphyte in mist-affected woodlands and montane forests in East Africa and appears to be a common species.

Key words: Afromontane rainforests, *Collemataceae*, lichen, *Mcm7*, mrSSU, nrITS, nrLSU, taxonomy

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Introduction

The *Collemataceae* (‘jelly lichens’) is a large lichen family having a cyanobacterial photobiont (*Nostoc*). A revised classification of the family distinguishes 10 genera, of which *Leptogium* (Ach.) Gray remains the largest, despite extensive species transfer to the re-erected genera *Scytinium* (Ach.) Gray and *Pseudoleptogium* Müll. Arg. (Otálora *et al.* 2014). *Leptogium* comprises, in its present form, c. 100 mainly epiphytic species from wet tropical to temperate regions of the world (Jørgensen 1973a, 1975, 1997; Otálora *et al.* 2013).

Leptogium species with a *Mallotium*-type thallus (i.e. those having a tomentum of spherical- or cylindrical-celled hyphal hairs on one or both thallus surfaces) are retained in *Leptogium* in the classification of Otálora *et al.* (2014), but were recovered as polyphyletic. While species with spherical-celled hyphal hairs, the focal group of the present study, form a monophyletic group, those with cylindrical-celled hairs occur in three distinct groups

(Otálora *et al.* 2013). Over 50 species with a *Mallotium*-type thallus are currently accepted (Kitaura & Marcelli 2013), including a large number of recently described taxa (Jørgensen 1973a, b, 1997; Awasthi & Akhtar 1977; Jørgensen & Kashiwadani 2008; Jørgensen & Olley 2010; Kitaura & Marcelli 2012, 2013; Kitaura *et al.* 2014, 2015). Among these, only seven species with spherical-celled hairs are known to date (Kitaura & Marcelli 2013), of which *L. burgessii* (L.) Mont., *L. digitatum* (A. Massal.) Zahlbr., *L. hibernicum* M. E. Mitch. ex P. M. Jørg., and *L. laceroides* B. de Lesd. are included in *Macrolichens of East Africa* (Swinscow & Krog 1988). *Leptogium ethiopicum* C. W. Dodge (Dodge 1964) is treated as a possible synonym of *L. burgessii* in Jørgensen (1973a) and Aragón *et al.* (2005), but has been recently accepted as a distinct species (Kitaura & Marcelli 2013). The remaining two, *L. mantiqueirensis* Kitaura & Marcelli and *L. nylanderii* Kitaura & Marcelli, were recently described from South America, whereupon the former was additionally reported for South Africa (Kitaura & Marcelli 2013). The similarly neotropical *L. velutinum* P. M. Jørg. has hairs of an intermediate type, described as isodiametric at the base and short cylindrical towards the tips.

In lichen-forming fungi, a situation with high levels of homoplasy, few discrete morphological characters working at species

T. Bjelland: Rådgivende Biologer AS, Bredsgården, Bryggen, N-5003 Bergen, Norway.
M. Bendiksbj (corresponding author) and A. Frisch: NTNU University Museum, Norwegian University of Science and Technology, NO-7491 Trondheim, Norway.
Email: mika.bendiksbj@ntnu.no

level, and insufficient material available for study, has frequently resulted in broadly circumscribed and morphologically variable species with wide distributions. In recent years, a growing number of publications have demonstrated the presence of high genetic diversity and pseudocryptic lineages in such widespread species (e.g. the reviews by Crespo & Perez-Ortega 2009; Crespo & Lumbsch 2010; Lumbsch & Leavitt 2011). Moreover, genetic divergence is often seen to be positively correlated with geographical distance or dispersal barriers (e.g. Argüello *et al.* 2007; Sérusiaux *et al.* 2011; Leavitt *et al.* 2012, 2013; Amo de Paz *et al.* 2012; Del-Prado *et al.* 2013; Moncada *et al.* 2014; Alors *et al.* 2016).

Several specimens identified as *L. hibernicum* from extra-European countries were sequenced and compared with Norwegian and Western European specimens during a population genetic study of *Leptogium hibernicum* (M. Bendiksby, T. Bjelland, A. Frisch, M. H. Jørgensen & M. Görts, unpublished data). Initial analyses showed that specimens identified as *L. hibernicum* from East Africa form a strongly supported phylogenetic clade, clearly distinct from the species in Europe and eastern North America. After careful morphological examination, the East African material is described here under the name *Leptogium krogiae* and compared with other *Leptogium* species with spherical-celled hairs. We show the phylogenetic position of *L. krogiae* within the *Collemataceae* by Bayesian and maximum likelihood phylogenetic analyses of DNA sequence alignments containing both mitochondrial and nuclear DNA sequence data.

Material and Methods

Lichen sampling

Sequence data of the nuclear ribosomal internal transcribed spacer (nrITS) and large subunit (nrLSU), the mitochondrial ribosomal small subunit (mrSSU) and the DNA replication licensing factor mini-chromosome maintenance complex (*Mcm7*) were generated for 23 specimens identified as *L. hibernicum* from East Africa (Ethiopia, Kenya, Tanzania), Europe, and North and South America, and for one specimen of *L. satuminum*

(Dicks.) Nyl. from Norway. The material was received on loan from the following herbaria: BG, BM, E, O, and S. Additional sequence data were added from GenBank, most of which were also used in previous phylogenies of the *Collemataceae* by Otálora *et al.* (2013, 2014; Table 1). Based on their results, we selected all specimens of taxa presumed to be close relatives of *L. hibernicum* (i.e. section *Mallotium* with spherical-celled hairs). These include *L. hibernicum*, *L. digitatum*, *L. laceroides* and *L. velutinum*. No DNA sequence data are yet available for *L. burgessii*, *L. ethiopicum*, *L. mantiqueirensis* and *L. nylanderii* which are clearly differentiated morphologically from both *L. hibernicum* and *L. krogiae*. Our DNA sequencing effort was therefore focused on the putative new species and its closest relative, *L. hibernicum*. We also included a broad sampling from the *Collemataceae* and, based on Otálora *et al.* (2013, 2014), used *Staurolemma omphalarioides* (Anzi) P. M. Jørg. & Henssen (*Pannariaceae*) as the ultimate outgroup.

DNA extraction and PCR amplification

The lichen samples were crushed using a Retsch TissueLyser II. Total genomic DNA was extracted using the E.Z.N.A. SP Plant DNA Mini Kit (Omega Bio-tek, Inc., Norcross, Georgia, USA) following the manufacturer's instructions. The DNA regions were PCR amplified using PuReTaq Ready-To-Go PCR beads (GE Healthcare Life Sciences, Little Chalfont, Buckinghamshire, UK), in accordance with the manufacturer's instructions. The following primer sets were used: 1) nrITS: ITS4 and ITS5 (White *et al.* 1990), 2) nrLSU: LIC24R (Miadlikowska & Lutzoni 2000) and nrLSU-hypR2 (Bendiksby & Timdal 2013), 3) mrSSU: mtSSU1 and mtSSU3R (Zoller *et al.* 1999) and 4) *Mcm7*: our newly designed primers MCM7-FLH (GTCACACTACGAAACAGTTCACC) and MCM7-RLH (GACGCCACCGATCAGGAGTAG). In older specimens, higher PCR success was achieved by amplifying shorter fragments using the following internal primers: 1) mrSSU: mtSSU-hypF and mtSSU-hypR (Bendiksby & Timdal 2013) and 2) nrITS: ITS-lichF and ITS-lichR (Bendiksby & Timdal 2013).

The samples were run on a BIO RAD T100 Thermal Cycler with the following settings: initial denaturation at 95 °C for 10 min, and 31 (nrITS, nrLSU, mrSSU) or 35 (*Mcm7*) cycles at 95 °C for 30 s, 60 °C (nrITS, nrLSU, mrSSU) or 56 °C (*Mcm7*) for 30 s and 72 °C for 30 s, followed by a final extension at 72 °C for 5 min. All PCR products including negative controls were visualized by electrophoresis on a 2% agarose gel. PCR products were purified with ExoProStar 1-step, Enzymatic PCR and Sequence Reaction Clean-up Kit (GE Healthcare Life Sciences) and sent for sequencing to Eurofins Genomics GmbH (Ebersberg, Germany) with the same primers as used for the PCR reaction.

Phylogenetic analyses

Sequences were aligned using the general MAFFT settings as implemented in the Guidance Web Server (Penn *et al.* 2010) and manually corrected. The nrITS region was included only for *Leptogium hibernicum* s. lat.

TABLE 1. *Specimens used in this study with voucher information and GenBank Accession numbers. New sequences are shown in bold.*

| Taxon | Voucher | GenBank Accession numbers | | | |
|-----------------------------------|--|---------------------------|-----------------|-----------------|-----------------|
| | | mrSSU | <i>Mcm7</i> | nrLSU | nrITS |
| <i>Collema furfuraceum</i> | Norway, <i>Otálora</i> 819 (MA) | EU982608 | JX992982 | EU982608 | – |
| <i>C. nigrescens</i> | Spain, <i>Aragon</i> 80/04 (MA) | EU982563 | JX992989 | EU982604 | – |
| <i>C. subconveniens</i> | New Zealand, <i>Wedin</i> 9225 (S) | JX992937 | JX992996 | JX992880 | – |
| <i>Enchylium bachmanianum</i> | Sweden, <i>Nordin</i> 1521 (UPS) | JX992914 | JX992974 | JX992862 | – |
| <i>E. polycarpon</i> | Peru, <i>Tehler</i> s. n. (S) | JX992935 | JX992994 | – | – |
| <i>E. tenax</i> | Spain, <i>Etayo</i> 20214 (MA) | EU982556 | JX992998 | EU982597 | – |
| <i>Lathagrium auriforme</i> | Norway, <i>Nordin</i> 4621 (UPS) | JX992913 | JX992973 | JX992861 | – |
| <i>L. cristatum</i> | Norway, <i>Lofall</i> bpl-L10515 (O) | DQ917409 | JX992979 | DQ917408 | – |
| <i>L. fuscovirens</i> | Sweden, <i>Tibell</i> 23588 (UPS) | JX992923 | JX992983 | JX992869 | – |
| <i>Leptogium austroamericanum</i> | USA, <i>Yahr</i> 4357 (DUKE 30733) | EU982582 | JX993001 | EU982621 | – |
| <i>L. azureum</i> | Chile, <i>Cornejo</i> 26507 (MA) | JX992939 | JX993002 | JX992882 | – |
| <i>L. brebissonii</i> | Spain, <i>Otálora</i> 880 (MA) | EU982583 | JX993005 | EU982622 | – |
| <i>L. burnetiae</i> | Spain, <i>Aragon</i> 141/97 (MA) | EU982584 | – | EU982623 | – |
| <i>L. corticola</i> | USA, <i>Nordin</i> 4084 (UPS) | EU982585 | JX993008 | EU982624 | – |
| <i>L. dactylinum</i> | Mexico, <i>Nordin</i> 3916 (UPS) | JX992946 | JX993011 | JX992888 | – |
| <i>L. denticulatum</i> | Argentina, <i>Wedin</i> 8698 (S) | JX992948 | JX993013 | JX992890 | – |
| <i>L. digitatum</i> | Colombia, <i>Otálora</i> 010109 (S) | JX992950 | JX993016 | – | – |
| <i>L. furfuraceum</i> | Spain, <i>Aragon</i> 175/97 (MA) | EU982553 | JX993017 | EU982594 | – |
| <i>L. hibernicum</i> -LH014 | Hordaland, Norway, <i>Bjelland</i> TB_RB_4 (BG) | KX013750 | KX013732 | KX013758 | KX013723 |
| <i>L. hibernicum</i> -LH021 | Scotland, <i>Jørgensen</i> s. n. (BG-L-64038) | KX013700 | KX013658 | – | KX013616 |
| <i>L. hibernicum</i> -LH024 | Kerry, Ireland, <i>Jørgensen</i> s. n. (BG-L-64037) | KX013751 | KX013733 | KX013759 | KX013724 |
| <i>L. hibernicum</i> -LH027 | Madeira, <i>Jones</i> s. n. (BG-L-93567) | – | KX013734 | – | – |
| <i>L. hibernicum</i> -LH044 | Chile, <i>Santesson</i> s. n. (S-L-36254) | KX013756 | – | – | KX013635 |
| <i>L. hibernicum</i> -LH045 | North Carolina, USA, <i>Egan</i> s. n. (S-L-44011) | – | – | – | KX013615 |
| <i>L. hibernicum</i> -LH047 | Chile, <i>Santesson</i> s. n. (S-L- 36182) | KX013757 | – | – | KX013636 |
| <i>L. hibernicum</i> -LH049 | Arizona, USA, <i>Nash</i> s. n. (S-L-64028) | KX013755 | – | – | KX013637 |
| <i>L. hibernicum</i> -LH031 | France, <i>Dahl</i> s. n. (O) | KX013752 | KX013735 | – | KX013725 |
| <i>L. hibernicum</i> -LH062 | Scotland, <i>Coppins</i> s. n. (E-L-00665882) | KX013753 | KX013736 | KX013761 | KX013721 |
| <i>L. hibernicum</i> -LH063 | Azores, <i>Purvis & James</i> s. n. (BM-000747636) | KX013754 | KX013737 | KX013760 | KX013722 |
| <i>L. hildenbrandii</i> | Rumania, <i>Halda & Palice</i> s. n. (UPS) | JX992951 | – | JX992892 | – |
| <i>L. juressianum</i> | South Africa, <i>Nordin</i> 11812 (UPS) | JX992954 | JX993019 | JX992894 | – |
| <i>L. krogiae</i> -LH026 | Kenya, <i>Jørgensen</i> s. n. (BG-L-64040) | KX013740 | – | – | KX013710 |
| <i>L. krogiae</i> -LH032 | Ethiopia, <i>Krog</i> s. n. (O-L-92741) | KX013741 | KX013738 | – | KX013711 |
| <i>L. krogiae</i> -LH033 | Tanzania, <i>Krog</i> s. n. (O-L-188654) | KX013742 | KX013726 | – | KX013712 |
| <i>L. krogiae</i> -LH034 | Tanzania, <i>Krog</i> s. n. (O-L-188660) | KX013743 | KX013727 | KX013762 | KX013713 |

TABLE 1 (continued).

| Taxon | Voucher | GenBank Accession numbers | | | |
|-----------------------------------|--|---------------------------|-----------------|-----------------|-----------------|
| | | mrSSU | <i>Mcm7</i> | nrLSU | nrITS |
| <i>Leptogium krogiae</i> -LH035 | Kenya, <i>Krog</i> s. n. (O-L-188661) | KX013744 | KX013728 | - | KX013714 |
| <i>L. krogiae</i> -LH036 | Kenya, <i>Krog</i> s. n. (O-L-188668) | KX013745 | KX013739 | - | KX013715 |
| <i>L. krogiae</i> -LH037 | Kenya, <i>Krog</i> s. n. (O-L-188664) | KX013746 | KX013729 | - | KX013716 |
| <i>L. krogiae</i> -LH039 | Ethiopia, <i>Krog</i> s. n. (O-L-092838) | KX013748 | KX013731 | KX013763 | KX013717 |
| <i>L. krogiae</i> -LH065 | Tanzania, <i>Swinscow</i> s. n. (BM-001085148) | - | - | - | KX013718 |
| <i>L. krogiae</i> -LH067 | Kenya, <i>Swinscow</i> s. n. (BM-001085150) | - | - | - | KX013719 |
| <i>L. krogiae</i> -LH068 | Kenya, <i>Swinscow</i> s. n. (BM-001085151) | KX013748 | - | - | KX013720 |
| <i>L. laceroides</i> | Colombia, <i>Otálora</i> 020109 (S) | JX992955 | JX993039 | JX992896 | - |
| <i>L. marginellum</i> | Ecuador, <i>Tehler</i> 8676 (S) | JX992957 | JX993024 | JX992899 | - |
| <i>L. papillosum</i> | Argentina, <i>Wedin</i> 8750 (S) | JX992961 | JX993027 | JX992902 | - |
| <i>L. pedicellatum</i> | South Korea, <i>Thor</i> 17186 (S) | JX992962 | - | JX992903 | - |
| <i>L. phyllocarpum</i> | Costa Rica, <i>O'Brien</i> 03051203 (DUKE) | EU982589 | JX993028 | EU982628 | - |
| <i>L. pseudofurfuraceum</i> | Argentina, <i>Aragon</i> 2250/05 (MA) | EU982562 | JX993031 | EU982603 | - |
| <i>L. saturninum</i> | Rogaland, Norway, <i>Johnsen</i> s. n. (BG-L-81192) | KX027280 | KX027283 | - | - |
| <i>L. sp.</i> | New Zealand, <i>Wedin</i> 8751 (S) | JX992952 | JX993020 | JX992895 | - |
| <i>L. sp.</i> | Kenya, <i>Krog</i> 4K20/141 (O-L-188772) | KX013747 | KX013730 | - | - |
| <i>L. velutinum</i> | Ecuador, <i>Prieto</i> 2010 (UPS) | JX992972 | JX993041 | JX992912 | - |
| <i>Pseudoleptogium diffractum</i> | Sweden, <i>Nordin</i> 2529 (UPS) | JX992949 | JX993015 | JX992891 | - |
| <i>Rostania multipunctata</i> | Greece, <i>Nordin</i> 3160 (UPS) | JX992930 | JX992988 | JX992874 | - |
| <i>R. occultata</i> | Sweden, <i>Nordin</i> 5407 (UPS) | JX992931 | JX992990 | JX992876 | - |
| <i>Scytinium fragrans</i> | Sweden, <i>Thor</i> 22081 (UPS) | JX992922 | JX992981 | JX992868 | - |
| <i>S. lichenoides</i> | Spain, <i>Aragon</i> 1301/02 (MA) | DQ923120 | JX993021 | DQ917412 | - |
| <i>S. palmatum</i> | Sweden, <i>Nordin</i> 5369 (UPS) | JX992959 | JX993025 | JX992900 | - |
| <i>Staurolemma omphalarioides</i> | Canary Islands, Tenerife, <i>Hafellner & Hafellner</i> 41399 (UPS) | - | JX993043 | - | - |
| <i>S. omphalarioides</i> | Spain, <i>Aragon</i> 83/04 (MA) | EU982560 | - | EU982601 | - |

(i.e. the specimens from Europe, the northern Atlantic and the Americas) and *L. krogiae*. This region is highly variable across the *Collema* and available for only a small number of the species included in our phylogeny. By excluding all nrITS sequences outside the *L. hibernicum-L. krogiae* clade, we were able to make use of the full phylogenetic signal of the nrITS for our target species.

Prior to concatenation, we tested the four single-gene alignments for conflicting tree topologies. Bayesian Inference (BI) and Maximum Likelihood (ML) analyses were performed using the same settings for the single-gene and the concatenated four-gene alignments. Serious conflict was assumed when deviant tree topologies were supported by $\geq 70\%$ bootstrap values (BS) and ≥ 0.95 posterior probabilities (PP). A partitioned dataset was used for the final phylogenetic analyses to enable independent parameter estimation for the four DNA regions. The *Mcm7* alignment was further partitioned according to codon positions to allow for the higher evolutionary rates of the 3rd codon position.

The BI and ML phylogenetic analyses were performed on the CIPRES Science Gateway (Miller et al. 2010). For the BI analysis, we used MrBayes 3.2.6 (Ronquist & Huelsenbeck 2003). A GTR-I Γ model of sequence evolution was applied to the partitioned dataset and the model parameters were estimated during the run for each gene partition separately starting from a default flat Dirichlet distribution. The analysis was run for 15 000 000 generations in eight chains and every 500th generation was sampled. The first 50% of trees were discarded as burn-in and the PPs summarized on a 50% majority-rule consensus tree. For the ML analysis, we used the RAxML-HPC black box with rapid bootstrapping and full ML analysis under the GTR+GAMMA approximation (i.e. not allowing for a proportion of invariable sites, I). The analysis was automatically stopped after 252 bootstrap replicates using the bootstrapping option implemented in RAxML 3.2.7 (Pattengale et al. 2009).

Morphology

The morphology of the lichen specimens was examined using a Zeiss stereomicroscope. Sections for anatomical examination were cut by hand, mounted in water, and examined with a Zeiss Axioskop 2 microscope. With the exception of the lobe and apothecium sizes, all measurements were made in water.

Results

Phylogenetic analysis

New sequences generated in this study included 21 nrITS, 6 nrLSU, 20 mrSSU, and 16 *Mcm7* (Table 1).

The final alignment of 59 accessions and 2704 nucleotide positions contained 19 nrITS (453 nucleotide positions), 39 nrLSU (959), 55 mrSSU (815), and 48 *Mcm7* (477) sequences, including the outgroup, *Staurolemma omphalarioides*.

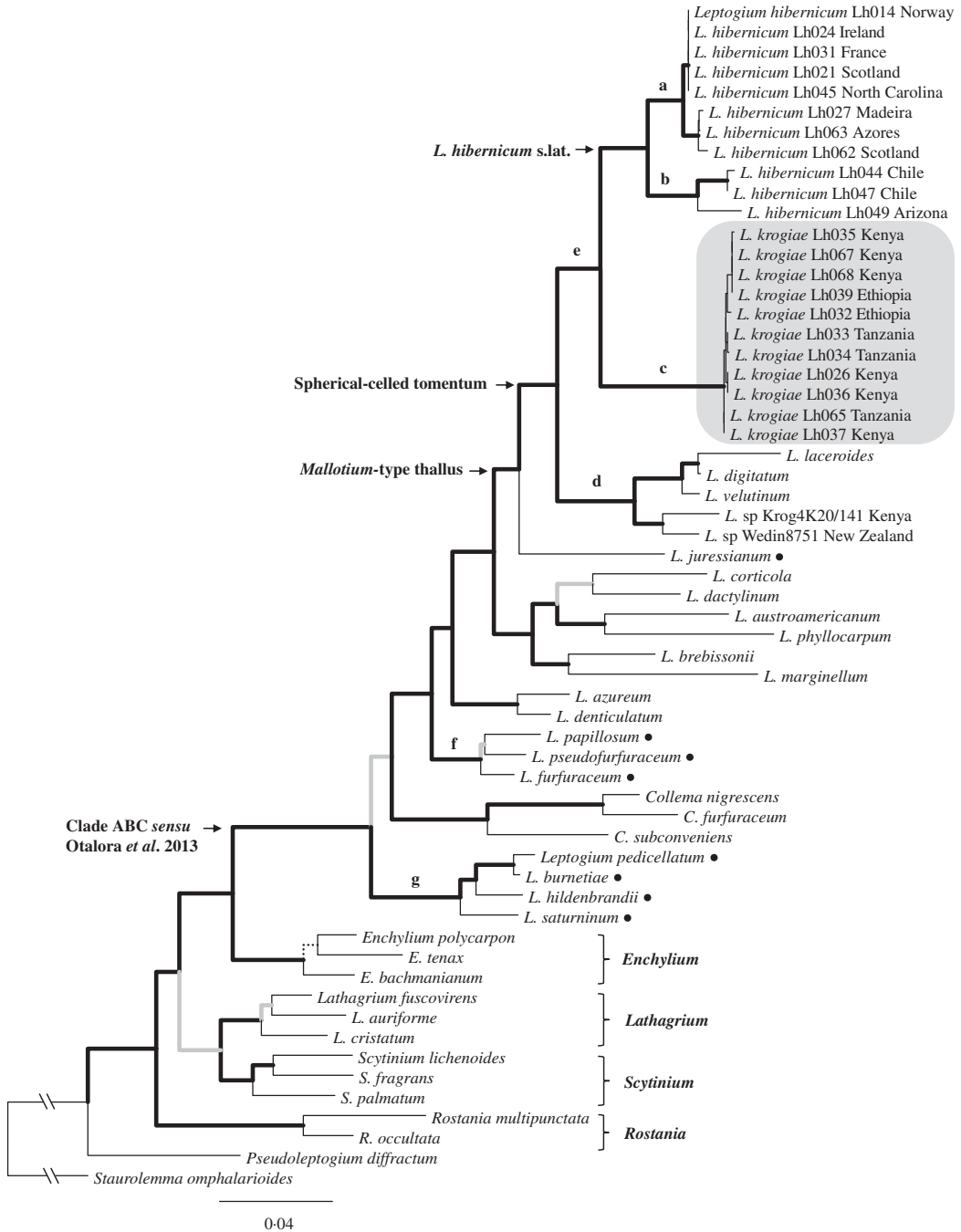
There were no supported conflicts between the BI and ML analyses or between the separate single-gene analyses. We therefore performed the final analyses on a concatenated dataset of all four DNA regions which resulted in a well-resolved and overall strongly supported phylogenetic hypothesis (Fig. 1). All included accessions of *L. hibernicum* s. lat. group with high support. This clade contains two strongly supported subclades that correlate with geographical distance (Europe, the northern Atlantic and the eastern USA vs. Chile and the south-western USA; clades a and b, respectively). There is less genetic variation among the included accessions of *L. krogiae*, which forms a strongly supported sister (clade c) to *L. hibernicum* s. lat. Clade d comprises *L. digitatum*, *L. laceroides*, *L. velutimum* and two specimens collected as *L. hibernicum* from Kenya and New Zealand (*Krogh* 4K20/141 and *Wedin* 8751, respectively) that do not group with the other accessions of *L. hibernicum* or *L. krogiae*. Clade e, including subclades a, b, and c, together with its sister clade d, consists of species with spherical-celled hyphal hairs, with the exception of *L. velutimum* which has intermediate hairs.

Our tree topology is largely consistent with previous studies by Otálora et al. (2013, 2014), except for the deviating position of *Rostania Trevis.*, here falling out closer to the base of the tree (Fig. 1). Included specimens of *Collema* nest within *Leptogium* but the supporting branch receives PP < 0.95. Species having a tomentum formed of hyphal hairs with cylindrical cells are found in three places in our tree topology: 1) *L. juressianum* Tav. being basally attached to the species with spherical-celled hyphal hairs, 2) *L. furfuraceum* (Harm.) Sierk, *L. papillosum* (B. de Lesd.) C. W. Dodge, and *L. pseudofurfuraceum* P. M. Jørg. & A. K. Wallace (clade f) and 3) *L. burnetiae* Dodge, *L. hildenbrandii* (Garov.) Nyl., *L. pedicellatum* P. M. Jørg., and *L. saturninum* (clade g).

The Species

Leptogium krogiae Bjelland, Frisch & Bendiksby sp. nov.

Mycobank No.: MB 817371



Leptogium species similar to *L. hibernicum*, but differing in the thinner thallus (190–210 µm vs. 390–480 µm), shallowly striate instead of deeply furrowed lobes, and only rarely distinctly flattened isidia. Apothecia stipitate with wrinkled pedicel, subhymenial euparaplectenchymatous layer, and excipular cortex of one cell layer. Ascospores submuriform, 32–38 × 11–14 µm, 8 per ascus.

Type: Tanzania, Iringa region, Iringa District, Mufundi escarpment, 8°40'S, 35°20'E, 1750–1800 m, August 1989, *H. Krog* no 4T 15/027 (O—holotype).

(Fig. 2A & C)

Thallus brownish or bluish grey, dry lobes to 1 cm broad, wet lobes swollen to 190–210 µm thick; quadratic cells of cortex 5–6 µm thick; thallus plane to undulate, transversely wrinkled-striate only on the thickest portions, usually in the middle of the thallus; columnar hyphae present, straight to sinuose, 2.5–3.0 µm wide; *isidia* mainly laminal, scattered to agglomerated, grey to brown, granular to cylindrical to coralloid, occasionally flattened, and sometimes pubescent-tomentose close to the thallus surface. Lower surface paler, uniformly pubescent-tomentose; hyphal hairs unbranched to irregular branched; 80–110 µm long, with (5–)9–15 globose cells. *Photobiont* *Nostoc*; filaments often long (20–30 cells), *c.* 2.5 µm diam.

Apothecia very rare, up to 3 mm diam., stipitate; pedicel with longitudinal wrinkles in older apothecia; mature disc concave, red-brown; thalline exciple bearing lobules and sometimes numerous apical upright isidia (one specimen). *Hymenium* 180–220 µm high; subhymenium and hypothecium not clearly separated, 60 µm thick, yellowish; subhymenial euparaplectenchymatous layer 160–180 µm thick, of 10 cell layers (Fig. 2C); excipular cortex of one cell layer. *Ascospores* 8 per ascus, colourless, ellipsoid with acute ends, submuriform, 32–38 × 11–14 µm.

Etymology. Named after the late Norwegian lichenologist Hildur Krog to commemorate her

major contributions to the exploration of East African lichens.

Ecology and distribution. Corticolous in mist-affected woodland and montane forest at altitudes of 1800–3300 m, scattered, not uncommon (Jørgensen 1973a; Swinscow & Krog 1988; Krog 1991, 2000; Alstrup & Christensen 2006; all as *L. hibernicum*). Confirmed specimens have been seen from central Ethiopia through the Kenyan highlands to the Iringa region in south-western Tanzania. *Leptogium krogiae* seems to be common in the Taita Hills in south-eastern Kenya (J. Rikkinen, pers. comm.).

Notes. *Leptogium krogiae* (Fig. 2A) is morphologically close to *L. hibernicum* (Fig. 2B), with which it has been identified in the past (Jørgensen 1973a; Swinscow & Krog 1988; Krog 1991, 2000; Alstrup & Christensen 2006). Both species share the brownish to bluish grey lobes with predominantly laminal isidia, short tomentum of spherical-celled hyphal hairs mainly on the lower surface, and lobe striation. While apothecia are present in some of the specimens of *L. krogiae* investigated, fertile specimens are unknown for *L. hibernicum* (Kitaura & Marcelli 2013; M. Bendiksby, T. Bjelland, A. Frisch, M. H. Jørgensen & M. Gørts, unpublished data). Morphological differences are mainly observed in the degree of striation of the upper lobe surface, lobe thickness, the length of the *Nostoc* filaments, and isidium shape.

In *Leptogium krogiae*, the striation is typically rather shallow and scattered compared to the deeply furrowed lobes of *L. hibernicum*. According to P. M. Jørgensen (pers. com.), the degree of striation of the lobes may reflect the thallus thickness which, when wet, is 390–480 µm in *L. hibernicum* (Aragón *et al.* 2005) and 190–210 µm in *L. krogiae*. We have

FIG. 1. Phylogenetic relationships among *Leptogium* species with spherical-celled tomentum based on the best RAxML tree using a concatenated alignment of four genetic regions (nrITS, nrLSU, mrSSU, *Mcm7*) with particular focus on the phylogenetic position and genetic delimitation of *Leptogium hibernicum* s. lat (clades a + b) and the new species, *L. krogiae* (clade c; grey box). Thickened branches indicate Bayesian posterior probabilities PP ≥ 0.95 and RAxML bootstrap values BS ≥ 70%. Wide, light grey branches indicate BS ≥ 70% and PP < 0.95. The dashed branch indicates PP ≥ 0.95 (BS < 70%). The gap in the root branch spans 65% of the root branch length. Clades discussed in the text are indicated with the letter a–g. Species with cylindrical-celled tomentum are indicated with a black circle.

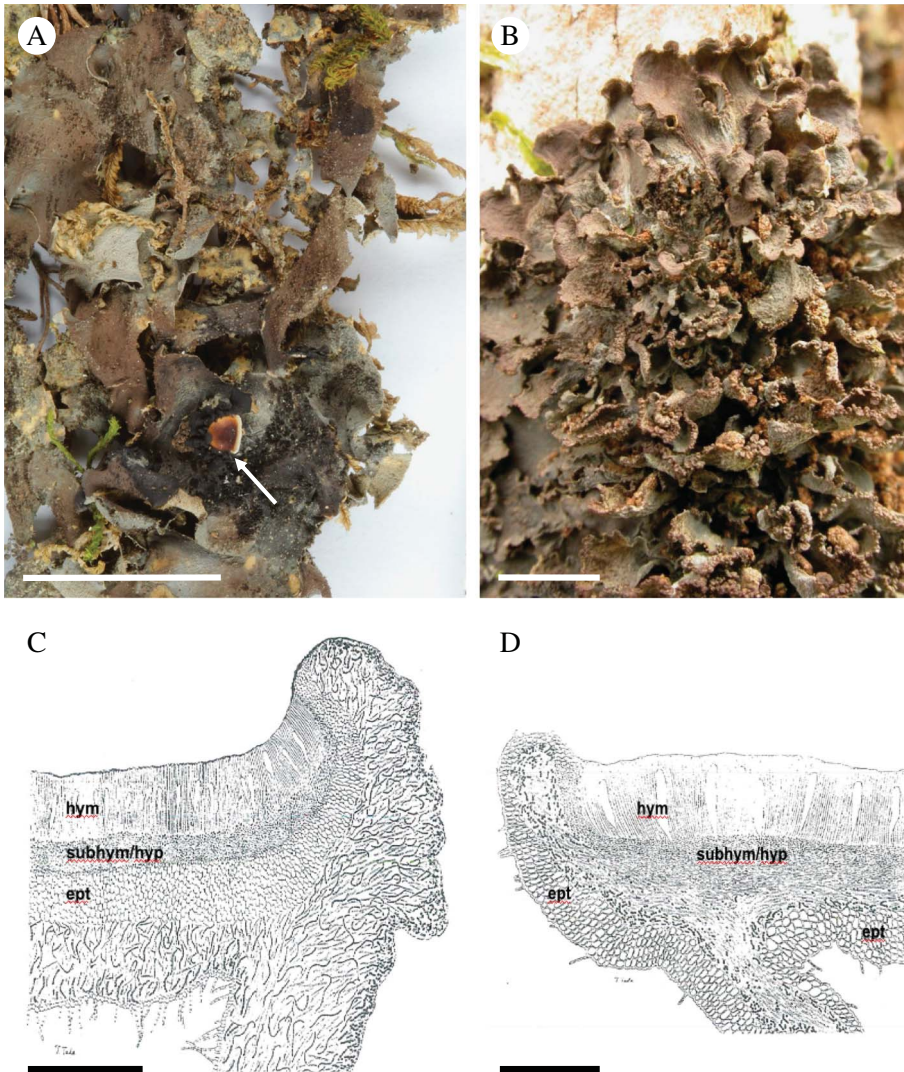


FIG. 2. A, *Leptogium krogiae* Bjelland, Frisch & Bendiksbj, sp. nov. (O, holotype; photograph Lena Ohnheiser), apothecium indicated by arrow. B, *Leptogium hibernicum* s. lat. clade (typical specimen) (see Fig. 1; photograph Einar Tindal). C, *Leptogium delavayi*, apothecium section (similar to *L. krogiae*). D, *Leptogium pedicellatum*, apothecium section (similar to *L. burgessii* and *L. ethiopicum*), showing different location of the euparaplectenchyma. Illustrations C and D from Asahina (1935) with tissue descriptions and approximate scale bars superimposed. Abbreviations: ept = euparaplectenchyma, hym = hymenium, subhym = subhymenium, hyp = hypothecium. Scales: A & B = 1 cm; C & D = c. 1 mm. In colour online.

also observed marked differences in the length of the *Nostoc* filaments, which on average consist of 12–16 cells in *L. hibernicum* (Aragón *et al.* 2005) and 20–30 cells in *L. krogiae*. The shape of the isidia in *L. krogiae* varies from

granular to cylindrical to coralloid. The isidia in *L. krogiae* are more rarely flattened compared to those in *L. hibernicum*.

No DNA sequence data are yet available for *L. burgessii*, *L. ethiopicum*, *L. mantiqueirense*

and *L. nylanderi*, but these species are morphologically clearly differentiated from both *L. hibernicum* and *L. krogiae*. Neither *L. burgessii*, *L. ethiopicum*, *L. mantiqueirense* nor *L. velutinum* have isidia. Furthermore, *L. burgessii* differs from *L. krogiae* in the absence of a subhymenial euparaplectenchymatous layer (see Fig. 2C vs. 2D), the thick euparaplectenchymatous amphithecium, thinner lobes (40–50 µm) and a smooth to slightly rugulose surface. *Leptogium ethiopicum* was considered a possible synonym of *L. burgessii* (Jørgensen 1973a) but was recently accepted as an independent taxon (Kitaura & Marcelli 2013). The type and only specimen of the species from the Chokke Mountains in Ethiopia (FH-Dodge!) is difficult to interpret, but clearly differs from *L. krogiae* in the absence of a subhymenial euparaplectenchymatous layer (see Fig. 2C vs. 2D). Further differences include the thick euparaplectenchymatous amphithecium, thinner lobes (80–90 µm), absence of striation on the upper lobe surface, shorter tomentum (10–20 µm) and presence of degraded lobules on the rim of the apothecial margin rather than isidia.

Leptogium velutinum differs from *L. krogiae* in the absence of isidia and a subhymenial euparaplectenchymatous layer, thinner lobes (50–55 µm), a smooth surface (rugulose under ×20 magnification) and a velvety lower side, and the tomentum hairs have isodiametric cells at their base and cylindrical cells at their apex (Kitaura et al. 2014). *Leptogium nylanderi* differs from *L. krogiae* by having a subhymenial colloplectenchymatous layer, a euparaplectenchymatous amphithecium and an apothecium with granular isidia and thinner lobes (70–90 µm) (Kitaura & Marcelli 2013). *Leptogium laceroides* differs from *L. krogiae* in the absence of a subhymenial euparaplectenchymatous layer, thinner lobes (70–100 µm) and by having a smooth surface (Kitaura & Marcelli 2013).

Only two additional *Leptogium* species with both spherical-celled hairs and a thick subhymenial euparaplectenchymatous layer (Fig. 2C) are described at a global level. 1) *Leptogium digitatum* is easily distinguished by the deeply ridged lobes bearing a dense tomentum on both surfaces, while the

tomentum is largely confined to the lower lobe surfaces in *L. krogiae*; isidia and lobules are absent and the ascospores are smaller (25–30 × 10–13 µm). 2) *Leptogium mantiqueirense* differs from *L. krogiae* in the non-isidiate and non-lobulate thallus with smooth margins, larger apothecia (to 6 mm diam.) bearing a basally fused collar of lobules at the edge of the otherwise smooth margin, smaller ascospores (22–30 × 10–13 µm) and anatomical details such as a thinner thallus (100–190 µm), shorter tomentum (5–20(–35) µm), lower subhymenium (12–25 µm), lower hymenium (110–150 µm) and a subhymenial euparaplectenchymatous layer (85–125 µm). A comparison of diagnostic characters of *Leptogium* species with spherical-celled hyphal hairs is presented in Table 2.

Selected specimens examined. Ethiopia: Sidamo (Shewa) Province: Wondo Gennet, 20 km S of Shashemenne, elev. 1800–2000 m, on trees in mixed forest, i 1972, *Krog* s. n. (O); between Shashemenne and Kofele, elev. 2400 m, on trees in mixed forest, i 1972, *Krog* s. n. (O); *ibid.*, *Krog* E27/36 (O). *Arussi Province:* W slope of Mt. Chilalo, E of Asella, elev. 3000–3100 m, on trees in *Hagenia-Hypericum* forest, i 1972, *Krog* E30/41, E30/43, E30/44 (O).—**Kenya:** S slope of Mt. Kenya, *Arundinaria alpina* bamboo thicket ab. Kamaweti Forest Station, elev. 2400–2700 m, on *Nuxia* trees, 29 xi 1971, *Pöcs* 6601/K & *Fadew* (BG). *Rift Valley Province:* Elgeyo Marakwet District, Chebiemit forest 1 km W of Cheptongei, 0°57'N, 35°29'E, elev. 2300 m, on trees, 1973, *Krog* 2K11/121 (O); *ibid.*, forest 2 km NE of Kapcherop, 1°04'N, 35°20'E, elev. 2200 m, on fallen forest tree, 1973, *Krog* 2K14/113a (O); Kajiado District, Ngong Hills, 1°27'S, 36°37'E, elev. 2300 m, on shrubs and rocks, 1972, *Krog* K45/141 (O). *Central Province:* Mt. Elgon, S of Suam River valley to Kapchalwa Gate, 1°15'N, 34°50'E, elev. 2500–3100 m, 1973, *Ryvarden* 9360 (O); Nyeri District, Mt. Kenya Safari Club, 0°02'S, 37°07'E, elev. 2100 m, 1976, *Krog* 4K19/143 (O); Nyeri District, Mt. Kenya, S slope, Regati Forest Station, 0°20'S, 37°15'E, elev. 2200–2300 m, 1973, *Ryvarden* 9827 (O); Kirinyaga District, Mt. Kenya, near Castle Forest Station, 0°23'S, 38°18'E, elev. 1900 m, on trees, 1972, *Krog* K49/128 (O); Kirinyaga District, Aberdare Mts., 10 km W of Tusha, ericaceous zone, 0°31'S, 36°44'E, elev. 2900 m, on trees, 1974, *Krog* 3K31/184 (O); Kirinyaga District, Mt. Kenya, 2 km NW of Irangi Forest Station, moist deciduous forest near River Ena, 0°20'S, 37°28'E, 2000 m, 1972, *Krog* K48/152, K48/153, K48/154 (O); Kirinyaga District, Mt. Kenya, by Thiba Fishing Camp, 0°24'S, 37°18'E, elev. 1850 m, on tree, 1972, *Krog* K52/116 (O). *Eastern Province:* Meru District, Mt. Kenya, open forest on E side of Themwe, 0°09'S, 37°34'E, elev. 2100 m, on rocks and trees, 1974, *Krog* 3K16/288 (O).—**Tanzania:** *Tanga Province:* Lushoto District, West Usambara Mts., along boundary of Mazumbai University Forest Reserve,

TABLE 2. Taxonomic characteristics of *Leptogium* section *Mallotium* with spherical-celled or velvet-like hairs (authors' data; Kitaura & Marcelli 2013; Kitaura et al. 2014).

| Species | Lobes | Upper surface | Isidia | Columnar hyphae | Apothecium | Euplectenchyma in apothecia | Hairs |
|---------------------------|--|---------------------------------------|---|----------------------|-----------------------------------|--|----------------------|
| <i>L. burgessii</i> | Rounded and smooth; 40–50 µm thick | Smooth to slightly rugulose | Absent | Straight or inclined | Apothecium with lobules | Amphithecial | Mostly lower surface |
| <i>L. digitatum</i> | Rounded and crisped, 190–210 µm thick | Rugulose | Absent | Sinuuous | Apothecium with lobules | Subhymenial | Both surfaces |
| <i>L. ethiopicum</i> | Rounded and crisped; 80 µm thick | Smooth | Absent | Straight | Apothecium with lobules | Amphithecial | Both surfaces |
| <i>L. hibernicum</i> | Rounded and usually smooth; 390–480 µm thick | Wrinkled | Granular, cylindrical, coralloid to flattened | Sinuuous | Not known | | Mostly lower surface |
| <i>L. krogiae</i> | Rounded and usually smooth; 190–210 µm thick | Smooth to slightly wrinkled | Granular, cylindrical to coralloid | Straight to sinuous | Apothecium with lobules | Subhymenial | Mostly lower surface |
| <i>L. laceroides</i> | Rounded and usually smooth; 70–100 µm thick | Smooth | Cylindrical to flattened | Straight | Apothecium with irregular lobules | Amphithecial | Mostly lower surface |
| <i>L. mantiqueirensis</i> | Rounded and smooth | Rugulose | Absent | Sinuuous | Apothecium with lamellar collar | Amphithecial | Mostly lower surface |
| <i>L. nylanderi</i> | Rounded and isidiate; 70–90 µm thick | Slightly rugulose to smooth | Cylindrical to flattened | Inclined | Apothecium with granular isidia | Amphithecial and colloplectenchyma subhymenial | Mostly lower surface |
| <i>L. velutinum</i> | Rounded and smooth; 50–55 µm thick | Smooth to naked eye, rugulose at ×20. | Absent | Straight or inclined | Apothecium without ornamentations | Amphithecial | Mostly lower surface |

in montane rainforest between Sagara Ridge and motor road, 4°49'S, 38°30'E, elev. 1500–1900 m, 1988, *Krog* 2T13/6 (O); *ibid.*, Usambara Mts., Magamba c. 4 km N of Lushoto, 4°50'S, 38°15'E, elev. 1600–2000 m, 1973, *Ryvarden* 10922 (O); *ibid.*, West Usambara Mts., Mazumbai University Forest Reserve, Sagara Ridge, 4°49'S, 38°30'E, elev. 1850–1980 m, in mossy elfin forest and *Philippia* heath with rock outcrops, 1988, *Krog* 2T09/18 (O); *ibid.*, along motor road from Mazumbai University

Forest Station to the edge of the forest reserve, 4°48'S, 38°30'E, elev. 1500–1560 m, 1988, *Krog* 2T08/023 (O). *Southern Highlands Province*: Iringa District, Mufindi escarpment, 8°40'S, 35°20'E, elev. 1750–1800 m, 1989, *Krog* 4T15/027, 4T15/28 (O); Iringa District, Buffalo Hill at edge of Mufindi escarpment, on trees in montane rainforest and at forest edge, 8°40'S, 35°20'E, elev. 1850–1900 m, 1989, *Krog* 4T10/168, 4T10/170, 4T10/173 (O).

Key to *Leptogium* species with spherical-celled hairs

- 1 Thallus isidiate 2
- Thallus not isidiate 5

- 2(1) Upper surface distinctly wrinkled, thallus very thick when wet (c. 400 µm thick) **L. hibernicum**
- Upper surface smooth to slightly wrinkled, wet lobes ≤200 µm thick 3

- 3(2) Upper surface slightly wrinkled (wet lobes c. 200 µm thick) **L. krogiae**
- Upper surface smooth (wet lobes <100 µm thick) 4

- 4(3) Laminal lobules present **L. laceroides**
- Laminal lobules absent **L. nylanderii**

- 5(1) Apothecium with euparaplectenchymatous proper exciple 6
- Apothecium without euparaplectenchymatous proper exciple 7

- 6(5) Hairs equally abundant on upper and lower surface **L. digitatum**
- Hairs predominantly on lower surface **L. mantiqueirensis**

- 7(5) Hairs with isodiametric cells at base and short-cylindrical towards the tips **L. velutinum**
- Hairs with spherical cells throughout **L. burgessii/L. ethiopicum**

Discussion

We found three distinct phylogenetic lineages within *L. hibernicum* having discrete distributions (Fig. 1: clades a–c). *Leptogium hibernicum* in the strict sense (clade a) in our study is confined to Western Europe, some islands of the northern Atlantic (Azores, Madeira) and eastern North America. An extended study on the phylogeography of *L. hibernicum* in Western Europe (M. Bendiksby, T. Bjelland, A. Frisch, M. H. Jørgensen & M. Görts, unpublished data) supports this pattern. Three specimens from Chile and the south-western USA form a second lineage (clade b). We have not

studied these specimens in detail, and more material is needed for drawing solid taxonomic conclusions. However, our preliminary phylogenetic results suggest that this clade represents one or more as yet unrecognized species closely related to *L. hibernicum* s. str. (clade a).
 A third strongly supported and distinct lineage (clade c) is described here as *L. krogiae*. We have seen a large number of specimens of this species from East Africa but a wider distribution within Africa is suggested by reports of *L. hibernicum* from southern Africa (Swinscow & Krog 1988) and the availability outside East Africa of habitats suitable for the species. Another example of geographically

disjunct lineages within *Leptogium* species was given recently by Otálora *et al.* (2010), who recovered the populations of *L. furfuraceum* from Europe and *L. pseudofurfuraceum* from North America as sister clades, as was the case with *L. furfuraceum* from Africa and *L. pseudofurfuraceum* from South America.

Further extra-European specimens of *L. hibernicum* included in our study proved unrelated to that species though they all fall within the clade covering species with a spherical-celled tomentum (Fig. 1). *Leptogium* sp. Krogh 4K20/141 from Kenya is morphologically close to *L. laceroides* (P. M. Jørgensen, pers. com.) but differs genetically from a second specimen of that species in our phylogeny collected in Colombia which is geographically closer to the type locality of *L. laceroides* in Mexico. *Leptogium* sp. Wedin 8751 from New Zealand, included as *L. hibernicum* in the studies of Otálora *et al.* (2013, 2014), was not available for study.

In the classification of Otálora *et al.* (2014), the selected lectotype of *Leptogium*, *L. lacerum* (Ach.) Gray (= *L. lichenooides* (L.) Zahlbr.), falls within the reinstated genus *Scytinium* (Jørgensen *et al.* 2013). In order to sustain taxonomic stability and avoid the need for recombining the bulk of *Leptogium* species into *Mallotium* (Ach.) Gray, Jørgensen *et al.* (2013) proposed conserving *Leptogium* with a new type, *L. azureum* (Sw. ex Ach.) Mont. We prefer to follow the present classification and describe the new species in *Leptogium* rather than *Mallotium* until a decision is made on this proposal.

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