A Note on the Lichen Genus Ramalina (Ramalinaceae, Ascomycota) in the Hengduan Mountains in China

Soon-Ok Oh¹, Xin Yu Wang², Li Song Wang², Pei Gui Liu² and Jae-Seoun Hur¹*

¹Korean Lichen Research Institute, Sunchon National University, Sunchon 540-742, Korea
²Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences, Heilongtan, Kunming, Yunnan 650204, China

Abstract On the basis of extensive field investigation and a series of herbarium specimen identifications, we present and discuss the descriptions and distribution of 22 species of Ramalina found in the Hengduan Mountains of southwestern China. In this revisionary study, representatives of the Ramalina genus, including R. americana, R. confirmata, R. dendroiscoides, R. obtusata, R. pacifica, R. pentecostii, R. peruviana, R. shinanoana, and R. subcomplanata are found for the first time in this area. In addition, R. holstii is reported for the first time China. Finally, a newly described species identified as Ramalina hengduanshanensis S. O. Oh & L. S. Wang is reported. It is characterized as growing from a narrow holdfast, solid, sparsely or richly and irregularly dichotomously branched, palmate and flattened lobes with distinctly dorsiventral appearance, surface rugose to reticulate, surface rugosely cracked, dense chondroid tissue, helmet shaped soralia at the tip. The species grows on rock and tree at the highest elevations in this area. Although very few lichen species belonging to the genus Ramalina have been collected above 4,000 m, this new species is found at this elevation. We present detailed morphological, anatomical, and chemical descriptions of this species along with molecular phylogenetic analysis of the internal transcribed spacer rDNA sequences.

Keywords Hengduanshan, Key, Molecular phylogeny, New species

The Hengduan Mountains are the biggest series of parallel north-south ranges in China, extending from Sichuan and Yunnan provinces to eastern Tibet, lying between 22°-32°05’ N and 97°-103° E. Alternating high ridges and deep valleys range from 2,000 to 6,000 m above the sea level. The genus Ramalina belongs to the family Ramalinaceae and is a widespread fruticose lichen genus containing about 200 species worldwide, with about 50 taxa described to date in China [1]. The genus Ramalina has been studied in the Northern Hemisphere for over two hundred years and several hundred taxa have been named [2]. In the Southern Hemisphere, by contrast, very little research has been undertaken. The genus Ramalina was first described by Acharius when it was distinguished from Parmelia by its cartilaginous thallus and apothecial characteristics [3]. It was later subjected to monographic treatment on a global basis by Nylander [4]. Although several groups within the genus have been revised by Kashiyadani and other lichenologists [5-11], certain species found in Japan and its neighboring countries still require more precise study. Recently, Kashiyadani et al. [12, 13] have described seven new taxa, including R. alquistii Vain., R. hokkaidensis Kashiw., R. intermedia (Delise ex Nyl.) Nyl., R. obtusata (Arnold) Bitt., R. pertusa Kashiw., Ramalina pentecostii Krog & Swinsc., and R. sekika from China, and in addition have revised the genus Ramalina in Taiwan. Zhao et al. [14] described two new species, R. americana Hale. and R. aspera Räsänen Ann. from the Qinling Mountains in China. Ohmura et al. [15] revised three species, R. pollinaria (Westr.) Ach., R. sekika Asahina, and R. yasudae Räsänen based on morphological and chemical characteristics as well as a molecular phylogenetic analysis using internal transcribed spacer (ITS) rDNA sequences. Since 1991, there have been few or no studies pertinent to China, and no revisional work had been carried out in the Hengduan Mountains before the present study. As a result, many taxonomic and...
nomenclatural problems remain in the description of the Ramalina genus in China. Therefore, the purpose of the present study is to revise the taxonomy of the Ramalina species found in the Hengduan Mountains.

MATERIALS AND METHODS

Characterization of phenotype. A total of 354 specimens were collected from Sichuan, Tibet, and Yunnan provinces of China between 1980 and 2010 and stored at the Cryptogamic Herbarium of the Kunming Institute of Botany (HKAS) at the Chinese Academy of Sciences. Description of the external morphology was based on air-dried material observed under a dissecting microscope (C-PS 1068908; Nikon, Tokyo, Japan). To evaluate anatomical variation within the thallus and the apothecia, hand-cut sections were prepared. The anatomy of the specimens was examined with standard light microscopic procedures (BX 50F4; Olympus, Tokyo, Japan). The microcrystallization and color tests of lichen substances were conducted as described previously [16]. Thin-layer chromatography (TLC) was carried out by a standardized method [17] using a DC Platten Kieselgel 60 F254 instrument (Merck, Darmstadt, Germany) and a three solvent system: Solvent A (benzene : dioxane : acetic acid = 180 : 45 : 5), Solvent B (hexane : methyl tert-butyl ether : formic acid = 140 : 72 : 18), Solvent C (toluene : acetic acid = 200 : 30).

Molecular phylogeny. Sequences of the ITS were amplified from selected specimens of Ramalina and a total of 17 new sequences were generated for this study. DNA was extracted using the QIAGEN DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) amplification of the ITS1, ITS2, and 5.8S rDNA region was performed using the previously described primer sets ITS4 [18] and ITS5 [18]. Amplifications were performed using the AmpliTaq DNA polymerase with buffer conditions recommended by the manufacturer and the following reaction parameters: 30 cycles of 45 sec denaturation at 94°C, 45 sec annealing at 55°C, and 1 min extension at 72°C, followed by 1 cycle of 5 min extension at 72°C. The PCR products were electrophoresed on an agarose gel to verify product size, and the remaining product was then purified using the PCR Purification Kit (Qiagen) and was sequenced.

The sequences obtained from each sample were aligned with selected sequences of Ramalina from GenBank, by using Usnea diffracta (DQ232663, DQ394374) as the outgroup. Details of the GenBank accession numbers are presented in Table 1. Alignments for each sequence were assembled separately in BIOEDIT 7.0.9 [19] and the sequences were initially aligned using CLUSTALX 1.83 [20]. The ITS rDNA phylogenetic analysis was conducted using neighbor-joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) methods in MEGA ver. 5 [21]. For the NJ analysis, the Tamura-Nei model was used.

Table 1. Voucher specimens and GenBank accession numbers of the internal transcribed spacer sequences used in the phylogenetic analysis

| Lichen species | Species and herbarium accession No. | GenBank accession No. |
|----------------|------------------------------------|-----------------------|
| Usnea diffracta | -                                  | DQ232663              |
| Usnea diffracta | -                                  | DQ394374              |
| Ramalina americana | -                              | AF109235              |
| R. americana | 06-26100                           | JF923601              |
| R. aspera | 06-26284                           | HQ845884              |
| R. aspera | KoRI No. 003908                    | JF937044              |
| R. aspera | KoRI No. 004051                    | JF937043              |
| R. calicaris | KoRI No. 003686                    | JF937045              |
| R. calicaris | KoRI No. 003714                    | JF937042              |
| R. conduplicans | 06-26202                          | JF937000              |
| R. conduplicans | -                                 | AB362789              |
| R. conduplicans | -                                 | DQ394391              |
| R. confirma | 06-26195                           | JF937009              |
| R. confirma | 07-28833                           | JF937006              |
| R. fraxinea | -                                  | AF249907              |
| R. fraxinea | -                                  | AY462054              |
| R. hengduanshanensis | 02-21466                   | JF937006              |
| R. pollinaria | 07-29001                           | JF937012              |
| R. pollinaria | -                                  | AB362794              |
| R. pollinaria | -                                  | AB362795              |
| R. pollinaria | -                                  | EF32560               |
| R. sinensis | 06-26194                           | JF937011              |
| R. sinensis | KoRI No. 003711                    | JF937050              |
| R. sinensis | KoRI No. 003747                    | JF937053              |
| R. sinensis | KoRI No. 003780                    | JF937051              |
| R. sinensis | KoRI No. 003903                    | JF937052              |
| R. sinensis | KoRI No. 004049                    | JF937049              |
| R. sinensis | -                                  | AB362797              |
| R. yasudae | -                                  | AB362799              |
| R. yasudae | -                                  | AB362800              |
| R. yasudae | -                                  | AB362801              |
| R. yasudae | -                                  | AB362802              |
| R. yasudae | -                                  | AB362803              |
| R. yasudae | -                                  | AB362804              |
| R. yasudae | -                                  | AB362809              |
| R. yasudae | -                                  | AB362810              |
| R. yasudae | -                                  | AB362811              |
| R. yasudae | -                                  | AB362812              |
| R. yasudae | -                                  | DQ394394              |
| R. yasudae | -                                  | EF544573              |

Accession numbers in bold font represent new sequences generated in this study.

One thousand bootstrap replications were performed to assess confidence values for each tree. The MP analysis was obtained using the subtree pruning regrafting method with a search level of 1, in which the initial trees were obtained with the random addition of sequences (10 replicates). All positions containing gaps and missing data were eliminated from the dataset (Complete Deletion option). The ML analysis was performed using parameters that included uniform rates among sites, the nearest
neighbor interchange heuristic method, and all gaps being treated as missing data.

**RESULTS AND DISCUSSION**

The newly derived sequence of *Ramalina hengduanshanensis* was aligned with sequences obtained from GenBank as listed in Table 1. The ML tree based on the *Ramalina* sequences is shown in Fig. 1. The operational taxonomic units (OTUs) of all species formed a monophyletic clade (> 95% in ML, NJ, and MP). The *R. pollinaria* clade was not related to the OTUs of *R. yasudae*, suggesting that *R.

![Fig. 1. Phylogeny of Ramalina species as inferred from rDNA analysis to investigate the phylogenetic placement of R. hengduanshanensis. Maximum likelihood tree obtained using the program MEGA ver. 5. Thick branches indicate bootstrap support > 95% and support values given (bootstrap values from maximum likelihood neighbor-joining maximum parsimony). Characters are indicated as follows: A, apothecial clade; S, soralial clade; P, pseudocyphellae clade.](image-url)
pollinaria and \textit{R. yasudae} should be treated as different species \cite{15}. \textit{R. hengduanshanensis} resembles \textit{R. sinensis} in having similar branches. However, it can be distinguished from the latter species by the absence of soralia. \textit{R. hengduanshanensis} also resembles \textit{R. pollinaria} upon molecular analysis, but it can be distinguished from the latter species by the sublabiliform powdery soralia at the apices of the branches. Neither the \textit{R. pollinaria} clade nor the \textit{R. sinensis} clade was related to the OTUs of \textit{R. hengduanshanensis}. The support value for the branching of the clade, including \textit{R. hengduanshanensis} and the other species, is relatively lower compared to that of other monophyletic clades (61\%, 44\%, and 59\% by ML, NJ, and MP, respectively). The monophyly of other species could not be confirmed within the clade. Hence, \textit{R. hengduanshanensis} was determined to be a different species by both morphological and molecular phylogenetic characteristics, and therefore, is reported as such.

\section*{Key to the \textit{Ramalina} species in the Hengduan Mountains in China}

1. Thallus hollow .................................................................... 2
1a. Thallus solid ..................................................................... 3
2. Saxicolous, soredia absent \textit{--- R. almquistii} 2a. Corticolous, soredia present \textit{--- R. obtusata} 2b. Corticolous, soredia absent ..................................................................... 4
3. Soredia present .................................................................... 4
4. Soredia absent .................................................................... 14
4a. Pseudocyphellae absent \textit{--- R. hengduanshanensis} 4a. Pseudocyphellae present ..................................................................... 5
5. Marginal, linear pseudocyphellae ..................................................................... 6
5a. Punctiform, ellipsoid, rounded pseudocyphellae ..................................................................... 7
6. Cracked chondroid tissue, soralia parietal, marginally in cracks between the upper and lower cortex \textit{--- R. pentecostii} 6a. Not cracked chondroid tissue \textit{--- R. pacifica} 7. Soralia very irregular in shape, at or close to the lobe tips ..................................................................... 8
7a. Soralia round, elliptical, or elongate; branch tips tapering ..................................................................... 9
8. Branches narrow or broad, 0.5--3 mm wide; soredia mainly on the lower surface of the branch tips, not developing within hood-shaped expansions; soralia subterminal, terminal ..................................................................... \textit{R. pollinaria} 8a. Branches narrow, 1 mm wide; soredia terminal, laminal, bilateral laciniae \textit{--- R. yasudae} 9. Isidia-like branchlets ..................................................................... 10
9a. Lacking isidia-like branchlets ..................................................................... 11
10. Chondroid tissue cracked \textit{--- R. hossei} 10a. Chondroid tissue not cracked \textit{--- R. peruviana} 11. Soredia granular, usually concentrated at the branch tips ..................................................................... 12
11a. Soredia marginal or laminal, not concentrated at the branch tips ..................................................................... 13
12. Common holdfast, shrubby, weakly canaliculated; chondroid tissue not cracked \textit{--- R. dendriscoides} 12a. Delimited holdfast; chondroid tissue cracked \textit{--- R. intermedia} 13. Soralia mainly elliptical farinose, on the branch margins, and occasionally on the surface; branches mostly 0.5--3 mm wide ..................................................................... \textit{R. farinacea} 13a. Soralia subgranular, laminal, subterminal; finely dissected branchlets ending in nodules; branches mostly 1 mm wide ..................................................................... \textit{R. shinanoana} 14. Apothecia on the branch surface or margins \textit{--- R. pollinaria} 15. Warts or tubercles abundant on the branches; branches broad, up to 5 mm wide \textit{--- R. aspera} 15a. Warts or tubercles abundant on the margin; Branches narrow, up to 2 mm wide \textit{--- R. holstii} 16. Branches canaliculated ..................................................................... 17
17. Branches with depressions and ridges, or with long grooves, but without tubercles ..................................................................... 19
17a. Pseudocyphellae rare, chondroid tissue not cracked, spore ellipsoid \textit{--- R. calicaris} 18. Narrow curved spore ca. 12.5 \textmu m long and 3.8 \textmu m wide \textit{--- R. confirma} 19. Broad curved spore ca. 15 \textmu m long and 5 \textmu m wide \textit{--- R. subcomplanata} 19a. Laciniae 1--1.5 cm wide; palmately branched; small curved spores 5 \textmu m long and 2.5 \textmu m wide \textit{--- R. sinensis} 19a. Laciniae 1--5 mm wide ..................................................................... 20
20. Ellipsoid, short spores 12.5 \textmu m long and 5 \textmu m wide \textit{--- R. americana} 20a. Broad spores 15 \textmu m long and 6 \textmu m wide ..................................................................... 21
21. Fusiform pseudocyphellae common, short branch ..................................................................... \textit{R. conduplicans} 21a. Ellipsoid pseudocyphellae rare, long branch \textit{--- R. fraxinea} \section*{The new species.}
\textit{Ramalina hengduanshanensis} S. O. Oh \& L. S. Wang sp. nov. (Fig. 2)

MycoBank no.: MB 809857.
Type: Sichuan Prov., Xiangcheng County, Mt. Daxue, 12 Sep 2002, L. S. Wang, 18823 (KUN: holotype).

\section*{Brief description of morphology and anatomy:}
Habitat: corticolous, saxicolous, fruiticose; Growth form: caespitose, growing from a narrow holdfast; Thallus: solid, sparsely, or richly and irregularly dichotomously branched, erect, palmate, and flattened lobes with distinctly dorsiventral appearance, surface rugose to reticulate, surface rugosely cracked, up to 1.5 mm wide, up to 3--5 cm high; Cortex: distinct, ca. 12.5 \textmu m thick; Medulla: lacking, dense; Chondroid tissue: irregular in thickness, ca. 75 \textmu m thick, not cracked; Pseudocyphellae: absent; Soralia: apical on lower side, helmet-shaped; Soredia: granular; Apothecia: not seen.

\section*{Chemistry:}
Medulla K--; C--. TLC: usnic acid

\section*{Etymology:}
The specific epithet "hengduanshanensis" refers to the Hengduan Mountains.
**Remark:** *Ramalina hengduanshanensis* resembles *Ramalina sinensis* in having similar branches. However, it can be distinguished from the latter species by the absence of soralia. *R. hengduanshanensis* also resembles *R. pollinaria*, although it can be distinguished from the latter species by the sublabriform powdery soralia at the apices of branches. Most lichens have specific requirements with regard to moisture, light, and temperature, effectively restricting their distribution range. In the Hengduan Mountains, the variation in altitude is from 2,000 m to almost 6,000 m. At about 2,000 m *R. dendriscoideus* is found, which is another species mostly collected between 2,000 and 3,500 m, up to 4,000 m. From about 4,000 m upward, the new species *R. hengduanshanensis* is found. Since very few lichens have been collected above 4,000 m, the upper distribution limit for these species is not yet known.

**Selected specimens examined:** Sichuan Prov.: Kangding Co., Mt. Zheduo, on rock, elev. 4,000 m, 7 Sep 1996, L. S. Wang, 96-16339; Xiangcheng Co., Mt. Daxue, on bark of *Larix* sp., N 31°13', E 102°46', elev. 3,800 m, 2 Jun 2006, L. S. Wang, 06-26065; Tibet: Basu Co., Ranwu village, on bark of *Juniperus* sp., N 29°29', E 096°42', elev. 4,050 m, 19 Aug 2007, L. S. Wang, 07-28103.

**Other Ramalina spp.**

*Ramalina almquistii* Vain., Ark. Bot. 8: 17 (1909).

**Brief description of morphology and anatomy:** Thallus saxicolous, fruticose, shrubby and fistulose thallus forming indistinct holdfast at the base, absence of pseudocyphellae and soredia, chondroid tissue not cracked, common apical-terminal apothecia, shortly fusiform ascospores are 18 μm long and 8 μm wide in size.

**Chemistry:** Medulla K−, C−. TLC: usnic acid, divaricatic acid.

**Ecology and distribution:** This species has been previously reported in China [13], Kuril Islands [22], Japan [23], and South Korea [24]. In China, it was found growing on rock in mountainous regions at altitudes ranging from 3,230 to 3,700 m.

**Selected specimens examined:** Yunnan Prov.: Huize Co., Dahai, on rock, elev. 3,700 m, L. S. Wang, 96-17149, L. S. Wang, 96-16702, L. S. Wang, 96-16721; Binchuan Co., Mt. Jizhu, Jinding, on rock, alt. 3,200 m, L. S. Wang, 02-21070 and on rock, elev. 3,230 m, L. S. Wang, 06-26143.

*Ramalina americana* Hale. Bryologist 81: 599 (1978).

**Brief description of morphology and anatomy:** Thallus corticolous, fruticose, shrubby, delimited holdfast, solid, with strong ridges and channels, flat, side branches common, pseudocyphellae rare, laminal and marginal, chondroid tissue not cracked, apothecia common, subterminal, ellipsoidal ascospores are 12.5 μm long and 5 μm wide in size.

**Chemistry:** Medulla K−, C−. TLC: usnic acid.

**Ecology and distribution:** This species has been previously reported in North America [25] and China [14] where it grows on bark in mountainous regions at altitudes ranging from 1,900 to 2,900 m.

**Selected specimens examined:** Yunnan Prov.: Gongshan Co., Binzhongluo, Zhongdi village, elev. 3,800 m, L. S. Wang, 05-24282; Xuchiong Co., Mt. Zixi, N 25°04', E 101°24', elev. 2,300 m, L. S. Wang, D. L. Niu, Luo Heng, 05-25253; Songming Co., A-ziying village, Shujie, elev. 2,200 m, L. S. Wang, Xin-yu Wang, Meng-meng Liang, 10-31340.

**Remark:** Small specimens of *R. sinensis* are difficult to distinguish from *R. americana*. The latter species is more abundantly branched and never produces fan-shaped thalli.

*Ramalina aspera* Räsänen Ann. Bot. Soc. Zool. Bot. Fenn. Vanamo 20: 5 (1944).

**Brief description of morphology and anatomy:** Thallus corticolous, fruticose, sparingly branched, growing from a narrow holdfast, flat, branches solid, pseudocyphellae common, laminal, raised, tuberculate, chondroid tissue not cracked, apothecia common, subterminal, straight or slightly curved, shortly fusiform ascospores are 13 μm long and 5 μm wide in size.

**Chemistry:** Medulla K−, C−. TLC: usnic acid, divaricatic acid.

**Ecology and distribution:** This species has been previously reported in North America [25] and China [14] where only two collections have been made on bark in mountainous regions at 3,750 m.

**Selected specimens examined:** Sichuan Prov.: Kangding Co., Mt. Jiaozixue, N 26°03', E 102°05', elev. 3,245 m, L. S. Wang, 06-26143.
Yunlong Co., Mt. Ziben, N 25°44', E 99°03', L. S. Wang, 00-18924; Luquan Co., Zhuanglong village, Mt. Jiaozixue, N 26°04', E 102°50', elev. 2,567 m, L. S. Wang, S. O. Oh, 06-26100; Lijiang Co., Mt. Laojun, N 25°59', E 99°53', elev. 2,955 m, L. S. Wang, S. O. Oh, CH050258.

**Remark:** *R. aspera* might be confused with *R. complanata*, which differs in having a more flattened surface of the branches with cracked chondroid tissue and in containing salazinic acid.

**Ramalina calicaris** (L.) Fr. Fries, Sched, Critic: 17 (1824).

**Brief description of morphology and anatomy:** Thallus corticolous, fruticose, shrubby, growing from a delimited holdfast, branches solid, flat, channeled towards base, pseudocyphellae rare, lateral, marginal, tuberculate, chondroid tissue not cracked, apothecia common, terminal or subterminal, elliptical ascospores are 12.5 μm long and 5 μm wide in size.

**Chemistry:** Medulla K—, C—. TLC: usnic acid, homosekikaic acid, sekikaic acid.

**Ecology and distribution:** This species has been previously reported in Europe [26] and China [14] where it grows on bark at elevations between 1,350 and 3,500 m.

**Selected specimens examined:** Sichuan Prov.: Huili Co., Mt. Longzhou, on bark of *Rhododendron* sp., elev. 3,000~3,500 m, L. S. Wang, 97-17946; Yunnan Prov.: Baoshan Pref., Longlin Co., Mt. Liang, elev. 2,300 m, L. S. Wang, 26-2002; Chuxiong Co., Mt. Zixi, N 25°04', E 101°24', elev. 2,330 m, L. S. Wang, 05-25270; Dali Co., Mt. Cang, N 25°42', E 100°06', elev. 3,200~3,465 m, L. S. Wang, S. O. Oh, D. L. Niu, 06-26855; Dongchuan Co., Yaoshan village, Mt. Jiaozi xue, elev. 2,322 m, L. S. Wang, 07-27781; Lijiang Pref., Mt. Tiejia, elev. 2,600 m, L. S. Wang, 85-0226; Yongde Co., Wumulong village, Mt. Daxue, elev. 3,500 m, L. S. Wang, 07-27631.

**Remark:** *R. complanata* is widely distributed in eastern Asia, having been collected in China, Japan, Korea, and the Himalayas. This species is highly variable morphologically and chemically. The holotype of this species is a fragmental specimen having dorsiventral main lobes with sparse secondary branches tapering towards the apices. Most of the specimens from China have wide and canaliculated main branches.

**Ramalina confirmata** (Nyl.) Elix Lichenes Australasici Excipicati 5: 219 (1990).

**Brief description of morphology and anatomy:** Habitat: corticolous, fruticose; Growth form: erect to subpendulous; Thallus: branching subdichotomous, sparse to moderate, branches compressed, narrow and canaliculated to broad and flat, apices attenuate or blunt, surface smooth to rugose, up to 1.5 mm wide, up to 6 cm high; Cortex: distinct, ca. 25 μm thick; Medulla: dense; Chondroid tissue: discontinuous, cracked, ca. 75 μm thick; Pseudocyphellae: short linear pseudocyphellae; Soralia: absent; Apothecia: common, marginal, subterminal, disc concave to plane, margin entire; Ascospores: elliptoid, straight, or curved, 12.5 μm long and 3.8 μm wide.

**Chemistry:** Medulla K—, C—. TLC: usnic acid.

**Ecology and distribution:** This species has previously been reported in Australia [3]. In China, it was collected on bark of various species (*Quercus, Salix*) at elevations between 1,460 and 3,400 m.

**Selected specimens examined:** Sichuan Prov.: Dukou Co., Dabaoding, on bark of *Quercus* sp., elev. 2,000 m, L. S. Wang, 83-221; Tibet: Shangri La, Mulang 88; Yunnan Prov.: Gongshan Co., Bingxiongluo, Mt. Songtазве, N 28°09', E 98°33', elev. 2,500 m, L. S. Wang, 00-19205; Lijiang Co., Mt. Laojun, on bark of *Salix* sp., N 26°39', E 99°46', elev. 3,000 m, L. S. Wang, 05-25104; Pingbian Co., Mt. Dawei, N 22°58', E 103°42', elev. 1,530 m, L. S. Wang, 05-23909; Yunlong Co., Caojian village, Ziben Mt., on bark of *Salix* sp., N 27°44', E 99°03', elev. 3,000 m, L. S. Wang, 05-24390; Bingchuan Co., Mt. Jizu, on bark of *Quercus* sp.,
Gongga Mt., N 29° in Brazil [4] and East Africa [27]. In China, only two this species has been reported Ecology and distribution: Chemistry: Brief description of morphology and anatomy: Habitat: corticolous, fruticous; Growth form: shrubby, developing from a restricted or common holdfast; Thallus: richly and irregularly branched, up to 3 cm high, solid, less than 1 mm wide, weakly canaliculated in main branches, sorediate; Cortex: distinct, ca. 10 µm thick; Medulla: dense; Chondroid tissue: not cracked, discontinuous, dissected by the medullary hyphae, ca. 12.5–65 µm thick; Pseudocyphellae: small, elliptic, flat; Soralia: terminal, apical, restricted to short, secondary branches; Soredia: granular; Apothecia: not seen. Chemistry: Medulla K−, C−. TLC: salazinic acid. Ecology and distribution: This species has been reported in Brazil [4] and East Africa [27]. In China, only two collections were made on bark at elevations between 1,840 and 2,450 m. Selected specimens examined: Sichuan Prov.: Luding Co., Gongga Mt., N 29°20′, E 101°30′, elev. 2,450 m, L. S. Wang, 96-17327; Yunnan Prov.: Luchun Co., Fengshuling, N 23°59′, E 102°26′, elev. 1,840 m, L. S. Wang, 05-23992. Remark: R. dendriscoides has been confused with R. persiviana, a species with very similar subterete branches and mode of branching, with a primary difference being the lateral rather than mainly apical soralia. In addition, R. persiviana is known to produce sekikaic acid. R. tenella, a coastal species, also resembles R. dendriscoides in its apical, subapriculate soralia and in the production of salazinic acid, but it has shorter and distinctly flattened main branches. Ramalina farinacea (L.) Ach. Lichenogr. Univers.: 602 (1810). Brief description of morphology and anatomy: Habitat: corticolous, fruticose; Growth form: coarsely tufted, rarely monopodial, subpendulous; Thallus: laciniae compressed, twisted, longitudinally and partly reticulate, variable in width, more or less irregularly tapered towards the base and the apices, planar or variously channeled, up to 1–3 mm wide, more than 7 cm high; Cortex: distinct ca. 12.5 µm thick; Medulla: loose; Chondroid tissue: discontinuous, not cracked, ca. 50 µm thick; Pseudocyphellae: frequent, pale, rounded or ovate-elongate; Apothecia: usually frequent, marginal or laminal, disc concave to plane; Ascospore: ellipsoid, 15 µm long and 6.25 µm wide. Chemistry: Medulla K−, C−. TLC: usnic acid. Ecology and distribution: This species has been reported previously in Europe [26], the distribution range now includes China where it grows on various barks (Quercus, Pinus, and Rhododendron) at elevations between 1,760 and 3,500 m. Selected specimens examined: Sichuan Prov.: Huili Co., Mt. Longzhou, on bark of Rhododendron sp., elev. 3,000–3,500 m, L. S. Wang, 96-18036; Yunnan Prov.: Dali Co., Mt. Cang, on bark of Quercus sp., elev. 2,500 m, L. S. Wang, Y. Q. Xiao, 04-23545 and 04-32420, on bark of Pinus armandii, N 25°4′, E 100°6′, elev. 3,400 m, L. S. Wang, 05-24657 and 05-24658; Songming Co., Guo dong, Mt. Zhaobi, elev. 2,400 m, Y. G. Su, 11; Yunnan Prov., on bark of Cinnamomum sp., N 23°07′, E 102°44′, elev. 1,760 m, L. S. Wang, 05-23940. Remark: The narrow-lobed forms of R. fraxinea can resemble R. calicaris. The latter species has smoother, less wrinkled lobes, with uncommon pseudocyphellae. The spores are straight, and are longer and wider than R. calicaris. Ramalina holstii Krog & Swinsc. Sp. Nov. T. D. V. Nor J Bot 22: 275 (1975). Brief description of morphology and anatomy: Habitat: corticolous, fruticose; Growth form: shrubby branches developing from a delimited holdfast; Thallus: greenish yellow, solid, up to 2 mm wide, up to 3–5 cm high, flat or partially canaliculated; Cortex: distinct, 10–12.5 µm thick; Medulla: loose; Chondroid tissue: not cracked, layer discontinuous, 20–70 µm thick; Pseudocyphellae: predominantly...
marginal, tuberculate common; Apothecia: lateral, marginal, on genitalic branches, disc concave to flat, reticulately ridged, common; Hymenium: ca. 30 µm thick; Subhymenium: ca. 12.5−17.5 µm thick; Ascospore: fusiform, slightly curved, two-celled, 12.5 µm long and 2.5−3.7 µm wide.

**Chemistry:** Medulla K−, C−. TLC: salazinic acid.

**Ecology and distribution:** This species has been reported previously in East Africa [27]. In China, only two collections were made on bark at elevations between 2,000 and 3,800 m.

**Selected specimens examined:** Yunnan Prov.: Gongshan Co., Binzhongluo to Tongda Yakou, on bark of Abies sp., N 28°05', E 98°41', elev. 3,800 m, L. S. Wang, 99-18503; Lijiang Co., Shigu to Xionggu village, elev. 2,000 m, L. S. Wang, 09-30062.

**Remark:** Ramalina specimens with tuberculate pseudocyphellae and salazinic acid are often identified as *R. denticulata* (Eschw.) Nyl., described in Brazil [4]. The latter species has predominantly laminal pseudocyphellae and larger spores, 5−6 µm long and 15−17 µm wide, and is lacking in cryptochlorophoric acid.

**Ramalina hossei** Vain. Ann. Soc. Zool-Bot. Fenn, Vanamo 1: 36 (1921).

**Brief description of morphology and anatomy:** Thallus corticolous, fruticose, tufted, richly branched near the base, narrow branches growing from a common holdfast, solid, anisotomic, dichotomous or irregular, often with short side branches ending in granular soredia or tiny branchlets, pseudocyphellae common, orbicular, more or less raised, punctiform, granular soredia, chondroid tissue cracked, apothecia rare, laminal or submarginal, short fusiform ascospores of 15 µm long and 5 µm wide in size, slightly curved.

**Chemistry:** Medulla K−, C−. TLC: usnic acid, homosekikaic acid, sekikaic acid.

**Ecology and distribution:** This species has been previously reported in Taiwan [12] and Bhutan [8]. In China, it was collected on various barks (Abies, Rhododendron etc.) at elevations between 1,900 and 4,000 m.

**Selected specimens examined:** Sichuan Prov.: Huili Co., Mt. Longzhou, elev. 3,000−3,500 m, L. S. Wan, 97-17905; Yunnan Prov., Caojian Co., Mt. Ziben, L. S. Wang, 00-18886; Dali Co., Mt. Cang, elev. 3,400 m, L. S. Wang, Y. Q. Xiao, 04-23445; Deqen Co., Mt. Baimaxue, elev. 3,400 m, L. S. Wang, 93-13568 and on rock, elev. 3,400 m, L. S. Wang, 93-13436; Lijiang Co., Jiubei village, Mt. Laojun, on bark of Rhododendron sp., N 26°39', E 99°46', elev. 3,450 m, L. S. Wang, S. O. Oh, D. L. Niu, 06-26468 and on bark of Abies sp., N 26°39', E 99°46', elev. 3,600 m, L. S. Wang, 05-24738; Zhongdian Co., Dabaosi, on bark of Rhododendron sp., N 27°46', E 99°46', elev. 3,650 m, L. S. Wang, 07-25807 and 04-23381.

**Remark:** *R. intermedia* might be confused with *R. polatinaria*, as they have similar branching pattern and soredia. However, *R. intermedia* can be easily distinguished from the latter species by its laciniae that develop from a narrow holdfast and by having homosekikaic and sekikaic acid as major chemical substances. *R. intermedia* resembles *R. exilis* in having similar branches with soredia. However, it can be distinguished from the latter species by the laminal and marginal soredia, the conspicuously cracked chondroid tissue below the cortex, and by the presence of homosekikaic and sekikaic acids.

**Ramalina obtusta** (Arnold) Bitt. Pingsheim Jahrb. Wiss. Bot. 36: 435 (1901).

**Brief description of morphology and anatomy:** Thallus corticolous, fruticose, shrubby, shortly tufted, growing from a narrow holdfast, fistulose thallus, inflated and more or less pellucid thallus without side branches, consisting of
one single or several palmate or unorientated laciniae, pseudocyphellae absent, farinose soredia, soralia common, mainly developed within terminal or subterminal vesicles, labriform, helmet-shape, chondroid tissue not cracked, apothecia unknown.

**Chemistry:** Medulla K−, C−. TLC: obtusatic acid, evertnic acid.

**Ecology and distribution:** This species has been previously reported in China [13], Europe [26], and North America [25]. In China, the only two collections were made on bark at elevations between 2,000 and 3,000 m.

**Selected specimens examined:** Sichuan Prov.: Nanping Co., Jiuzhaiqiao, on bark, elev. 2,000 m, L. S. Wang, 86-2525; Yunnan Prov., Binchuan Co., Mt. Jizhu, elev. 3,000 m, L. S. Wang, 96-15946.

**Remark:** *R. polnaria* is very similar but does not develop inflated tips. *R. obtusata* can be distinguished by the soralia that are very irregular in size and shape appearing where the branch tips. *R. intermedia* is much more slender, dissolving into irregular sorediate patches near the tips.

**Ramalina pacifica** Asah. J. Jpn. Bot. 15: 213 (1939).

**Brief description of morphology and anatomy:** Habitat: corticolous, fruticose; Growth form: subpendulous to pendulous, tufted; Thallus: branching dichotomously, sparse to moderately dense, branches compressed, flat, solid, usually with longitudinal striae or cracks on the surface, sorediate, up to 1 mm wide, up to 4 cm high; Cortex: distinct, ca. 12.5 μm thick; Medulla: dense; Chondroid tissue: under the cortex not continuous, not cracked, 25–60 μm thick; Pseudocyphellae: linear pseudocyphellae slightly or strongly developed; Soralia: marginal, ellipsoid, well-delimited; Apothecia: rare, stipitate, apical; Disc: flat to convex; Hymenium: ca. 50 μm thick; Subhymenium: ca. 25 μm thick; Ascospore: fusiform or ellipsoid, 12.5 μm long and 7.5 μm wide.

**Chemistry:** Medulla K−, C−. TLC: usnic acid, salazinic acid.

**Ecology and distribution:** This species has been reported in China [13] and in East Africa [27]. In China it was collected on various barks (*Quercus, Rhododendron*) at an elevation of 3,300 m.

**Selected specimens examined:** Sichuan Prov.: Xiaojin Co., Shuangqiaogou, on trunk, elev. 3,300 m, L. S. Wang, 96-17717.

**Remark:** *R. pentecostii* may be confused with *R. disparate* because they both have similar soralia branches. It can be distinguished from the latter species in its parietal, mainly marginal soralia that never spread irregularly on to the lamina, and in the lack of medullary lichen substances.

**Ramalina peruviana** Ach. Lich. Univ.: 1599 (1810).

**Brief description of morphology and anatomy:** Thallus corticolous, fruticose, subpendulous, tufted thallus growing from a common holdfast, solid, pseudocyphellae rare, narrowly elongated, marginal or laminal soralia with granular soredia and tiny branchlets, chondroid tissue not cracked, apothecia not seen in Chinese materials.

**Chemistry:** Medulla K−, C−. TLC: usnic acid, homosekikaic acid, sekikaic acid.

**Ecology and distribution:** This species has previously been reported in China [13], but is also widely distributed throughout the world, including reports from Australia [3], Brazil [4], Chile [28], East Africa [27], Indonesia [29], Japan [23], South Korea [24], and North America [25]. In China, it was collected on various barks (*Camellia, Rhododendron*) at elevations between 2,000 and 3,450 m.

**Selected specimens examined:** Sichuan Prov.: Hueli Co., Mt. Longzhou, on bark of *Rhododendron* sp., elev. 3,450 m, L. S. Wang, 97-24264; Miyi Co., Malong village, Mt. Beipo, elev. 2,800 m, L. S. Wang, 83-995; Yunnan Prov.: Lincang Co., Nouling village, on bark of *Camellia* sp., N 23°19', E 99°22', elev. 2,000 m, Cong-ren Yang, 05-33; Qiaojia Co., Dagiao, elev. 2,700 m, L. S. Wang, 96-16688.

**Remark:** *R. dendriscoides* Nyl. resembles *R. peruviana* in its mode of branching, but it differs in having most of its soralia situated apically on short lateral branchlets, and in containing salazinic acid.

**Ramalina pollinaria** (Westr.) Ach. Lich. Univ.: 608 (1810).

**Brief description of morphology and anatomy:** Thallus corticolous, fruticose, pendulous, sparingly branched, growing from a narrow holdfast, solid, flattened or partly terete,
especially towards the aices, extensively branched and dissected, with numerous small nodulate proliferations, palmate margins, irregular in thickness in cross section, pseudocyphellae common, ellipsoid, laminal, terminal soralia, labiform or developed in terminal helmet-shaped soralia with granular soredia, chondroid tissue not cracked, apothecia not seen.

**Chemistry:** Medulla K−, C−. TLC: usnic acid.

**Ecology and distribution:** This species has previously been reported in China [13], but is also widely distributed throughout the world, including reports from East Africa [26], Europe [27], the Kuril Islands [22], and North America [25]. In China, it was collected on various barks (Quercus, Sorbus) at elevations between 2,900 and 3,540 m.

**Selected specimens examined:** Sichuan Prov.: Kangding Co., Mt. Zhedou, on rock, N 30°02', E 101°49', elev. 3,800 m, L. S. Wang, 07-29001; Yunnan Prov., Dongchuan Co., Hongtudi village, on bark of Quercus sp., elev. 2,900 m, L. S. Wang, 09-30680; Luquan Co., Jiaozixue Mt., on bark of Sorbus sp., N 26°03', E 102°05', elev. 3,245 m, L. S. Wang, 93-13569; Luquan Co., Napa Lake, on branch, N 27°56', E 99°36', elev. 3,540 m, L. S. Wang, S. O. Oh, D. L. Niu, 06-26646 and 06-26653.

**Remark:** *R. polypompha* may also resemble *R. pollinaria*, but the former species has a roughened cortex, coarser soredia, and no medullary substance. Flattened specimens can be confused with *R. farinacea*, but the soralia of *R. pollinaria* are at least partly laminal and the medullary chemistry is different. *R. pollinaria* also resembles *R. chihuahuana* that differs in having marginal soralia and in producing dikaryotic tissue.

**Ramalina shinanoana** Kashiw. Bull. Natl. Sci. Mus. Ser. B Bot. 12: 122 (1986).

**Brief description of morphology and anatomy:** Thallus corticolous, fruticose, growing from a narrow holdfast, solid, the finely dissected branchlets ending in nodules, pseudocyphellae common, ellipsoid, lateral, laminal soralia with subgranular soredia, chondroid tissue not cracked, apothecia rare, laminal, broadly fusiform ascospores are 15 µm long and 6 µm wide.

**Chemistry:** Medulla K−, C−. TLC: homosekikaic acid, sekikaic acid.

**Ecology and distribution:** This species has previously been reported in China [12, 14], and is also widely distributed in Europe [26], the Kuril Islands [22], Japan [23], North America [25], and South Korea [24]. In China, it was collected on various barks (Quercus, Rhododendron, Pinus, etc.) at elevations between 1,800 and 4,300 m.

**Selected specimens examined:** Sichuan Prov.: Daofo Co., Rengu village, on bark of Picea sp., N 30°46', E 101°18', elev. 3,950 m, L. S. Wang, 07-28313; Tibet: Jiayu Co., Zhubenzada, Mu Zang, 67; ZhangMu to Lixing, elev. 2,500–2,700 m, Shu-Kun Luo 50; Yunnan Prov.: Bingchuan Co., Mt. Jizu, on bark of Quercus sp., N 25°58', E 100°21', elev. 3,200 m, L. S. Wang, 06-26194; Dali Co., Mt. Cang, on bark of Pinus armandii, N 25°41', E 100°06', elev. 3,400 m, L. S. Wang, 05-24661; Deqen Co., Mt. Baimaxue, elev. 3,400 m, L. S. Wang, 93-13569; Luquan Co., Mt. Jiaozixue, elev. 3,750 m, L. S. Wang, S. O. Oh, 06-26101; Zhongdian Co., Napa Lake, on bark of Rhododendron sp., N 27°56', E 99°36', elev. 3,540 m, L. S. Wang, S. O. Oh, D. L. Niu, 06-26611.

**Remark:** *R. sinensis* is highly variable in lobe shape and branching, though specimens commonly found in China...
typically have palmate and loosely tufted lobes. It might be confused with \textit{R. fraxinea}, a species that differs in having branches without dorsiventral appearance, the ellipsoid pseudocyphellae, and distinctly curved ascospores.

**Ramalina subcomplanata** (Nyl.) Kashiw. Bull. Natl. Sci. Mus., Ser. B. Bot. 12: 92 (1986).

**Brief description of morphology and anatomy:** Thallus corticolous, fruticose, growing from a narrow holdfast, dichotomously or irregularly branched, solid, surface angular or flat, rarely canaliculated, pseudocyphellae common, marginal or ventral side, more or less convex, chondroid tissue cracked, apothecia subterminal, rarely lateral, broadly ellipsoid ascospores that are 15 \( \mu \text{m} \) long and 5 \( \mu \text{m} \) wide.

**Chemistry:** Medulla K−, C−. TLC: usnic acid, homosekikaic acid, sekikaic acid.

**Ecology and Distribution:** This species has been reported in China [14], where it grows on various barks (Rhododendron, Salix etc.) at elevations between 1,700 and 3,540 m.

**Selected specimens examined:** Yunnan Prov.: Long-chuan Co., Tongbiguang, elev. 1,700 m, L. S. Wang, 97-18101; Zhongdian Co., Napa Lake, on bark of \textit{Rhododendron} sp., N 27°56′, E 99°36′, elev. 3,540 m, L. S. Wang, S. O. Oh, D. L. Niu, 06-26607; Yunlong Co., Caojian village, Mt. Ziben, on bark of \textit{Salix} sp., N 27°44′, E 99°03′, elev. 3,000 m, L. S. Wang, 05-24388 and N 25°45′, E 99°06′, elev. 2,800 m, L. S. Wang, Qiang Ren, 02-22438.

**Remark:** \textit{R. subcomplanata} resembles \textit{R. calicaris}, though it can be distinguished from the latter species by the strongly pseudocyphellate lobes and by the distinctly cracked chondroid tissue. In \textit{R. calicaris}, the pseudocyphellae are very rare or completely lacking and the chondroid tissue forms no hyphal bundles.

**Ramalina yasudae** Rässänen J. Jpn. Bot. 16: 87 (1940).

**Brief description of morphology and anatomy:** Thallus saxicolous, fruticose, caespitose, erect, growing from a common holdfast, solid, simple or sparingly furcate branches towards the apices, mostly flattened, pseudocyphellae rare, soralia terminal and laminal with granular soredia, chondroid tissue not cracked, apothecia not seen.

**Chemistry:** Medulla K−, C−. TLC: evernic acid, obtusatic acid.

**Ecology and distribution:** This species has been reported in China [15], and is also distributed in Japan [23] and South Korea [24]. In China it was collected on rock or in China [14], where it grows on various barks (\textit{Rhododendron}, Salix etc.) at elevations between 1,700 and 3,540 m.

**Selected specimens examined:** Sichuan Prov.: Kangding Co., Liqiu to Shade, on rock, elev. 3,200 m, L. S. Wang, 96-17546; Kangding Co., Mt. Zhedou, on rock, elev. 3,800 m, L. S. Wang, 07-28995; Mulii Co., Yala Shaoxiangliangzi, elev. 3,800 m, L. S. Wang, 83-1734; Xiaojin Co., Changpinggou, elev. 3,100 m, L. S. Wang, 96-17784; Yunnan Prov.: Deqin Co., Mellishi village, Suola Ya-kou, N 28°38′, E 98°36′, elev. 4,000 m, L. S. Wang, 00-19764; Zhongdian Co., Wengshuei village, Mt. Daxue, N 28°30′, E 99°49′, elev. 3,800 m, L. S. Wang, 00-19843.

**Remark:** \textit{R. yasudae} may be confused with \textit{R. pollinaria} because they both have similar sorediate branches. However, it can be distinguished from the latter species by bilateral laciniae, terminal and laminal soralia, and by granular and slightly corticated soredia.

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