

## Notes on Eight Threatened Species of Lichens in Japan

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**Abstract** New localities and/or molecular data are provided for the following eight threatened species of lichens in Japan: *Chiodecton congestulum*, *Flavopunctelia soledica*, *Icmadophila japonica*, *Lethariella togashii*, *Pseudocyphellaria argyracea*, *Thysanothecium scutellatum*, *Usnea filipendula*, and *U. nipparensis*. ITS rDNA sequences were successfully obtained from all species except for *C. congestulum*, and were compared with the known sequences retrieved from GenBank.

**Key words**: distribution, ITS rDNA, lichenized Ascomycota, Red List.

### Introduction

The current Red List of Japanese lichens is comprised of 152 taxa: 152 species, 1 subspecies, and 3 varieties (Ministry of the Environment, 2007; Inoue *et al.*, 2010). Molecular data of these lichens are of potentially great value for taxonomic, phylogenetic, phytogeographic, and ecological purposes.

During recent field surveys in Japan, fresh materials of eight threatened species were collected in 2009–2010 from both new and already known localities, and the sequences of internal transcribed spacer of ribosomal DNA (ITS rDNA) were successfully obtained from all extracted DNA samples except for *Chiodecton congestulum*. The purpose of this study is to provide information of new localities, and/or the present

situation of each species as well as molecular data.

### Materials and Methods

All collections examined are stored at the National Museum of Nature and Science, Tokyo (TNS) unless otherwise indicated. Two specimens of *Chiodecton congestulum* are stored at Uppsala University (UPS).

Lichen substances were examined by thin layer chromatography (TLC) (Culberson and Johnson, 1982). Only the solvent B system was used here.

Materials for molecular analysis and the associated GenBank accession numbers are shown in Table 1. DNA was extracted from 5–10 mg of lichen thalli by using a FastDNA SPIN Kit (MP Biomedicals) following the method of Ohmura *et*

Table 1. Specimen vouchers for DNA analysis and the GenBank accession numbers obtained in this study

Species	Collection number of Y. Ohmura (in TNS)	GenBank accession no.
<i>Flavopunctelia soledica</i>	7164	AB623069
<i>Icmadophila japonica</i>	6762	AB623070
<i>Lethariella togashii</i>	6735	AB623071
<i>Pseudocyphellaria argyracea</i>	6418	AB623072
<i>Thysanothecium scutellatum</i>	6773	AB623073
<i>Usnea filipendula</i>	6771	AB623074
<i>U. nipparensis</i>	6282	AB623075

*al.* (2006).

PCR amplification of the ITS rDNA region (including ITS1, 5.8S rDNA, and ITS2) was performed using ITS1F (Gardes and Bruns, 1993) as the 5' primer and LR1 (Vilgalys and Hester, 1990) as the 3' primer. PCR reactions were performed according to the method of Ohmura *et al.* (2006) by using PuReTaq Ready-To-go PCR beads (GE Healthcare) and a thermal cycler (Gene Amp PCR system 9700, Applied Biosystems). PCR products were purified using an ExoSAP-IT (GE Healthcare). Sequencing was carried out using a BigDye Terminator ver. 3.1 Cycle Sequencing Kit on an ABI Prism 3130x genetic analyzer (Applied Biosystems) according to the manufacturer's instructions. DNA strands were assembled and manually corrected using ATGC ver. 6.03 (Genetyx). The obtained sequences in this study were compared with the known sequences of related species registered in GenBank by using BLAST, a homology search program (Altschul *et al.*, 1997).

### Results and Discussion

**Chiodecton congestulum** Nyl., Bull. Soc. Linn. Normandie, sér. 2, 2: 106 (1868).

*Chiodecton congestulum* was well revised taxonomically by Thor (2002). This species has been reported from India, Fiji, Australia, Malaysia, and Japan (Thor, 2007). In Japan, it has been known from western Honshu, Kyushu, Ogasawara Islands, and Yaeyama Islands (Thor, 2002). New localities were found at Mt. Horaiji in Aichi Prefecture and Nagatani Shrine in Kochi Prefecture.

Fresh materials of this species were also collected from Miyajima Island in Hiroshima Prefecture. Although DNA was extracted from the specimens, the ITS rDNA sequence could not be obtained.

As reported by Thor (2002), this species grows on rocks and tree barks. The specimens collected from Aichi and Hiroshima Prefectures were saxicolous, and those collected from Kochi Prefecture were corticolous.

Specimen examined. **Japan.** Honshu. Prov. Mikawa (Pref. Aichi): Mt. Horaiji, Kadoya, Horai-cho, Minamishitara-gun, on rock, elevation about 315 m, 5 December 1996, Y. Ohmura 2840. Prov. Aki (Pref. Hiroshima): Mt. Misen, Miyajima Island, Hatsukaichi-city, on rock (granite), elevation 259 m, 12 January 2010, M. Kono (41 and 42), Y. Ohmura & Y. Koda. Shikoku. Prov. Tosa (Pref. Kochi): Nagatani Shrine, Nagasawa, Tsuno-mura Village, on coniferous tree, elevation 590–610 m, 30 October 2006, G. Thor 21515 (UPS, det. G. Thor, not seen); ditto, on stump, G. Thor 21516 (UPS, det. G. Thor, not seen).

**Flavopunctelia soredica** (Nyl.) Hale, Mycotaxon, 20: 682 (1984).

*Flavopunctelia soredica* is a well-known foliose species of Parmeliaceae and is easily distinguished from similar species on the basis of the presence of soralia along the margin of lobes and usnic and lecanoric acids as major substances in the cortex and medulla respectively. Although *F. soredica* is widely distributed in the Northern Hemisphere, its presence is restricted in central Honshu, Japan, where it has been found on barks of *Betula*, *Castanea*, *Chamaecyparis*, *Larix*, *Pinus*, *Prunus*, and *Zelkova* (Asahina, 1941; Kurokawa and Kashiwadani, 1988; Saitama Museum of Natural History, 1988; Kashiwadani, 1991; Harada *et al.*, 2003; Morita and Harada, 2006). Additional specimens were collected from Arakoda (Saku-city) at 730 m alt. and Sugadaira (Ueda-city) at 1577 m alt. in Nagano Prefecture. They were found on barks of *Larix* and *Quercus*. Although the known localities are restricted to a narrow region in Japan, *F. soredica* appears to grow well in these conditions.

ITS rDNA sequences were successfully obtained from two specimens that were both collected from the same locality of Sugadaira but from different trees: *Betula ermanii* and *Quercus crispula*. Each ITS rDNA sequence is 495 bp in length, and there are no differences between them. The known *F. soredica* sequence from New York, U.S.A. [GenBank accession no. AY773128

(Thell *et al.*, 2005)] is different in only two residues from Japanese materials (identity=493/495 bp, 99%).

Specimens examined. **Japan.** Honshu. Prov. Shinano (Pref. Nagano): along the route from Sugadaira Pasture to Mt. Azumaya, Ueda-city, on bark of *Quercus crispula*, elevation 1577 m, 30 May 2010, Y. Ohmura (7119 and 7164) & A. Okamura; ditto, on bark of *Betula ermanii*, Y. Ohmura (7121 and 7167) & A. Okamura; Arakoda, Saku-city, on bark of *Larix leptolepis*, elevation about 730 m, 17 July 1972, H. Kashiwadani 10332.

***Icmadophila japonica*** (Zahlbr.) Rambold & Hertel, Biblioth. Lichenol., 53: 230 (1993). = *Glossodium japonicum* Zahlbr., Bot. Mag. Tokyo, 41: 336 (1927).

*Icmadophila japonica* is a well-known species of lichens under the name of *Glossodium japonicum* Zahlbr. by Japanese lichenologists (see Rambold *et al.*, 1993). This species generally grows on decayed wood of coniferous trees in an old-growth forest, and its distribution is restricted to Far East Russia and Japan. In Japan, it grows widely from Hokkaido to Kyushu (Sato, 1967), but the populations in Japan are decreasing according to recent field surveys (Inoue *et al.*, 2010).

ITS rDNA sequence was successfully obtained from one specimen collected from Mt. O-akan, Hokkaido. The sequence of ITS rDNA region is 466 bp in length. No reference ITS rDNA sequence of *Icmadophila* has been registered in GenBank.

Further molecular phylogenetic analysis based on ITS rDNA sequences and other appropriate markers is needed to examine the independence of *Glossodium* and *Icmadophila* (s. str.) since morphology of the ascomata is quite different between them.

Specimens examined. **Japan.** Hokkaido. Prov. Kushiro: Mt. O-akan, on decayed stump, elevation 494 m, 10 August 2009, Y. Ohmura 6762.

***Lethariella togashii*** (Asahina) Krog, Norw. J.

Bot., 23: 95 (1976).

*Lethariella togashii* was revised taxonomically by Asahina (1952), Krog (1976), and Obermayer (1997). The distribution of this species is restricted to Far East Russia and Japan (Skirina, 2006). In Japan, it has been collected from Hokkaido and central Honshu (e.g. around Lake Yamanaka, Mt. Fuji), but the habitats in the latter locality were heavily damaged and the populations are almost disappeared (Kashiwadani and Inoue, 2000).

The ITS rDNA sequence was successfully obtained from one specimen collected at Mt. O-akan, Hokkaido. The sequence of the ITS rDNA region is 501 bp in length. According to the BLAST result, the top scores of known related species were obtained with *Letharia columbiana* (Nutt.) J.W. Thomson [AF115762 (Thell *et al.*, unpublished); identity=434/461 bp, 94%], *L. vulpina* (L.) Hue [GQ398408 (Altermann *et al.*, unpublished); identity=428/456, 93%], *Lethariella cashmeriana* Krog. [DQ980014 (Crespo *et al.*, 2007); identity=521/457, 92%], and *L. sernanderi* (Motyka) Obermayer [AF297744 (Kroken, unpublished); identity=420/459, 91%]. Although large-scale phylogenetic analysis based on three ribosomal DNA regions and RPB1 gene supports a monophyletic clade consisting of *Letharia columbiana* and *Lethariella cashmeriana* (Crespo *et al.*, 2007), a narrower scale analysis has not yet been performed to confirm generic independence between them. The genus *Letharia* is morphologically separated from *Lethariella* mainly by the lacerate axis. The yellow-colored thallus due to vulpinic acid is distinct in *Letharia* spp., but this substance is also present as a major substance in *Lethariella togashii*. The delimitation and independence between these genera should be revised in the further researches.

Specimen examined. **Japan.** Hokkaido. Prov. Kushiro: Mt. O-akan, on bark of *Picea glehnii*, elevation 728 m, 10 August 2009, Y. Ohmura 6735.

***Pseudocyphellaria argyracea*** (Delise) Vain.,

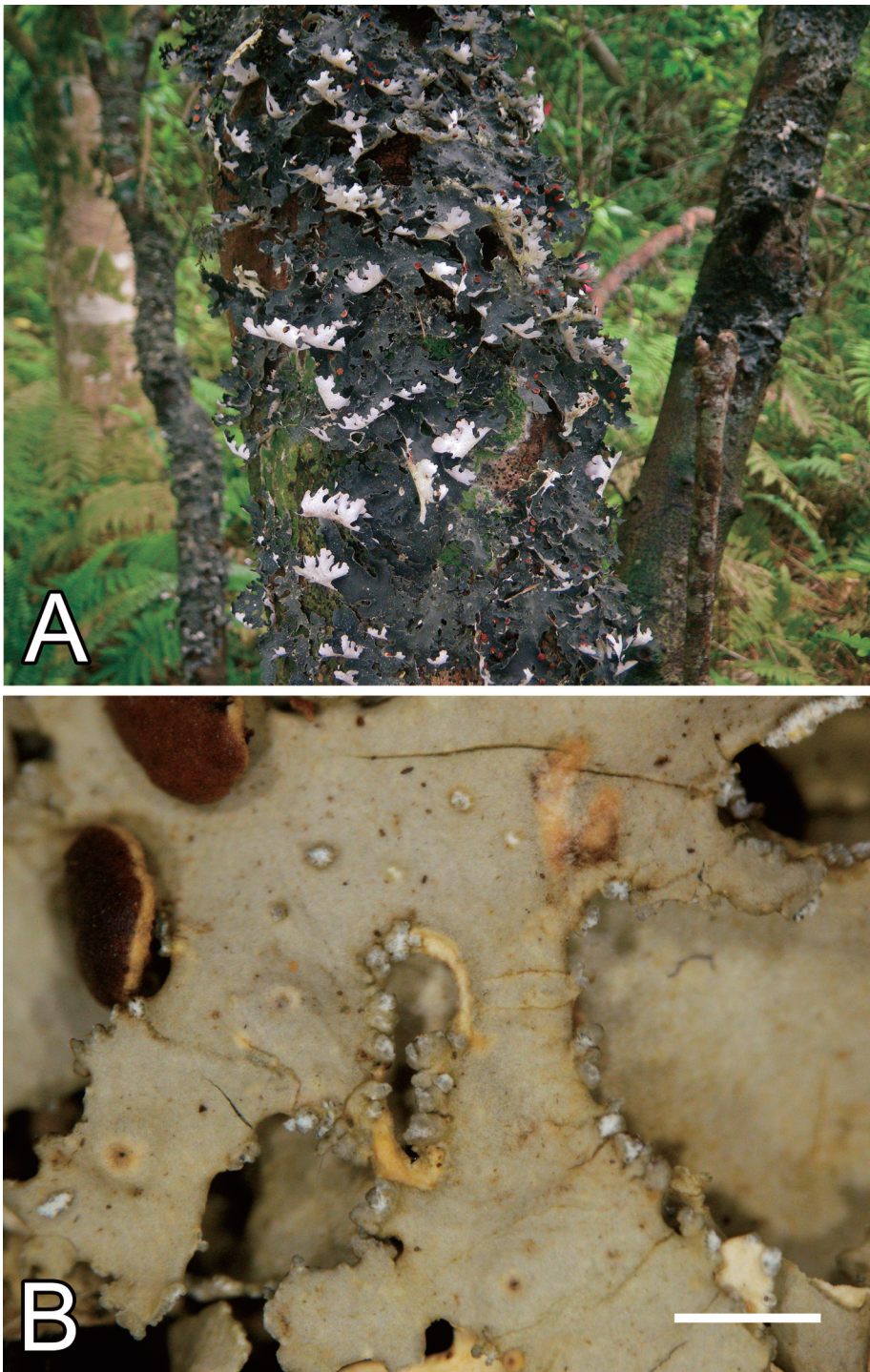


Fig. 1. *Pseudocyphellaria argyracea*. A. Habitat in Kita-iwo Island. B. Isidia along the margins of lobes and on pseudocyphellae (Y. Ohmura 6418). Scale=1 mm.

Hedwigia, 37: 35 (1898). [Fig. 1]

*Pseudocyphellaria argyracea* is characterized by the rosette-forming to irregularly spreading foliose thallus with laminal white soralia and pseudocyphellae on the upper surface, and the simple to coralloid isidia on pseudocyphellae and the margins of lobes. This species is widely distributed in the Palaeotropics from East Africa to India, Japan, New Zealand, South America, and the islands of the Pacific Ocean (Galloway *et al.*, 2001). In Japan, it has been collected from Kyushu and Bonin Islands (= Ogasawara Islands) (Kurokawa, 1957, 1969; Kurokawa and Kashiwadani, 1988). Rich populations of this species are newly found in Kita-iwo Island of Sulphur Islands.

The ITS rDNA sequences were obtained from five specimens of Kita-iwo Island. They are 455 bp in length and no differences are found among the sequences. The identity with the known sequence of this species from Réunion in the Indian Ocean is 93% (427/456 bp) [EU558727 (Högnabba *et al.*, unpublished)].

Specimens examined. **Japan.** Sulphur Islands: Kita-iwo Island, on bark of *Eurya japonica*, elevation 522 m, 18 June 2009, Y. Ohmura 6394; ditto, elevation 448 m, Y. Ohmura 6418 and 6419; ditto, on bark of *Elaeocarpus sylvestris* var. *pachycarpus*, elevation 490 m, Y. Ohmura 6404; Sakakigamine, Kita-iwo Island, on tree trunk, elevation about 750 m, 19 June 2009, T. Yamaguchi s. n. (Herb. Y. Ohmura 6452).

***Thysanothecium scutellatum*** (Fr.) D.J. Galloway, Nova Hedwigia, 36: 390 (1982).

*Thysanothecium scutellatum* is a well-known fruticose species of Cladoniaceae. It has been reported from Oceania, Southeast Asia, and East Asia (Galloway and Bartlett, 1982; Wei *et al.*, 1994; Kashiwadani, 2008). In Japan, it has been known from central to western Honshu, Shikoku, and Kyushu where it grows on barks of *Cryptomeria*, *Pinus*, *Washingtonia*, and decayed stump elevation at 5 to 700 m (Asahina, 1956; Okamoto and Iwatsuki, 1992; Kurokawa and Kashiwadani, 1988; Kashiwadani, 1998, 2008;

Okamoto, 2010).

The ITS rDNA sequence was successfully obtained from one specimen collected in Miyajima Island, Hiroshima Prefecture, and it is 551 bp in length. According to the BLAST result, the top score of known related species is obtained with *Notocladonia cochleata* (Müll. Arg.) S. Hammer (= *Ramalea cochleata* Müll. Arg.) [AF453267 (Stenroos *et al.*, 2002); identity=514/525 bp, 97%]. As discussed by Hammer (2003), the genus *Notocladonia* is related to *Thysanothecium* as well as *Cladia* and *Squamella* in the morphology. The identity of ITS rDNA sequences between *T. scutellatum* and *N. cochleata* is very high. The main morphological difference between the genera is the branching patterns of podetia. Further phylogenetic studies of these genera are needed based on the ITS rDNA sequences and other appropriate markers.

Specimens examined. **Japan.** Honshu. Prov. Mikawa (Pref. Aichi): Mt. Horaiji, Kadoya, Horai-cho, Minamishitara-gun, on base of decayed stump, elevation about 450 m, 5 December 1996, Y. Ohmura 2843. Prov. Yamashiro (Kyotofu): Takenouchi-cho, Sakyo-ku, Kyoto-city, on bark of *Cryptomeria japonica*, elevation about 140 m, 6 December 2005, H. Kashiwadani 47808 and 47809. Prov. Ise (Pref. Mie): Kitayama, Ise-city, on bark of *Cryptomeria japonica*, 15 June 1976, C. Chuma 3786. Prov. Aki (Pref. Hiroshima): Miyajima Island, on bark of *Pinus densiflora*, elevation about 5 m, 10 January 1993, Y. Ohmura 337; ditto, 19 March 1995, Y. Ohmura 936; ditto, on bark of *Pinus thunbergii*, elevation 2 m, 26 August 2009, Y. Ohmura 6773.

***Usnea filipendula*** Stirt., Scott. Natural. 6: 104 (1881).

*Usnea filipendula* is characterized by the fruticose thallus with a jet blacked base and the elongated terminal branches with convex soralia, and the presence of salazinic acid as a major substance. For a detailed description, see Ohmura (2001). This species is widely distributed in circumpolar boreal to northern temperate regions (Thomson, 1984). In Japan, however, it has been

known only from two localities on Hokkaido (Ohmura and Onimaru, 2010).

The ITS rDNA sequence of the Japanese specimen is 492 bp in length. It is 100% identical to the sequence from Sweden (492/492 bp) [AJ457149 (Articus *et al.*, 2002)].

Specimens examined. **Japan.** Hokkaido. Prov. Kitami: Hinami, Bihoro-cho, Abashiri-gun, on bark of *Abies sachalinensis*, elevation 330 m, 11 August 2009, Y. Ohmura 6771.

**Usnea nipparensis** Asahina, Lich. Jap. 3: 91 (1956).

*Usnea nipparensis* is easily distinguished from other related Japanese *Usnea* species by the rounded soralia which are distinctly stipitate and larger than branch diameter, and the presence of caperatic acid as a major substance. For a detailed description, see Ohmura (2001). This species is distributed in Japan, Korea, Taiwan, India, and Nepal. In Japan, it has been known from central Honshu.

A new locality was found in Nishizawa Valley, Yamanashi Prefecture. The ITS rDNA sequences were successfully obtained from two specimens, and they are both 494 bp in length. The sequences are 100% identical with that from Yatsugatake Mts., Nagano Prefecture in Japan (494/494 bp) [AB051652 (Ohmura, 2002)].

Specimens examined. **Japan.** Honshu. Prov. Kai (Pref. Yamanashi): Nishizawa Valley, Mitomi-Kawaura, Yamanashi-city, on bark of *Larix kaempferi*, elevation 1160 m, 8 February 2009, Y. Ohmura (6274 and 6282), A. Crespo & P.K. Divakar.

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