



# From cradle to grave? A global hotspot and new species of the genus *Lobaria* discovered in the Himalayas and the Hengduan Mountains

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## Key words

East Asia  
evolution  
lichen-forming ascomycetes  
*Lobaria*  
multi-locus phylogeny  
new taxa

**Abstract** In this study, the East Asian diversity of green-algal *Lobaria* was evaluated by applying both morphological and phylogenetic approaches. A multi-locus phylogenetic analysis of 72 green-algal *Lobaria* specimens was performed using a three-locus and time-calibrated species-tree approach. The analyses demonstrate that pairs of sexually and vegetatively reproducing lineages split into highly supported monophyletic clades. Taxonomically, 11 green-algal *Lobaria* species were identified as new to science, while 10 were previously described species. The species differentiated during the Pliocene and Pleistocene. The coincidence of paleoclimatic events with estimated dates of divergence support a bioclimatic hypothesis for species evolution in the green-algal *Lobaria*. Molecular phylogenies, a summary of diversity, detailed new species descriptions and geographical analyses are provided. Special recognition of species with a long evolutionary history, which merit high conservation priority, will be critical for preserving geographically restricted endemics in the Himalayas and the Hengduan Mountains, where habitat loss is driving rapid declines.

**Citation:** Yang MX, Wang LS, Miao CC, et al. 2022. From cradle to grave? A global hotspot and new species of the genus *Lobaria* discovered in the Himalayas and the Hengduan Mountains. *Persoonia* 48: 150–174. <https://doi.org/10.3767/persoonia.2022.48.04>. Effectively published online: 14 May 2022 [Received: 7 November 2021; Accepted: 24 January 2022].

## INTRODUCTION

The lichenized ascomycetous genus *Lobaria* belongs to the subfamily *Lobarioideae* of the family *Peltigeraceae*, and forms an obligate symbiotic association with algae and/or cyanobacteria (photobionts) (Ahmadjian 1993, Honegger 2012, Lücking et al. 2017, Widhelm et al. 2019, Simon et al. 2020). The genus *Lobaria* is characterised by a large, foliose, foveolate thallus. The majority of species have neither cyphellae nor pseudocyphellae (Yoshimura 1998a, b, Wedin & Wiklund 2004, Wedin et al. 2009). Photobionts of *Lobaria* are either green-algal and belong to *Symbiochloris reticulata* (Dal Grande et al. 2014, Škaloud et al. 2016) or cyanobacterial and belong to the genus *Nostoc* (Cornejo & Scheidegger 2015). Based on a three-gene phylogeny (mtSSU, nuLSU, ITS data) and morpho-chemical and ecological features, Moncada et al. (2013) classified seven clades of *Lobaria* s.lat. as different genetic lineages, including the actual genus *Lobaria* in a narrower sense. *Lobaria* s.str. largely consists of three species groups: the circumboreal green-algal *L. linita*, the cyanobacterial *L. retigera* group, and the green-algal *L. pulmonaria* group.

The distribution of the genus *Lobaria* ranges from tropical to temperate regions (Miadlikowska & Lutyoni 2004, Lücking et al. 2009, Cornejo & Scheidegger 2010, Miadlikowska et al. 2014). The type species, *L. pulmonaria*, has an extremely wide distribution in the Northern Hemisphere (Hilmo et al. 2012), covering Asia (Yoshimura 1971, Cornejo & Scheidegger 2015, Devkota et al. 2017), Europe (Nadyeina et al. 2014) and North America (Jordan 1973). In the Southern Hemisphere, this species occurs in East African mountain systems and in South Africa (Schiefelbein & Thell 2018). Endemic species of green-algal *Lobaria* are known from the west coast of North America (*L. oregana*), the Macaronesian Islands (*L. immixta*, *L. macaronesica*) and East Asia.

The following species of green-algal *Lobaria* have been reported in East Asia: *Lobaria gyrophorica*, *L. kazawaensis*, *L. orientalis*, *L. sachalinensis*, *L. spatulata* and *L. tuberculata* (Yoshimura 1971, Moncada et al. 2013, Cornejo & Scheidegger 2015, Cornejo et al. 2018) across a relatively wide distribution area; *L. chinensis* and *L. isidiophora* in Taiwan; *L. yoshimurae* in Taiwan and Japan (Kurokawa 1978, Lai 2000); and *L. meridionalis* in the Philippines (Yoshimura 1971, Cornejo & Scheidegger 2015). Additionally, *L. pindarensis* occurs in the western and central Himalayas (Devkota et al. 2017), and *L. yunnanensis* and *L. yulongensis* have been reported in the Hengduan Mountains (Yoshimura 1971, Chen 1995, Cornejo et al. 2018). *Lobaria yulongensis* has been reported in Yunlongshan in Yunnan (Chen 1995). This species is characterised by numerous lobules at the margins, especially at the ridge of the upper surface (Chen 1995). *Lobaria yoshimurae* is known to occur in Japan (Kurokawa 1978) and Taiwan (Lai 2000). This species can be identified by the absence of isidia and lobules, and the presence of gyrophoric acid.

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The mountain ecosystems of the Himalayas and the Hengduan Mountains regions bordering the Qinghai-Tibetan Plateau (QTP) harbour some of the world's richest floras, yet unlike other biodiversity hotspots they are temperate rather than tropical or Mediterranean in climate (Xing & Ree 2017). The fossil record and reconstructed biogeographic histories show that the core QTP region has been an evolutionary centre (Deng et al. 2011, Tseng et al. 2013, Wang et al. 2014, Robert et al. 2020), even termed a 'cradle of evolution' of cold-adapted mammals and forest-dwelling ground beetles (Schmidt et al. 2012). Compared with other mountain ranges bordering the QTP, the Hengduan Mountains are younger, having uplifted over the last 8 million years (Myr), and they had a very high species diversification rate during this period (Xing & Ree 2017). During the same period, the rate of *in situ* diversification remained relatively high in the Himalayas (Xing & Ree 2017). The tectonic uplift of the two mountain systems created environmental conditions, such as new habitats and barriers to population migration and dispersal, that increased the rate at which resident species diverge and evolve to form new ones, thus acting as cradles for biodiversity (Rangel et al. 2018). Moreover, many studies on plants (Xing & Ree 2017, Yu et al. 2020) and birds (Martin et al. 2015) have demonstrated that species dispersal between the Himalayas and the Hengduan Mountains increased significantly over the last 2 Myr. Despite an increasing interest of biogeographers, the biota in these two regions remains poorly understood. The diversity of lichenized species in particular is very high in both regions, and discoveries of new species have been published recently (Aptroot & Feijen 2002, Cornejo & Scheidegger 2015, Devkota et al. 2017, Liu et al. 2017, Wang et al. 2017, Cornejo et al. 2018, Yang et al. 2019).

Here, we studied 476 green-algal *Lobaria* specimens collected in the Himalayan regions, Hengduan Mountains, QTP, and other regions of East Asia (Taiwan, Primorsky Krai, Kurile Islands and Sakhalin Island) where the presence of green-algal *Lobaria* has been reported. In this phylogenetic study, we included all reported species in East Asia, except *L. yulongensis* and *L. yoshimurae* (Kurokawa 1978), which we only studied morphologically. Our primary aim was to investigate the phylogenetic relationships between specimens of green-algal *Lobaria* collected in the Himalayas and the Hengduan Mountains, and previously described taxa from East Asia. We also investigated whether there is evidence that geographical and climatic events in the Himalayas and Hengduan Mountains influenced the speciation of green-algal *Lobaria*. We conducted morphological studies and phylogenetic analyses of DNA sequences. To obtain divergence time estimates, we used a molecular-clock-calibrated phylogeny.

## MATERIALS AND METHODS

### Sampling specimens

In total, 476 thalli of green-algal *Lobaria* were collected in 2016–2019 by the authors or studied in the Lichen Herbarium of the Kunming Institute of Botany (KUN-L). The identification of *Lobaria* species based on morphology alone is difficult, especially when thalli of green-algal *Lobaria* are not well developed, and molecular information from a multilocus phylogenetic approach is required to unravel the genus' species diversity. From all specimens of green-algal *Lobaria*, we therefore excluded chemically identical specimens sharing the same ITS haplotype. This resulted in a set of 72 specimens for phylogenetic analyses and time-estimates that covered all of the originally found ITS haplotypes of green-algae-containing specimens: 12 *Lobaria* specimens were examined from Bhutan, three from Nepal, eight from Yunnan, 25 from Xizang, three from Sakhalin Island, three from Kurile Islands, six from Primorsky Krai, one from Qinghai, seven from Taiwan and four selected outgroup species (from Madeira, Switzerland and Russia). Twenty-two specimens from Nepal, Primorsky Krai, Taiwan, the Kurile Islands and Sakhalin Island, had already been included in a previous study (Cornejo et al. 2018).

All dried specimens and DNA extracts were stored frozen (-20 °C) at the Swiss Federal Institute for Forest, Snow and Landscape Research WSL. We initially identified all specimens using morphological characteristics. From each collection site, we used at least three specimens that fulfilled all diagnostic traits of a specific species, following Räsänen (1952) and Yoshimura (1969, 1971). We verified the chemical diagnostic features with thin-layer chromatography, following the methods used by Cornejo et al. (2009). We examined the specimens using standard microscopic techniques and hand-sectioned them under a Carl Zeiss Axio Lab.A1 dissecting microscope (Carl Zeiss Microscopy, LLC; White Plains, NY, USA) in water. Anatomical descriptions are based on observations using a Wild Heerbrugg Plant 1× dissecting microscope. We took macrophotographs with a Nikon D-300s camera (Tokyo, Japan) equipped with a Zuiko Macro 38 mm F/2.8 macrolens (Olympus, Bethlehem, PA, USA) on a 10.5 cm extension tube. We used the program Zerene Stacker ([zerenesystems.com/cms/home](http://zerenesystems.com/cms/home)) for focus stacking. The descriptions of species are arranged in alphabetical order of the epithets. Specimens used in this study, along with the corresponding voucher information, are shown in Table 1.

**Table 1** Localities, fungaria and collectors of specimens used for this phylogenetic study.

| Locality                | Specimen Nr.   | Fungarium   | Collector(s)                              |
|-------------------------|--|-------------|---|
| Paro District, Bhutan   | 40459, 40473, 40476, 40486, 40494, 40506, 40538, 40544, 40552, 40623, 40629, 40635   | Scheidegger | C. Scheidegger                            |
| Sakhalin Island, Russia | RS/104, RS/113a, RS/121  | Scheidegger | C. Scheidegger, S. Chabanenko, Taran      |
| Kurile Islands, Russia  | RIT/04c, RIT/04d, RIT/08a  | Scheidegger | S. Chabanenko                             |
| Nepal                   | NE23/02a, NE40/01b, NE64/06a   | Scheidegger | C. Scheidegger, S. Devkota                |
| Primorsky Krai, Russia  | 010/1, 004/15, 373/1, 374/1, 377/1, 377/2  | Scheidegger | S. Chabanenko                             |
| Qinghai, China          | 20-67877   | KUN-L       | L.S. Wang et al.                          |
| Taiwan, China           | CT3/02b, CT3/02e, CT16/01f, TW2/01_8, TW2/26_2, TW3/01_10, TW3/02_6  | KUN-L       | J.-T. Yang, F. Dal Grande, C. Scheidegger |
| Xizang, China           | 16-52050, 16-53208, 16-53682, 18-59984, 18-62299, 18-62315, 19-63350, 19-64420, 19-64424, 19-64427, 19-64430, 19-64441, 19-64448, 19-64564, 19-65719, XY19-501, XY19-505, XY19-525, XY19-526, XY19-1007, XY19-1049, XY19-1105, XY19-1114, XY19-1119, XY19-1128 | KUN-L       | L.S. Wang, M.X. Yang et al.               |
| Yunnan, China           | 40008, 40081, 40112, 40332, 40362, 40396, 40411, CT10/02e  | Scheidegger | C. Scheidegger, L.S. Wang, M.X. Yang      |

**Table 2** Overview of 72 green-algal *Lobaria* specimens arranged according to the retrieved clades in the species-tree, with the respective voucher information from GenBank. Sequences that were newly obtained in this study are indicated in **boldface**.

| Taxon                    | Specimen ID           | Lineage | Chemical feature                                      | Diaspores/<br>Apothecia   | GenBank number       |                      |                      | Note |
|--------------------------|-----------------------|---------|---|---|----------------------|----------------------|----------------------|------|
|                          |                       |         |   |   | ITS                  | <i>EF-1a</i>         | <i>RPB2</i>          |      |
| <i>L. pindarensis</i>    | 18-59984              | 1       | Gyrophoric, norstictic, stictic, constictic acid      | Cylindrical isidia  | <b>MW269755</b>      | <b>MW393957</b>      | <b>MW393992</b>      |      |
|                          | 19-64424              |         |   |   | <b>MW343613</b>      | <b>MW393958</b>      | <b>MW393994</b>      |      |
|                          | 19-64430              |         |   |   | <b>MW343615</b>      | <b>MW393960</b>      | <b>MW393996</b>      |      |
|                          | 19-64441              |         |   |   | <b>MW343614</b>      | <b>MW393961</b>      | <b>MW393997</b>      |      |
|                          | 40552                 |         |   |   | <b>MW343616</b>      | <b>MW393955</b>      | <b>MW393990</b>      |      |
|                          | NE23/02a              |         |   |   | KC494086             | KC602010             | KC602556             |      |
|                          | NE40/01b              |         |   |   | MF151243             | MF151196             | MF151701             |      |
|                          | NE64/06a              |         |   |   | MF151246             | MF151198             | MF151704             |      |
| <i>L. devkotaie</i>      | 18-62315              | 2       | Gyrophoric, stictic, constictic, hypoconstictic acid  | No isidia   | <b>MW343617</b>      | <b>MW393966</b>      | <b>MW394002</b>      | Type |
|                          | 19-64564              |         |   |   | <b>MZ724567</b>      | <b>MW393963</b>      | <b>MW393999</b>      |      |
|                          | XY19-1105             |         |   |   | <b>MZ724618</b>      | <b>MW393969</b>      | <b>MW394005</b>      |      |
| <i>L. spathulata</i>     | RIT/04c               | 3       | Gyrophoric acid                                       | Marginal lobules  | MF151252             | MF151202             | MF151723             |      |
|                          | RIT/04d               |         |   |   | MF151253             | MF151205             | MF151719             |      |
|                          | RIT/08a               |         |   |   | MF151254             | MF151206             | MF151709             |      |
| <i>L. rhizinata</i>      | 40332                 | 4       | Gyrophoric, norstictic, stictic, constictic acid      | No isidia or apothecia  | <b>MW343620</b>      | <b>MW393943</b>      | <b>MW393978</b>      |      |
|                          | 40362                 |         |   |   | <b>MW343621</b>      | <b>MW393944</b>      | <b>MW393979</b>      |      |
|                          | 40396                 |         |   |   | <b>MW343622</b>      | <b>MW393945</b>      | <b>MW393980</b>      |      |
|                          | 40411                 |         |   |   | <b>MW343623</b>      | <b>MW393946</b>      | <b>MW393981</b>      |      |
| <i>L. granulosa</i>      | 40629                 | 5       | Gyrophoric, norstictic, stictic, constictic acid      | Knob-like and multiple isidia in a group                                  | <b>MW343625</b>      | –                    | –                    |      |
|                          | 40635                 |         |   |   | <b>MW343626</b>      | –                    | –                    |      |
|                          | XY19-1128             |         |   |   | <b>MW343624</b>      | <b>MW393972</b>      | <b>MW394008</b>      |      |
| <i>L. bhutanica</i>      | 40476                 | 6       | Norstictic, stictic, constictic acid                  | No isidia   | <b>MW343627</b>      | <b>MW393949</b>      | <b>MW393984</b>      |      |
|                          | 40494                 |         |   |   | <b>MW343629</b>      | <b>MW393951</b>      | <b>MW393986</b>      |      |
|                          | 40506                 |         |   |   | <b>MW343630</b>      | <b>MW393952</b>      | <b>MW393987</b>      |      |
|                          | 40544                 |         |   |   | <b>MW343628</b>      | <b>MW393954</b>      | <b>MW393989</b>      |      |
| <i>L. gyrophorica</i>    | CT16/01f              | 7       | Gyrophoric acid                                       | No isidia   | MF151248             | MF151178             | MF151707             |      |
|                          | TW2/01_8              |         |   |   | MF151247             | MF151180             | MF151724             |      |
|                          | TW2/26_2              |         |   |   | MF151249             | MF151181             | MF151733             |      |
| <i>L. perelegans</i>     | 19-65719              | 8       | Gyrophoric, stictic acid                              | Knob-like, blastidiate and multiple isidia in a group                     | <b>MW343640</b>      | <b>MW393964</b>      | <b>MW394000</b>      | Type |
|                          | 40623                 |         |   |   | <b>MW343641</b>      | –                    | –                    |      |
| <i>L. multipartita</i>   | 19-63350              | 9       | Gyrophoric, stictic acid                              | Cylindrical isidia, marginal lobules                                      | <b>MW343637</b>      | <b>MW393956</b>      | <b>MW393991</b>      |      |
|                          | 40459                 |         |   |   | <b>MW343638</b>      | <b>MW393947</b>      | <b>MW393982</b>      |      |
|                          | XY19-501              |         |   |   | <b>MW343639</b>      | –                    | <b>MW394009</b>      |      |
|                          | XY19-525              |         |   |   | <b>MW343636</b>      | <b>MW393974</b>      | <b>MW394011</b>      |      |
| <i>L. tibetana</i>       | 18-62299              | 10      | Gyrophoric, stictic acid                              | Cylindrical and knob-like isidia  | <b>MW343631</b>      | <b>MW393965</b>      | <b>MW394001</b>      |      |
|                          | 40473                 |         |   |   | <b>MW343633</b>      | <b>MW393948</b>      | <b>MW393983</b>      |      |
|                          | 40538                 |         |   |   | <b>MW343632</b>      | <b>MW393953</b>      | <b>MW393988</b>      |      |
|                          | XY19-1007             |         |   |   | <b>MW343634</b>      | <b>MW393967</b>      | <b>MW394003</b>      |      |
| <i>L. orientalis</i>     | 004/15                | 11      | Gyrophoric, norstictic, stictic acid, constictic acid | No isidia   | MF151223             | MF151172             | MF151695             |      |
|                          | 010/1                 |         |   |   | MF151230             | MF151184             | MF151698             |      |
|                          | 40486                 |         |   |   | <b>MW343635</b>      | <b>MW393950</b>      | <b>MW393985</b>      |      |
| <i>L. isidiophora</i>    | TW3/01_10<br>TW3/02_6 | 12      | Gyrophoric, norstictic, stictic acid                  | Cylindrical isidia  | KC494095<br>KC494091 | KC602602<br>KR476673 | KC602542<br>KR476701 |      |
| <i>L. chinensis</i>      | CT3/02b               | 13      | Norstictic, stictic, constictic acid                  | No isidia   | MF151238             | MF151190             | MF151717             |      |
|                          | CT3/02e               |         |   |   | MF151237             | MF151191             | MF151732             |      |
| <i>L. costata</i>        | 40081                 | 14      | Gyrophoric, stictic acid                              | No isidia   | <b>MW343642</b>      | <b>MW393941</b>      | <b>MW393976</b>      | Type |
|                          | 40112                 |         |   |   | <b>MW343643</b>      | <b>MW393942</b>      | <b>MW393977</b>      |      |
|                          | 16-52050              |         |   |   | <b>MW343645</b>      | –                    | –                    |      |
|                          | 16-53682              |         |   |   | <b>MW343644</b>      | –                    | –                    |      |
| <i>L. ligulata</i>       | XY19-1114             | 15      | Stictic, constictic, hypoconstictic acid              | No isidia   | <b>MW343646</b>      | <b>MW393970</b>      | <b>MW394006</b>      | Type |
|                          | XY19-1119             |         |   |   | <b>MW343647</b>      | <b>MW393971</b>      | <b>MW394007</b>      |      |
| <i>L. verruculosa</i>    | 373/1                 | 16      | Stictic, constictic acid (norstictic acid)            | Cylindrical isidia  | MF151255             | MF151212             | MF151737             |      |
|                          | 374/1                 |         |   |   | MF151256             | MF151209             | MF151738             |      |
|                          | XY19-505              |         |   |   | <b>MW343648</b>      | <b>MW393973</b>      | <b>MW394010</b>      |      |
| <i>L. meridionalis</i>   | 377/1                 | 17      | Stictic, norstictic acid                              | Cylindrical isidia  | MF151257             | MF151207             | MF151734             |      |
|                          | 377/2                 |         |   |   | KC522960             | KC602631             | KC602588             |      |
| <i>L. kazawaensis</i>    | RS/104                | 18      | Retigeranic acid A+B                                  | Cylindrical isidia, rarely lobulate                                       | KC522950             | KC602604             | KC602545             |      |
| <i>L. sachalinensis</i>  | 20-67877              | 19      | Retigeranic acid A+B                                  | Cylindrical isidia  | <b>MW534714</b>      | –                    | –                    |      |
|                          | RS/113a               |         |   |   | KC522954             | KC602616             | KC602565             |      |
|                          | RS/121                |         |   |   | KC522955             | KC602617             | KC602566             |      |
| <i>L. wanglisongiana</i> | 16-53208              | 20      | Pannaric acid   | Cylindrical, blastidiate and multiple isidia in a group, marginal lobules | <b>MW343649</b>      | –                    | –                    | Type |
|                          | 19-64427              |         |   |   | <b>MW343650</b>      | <b>MW393959</b>      | <b>MW393995</b>      |      |
|                          | 19-64448              |         |   |   | <b>MW343651</b>      | <b>MW393962</b>      | <b>MW393998</b>      |      |
|                          | XY19-1049             |         |   |   | <b>MW343652</b>      | <b>MW393968</b>      | <b>MW394004</b>      |      |

Table 2 (cont.)

| Taxon                     | Specimen ID | Lineage | Chemical feature                         | Diaspores/<br>Apothecia        | GenBank number  |                 |                 | Note |
|---------------------------|-------------|---------|--|--------------------------------|-----------------|-----------------|-----------------|------|
|                           |             |         |  |                                | ITS             | <i>EF-1a</i>    | <i>RPB2</i>     |      |
| <i>L. yunnanensis</i>     | 40008       | 21      | No substances detected                   | No isidia                      | <b>MW343653</b> | <b>MW393940</b> | <b>MW393975</b> |      |
|                           | 19-64420    |         |  |                                | <b>MW343654</b> | –               | <b>MW393993</b> |      |
|                           | CT10/02a    |         |  |                                | KC522959        | KC602629        | KC602586        |      |
|                           | XY19-526    |         |  |                                | <b>MW343655</b> | –               | –               |      |
| <b>Outgroup specimens</b> |             |         |  |                                |                 |                 |                 |      |
| <i>L. immixta</i>         | X3          |         | Gyrophoric, norstictic, stictic acid     | Squamule-like flattened isidia | GU072764        | GU072881        | GU072839        |      |
| <i>L. macaronesica</i>    | PM12/13c    |         | Norstictic, stictic acid                 | Cylindrical isidia             | GU072746        | GU072884        | GU072863        |      |
| <i>L. pulmonaria</i>      | B13         |         | Norstictic, stictic acid                 | Soredia and isidioid soredia   | MF151265        | MF151214        | MF151740        |      |
| <i>L. tuberculata</i>     | RS/95       |         | 4-0-methylgyrophoric and gyrophoric acid | granular isidia                | KC522958        | KC602628        | KC602582        |      |

### DNA isolation, PCR amplification and sequencing

Genomic DNA was extracted from freshly collected and frozen herbarium specimens. About 15 mg of visually uncontaminated lichen thallus was sampled from each specimen for molecular analyses. Frozen lichen samples were lyophilized and disrupted with a stainless steel bead in a Retsch MM2000 mill (Düsseldorf, Germany) for 2 min at 30 Hz.

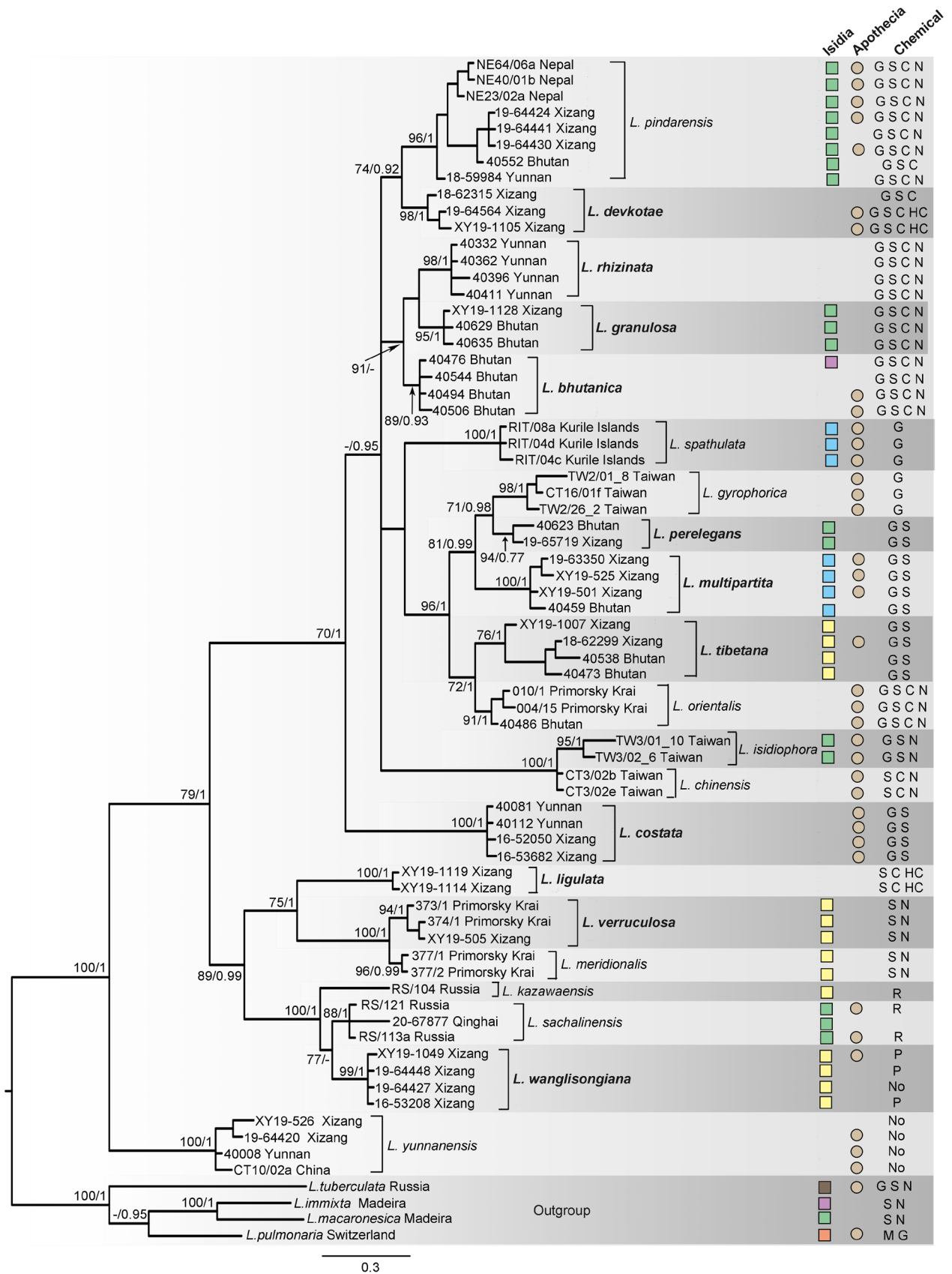
Genomic DNA was extracted using the Qiagen DNAeasy Plant Kit (QIAGEN, Hilden, Germany), following the manufacturer's Plant Tissue Mini protocol. Sequences were generated from three independent nuclear markers: the nuclear ribosomal internal transcribed spacer (ITS) region, a partial sequence of the putatively single-copy translation elongation factor-1 alpha gene (*EF-1α*), and an intron-containing portion of the putatively single-copy RNA polymerase II second largest subunit gene (*RPB2*). The fungus-specific primer ITS1F (Gardes & Bruns 1993) and the universal primer ITS4 (White et al. 1990) were used to amplify the ITS, as described in Cornejo et al. (2009). To make the PCR more specific and to amplify shorter fragments, the partial *RPB2* gene was amplified and cycle sequenced with the modified primer sets Lp-RPB-R (5'-CCCATGGCTTGCT-TACCCAT-3') and Lp-RPB-F (5'-CAAACCGCGTCAACTG-CATAAT-3') designed by Cornejo & Scheidegger (2015). The partial *EF-1α* gene (Johannesson et al. 2000) was amplified and cycle sequenced with the modified primer sets Lp-EF-1α-F (5'-RGACAAGRCTCACATCAACGTGGT-3') and Lp-EF-1α-R (5'-CCAGTGATCATGTTCTTGATGAAGT-3') (Cornejo & Scheidegger 2015).

All amplifications were performed with 1 µL DNA extract in a total of 15 µL containing the JumpStart™ REDTaq® Ready Mix™ PCR Reaction Mix (Sigma-Aldrich, St. Louis, MO, USA) and 100 nM of each primer. The cycling conditions for the ITS primer set designed in our lab were: (a) 2 min at 94 °C, (b) 10 cycles of (30 s at 94 °C, 45 s at 56 °C, 45 s at 72 °C), (c) 25 cycles of (30 s at 94 °C, 45 s at 52 °C, 45 s at 72 °C), (d) 10 min at 72 °C final extension. The cycling conditions for the *RPB2* primer set designed in our lab were: (a) 2 min at 94 °C, (b) 30 cycles of (60 s at 94 °C, 60 s at 49 °C, 60 s at 72 °C), (c) 10 min at 72 °C final extension. The cycling conditions for amplification of the *EF-1α* locus with primer designed in our lab were: (a) 2 min at 94 °C, (b) 35 cycles of (30 s at 94 °C, 30 s at 53 °C, 30 s at 72 °C), (c) 10 min at 72 °C final extension. The PCR products were visualised on 1.5 % agarose gel. The PCR products were then sent to Microsynth AG (Balgach, Switzerland) for sequencing with the same primers as the original PCR amplifications. Specimens used in this study, along with voucher information from GenBank accession numbers, chemical features and type of diaspores or apothecia are listed in Table 2.

### Phylogenetic analyses

Phylogenetic relationships were reconstructed using sequences of the three loci: ITS, *RPB2* and *EF-1α*, which were compiled for different analytical purposes. All sequences were assembled and edited using Geneious v. 7.1.9 (<https://www.geneious.com>). All newly produced sequences were checked using the BLASTN suite of the National Center for Biotechnology Information (NCBI) website (<http://www.ncbi.nlm.nih.gov/BLAST/>) to verify their close relatives in order to avoid possible contaminants. Matrices were aligned with MAFFT, using the web service (<http://mafft.cbrc.jp/alignment/server/index.html>). Sequences of each locus were aligned and analysed separately. Phylogenetic and molecular evolutionary analyses were conducted using MEGA v. 6 (Tamura et al. 2013). The ambiguously aligned regions were arranged manually for the phylogenetic analyses. The resulting alignment containing all three loci (ITS, *EF-1α* and *RPB2*) can be accessed at TreeBASE (study accession URL: <http://purl.org/phylo/treebase/phyloids/study/TB2:S28510>).

Single-gene analyses were conducted to test for potential incongruencies among the three-gene fragments using maximum likelihood (ML) analyses and Bayesian inference (BI). The three-gene fragments were combined using Geneious v. 7.1.9 for phylogenetic analysis, on the premise that no well-supported (BS > 70 %, Nuhn et al. 2013) conflict was detected. Maximum likelihood (ML) and Bayesian inference (BI) analyses were performed using RaxML v. 7.2.6 (Stamatakis 2006) and MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003), respectively. Sequences of ITS, *EF-1α* and *RPB2* were manually divided into three, seven and three data blocks (Appendix 1), respectively. Each exon was separated into three blocks based on the first, second and third codon. The intron-exon structure of *EF-1α* was adopted from *Lobaria* sequences deposited in GenBank by Cornejo et al. (2018). The positions of rDNA and ITS1/2 were inferred by comparison with sequences from eukaryote seed alignments downloaded from Rfam (<https://rfam.xfam.org>) and mapped against the alignment consensus using minimap2 (Li 2018). The best partition schemes and evolutionary models were selected using PartitionFinder v. 2.1.1 (Lanfear et al. 2016). For the best partition schemes and evolutionary models, see Appendix 1. Under ML optimization, the GTR+G model was selected for RAXML searches, and the bootstrap values were calculated with 1000 replicates. For BI analyses, four Markov Chain Monte Carlo (MCMC) chains were run simultaneously for 20 M generations with trees sampled every 100 generations. We considered the sampling of the posterior distribution to be adequate when the average standard deviation of split frequencies was < 0.01. Chain convergence was determined by checking the effective sampling size (ESS > 200) in Tracer v. 1.6 (Rambaut et al. 2014). By omitting the first 25 % of trees



**Fig. 1** Phylogram generated from a maximum likelihood (RaxML) analysis based on the combined sequence data of ITS, *RPB2* and *EF-1 $\alpha$*  of *Lobaria*. ML bootstrap support values/Bayesian posterior probability  $\geq 70\%$  / 0.95 are indicated. Names in **bolditalic** are new species. The abbreviations indicate species characteristics: for the isidia form: green square = cylindrical or knob-like and clustered in groups, purple square = marginal laminal lobules, yellow square = coralloid and clustered in groups, blue square = cylindrical and marginal spatulate, orange square = isidioid soredia, brown square = granular isidia; for the apothecia: light brown circle = apothecia present on specimens; and for chemical features: G = gyrophoric acid, S = stictic acid, C = constictic acid, N = norstictic acid, HC = hypoconstictic acid, R = retigeric acid, P = pannaric acid, M = 4-0-methylgyrophoric and No = no chemical feature.

as burn-ins using the 'sump' and 'sumt' commands, a majority rule consensus tree was generated. Clades were judged using both ML Bootstrap (MLB  $\geq 70\%$ ) and Bayesian posterior probabilities (BPP  $\geq 0.95$ ). The tree files were visualized with FigTree v. 1.4.3 (Rambaut 2012) and edited using Adobe Photoshop CS6 (Adobe Systems Incorporated, San Jose, CA, USA).

The genealogical concordance phylogenetic species recognition (GCPSR) method of Taylor et al. (2000) was used to delimit phylogenetic species. Following Dettman et al. (2003), two GCPSR-based criteria must be fulfilled when a phylogenetic species is defined:

1. genealogical concordance: the clade is well supported in the majority (2/3) of the single-locus genealogies;
2. genealogical non-discordance: the clade is well supported by at least one single-locus genealogy and is not contradicted in any other single-locus genealogy at the same level of support.

Such clades were assessed using both ML bootstrap (MLB  $\geq 70\%$ ) and Bayesian posterior probabilities (BPP  $\geq 0.95$ ). Each phylogenetic species had to be relatively distinct and well differentiated from the other species, and all individuals had to be placed within the phylogenetic species.

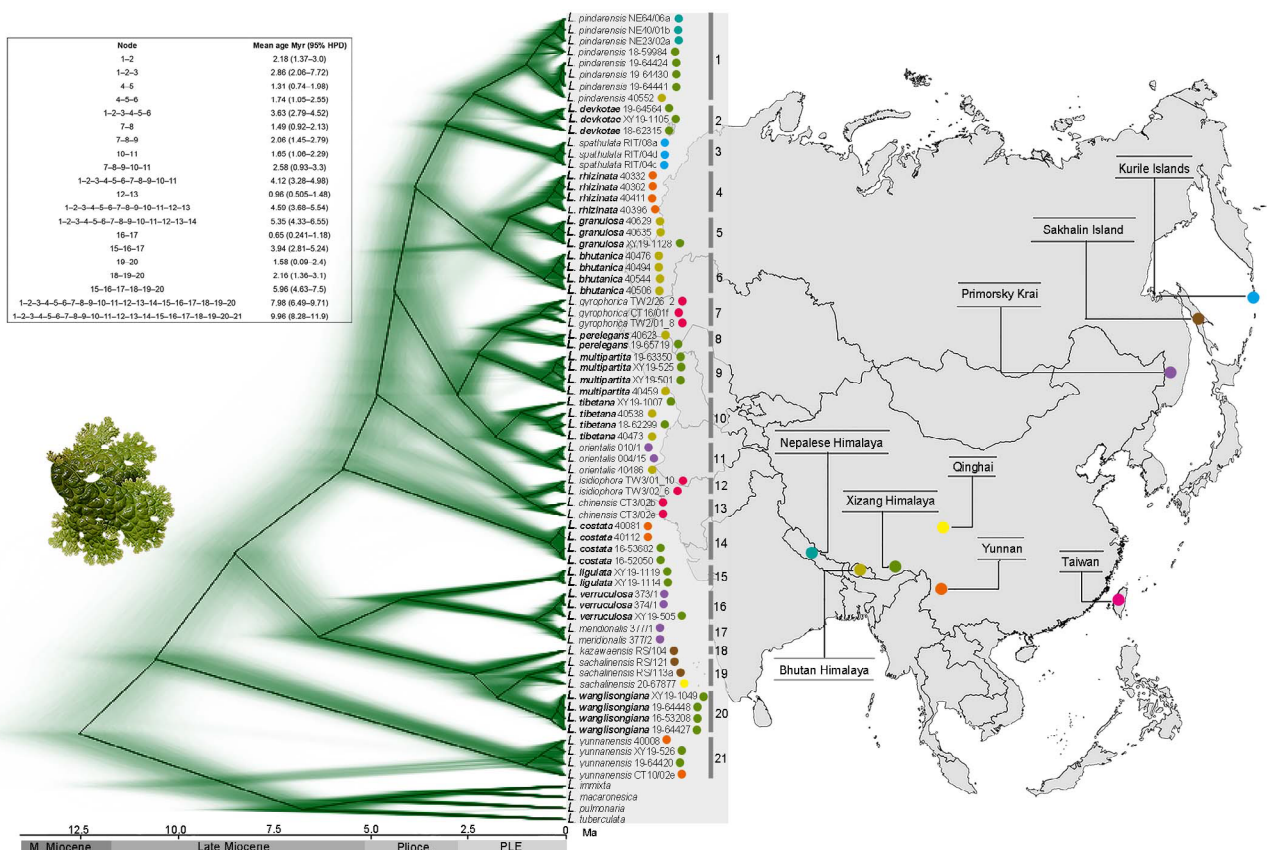
### Time-calibrated species-tree

We used the Bayesian method, implemented in the programs BEAUti and BEAST (both v. 1.8.4; Drummond & Suchard 2010; Drummond et al. 2012), to estimate a species-tree. We used the same dataset to estimate divergence time. We selected the best partition schemes and evolutionary models using PartitionFinder v. 2.2.1 (Lanfear et al. 2016; Appendix 1). We performed topologies with BEAST v. 1.8.4, using the uncor-

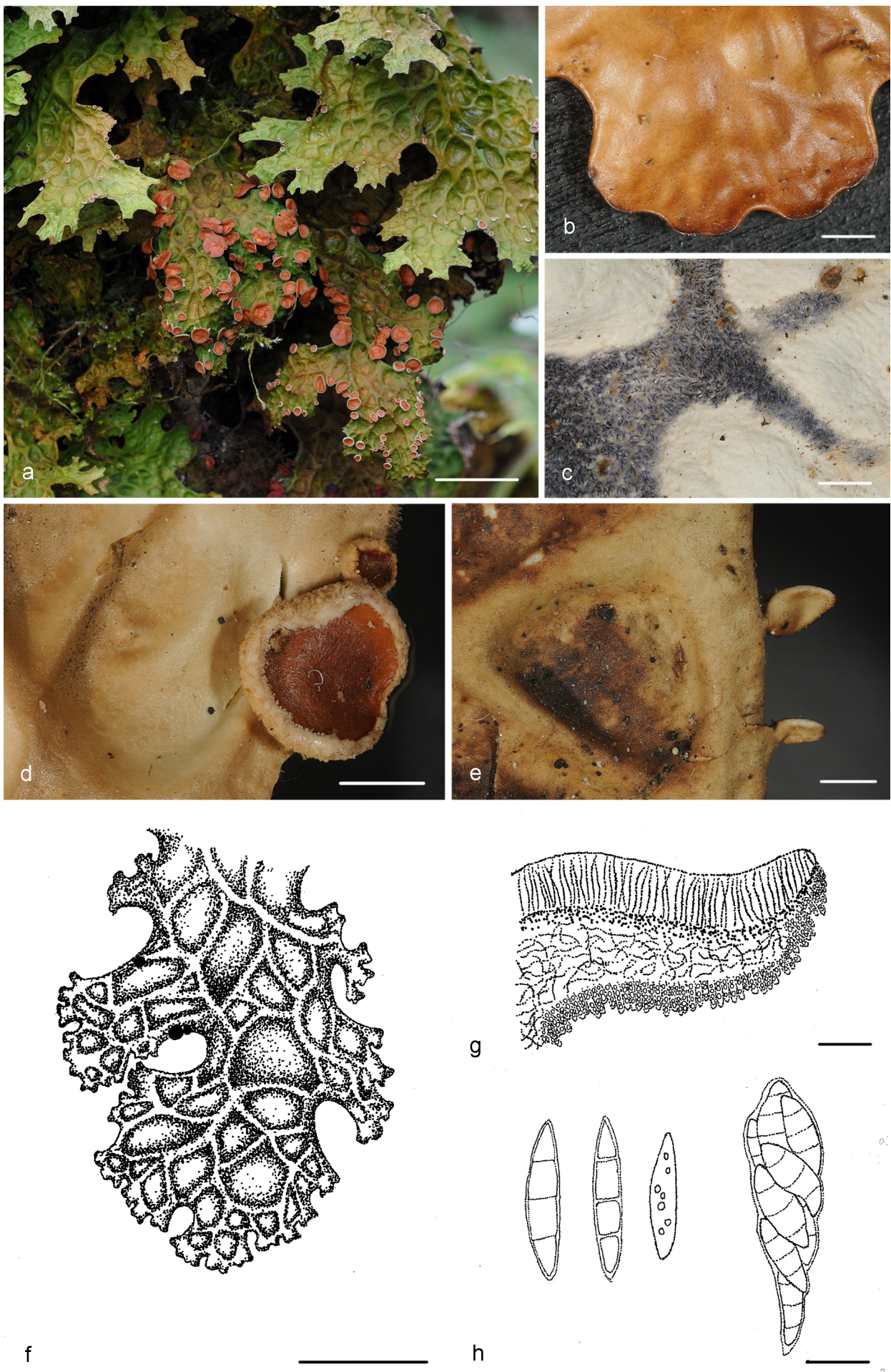
related lognormal-relaxed clock model, which draws the rate for each branch from an underlying lognormal distribution. The approach used partitioned data in BEAUti v. 1.8.4, analysed with unlinked substitution models across the loci. We selected a Yule speciation model for the species-tree prior, and we set the population size model to piecewise linear and constant root. We linked clock and tree parameters across partitions and used default values for the remaining priors. We ran three independent MCMC analyses of 25 million generations with a burn-in of 10% for each run. We assessed the effective sample sizes (ESS) of parameters of interest with Tracer (v. 1.6; Rambaut et al. 2014) to ensure that sample sizes were all greater than 200. We computed the maximum clade credibility tree, including posterior probabilities of branches, with TreeAnnotator (v. 1.8.4; included in the BEAST 1.8.4 package) from the sampled trees after exclusion of the burn-in. Additionally, we visualised sampled trees in DensiTree (v. 2.2.5; Bouckaert 2010) after exclusion of the burn-in, which provides a qualitative approach to represent all trees present in a species-tree analysis.

## RESULTS

Individual gene-trees obtained from Bayesian analyses with branch support by ML and BI analyses are shown in Appendix 2–4, where ML bootstrap support values/Bayesian posterior probabilities greater than 70% / 0.95 are indicated. A three-locus combined ML analysis is presented in Fig. 1. A total of 19 well-supported clades were identified (Table 2, Fig. 1), except for *Lobaria chinensis* and *L. kazawaensis*, the phylogenies were consistent with the individual ITS, *RPB2* and *EF-1 $\alpha$*  gene trees. Although *L. chinensis* and *L. kazawaensis* were not well-supported on the phylogenetic tree, they can be identified by



**Fig. 2** Time-calibrated species-trees based on the analysis of three loci under the hierarchical Bayesian model (BEAST) and represented with DensiTree graphical software. Branches are indicated by many lines and appear as a dense green area. Names in **boldface** are new species. Four outgroup-species of the genus *Lobaria* root the tree. The age estimates for the most recent common ancestor are listed in the table (top left). In the map to the right of the species-tree, different symbol colours indicate the collection sites of the species in the species-tree. Map created using the OmniGraffle software (v. 6.0.5; The Omni Group, Seattle, Washington, USA).



**Fig. 3** Morphological features of *Lobaria bhutanica* sp. nov. (a, d–h: Scheideg-40506; b–c: Scheideg-40476). a. Habit; b. lobe; c. ventral surface; d. apothecia; e. laminal lobules; f. thallus; g. hymenium; h. ascospores. — Scale bars: a, f = 1 cm; b–e = 1 mm; g = 50 µm; h = 10 µm.

morphological characteristics (Yoshimura 1971, Cornejo et al. 2018). From these clades, in total, 10 taxa were identified as previously described species reported from the Himalayas, Hengduan Mountains and other regions in East Asia: *L. chinensis*, *L. gyrophorica*, *L. isidiophora*, *L. kazawaensis*, *L. meridionalis*, *L. orientalis*, *L. pindarensis*, *L. sachalinensis*, *L. spatulata* and *L. yunnanensis*. The other 11 clades formed distinct branches not corresponding to any of the previously described species: *L. bhutanica*, *L. costata*, *L. devkotae*, *L. granulosa*, *L. ligulata*, *L. multipartita*, *L. perelegans*, *L. rhizinata*, *L. tibetana*, *L. verruculosa* and *L. wanglisongiana*. Cornejo et al. (2018) showed that specimens from Primorsky Krai morphologically and chemically related to *L. meridionalis* split into two well-supported monophyletic clades, and they mentioned these specimens as putatively undescribed species, *Lobaria* sp. 1a and *Lobaria* sp. 1b. Here, we report additional specimens identical with *Lobaria* sp. 1a from the Himalayas, which is described here as *L. verruculosa*. The two species *L. meridionalis* and *L. verruculosa* were well supported (96/0.99 and 94/1, respectively) in the tree resulting from the combined dataset (Fig. 1).

Overall, four species (*L. costata*, *L. pindarensis*, *L. rhizinata* and *L. yunnanensis*) of green-algal *Lobaria* were identified in the Hengduan Mountains, while two were new records for the area. Fourteen species (*L. bhutanica*, *L. costata*, *L. devkotae*, *L. granulosa*, *L. ligulata*, *L. meridionalis*, *L. multipartita*, *L. orientalis*, *L. perelegans*, *L. pindarensis*, *L. tibetana*, *L. verruculosa*, *L. wanglisongiana* and *L. yunnanensis*) were found in the Himalayas, 10 of which were new records for the region.

The time-calibrated species-tree is shown in Fig. 2. The most recent common ancestor of the green-algal *Lobaria* was estimated to have a mean age of c. 12.5 Myr, corresponding to the mid-Miocene. *Lobaria yunnanensis* was estimated to have diverged first (about 10 Myr ago) in the late Miocene. The other species are younger and speciated during the Pliocene and Pleistocene.

*Lobaria meridionalis*, *L. orientalis*, *L. sachalinensis*, *L. spatulata* and *L. verruculosa* were confirmed in the Kurile Islands, Sakhalin Island and Primorsky Krai of East Asia. *Lobaria chinensis*, *L. gyrophorica* and *L. isidiophora* were confirmed in Taiwan; *L. costata*, *L. pindarensis*, *L. rhizinata* and *L. yunnanensis* were identified in the Hengduan Mountains; and two species (*L. costata*, *L. rhizinata*) were newly found. Meanwhile, *L. bhutanica*, *L. costata*, *L. devkotae*, *L. granulosa*, *L. ligulata*, *L. multipartita*, *L. orientalis*, *L. perelegans*, *L. pindarensis*, *L. tibetana*, *L. verruculosa*, *L. wanglisongiana* and *L. yunnanensis* were identified in the Himalayas, while 10 species (*L. bhutanica*, *L. costata*, *L. devkotae*, *L. granulosa*, *L. ligulata*, *L. multipartita*, *L. perelegans*, *L. tibetana*, *L. verruculosa* and *L. wanglisongiana*) were newly found. Our findings highlight that the tectonic uplift of the Himalayas and the Hengduan Mountains systems created more favourable environmental conditions, such as new habitats and barriers to population migration and dispersal, that have increased the rate at which resident species diverge and evolve to form new ones. Compared with other green-algal *Lobaria* localities in East Asia, the Himalayas and Hengduan Mountains can therefore be considered cradles for green-algal *Lobaria* biodiversity.

## Taxonomy

***Lobaria bhutanica*** M.X. Yang & Scheid., *sp. nov.* — MycoBank MB 838325; Fig. 3

*Etymology.* 'bhutanica' refers to the known distribution of the species.

*Typus.* BHUTAN, Paro Distr., trekking path from Shinkarap to Thangthangkha, 12 Oct. 2017, C. Scheidegger, M.X. Yang, L.S. Wang, C.C. Miao (holotype Scheideg-40506).

Primary photobiont *Symbiochloris* cf. *reticulata*. Thallus medium-sized, 10–15 cm wide, lobes growing upwards, rotundate with shorter internodes near apex, axils rotundate; dorsal surface of thallus olive-ochre brown to yellowish brown in herbaria, reticulately ridged, shining; soredia and isidia absent, laminal lobules present on thallus margin; ventral surface of thallus blackish and densely tomentose in grooves between swellings, tomentose netted type, tomentum dark grey to black, 2–3 mm, sparsely rhizinate, rhizines blackish brown, up to 3 mm long, swelling pale nude.

Apothecia at margin or ridges, 0.5–1.5 cm diam, disc reddish brown, margin smooth; parathecium developed, united with edge of thalloid exciple; epithecium 10 µm thick; hypothecium 40 µm thick; hymenium c. 65–80 µm high; excipulum 35–50 µm thick. Ascospores colourless, fusoid, 3-septate at maturity, 25–40 × 4–5 µm. Pycnidia absent.

Chemical substances — Norstictic, gyrophoric, stictic and constictic acid.

Habitat — Scattered to gregarious on bark in forests, alt. 2300–3600 m.

*Additional materials examined.* BHUTAN, Paro Distr., trekking path from Gunitsawa to Shinkarap, alt. 2300–3100 m, 11 Oct. 2017, C. Scheidegger, M.X. Yang, L.S. Wang, C.C. Miao, Scheideg-40476, Scheideg-40494; trekking path from Shinkarap to Thangthangkha, alt. 3100–3600 m, 12 Oct. 2017, C. Scheidegger, M.X. Yang, L.S. Wang, C.C. Miao, Scheideg-40544.

Notes — This species is phylogenetically closely related to *L. rhizinata* and *L. granulosa*, and these three species also share a similar chemistry. However, the diagnostic characteristics for *L. bhutanica* are: absence of isidia and soredia, lobes rotundate, with shorter internodes near apex, axils rotundate, and the thin thalline margin and proper exciple are well connected in fully developed apothecia. The diagnostic characteristics for *L. rhizinata* are: absence of isidia and soredia, lobes growing upwards canalculated truncate, with longer internodes near apex, and absence of apothecia. The diagnostic characteristic for *L. granulosa* is: presence of knob-like isidia.

***Lobaria costata*** M.X. Yang & Scheid., *sp. nov.* — MycoBank MB 838330; Fig. 4

*Etymology.* 'costata' refers to the pronounced ridges on the lobes.

*Typus.* CHINA, Yunnan Prov., Lijiang Ci., Laojunshan Mt, along the downhill roadside, on bark, 5 July 2017, C. Scheidegger, L.S. Wang, M.X. Yang, C.C. Miao (holotype Scheideg-40081).

Primary photobiont *Symbiochloris* cf. *reticulata*. Thallus medium sized, up to 10 cm wide, irregularly laciniate-lobate, lobe with longer internodes near apex, apex more or less truncate or tapered; dorsal surface olive-ochre brown to yellowish brown in herbaria, reticulated ridge, distinctly reticulated, ridges prominent, narrow, glabrous and shining, foveae quadrangular, distinctly deepened, clear ridges look like protruding bones; soredia, isidia and laminal lobules absent; ventral surface of thallus blackish and densely tomentose in grooves between swellings, tomentose netted type, tomentum dark grey to black, 2–3 mm, sparsely rhizinate, rhizines blackish brown, up to 3 mm long, swelling pale nude.

Apothecia at margin or ridges, 0.5–3 cm diam, disc reddish brown, margin smooth, cortex thin at margin but connate with the parathecium, parathecium well developed; epithecium c. 10 µm thick; hypothecium 25 µm thick; hymenium c. 40–50 µm high; excipulum 40–50 µm thick. Ascospores colourless, fusoid, 3-septate at maturity, 25–35 × 5–6.5 µm. Pycnidia absent.

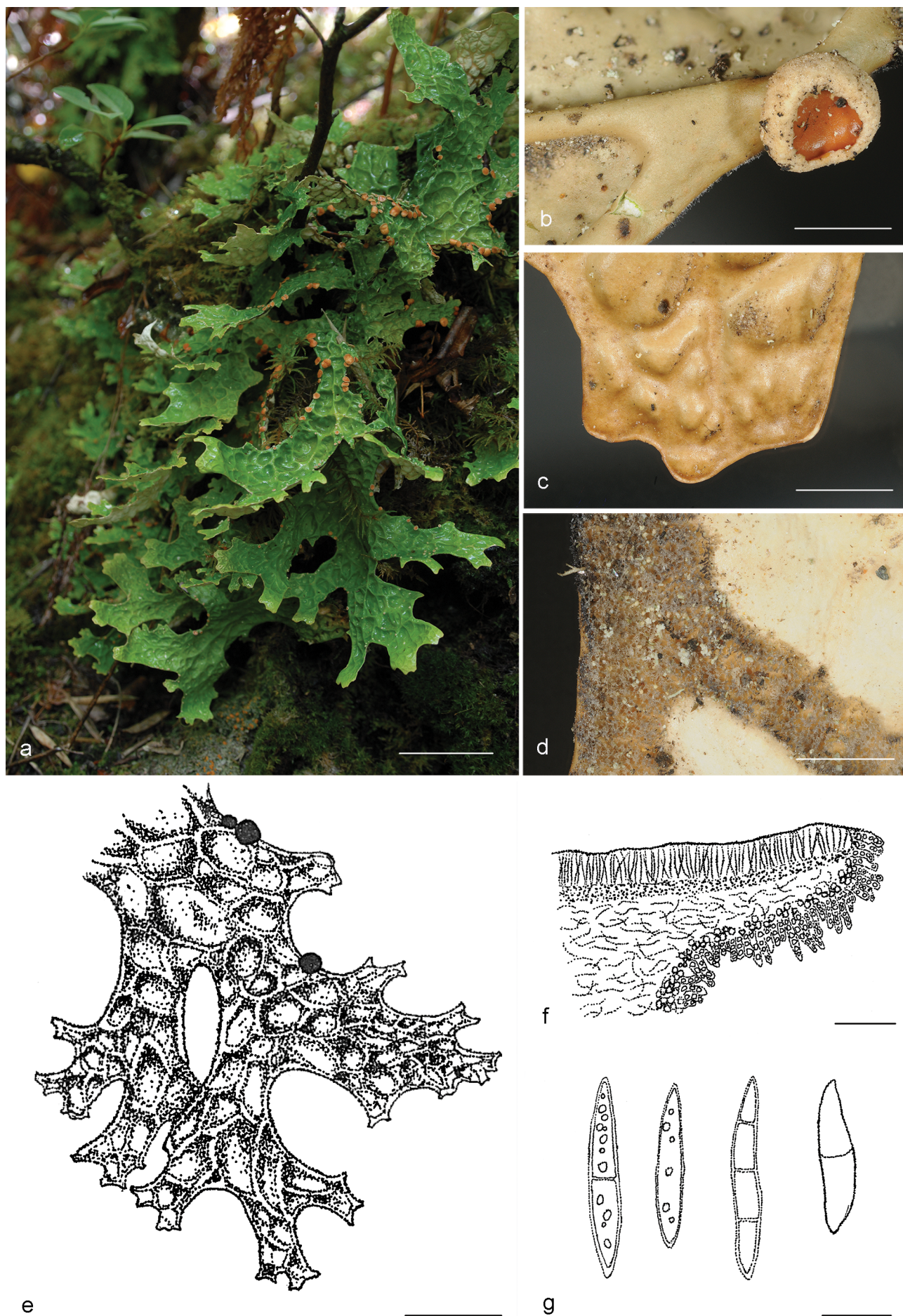
Chemical substances — Gyrophoric and stictic acid.

Habitat — Scattered to gregarious on bark in forests dominated by *Quercus* or on soil, alt. 2680–3675 m.





**Fig. 4** Morphological features of *Lobaria costata* sp. nov. (a: KUN-16-53628; b–c, e–f: Scheideg-40081, d: 19-65719). a. Habit; b. apothecia; c. ventral surface; d. thallus; e. hymenium; f. ascospores. — Scale bars: a = 1 cm; b–c = 1 mm; d = 0.5 cm; e = 50  $\mu$ m; f = 10  $\mu$ m.



**Fig. 5** Morphological features of *Lobaria devkotae* sp. nov. (holotype, KUN-19-64564). a. Habit; b. apothecia; c. lobe; d. ventral surface; e. thallus; f. hymenium; g. ascospores. — Scale bars: a–e = 1 cm; b–d = 1 mm; f = 50  $\mu$ m; g = 10  $\mu$ m.

**Additional materials examined.** CHINA, Yunnan Prov., Lijiang Ci., Laojunshan Mt, along the downhill roadside, alt. 3469.6 m, N26°64'92.7" E99°78'30.6", 5 July 2017, C. Scheidegger, L.S. Wang, M.X. Yang, C.C. Miao, Scheideg-40112; Xizang Prov., Dingqing Co., Jueen Vil., soil slope beside G324, alt. 3675 m, N31°14'52.78" E95°54'36.32", 2 Oct. 2016, L.S. Wang, M.X. Yang et al., KUN-16-53628; Bomi Co., Guxiang Vil., forest beside G318 road, alt. 2680 m, N29°53'58.02" E95°30'27.65", 24 Sept. 2016, L.S. Wang et al., KUN-16-52050.

**Notes** — *Lobaria costata* is characterised by a lobe with longer internodes near apex, apex more or less truncate or tapered, clear ridges look like protruding bones; soredia, isidia and laminal lobules absent. This species has a similar morphology to that of *L. yunnanensis*. However, *L. yunnanensis* is easily distinguished by dense irregularly laciniate-lobate and no chemistry (Yoshimura 1971), while *L. costata* has gyrophoric and stictic acid.

***Lobaria devkotae*** M.X. Yang & Scheid., *sp. nov.* — MycoBank MB 838326; Fig. 5

**Etymology.** 'devkotae' means in honour of Dr Shiva Devkota, who studied the related *L. pindarensis* in Nepal.

**Typus.** CHINA, Xizang Prov., Bayi Distr., on bark, 14 July 2019, L.S. Wang, M.X. Yang et al. (holotype KUN-19-64564).

Primary photobiont *Symbiochloris* cf. *reticulata*. Thallus medium to large, 10–20 cm wide, lobes growing upwards canaliculate

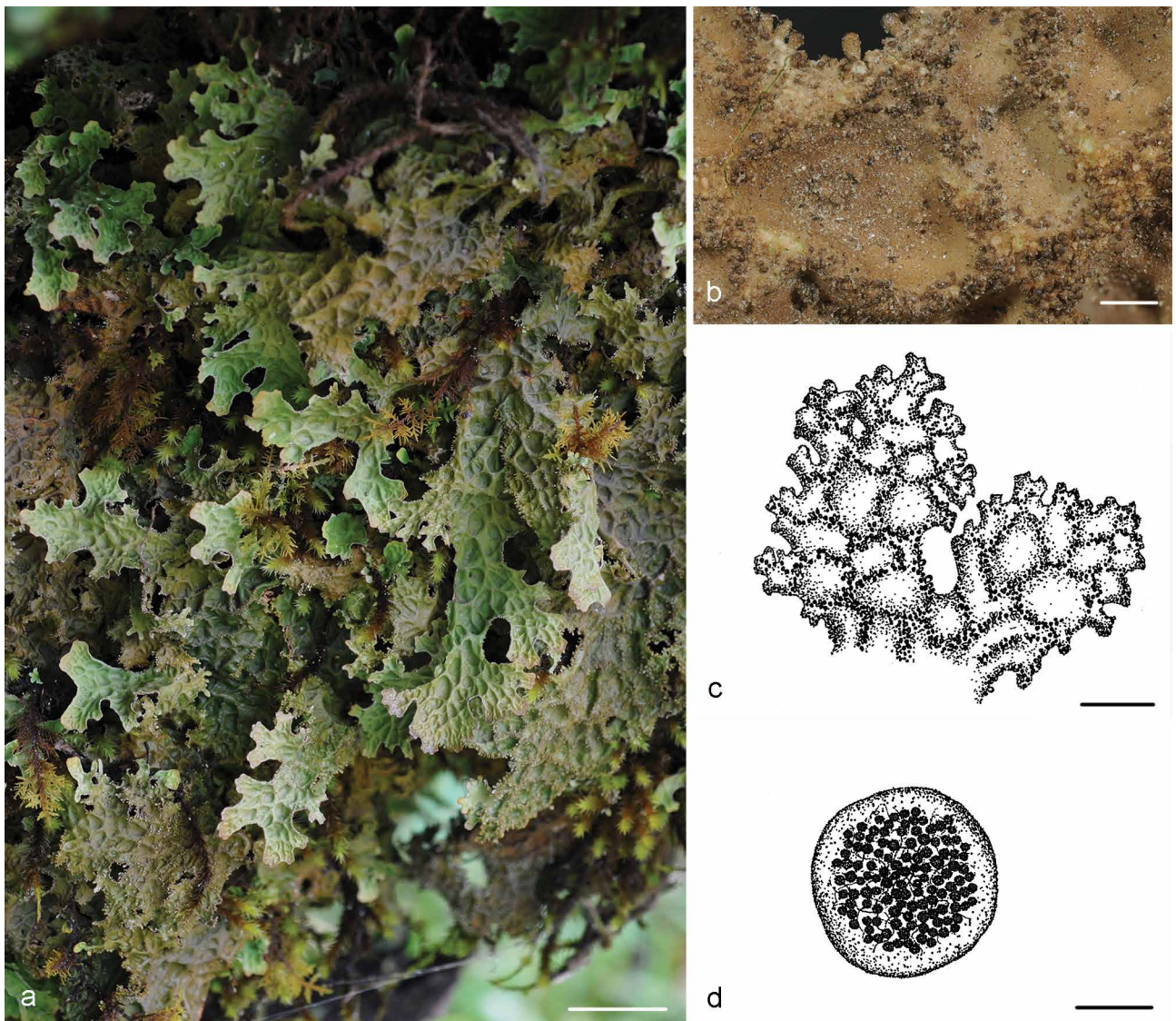
truncate, with shorter internodes near apex, closely adnate to substrate, elongate, acuminate to truncate, axils rotundate; dorsal surface vivid-green when fresh, olive-ochre brown to yellowish brown in herbaria, distinctly reticulate, ridges prominent, narrow, glabrous and shining, foveae quadrangular, distinctly deepened; soredia, isidia and laminal lobules absent; ventral surface pale brown to yellow-white, tomentum netted and in grooves between foveae, brown to black, 1–3 mm, rhizines scattered, 2–4 mm long.

Apothecia at margin or on ridges, 0.5–1.5 cm diam, disc reddish brown, margin crenulate, cortex thin at margin but connate with the parathecium; epitecium 10 µm thick; hypothecium 25 µm thick; hymenium c. 60–70 µm high; excipulum 50–70 µm thick. Ascospores colourless, fusoid, 3-septate at maturity, 30–40 × 5–6 µm. Pycnidia conspicuous, immersed on ridges and at margin; conidia rod-shaped.

**Chemical substances** — Gyrophoric, stictic, constictic and hypoconstictic acid.

**Habitat** — Scattered to gregarious on bark in forests dominated by *Quercus*; restricted to a few localities, rare, alt. 2892–3490 m.

**Additional materials examined.** CHINA, Xizang Prov., Bomi Co., alt. 3490 m, N29°48'31" E95°41'58", 10 Nov. 2018, L.S. Wang, M.X. Yang et al., KUN-18-62315; Bayi Distr., Lulang Town, alt. 2892 m, N29°49'344.28" E94°47'10.80", 19 July 2019, L.S. Wang, M.X. Yang, X.Y. Wang et al., KUN-XY19-1105.



**Fig. 6** Morphological features of *Lobaria granulosa* sp. nov. (holotype, KUN-XY19-1128). a. Habit; b. isidia; c. thallus; d. isidia cross-section. — Scale bars: a = 1 cm; b = 1 mm; c = 0.5 cm; d = 0.2 mm.

Notes — *Lobaria devkotae* is phylogenetically related to *L. pindarensis*, and these two species have common characteristics, such as a truncate or obtuse apex. However, *L. devkotae* can be easily distinguished from *L. pindarensis* by the lack of isidia and norstictic acid.

***Lobaria granulosa*** M.X. Yang & Scheid., *sp. nov.* — MycoBank MB 838307; Fig. 6

*Etymology.* We named this species after the short and globular isidia.

*Typus.* CHINA, Xizang Prov., Bayi Distr., Bayi Town, on bark, 20 July 2019, L.S. Wang, X.Y. Wang, M.X. Yang et al. (holotype KUN-XY19-1128).

Primary photobiont *Symbiochloris* cf. *reticulata*. Thallus medium-sized, 10–12 cm wide, pruinose present on the foveae of thallus, lobes truncate at apex, with shorter internodes near apex, axils rotundate; dorsal surface of thallus olive-ochre brown to yellowish brown in herbaria, reticulate ridged, prominent, narrow, pruinose and matte, foveae quadrangular,

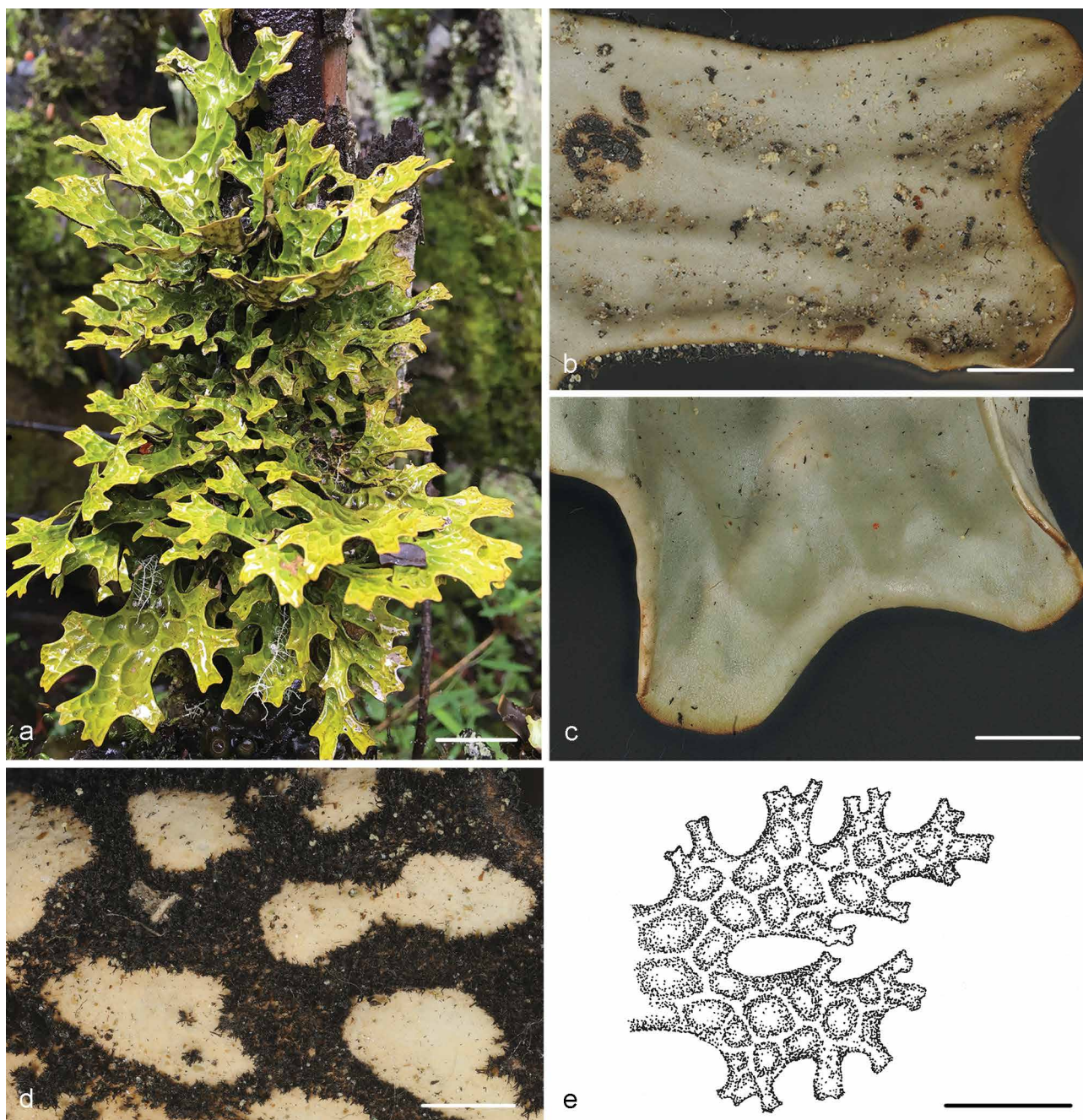
distinctly deepened; isidiate at ridges and margins, isidia sprout spherically on young lobes, knob-like and densely clustered on older lobes, or occasionally small lobed isidia mainly along margin, tips of isidia dark brown; ventral surface of thallus densely netted tomentose in grooves between nude, tomentum light to dark grey, 2–3 mm, pale swelling, rhizines sparse, 2–3 mm. Apothecia and pycnidia absent.

Chemical substances — Norstictic, gyrophoric, stictic and constictic acid.

Habitat — Scattered to gregarious on bark in forests, alt. 3034–3200 m.

*Additional material examined.* BHUTAN, Gasa Distr., Laya to Tongsheja (camp at river), alt. 3200 m, 22 Oct. 2017, C. Scheidegger, M.X. Yang, L.S. Wang, C.C. Miao, Scheideg-40629, Scheideg-40635.

Notes — *Lobaria granulosa* and *L. rhizinata* are phylogenetically closely related and share a similar chemistry. They are distinguished by the absence or presence of isidia.



**Fig. 7** Morphological features of *Lobaria ligulata* sp. nov. (a–b, e: KUN-XY19-1114; c–d: KUN-XY19-1119). a. Habit; b. dorsal surface; c. lobe; d. ventral surface; e. thallus. — Scale bars: a = 0.5 cm; b–d = 1 mm; e = 1 cm.

***Lobaria ligulata*** M.X. Yang & Scheid., *sp. nov.* — MycoBank MB 838331; Fig. 7

*Etymology.* 'ligulata' refers to the shape of the terminal lobes.

*Typus.* CHINA, Xizang Prov., Bayi Distr., Lulang Town, on bark, 19 July 2019, L.S. Wang, X.Y. Wang, M.X. Yang *et al.* (holotype KUN-XY19-1114).

Primary photobiont *Symbiochloris* cf. *reticulata*. Thallus small to medium, 5–10 cm, dense irregularly lacinate-lobate, lobes slender, antler-like, with longer internodes near apex, apex more or less truncate, axils rotundate; dorsal surface of thallus bright yellowish green when wet, olive-ochre green in herbaria, reticulate ridged, shining; soredia, isidia and laminal lobules absent;

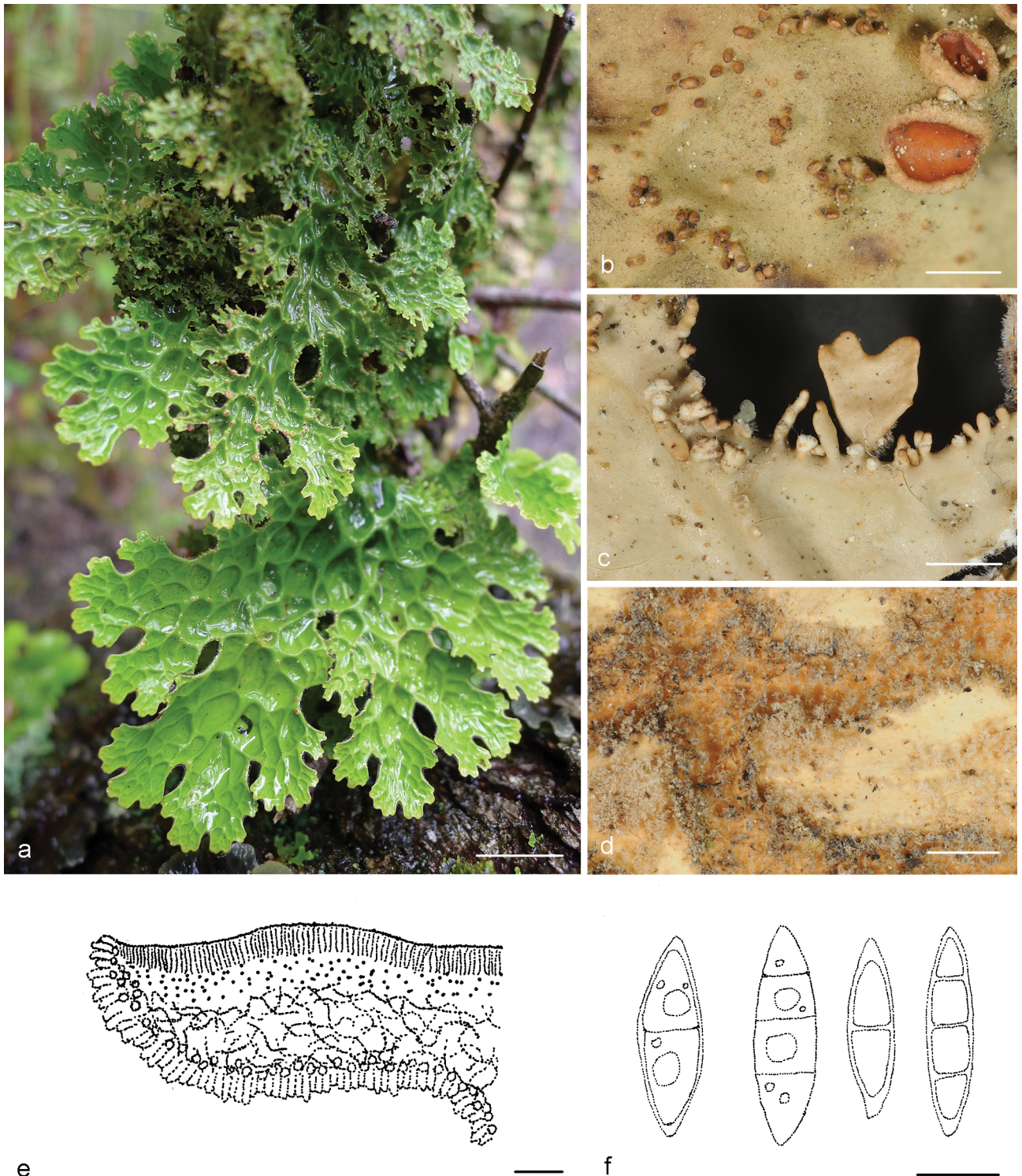
ventral surface of thallus blackish and densely tomentose in grooves between swellings, tomentose netted type, tomentum dark grey to black, 1–2 mm, sparsely rhizinate, rhizines blackish brown, up to 2 mm long, swelling pale nude.

Apothecia and pycnidia absent.

Chemical substances — Stictic, hypoconstictic and constictic acid.

Habitat — Scattered to gregarious on bark in forest, alt. 2818 m.

*Additional material examined.* CHINA, Xizang Prov., Bayi Distr., Lulang Town, alt. 2818 m, N29°53'01.14" E94°47'10.91", 19 July 2019, L.S. Wang, X.Y. Wang, M.X. Yang *et al.*, KUN-XY19-1114.



**Fig. 8** Morphological features of *Lobaria multipartita* sp. nov. (a: KUN-19-63350; b–f: KUN-XY19-525; c–d: Scheideg-40459). a. Habit; b. apothecia; c. isidia; d. ventral surface; e. hymenium; f. ascospores. — Scale bars: a = 1 cm; b–d = 1 mm; e = 50  $\mu$ m; f = 10  $\mu$ m.

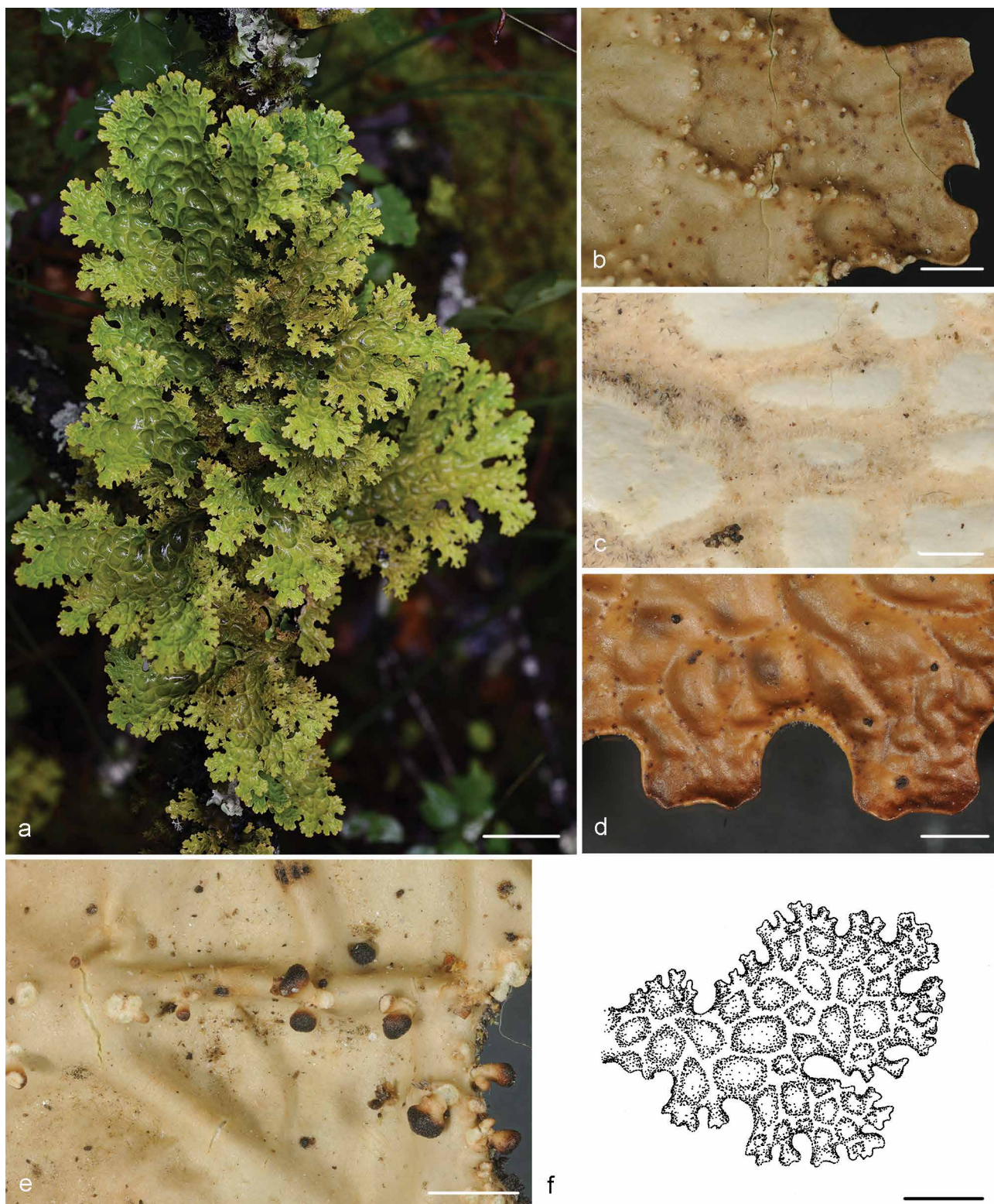
Notes — *Lobaria ligulata* is characterised by a relatively small thallus, dense lacinate-lobate, slender lobes, absence of soredia, isidia, laminal lobules and apothecia. This species is similar to *L. yunnanensis* (Yoshimura 1971), but *L. yunnanensis* differs in that it has apothecia and no chemical substances.

***Lobaria multipartita*** M.X. Yang & Scheid., *sp. nov.* — MycoBank MB 838328; Fig. 8

*Etymology.* 'multipartita' refers to the multifold lobation of the thallus.

*Typus.* CHINA, Xizang Prov., Bomi Co., Gu Vil., on route from Bomi to Linzhi, on bark, 19 July 2019, L.S. Wang, X.Y. Wang, M.X. Yang et al. (holotype KUN-XY19-525).

Primary photobiont *Symbiochloris* cf. *reticulata*. Thallus medium to large, up to 20 cm wide, divaricate or irregularly dichotomous, lobes > 5 mm long, 3–4 mm wide, apex more or less cut off or rounded, blunt or rounded joints; dorsal surface of thallus olive-ochre, reticulate ridged, pruina present on tips of lobes; isidiate at ridges and margins, isidia sprout spherically on young lobes, single isidia cylindrical to spatulate on mature lobes, always spatulate along margin, tips of isidia dark brown;



**Fig. 9** Morphological features of *Lobaria perelegans* sp. nov. (a–b: KUN-19-65719; c–e: Scheideg-40623). a. Habit; b. dorsal surface; c. ventral surface; d. lobe; e. isidia; f. thallus. — Scale bars: a = 1 cm; b–e = 1 mm; f = 0.5 cm.

ventral surface pale yellow or white, dense light brown to dark brown short tomentum in the grooves between the swellings, tomentum netted type, with sparse rhizines up to 3–4 mm.

Apothecia grow on reticulated ridges or margins, strongly constricted at base, disc light reddish brown to dark brown, 0.5–3 mm diam; epithecium c. 10 µm; hypothecium 30–50 µm thick; hymenium c. 40–50 µm high; excipulum 30–40 µm thick. Ascospores colourless, fusoid, straight to slightly curved, 3-septate at maturity, 20–25 × 5–6.5 µm. Pycnidia buried in the thallus close to the lobes; conidia rod-shaped, 6 × 1 µm.

Chemical substances — Gyrophoric and stictic acid.

Habitat — Scattered to gregarious on bark in forests, alt. 2300–3100 m.

*Additional materials examined.* BHUTAN, Paro Distr., Taktshang (Tiger's Nest), alt. 2300–3100 m, 9 Oct. 2017, C. Scheidegger, Scheideg-40459. — CHINA, Xizang Prov., Bomi Co., Gu Vil., on route from Bomi to Linzhi, alt. 2633 m, N29°54'02.18" E95°35'55.96", 18 July 2019, L.S. Wang, X.Y. Wang, M.X. Yang *et al.*, KUN-XY19-501; G318, 20 km from Bomi, alt. 2888 m, N29°47'06.21" E95°54'19.47", 13 July 2019 (KUN-19-63350).

Notes — This species has a similar morphology to that of *L. orientalis* and *L. gyrophorica*. *Lobaria multipartita* is easily distinguished from the two other species by its spatulate isidia

on the margins. Additionally, *L. orientalis* lacks isidia and has norstic, gyrophoric and stictic acid (Yoshimura 1971). *Lobaria gyrophorica* can be easily distinguished by its lack of isidia and presence of gyrophoric acid.

***Lobaria perelegans*** M.X. Yang & Scheid., *sp. nov.* — MycoBank MB 838329; Fig. 9

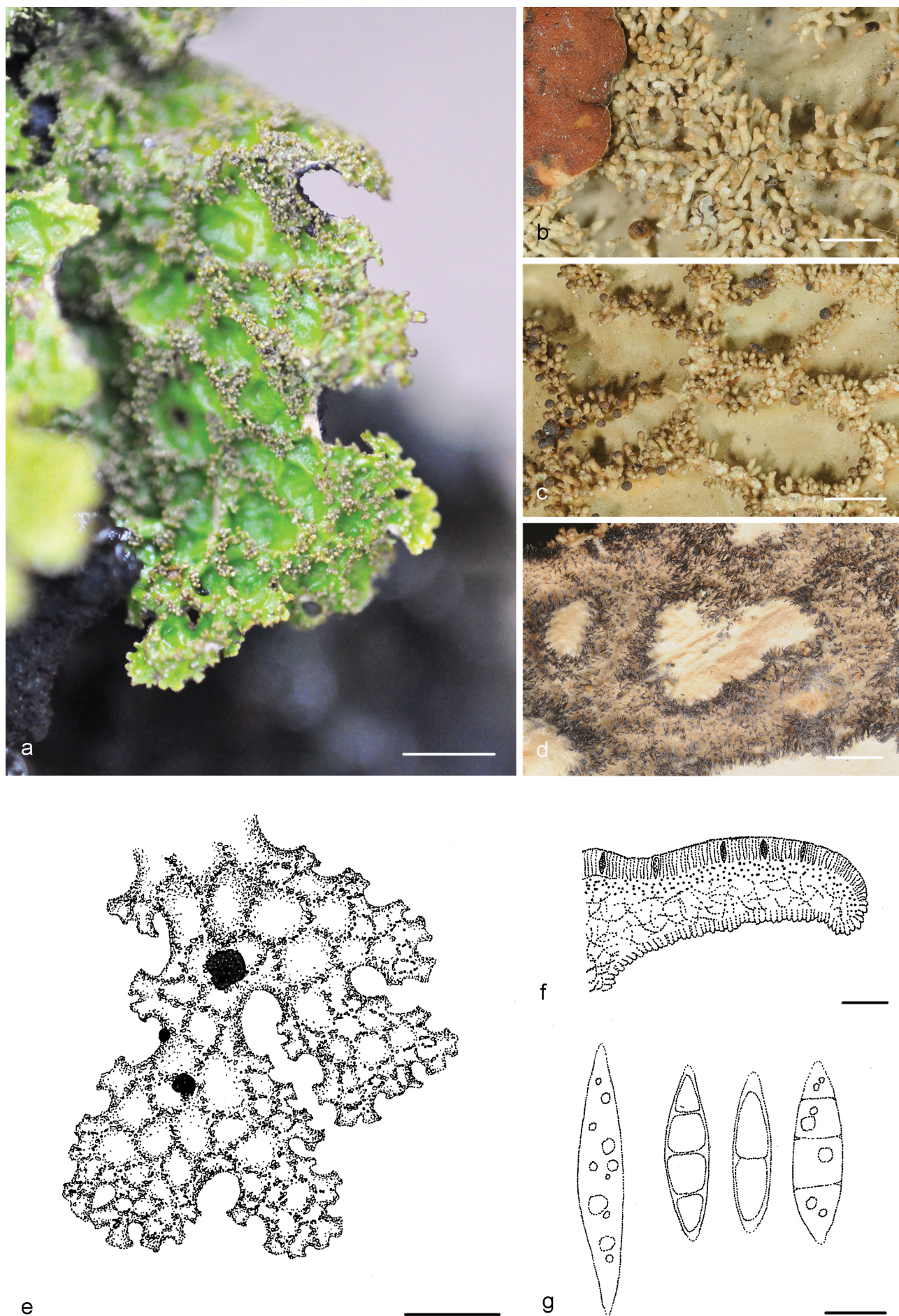
*Etymology.* 'perelegans' refers to the very elegant, symmetric subdivision of the lobes.

*Typus.* CHINA, Xizang Prov., Dingjie Co., Chentang Town, on bark, 28 July 2019, L.S. Wang, M.X. Yang *et al.* (holotype KUN-19-65719).

Primary photobiont *Symbiochloris cf. reticulata*. Thallus medium sized, 10–15 cm wide, reticulated ridge, lobe with shorter internodes near apex, apex more or less truncate; dorsal surface bright vivid-green when wet, olive-ochre green in fungaria, reticulated ridge; isidiate at ridged portions and margins, sometimes also present at foveae, knob-like isidia multiple in group or single isidia-scattered, always blastidiate, dark brown on the tips, often because of infections with *Tremella* sp.; ventral surface pale yellow or white, dense light brown short tomentose



**Fig. 10** Morphological features of *Lobaria rhizinata* sp. nov. (a, d: Scheideg-40411; b–c: Scheideg-40362). a. Habit; b. lobe; c. ventral surface; d. thallus. — Scale bars: a, d = 1 cm; b, c = 1 mm.



**Fig. 11** Morphological features of *Lobaria tibetana* sp. nov. (a: KUN-XY19-1007, b–f: KUN-18-62299). a. Habit; b, c. isidia and apothecia; d. ventral surface; e. thallus; f. hymenium; g. ascospores. — Scale bars: a, e = 1 cm; b–d = 1 mm; f = 50  $\mu$ m; g = 10  $\mu$ m.



in the grooves between the swellings, tomentose veined type, with sparse rhizines up to 3–4 mm.  
Apothecia and pycnidia absent.

Chemical substances — Gyrophoric and stictic acid.

Habitat — Scattered to gregarious on bark in forests, alt. 3028–3200 m.

*Additional material examined.* BHUTAN, Gasa Distr., Laya to Tongsheja (camp at river), alt. 3200 m, 22 Oct. 2017, C. Scheidegger, Scheideg-40623.

*Notes* — *Lobaria perelegans* is phylogenetically closely related to *L. multipartita* and *L. gyrophorica*. However, *L. perelegans* is characterised by symmetric subdivisions of the lobes, and knob-like or blastidiate isidia, while *L. gyrophorica* has irregularly dichotomous lobes and apices more or less cut off or rounded, and lacks isidia; *L. multipartita* has divaricate or irregularly dichotomous lobes, and cylindrical and spatulate isidia on the margins. Additionally, based on the species-tree, we found the existence of species-pairs: *L. gyrophorica* (sexual species) regularly forms apothecia, while *L. perelegans* (asexual species) regularly forms isidia and apothecia are absent.

***Lobaria rhizinata*** M.X. Yang & Scheid., *sp. nov.* — MycoBank MB 838327; Fig. 10

*Etymology.* 'rhizinata' refers to the characteristic rhizinae formed on the lower side of the thallus.

*Typus.* CHINA, Yunnan Prov., Shaggrila Ci., Tianbao Mt, on Rocky screes, 7 Aug. 2017, C. Scheidegger, M.X. Yang, L.S. Wang, C.C. Miao (holotype Scheideg-40411).

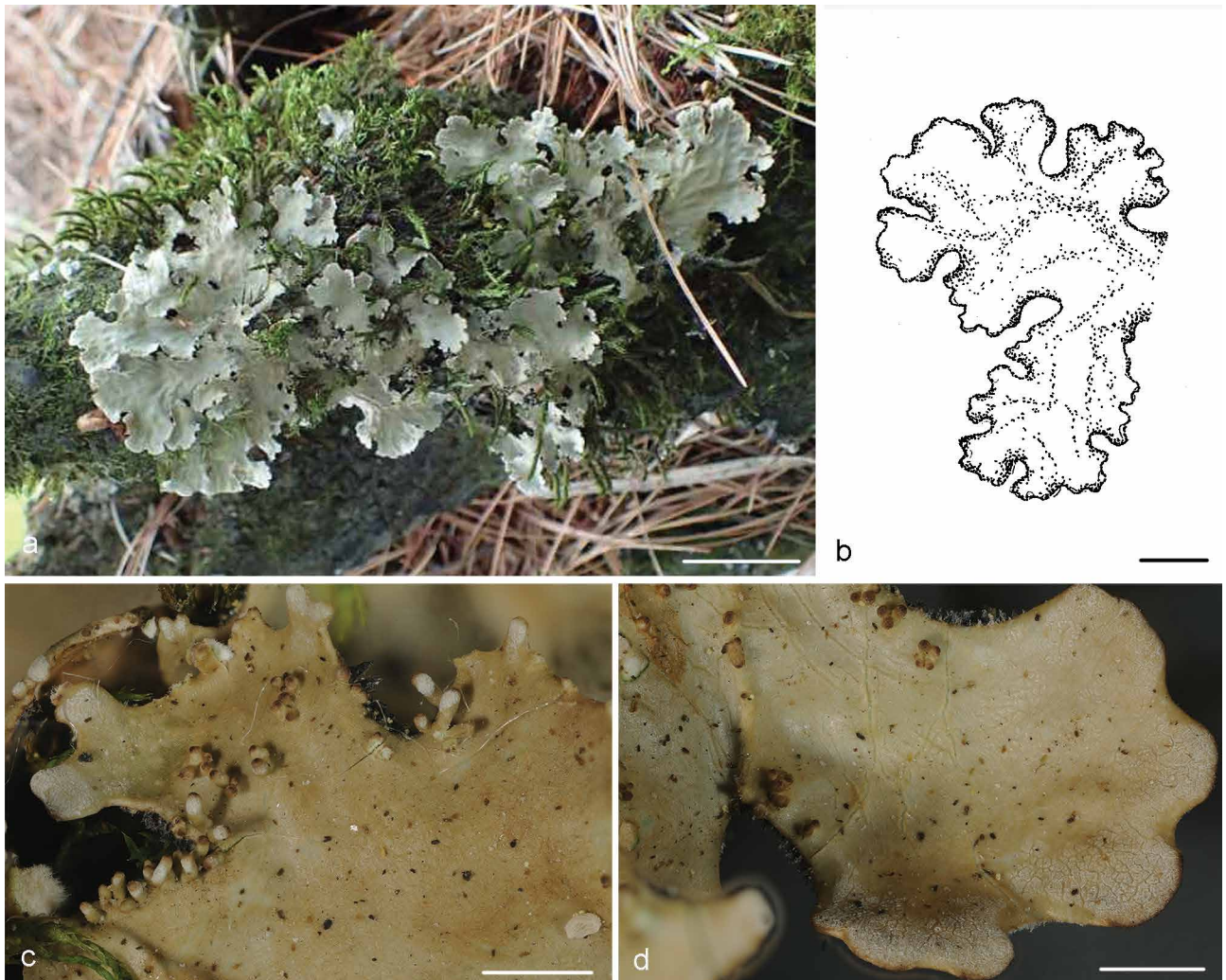
Primary photobiont *Symbiochloris* cf. *reticulata*. Thallus medium-sized, 10–12 cm wide, lobes growing upwards canalicate truncate, lobes growing horizontally and downwards raised from substrate, with longer internodes near apex, elongate, acuminate to truncate, axils tapered; dorsal surface olive-ochre brown to yellowish brown in herbaria, oil-shiny, irregularly ridged, distinctly reticulate, distinctly deepened; soredia, isidia and laminal lobules absent; ventral surface of thallus blackish brown and densely tomentose on older portions and in grooves between swellings, dark grey to black, 2–3 mm, sparsely rhizinate, rhizines blackish brown, up to 3 mm long, swelling pale nude. Apothecia and pycnidia absent.

Chemical substances — Norstictic, gyrophoric, stictic and constictic acid.

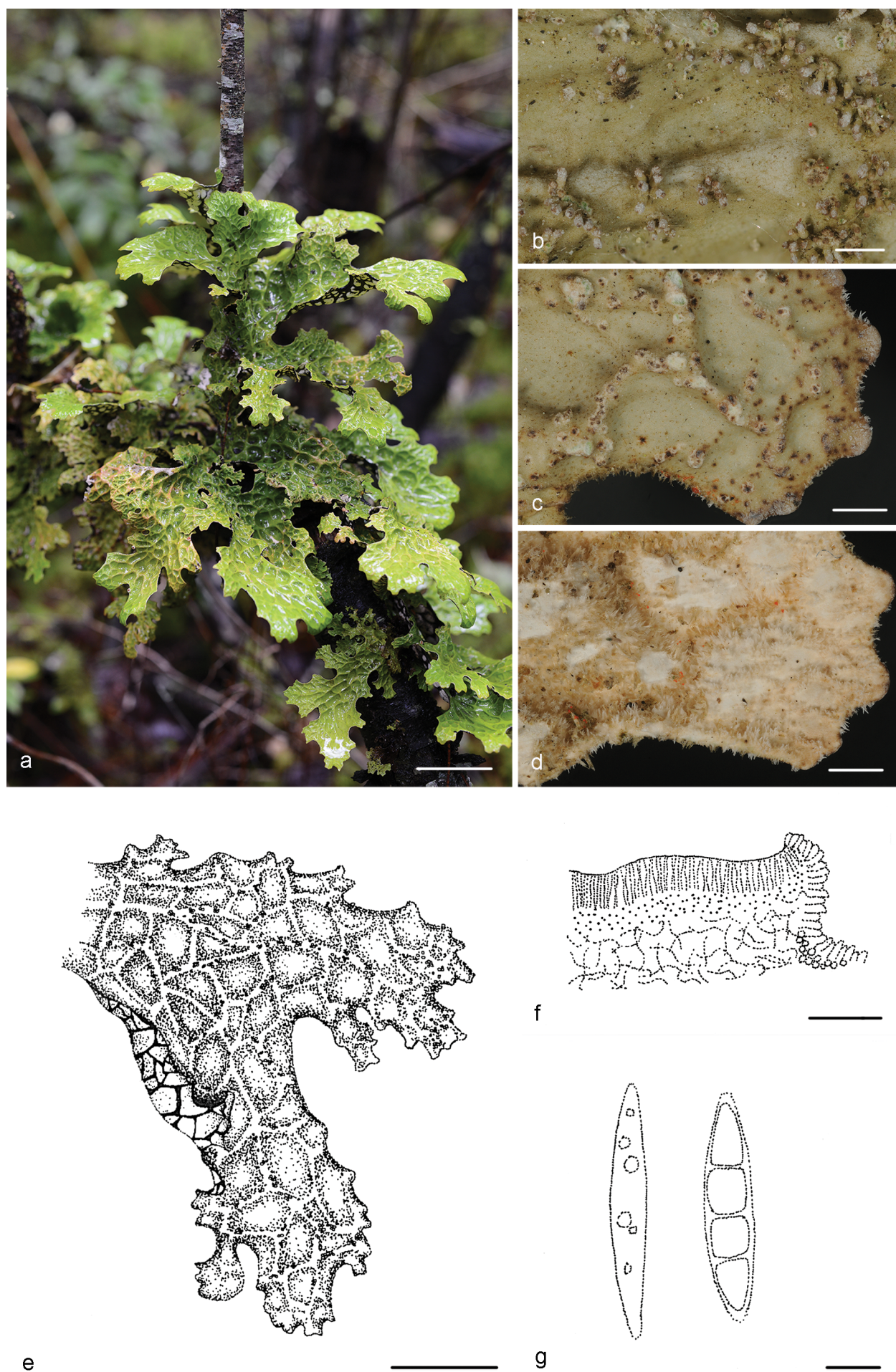
Habitat — Scattered to gregarious on rocky screes, alt. 3724–3736 m.

*Additional materials examined.* CHINA, Yunnan Prov., Shaggrila Ci., Tianbao Mt, Rocky screes, 7 Aug. 2017, alt. 3724.6 m, N27°60'30.6" E99°89'58.8", C. Scheidegger, M.X. Yang, L.S. Wang, C.C. Miao (Scheideg-40332); alt. 3728.7 m, N27°60'30.6" E99°89'60.2", 7 Aug. 2017, C. Scheidegger, M.X. Yang, L.S. Wang, C.C. Miao, Scheideg-40362; alt. 3734.1 m, N27°60'31.5" E99°89'64.3", 7 Aug. 2017, C. Scheidegger, M.X. Yang, L.S. Wang, C.C. Miao, Scheideg-40396.

*Notes* — *Lobaria rhizinata* is characterised by blackish brown rhizines formed on the lower side of the thallus, tapered axils of lobes, irregular ridges, and an absence of soredia, isidia, laminal lobules and apothecia. This species has a similar morphology to that of *L. ligulata*, which makes it difficult to distinguish these



**Fig. 12** Morphological features of *Lobaria verruculosa* sp. nov. (holotype, KUN-XY19-505). a. Habit; b. thallus; c. isidia; d. lobe. — Scale bars: a = 1 cm; b = 0.5 cm; c–d = 1 mm.



**Fig. 13** Morphological features of *Lobaria wanglisongiana* sp. nov. (holotype, KUN-XY19-1049). a. Habit; b. isidia; c. lobe; d. ventral surface; e. thallus; f. hymenium; g. ascospores. — Scale bars: a = 1 cm; b–d = 1 mm; e = 0.5 cm; f = 2 mm; g = 5  $\mu$ m.

two species in the field. However, the lobes of *L. rhizinata* are wider and acuminate to truncate, while *L. ligulata* is more densely lacinate-lobate and forms slender lobes. Additionally, *L. ligulata* lacks gyrophoric and norstictic acid.

***Lobaria tibetana*** M.X. Yang & Scheid., *sp. nov.* — MycoBank MB 838327; Fig. 11

*Etymology.* 'tibetana' refers to the known distribution of the species.

*Typus.* CHINA, Xizang Prov., Bomi Co., Prope Bomi Co., on bark, 18 July 2019, L.S. Wang, X.Y. Wang, M.X. Yang *et al.* (holotype KUN-XY19-1007).

Primary photobiont *Symbiochloris* cf. *reticulata*. Thallus medium to large, 10–20 cm wide, with shorter internodes near apex, apex more or less truncate; dorsal surface of thallus olive-ochre green in herbaria, distinctly reticulate ridged, ridges prominent, narrow, glabrous, foveae quadrangular, distinctly deepened, pruina present or absent on the lobes; isidia coralloid, clustered or single, cylindrical form at ridges and margins, often with black *Tremella* on the tips of isidia; ventral surface of thallus pale brown, densely netted type tomentum and rhizinate in grooves between swellings, rhizines and tomentum brown, both c. 3 mm long.

Apothecia very rare, mostly on ridges, 0.5–1.5 cm diam, disc reddish brown; parathecium poorly developed; epithecium 10 µm thick; hypothecium 25–40 µm thick; hymenium c. 40–50 µm high; excipulum 25 µm thick; ascospores fusoid, 3-septate at maturity, 25–30 × 5–6.5 µm; pycnidia absent.

Chemical substances — Gyrophoric and stictic acid.

Habitat — Scattered to gregarious on bark in *Abies* forests or on other trees, alt. 2300–3900 m.

*Additional materials examined.* BHUTAN, Paro Distr., Chele la pass and nun monastery, alt. 2300–3900 m, 10 Oct. 2017, C. Scheidegger, L.S. Wang, M.X. Yang, C.C. Miao, Scheideg-40473; trekking path from Shinkarap to Thangthangkha, alt. 3100–3600 m, 12 Oct. 2017, C. Scheidegger, L.S. Wang, M.X. Yang, C.C. Miao, Scheideg-40538. — CHINA, Xizang Prov. Bomi Co., alt. 3490 m, N29°48'31" E95°41'58", 10 Nov. 2018, L.S. Wang, M.X. Yang, X.Y. Wang *et al.*, KUN-18-62299.

Notes — *Lobaria tibetana* is characterised by the cylindrical or knob-like isidia and always has black *Tremella lobariacearum* growing on the tips of some of the isidia. This species is phylogenetically closely related to *L. orientalis*. However, *L. orientalis* lacks isidia (Yoshimura 1971) and contains norstictic acid, gyrophoric acid, stictic acid and constictic, while *L. tibetana* contains only gyrophoric acid and stictic acid.

***Lobaria verruculosa*** M.X. Yang & Scheid., *sp. nov.* — MycoBank MB 838332; Fig. 12

*Etymology.* 'verruculosa' refers to the form of the isidia.

*Typus.* CHINA, Xizang Prov., Bomi Co., on route from Bomi to Linzhi, on bark, 18 July 2019, L.S. Wang, X.Y. Wang, M.X. Yang *et al.* (holotype KUN-XY19-505).

Primary photobiont *Symbiochloris* cf. *reticulata*. Thallus medium, 10–15 cm, irregularly lobate, lobes apex wide circle, axils rotundate; dorsal surface of thallus smooth, reticulately ridged but not obviously so, grey green when wet, olive-ochre green in herbaria, pruina present on the margins of lobes; isidiate at ridged portions and margins, isidia normally verrucose, aggregated in clusters or rarely single cylindrical form at margins; ventral surface of thallus yellowish brown, densely tomentose in grooves between swellings, tomentum netted, brown, 1–2 mm, sparsely rhizinate, rhizines light brown to dark brown, up to 2 mm long, swelling pale nude. Apothecia and pycnidia absent.

Chemical substances — Stictic and constictic acid.

Habitat — Scattered to gregarious on bark in forest, always along with moss, alt. 2636 m.

Notes — *Lobaria verruculosa* is characterised by an apex wide circle of thallus, and verrucose aggregated isidia on upper surface. This species is phylogenetically closely related to *L. kazawaensis* and its isidia have a similar form, but *L. kazawaensis* contains retigeric acid while *L. verruculosa* contains stictic acid and constictic acid. Furthermore, the dorsal surface of *L. verruculosa* is smooth to reticulately ridged while *L. kazawaensis* is reticulately ridged on the dorsal surface.

***Lobaria wanglisongiana*** M.X. Yang & Scheid., *sp. nov.* — MycoBank MB 838333; Fig. 13

*Etymology.* 'wanglisongiana' refers to the eminent Chinese lichenologist Wang Li-Song.

*Typus.* CHINA, Xizang Prov., Bomi Co., Gu Vil., on route from Bomi to Linzhi, on bark, 19 July 2019, L.S. Wang, X.Y. Wang, M.X. Yang *et al.* (holotype KUN-XY19-1049).

Primary photobiont *Symbiochloris* cf. *reticulata*. Thallus medium to large size, 10–20 cm, lobes more or less sinuate along margin; dorsal surface of thallus dark green when wet, olive-ochre green in herbaria, reticulation weak, ridges indistinct, pruina present on thallus; isidiate in ridged portions and at margins, sometimes also at foveae, isidia coralloid, aggregated in groups or single, cylindrical along ridges and margins, always blastidiate, tip of isidia brown; ventral surface blackish and densely tomentose in grooves between the swellings, tomentose veined type, with sparse rhizines 1–3 mm long.

Apothecia very rare, mostly on ridges, 0.5–1 cm diam, disc reddish brown; parathecium developed, united with edge of thalloid exciple and surrounding medulla; epithecium 10 µm thick; hypothecium 10 µm thick; hymenium c. 25 µm high; excipulum 25 µm thick. Ascospores fusoid, 3-septate at maturity, 15–25 × 3.5–5 µm. Pycnidia absent.

Chemical substances — Pannaric acid or no chemistry.

Habitat — Scattered to gregarious on bark in forest dominated by *Quercus*, alt. 2680–3140 m.

*Additional materials examined.* CHINA, Xizang Prov., Bomi Co., Zhamu Town, alt. 2849 m, N29°47'06.25" E95°54'19.92", 13 July 2019, L.S. Wang, X.Y. Wang, M.X. Yang *et al.*, KUN-19-64427, KUN-19-64448; Songzong Vil., alt. 3140 m, N29°41'30.33" E96°12'11.42", 23 Sept. 2016, L.S. Wang, X.Y. Wang, M.X. Yang *et al.*, KUN-16-53176, KUN-16-53208, KUN-16-53233.

Notes — *Lobaria wanglisongiana* is phylogenetically closely related to *L. sachalinensis* and *L. kazawaensis*. While the new species has multiple blastidiate isidia often aggregated in groups, *L. sachalinensis* has no isidia and *L. kazawaensis* has cylindrical isidia (Yoshimura 1971).

#### KEY TO THE SPECIES OF THE GREEN-ALGAL LOBARIA S.STR. IN THE HIMALAYAS AND THE HENGDUAN MOUNTAINS

1. Thallus isidiate or lobulate . . . . . 2
1. Thallus without isidia and lobules . . . . . 14
2. Norstictic acid present . . . . . 3
2. Norstictic acid absent . . . . . 7
3. Gyrophoric acid absent . . . . . 4
3. Gyrophoric acid present . . . . . 5
4. Retigeric acid absent . . . . . *L. meridionalis*
4. Retigeric acid present . . . . . *L. sachalinensis*
5. Isidia knob-like . . . . . *L. granulosa*
5. Isidia cylindrical . . . . . 6
6. Cylindrical or occasionally small lobed isidia along the ridges; constictic acid present . . . . . *L. pindarensis*
6. Cylindrical isidia along the ridges and margins; constictic acid absent . . . . . *L. isidiophora*

|   |                          |
|---|--------------------------|
| 7. Gyrophoric acid present  | 8                        |
| 7. Gyrophoric acid absent   | 11                       |
| 8. Stictic acid present   | 9                        |
| 8. Stictic acid absent  | <i>L. spatulata</i>      |
| 9. Lobes apex rounded; rarely marginal lobules                          | <i>L. multipartita</i>   |
| 9. Lobes apex truncate  | 10                       |
| 10. Isidia knob-like, multiple in groups or scattered single isidia     | <i>L. perelegans</i>     |
| 10. Isidia coralloid, clustered or single                               | <i>L. tibetana</i>       |
| 11. Isidia present, lobules absent                                      | 12                       |
| 11. Isidia absent, lobules present                                      | <i>L. yulongensis</i>    |
| 12. Isidia verrucose aggregated; stictic acid present                   | <i>L. verruculosa</i>    |
| 12. Isidia coralloid or cylindrical; stictic acid absent                | 13                       |
| 13. Isidia coralloid; retigeric acid absent and pannaric acid present   | <i>L. wanglisongiana</i> |
| 13. Isidia cylindrical; retigeric acid present and pannaric acid absent | <i>L. kazawaensis</i>    |
| 14. Norstictic acid present   | 15                       |
| 14. Norstictic acid absent  | 18                       |
| 15. Gyrophoric acid present   | 16                       |
| 15. Gyrophoric acid absent  | <i>L. chinensis</i>      |
| 16. With longer internodes near apex                                    | <i>L. rhizinata</i>      |
| 16. With shorter internodes near apex                                   | 17                       |
| 17. Lobes apex truncate   | <i>L. bhutanica</i>      |
| 17. Lobes apex rounded, antler-like                                     | <i>L. orientalis</i>     |
| 18. Gyrophoric acid present   | 19                       |
| 18. Gyrophoric acid absent  | 22                       |
| 19. Stictic acid present  | 20                       |
| 19. Stictic acid absent   | 21                       |
| 20. With longer internodes near apex; constictic acid absent            | <i>L. costata</i>        |
| 20. With shorter internodes near apex; constictic acid present          | <i>L. devkotae</i>       |
| 21. Thelephoric acid present  | <i>L. gyrophorica</i>    |
| 21. Thelephoric acid absent   | <i>L. yoshimurae</i>     |
| 22. Lobes antler-like, stictic acid present                             | <i>L. ligulata</i>       |
| 22. Lobes tip acute, stictic acid absent                                | <i>L. yunnanensis</i>    |

## DISCUSSION

Our analyses revealed an unexpectedly high species richness in the lichen genus *Lobaria* in the Hengduan Mountains and the Himalayas, where the genus is widely distributed in old-growth forests. In our taxonomic study, a total of 21 species were identified based on a multilocus phylogenetic analysis. Surprisingly, only 10 species were previously known, based on morphological, chemical and reproductive characteristics. Eleven species were confirmed as new to science. These results indicate that the identification of *Lobaria* species based on morphology alone is difficult, and molecular information from a multilocus phylogenetic approach is required to unravel the genus' species diversity. It is likely that even more species from this region await description.

Our study confirms that green-algal *Lobaria* are widespread in the tropical to temperate regions in East Asia. In the Nepalese Himalayas, only one isidiate species (*L. pindarensis*) exists, while in the Hengduan Mountains and the eastern Himalayas (Bhutan, Eastern Xizang), isidiate and non-isidiate individuals form species rich photobiont-mediated guilds. This complexity is characteristic of several areas, where isidiate species co-occur with non-isidiate species (Dal Grande et al. 2014). The

second difference among them is the taxon diversity of the isidiate lichens. In the Nepalese Himalayas, there is a relatively high phenotypic diversity of the isidiate thalli, but they are all conspecific (*L. pindarensis*). In the Hengduan Mountains and the eastern Himalayas, however, we found several genetically distinct lineages, such as *L. tibetana* and *L. wanglisongiana* (Fig. 1), that share morphological traits and cannot be easily distinguished in the field. Future studies should clarify the role of different reproductive strategies of guild members (core and fringe species).

Divergence time estimates suggest that several major divergence events led to the evolution of extant taxa within *Lobaria*. The diversification of the green-algal species was estimated to have started in the mid-Miocene (12.5 Myr BP; Fig. 2). The divergence of *L. yunnanensis*, which is known to exist in Northwest Yunnan and the Xizang Himalayas, from other *Lobaria* species is estimated at around 10 Myr ago in the late Miocene. The next divergence of clades 1 to 20 is estimated at around 8 Myr ago. The diversified lineages of clades 1 to 14 speciated several times at around 5.35 Myr later during the end of the Miocene to Pliocene, while 15 to 20 after around 5.96 Myr. The widespread view is that considerable climatic change occurred during the late Miocene, around 8 Myr ago, leading to aridification in western Eurasia, although fossil records of mammal fauna suggest an increase in humidity in northern China of 700–1500 mm/yr at about the same time (Fortelius et al. 2006, Eronen et al. 2012). This coincides in time with evidence of stronger Indian summer monsoon circulation about 8 Myr ago in northern China, marking the start of a more humid phase, and subsequent drier conditions (< 700 mm/yr) from 4 Myr ago onwards (Zhisheng et al. 2001, Fortelius et al. 2006, Eronen et al. 2012). This highlights that the Indian summer monsoons have brought heavy summer rainfall to the southern part of the Himalayas, with decreasing precipitation in other areas of the Qinghai-Tibetan Plateau since the late Miocene. In addition, the Hengduan Mountains uplifted over the last 8 Myr, leading to novel environmental conditions including new habitats and population dispersal barriers, which increased the rate at which resident species evolved and diversified to form new ones. According to Heaney (1991), during the Miocene, tropical rain forests and temperate mountain forests in Southeast Asia extended to southern China and Japan, but since that time these forests have contracted. The impact of these climatic oscillations has been confirmed in various groups of plants (Yang et al. 2012, Zhou et al. 2013, Wen et al. 2014, 2016). In addition, our time-estimates show that the abundant species (*L. bhutanica*, *L. chinensis*, *L. gyrophorica*, *L. orientalis*, *L. perelegans*, *L. pindarensis*, *L. rhizinata*, *L. sachalinensis*, *L. tibetana* and *L. wanglisongiana*) appeared during the Pleistocene, from 0.68 Myr to 2.8 Myr ago, which supports the idea that dispersal between the Himalayas and the Hengduan Mountains significantly increased during the last 2 Myr. Furthermore, Fig. 1 shows strong evidence for the existence of species-pairs: *L. gyrophorica* (sexual) vs *L. perelegans* (asexual), newly reported in this study, and *L. chinensis* vs *L. isidiophora*, which has been reported previously (Cornejo & Scheidegger 2018). The divergence of *L. gyrophorica* and *L. perelegans* was estimated to have occurred during the Pleistocene (2.06 Myr ago) and support the idea of young lineages in a geologic timescale (Fig. 2).

Low-altitude regions and mountain gorges of the Hengduan Mountains and the Himalayas are considered to have been refugia during the glacial periods (Meng et al. 2015, Wang & Hua 2018). Species in this region responded to environmental changes by migrating north and south along the Hengduan Mountains, corresponding to a change in altitude (Hewitt 2004, Wang & Hua 2018, Hu et al. 2019). After migration and colonisa-

tion, populations were isolated for a long time in glacial refugia formed by Pleistocene glaciers, which promoted population differentiation and speciation (Meng et al. 2015, Ye et al. 2016). In addition, accurate species delimitation has critical implications for ecological and conservation studies.

Our study revealed an impressive diversity of green-algal *Lobaria* species in the study region. All species except *Lobaria pindarensis* have a narrow distribution area restricted to the eastern Himalayas (Xizang, Bhutan) and the Hengduan Mountains. Developing an effective conservation strategy for the geographically restricted endemic species of the forest landscapes in the study region is therefore a high priority. Based on our field investigations, it is clear that infrastructure construction and forest management activities considerably reduce and threaten *Lobaria* habitats in regions that have recently been under intensive development, especially in the forest landscape of Bomi (Linzi) where the reported species currently represent the highest diversity of green-algal *Lobaria* worldwide. Effective conservation strategies for the Himalayan and Hengduan Mountain forest landscapes must therefore ensure that these unique cradles of biodiversity do not turn into graves for biodiversity (Rangel et al. 2018), including the lichen genus *Lobaria*.

**Acknowledgements** We sincerely thank Xinyu Wang at the Lichen Fungarium of the Kunming Institute of Botany, CAS for providing sampling support, Christine Keller for providing chemical diagnostic features, Melissa Dawes for careful linguistic revision of the manuscript, and Markus Schlegel for guidance in the use of the PartitionFinder software. This work was supported by the Swiss National Science Foundation (grant JRP IZ70Z0\_131338/1 to CS), the National Natural Science Foundation of China (No. 31970022, 31670028), the Second Tibetan Plateau Scientific Expedition and Research Program (No. 2019QZK0503), and the China Scholarship Council.

**Declaration on conflict of interest** The authors declare that there is no conflict of interest.

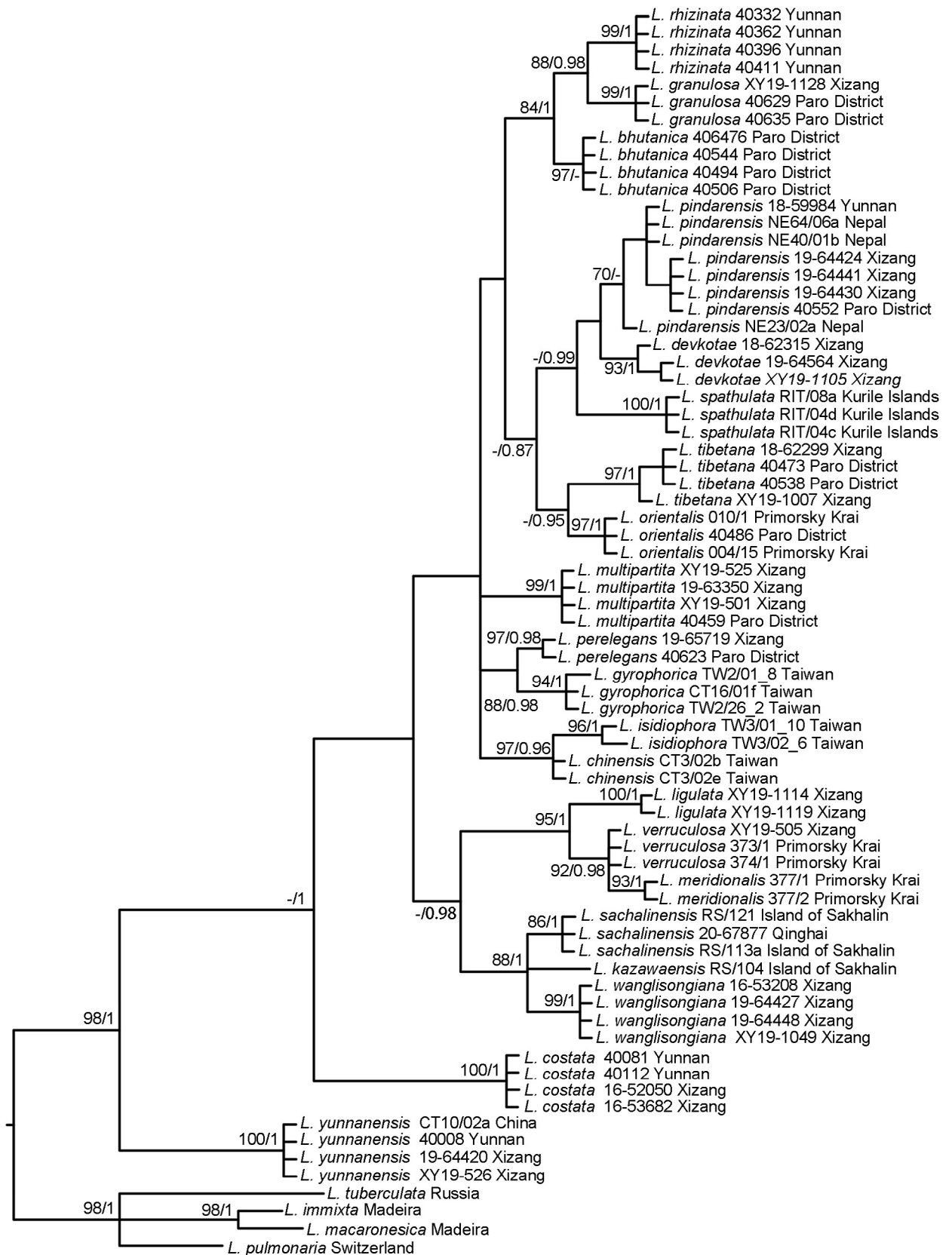
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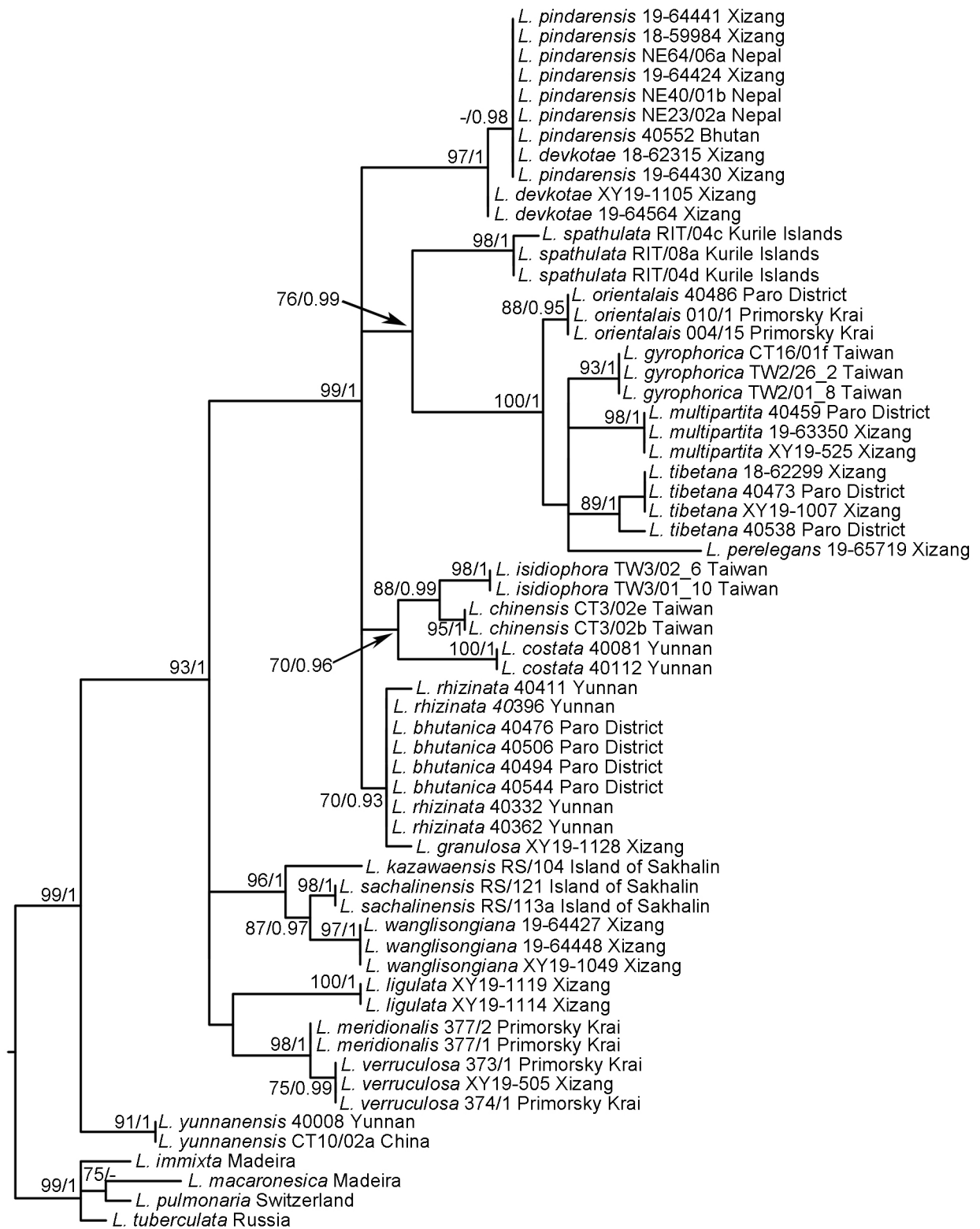
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**Appendix 1** The best partition schemes and nucleotide substitution models selected by PartitionFinder for both single gene and 3-gene dataset applied in phylogenetic analyses.

| Dataset     | MryBayes   |   | RAxML  |                                    |
|-------------|--|---|--|------------------------------------|
|             | Partition scheme   | Model   | Partition scheme   | Model                              |
| ITS         | ITS1, ITS2<br>5.8S   | TRN+G<br>K80+I  | ITS1, ITS2<br>5.8S   | GTR+G<br>GTR+I+G                   |
| <i>EF1α</i> | <i>EF1α</i> _introns<br><i>EF1α</i> -1_codon 1, <i>EF1α</i> -1_codon 2<br><i>EF1α</i> -1_codon 3   | K80+G<br>F81<br>K80                                     | <i>EF1α</i> _introns, <i>EF1α</i> -1_codon 3<br><i>EF1α</i> -1_codon 1, <i>EF1α</i> -1_codon 2   | GTR+G<br>GTR+G                     |
| <i>RPB2</i> | <i>RPB2</i> _codon 3<br><i>RPB2</i> _codon 1<br><i>RPB2</i> _codon 2   | K80+G<br>TRN+I<br>F81                                   | <i>RPB2</i> _codon 3<br><i>RPB2</i> _codon 1<br><i>RPB2</i> _codon 2   | GTR+G<br>GTR+I+G<br>GTR+G          |
| 3-gene      | ITS1, ITS2<br>5.8S<br><i>EF1α</i> -1_codon 1, <i>RPB2</i> _codon 1<br><i>EF1α</i> _introns<br><i>RPB2</i> _codon 2, <i>EF1α</i> -1_codon 2<br><i>EF1α</i> -1_codon 3<br><i>RPB2</i> _codon 3 | HKY+I<br>K80+I<br>TRN+I<br>K80+G<br>F81<br>K80<br>K80+G | ITS1, ITS2<br><i>RPB2</i> _codon 1, 5.8S, <i>EF1α</i> -1_codon 1<br><i>EF1α</i> -1_codon 3, <i>EF1α</i> _introns<br><i>EF1α</i> -2_codon 2, <i>RPB2</i> _codon 2<br><i>RPB2</i> _codon 3 | GTR+G<br>GTR+I+G<br>GTR+G<br>GTR+G |

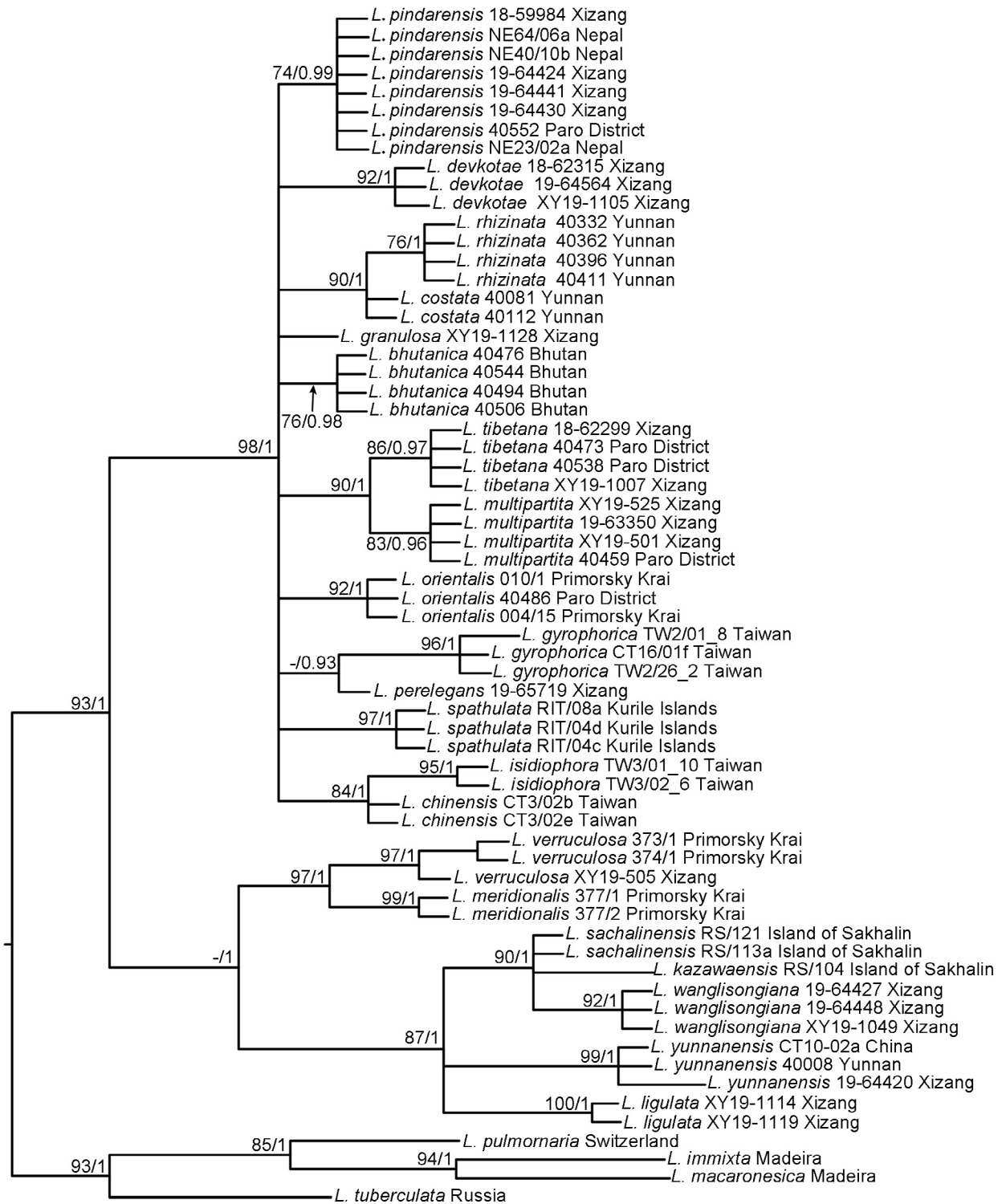


**Appendix 2** Phylogenetic tree of green-algal *Lobaria* inferred from BI analysis with branch support obtained by ML and BI analyses based on ITS sequences. Branch support values are indicated by numbers above branches (MLB/BPP). - represent MLB < 70 % or BPP < 0.95.



**Appendix 3** Phylogenetic tree of green-algal *Lobaria* inferred from BI analysis with branch support obtained by ML and BI analyses based on *EF-1 $\alpha$*  sequences. Branch support values are indicated by numbers above branches (MLB/BPP). - represent MLB < 70 % or BPP < 0.95.





**Appendix 4** Phylogenetic tree of green-algal *Lobaria* inferred from BI analysis with branch support obtained by ML and BI analyses based on *RPB2* sequences. Branch support values are indicated by numbers above branches (MLB/BPP). - represent MLB < 70 % or BPP < 0.95.