Folia Cryptog. Estonica, Fasc. 60: 47–55 (2023)
https://doi.org/10.12697/fce.2023.60.07

Usnea jezoformosana Y. Ohmura & P. Clerc, sp. nov. (Parmeliaceae, lichenized Ascomycota) from East Asia

Yoshihito Ohmura[1] & Philippe Clerc[2]

[1] Department of Botany, National Museum of Nature and Science, Amakubo 4-1-1, Tsukuba, Ibaraki, 305-0005, Japan
E-mail: ohmura-y@kahaku.go.jp

[2] Conservatoire et Jardin botaniques de la Ville de Genève, Case postale 71, CH-1292 Chambésy, Geneva, Switzerland
E-mail: philippe.clerc@ville-ge.ch

Abstract: *Usnea jezoformosana* is described as a new species from East Asia. The morphology of this taxon is very similar to *Usnea fragilescens* but differs by the presence of granular soredia (instead of farinose soredia). It differs furthermore chemically by the presence of protocetraric acid (major) and barbatic acid (major to trace). The monophyly and independence of the newly described species from *U. fragilescens* and related taxa were inferred by a molecular phylogenetic tree based on ITS rDNA sequences. *Usnea jezoformosana* was collected in subboreal forests of Hokkaido (Japan) and in Taiwan where it grew on tree barks, building wood or cliffs.

Keywords: chemistry, ITS rDNA, lichen, new species, *Usnea fragilescens*, Japan, Taiwan

INTRODUCTION

The genus *Usnea* Adans. belongs to the Parmeliaceae and contains more than 350 species (Lücking et al., 2017). Among them, the *Usnea cornuta* aggregate (Gerlach et al., 2019), previously called the *U. fragilescens* aggr. (Clerc, 1987a; Herrera-Campos et al., 2001), has been shown to be polyphyletic (Truong et al. 2013, Gerlach et al. 2019). It is, therefore, one of the most difficult taxonomic groups in *Usnea.* This group is characterized by the shrubby-erect to subpendulous thallus, the ± inflated branches constricted at the ramification points, minute soralia of various shapes, ± covered with isidiomorphs (Clerc, 1987a; Gerlach et al., 2019). The ratio of cortex, medulla and axis thickness (CMA) is of the *cornuta-* or *brasiliensis*-types (Truong et al., 2011; Gerlach et al., 2017), i.e., a thin cortex (4–7%), a thick, loose to dense medulla (30–38%), often heterogeneous (i.e., with a dense zone just below the cortex), and a thin axis (15–23%).

Ohmura et al. (2000) reported '*Usnea fragilescens* Hav. ex Lynge' as a new record for Japan. However, the specimens contained barbatic and protocetraric acids that have never been reported in this species. The ITS rDNA-based analyses showed that these specimens were genetically distinct from other taxa of the *U. cornuta* aggregate. Therefore, the aim of this paper is to describe a new species based on morphological, anatomical, chemical and genetic characters, highlighting the differences of this newly described species with *U. fragilescens* s.str.

MATERIAL AND METHODS

This study is based on the examination of 267 herbarium specimens of *Usnea cornuta* aggregate housed in the National Museum of Nature and Science (TNS), Tsukuba, Japan.

Morphological observations were made using a dissecting microscope and a bright field microscope. The ratios of cortex, medulla, and axis thickness (CMA) in relation to the total width of the branch were measured following Clerc (1984, 1987a). Statistical values are given as (minimum–) range including *mean* ± standard deviation (–maximum) (n = number of measurements). Cross sections of thallus were cut by hand with a razor blade and observed after mounting in water. Analyses of the anatomical structure of the cortex were made following the method of Ohmura (2001), on thin hand-cut sections and observed at ×1000 magnification with a bright field microscope.

Color spot tests with Pd, K, C and KC followed Orange et al. (2001).

Lichen substances were examined using thin layer chromatography (TLC) (Culberson & Johnson, 1982). Solvent B′ system (hexane:

methyl tert-butyl ether: formic acid, 140: 72: 18) was used for all TLC analyses.

DNA extraction followed a modified CTAB protocol (Hosaka, 2009). For DNA amplification, 10 µl of PCR mix contained 1 µl genomic DNA extraction, 0.25 µl of each primer (10 pmol/ µl) and 5 µl EmeraldAmp PCR Master Mix (TaKaRa Bio Inc.). PCR amplification of the ITS rDNA region (including ITS1, 5.8S rDNA and ITS2) was performed using the primer set of ITS1F (Gardes & Bruns, 1993) as the 5' primer and LR1 (Vilgalys & Hester, 1990) as the 3' primer. PCR cycling conditions were 94°C (3 min), followed by 11 cycles of 95°C (30 sec), 62°C to 52°C (30 sec) with annealing temperatures lowered by 1°C between cycles, and 72°C (1 min), followed by 30 cycles at 52°C annealing temperature and a final extension at 72°C (7 min). Sequencing was done on an ABI Prism 3130x genetic analyzer (Applied Biosystems) using the BigDye Terminator ver. 3.1 Cycle Sequencing Kit according to the manufacturer's instructions.

The sequences were aligned in MAFFT ver. 7 (Katoh et al., 2017) using the default settings. After removing sites with gaps, missing data and ambiguous data, the resulting alignment of 441 sites was used for the molecular phylogenetic analysis.

The maximum likelihood (ML) (Felsenstein, 1981) and neighbor-joining (NJ) (Saitou & Nei, 1987) analyses with the best nucleotide substitution model were performed. Kimura 2-parameter (Kimura, 1980) plus gamma distribution (K2P+G) was selected for the model. The bootstrap values (Felsenstein, 1985) with 1,000 replicates for ML and NJ were shown on the branches only when both are ≥ 50% simultaneously. All calculations were conducted in MEGA 11.0.13 (Tamura et al., 2021).

The sample data for molecular analyses and their DDBJ/EMBL/GenBank accession numbers for the obtained ITS rDNA sequences are shown in Table 1.

RESULTS AND DISCUSSION

Molecular analyses

A total of nine sequences of ITS rDNA (489 or 491 bp) for the Japanese and Taiwanese specimens of the newly described taxon with protocetraric (major) and barbatic acids (major to trace) was obtained in this study (Table 1). Two haplotypes were recognized in which there are one variable site and two sites with gaps in the alignment.

To examine the relationships with taxa related to the *U. cornuta* aggregate, our sequences mentioned above were analyzed with those used in Truong & Clerc (2016), those of *Usnea macaronesica* in Gerlach et al. (2020) and two new sequences of *Usnea pycnoclada* from Taiwan (this study; see Ohmura et al. 2010).

The ML tree is shown in Fig. 1. Basically, the tree is not in conflict with the tree shown in Truong & Clerc (2016). Our tree suggests that the Japanese and Taiwanese specimens called "*U. fragilescens*" by Ohmura et al. (2000) form a highly supported monophyletic clade (bootstrap values for ML/NJ = 100/100) not related to *U. fragilescens* or to other taxa of the *U. cornuta* aggr. Morphology and chemistry furthermore confirm the distinctness of this clade. Therefore, we propose to formally describe this clade as a new species. We were not able to find the sister clade due to the low support value of the internal branches of the tree. Phylogenetic analyses based on multi-loci sequences should be conducted in the future to infer the phylogenetic position of the new described species among the related taxa.

TAXONOMY

USNEA JEZOFORMOSANA Y. Ohmura & P. Clerc, sp. nov. (Fig. 2)

MycoBank no.: MB 849857

Diagnosis: Similar to *Usnea fragilescens* Lynge but differs by the presence of protocetraric (major) and barbatic acids (major to trace) in the medulla, by the presence of granular soredia and by its phylogenetic position.

Type: JAPAN, Hokkaido, Kushiro Prov.: Kiritappu Marsh, Yonbanzawa, Hamanaka-cho, Akkeshi-gun (N43°04′59″, E145°03′17″), on twig of *Salix caprea*, elevation about 5 m, 28 May 2012, Y. Ohmura 8884, K. Onimaru & Y. Takashima (TNS, holotype). %C/%M/%A=7/27/32 (0.9 mm diam.). Chemistry: usnic, barbatic (trace), 4-O-demethylbarbatic (trace), and protocetraric acids (major).

Description: Thallus fruticose, shrubby-erect to subpendent, up to 6.5 cm long, grayish green in

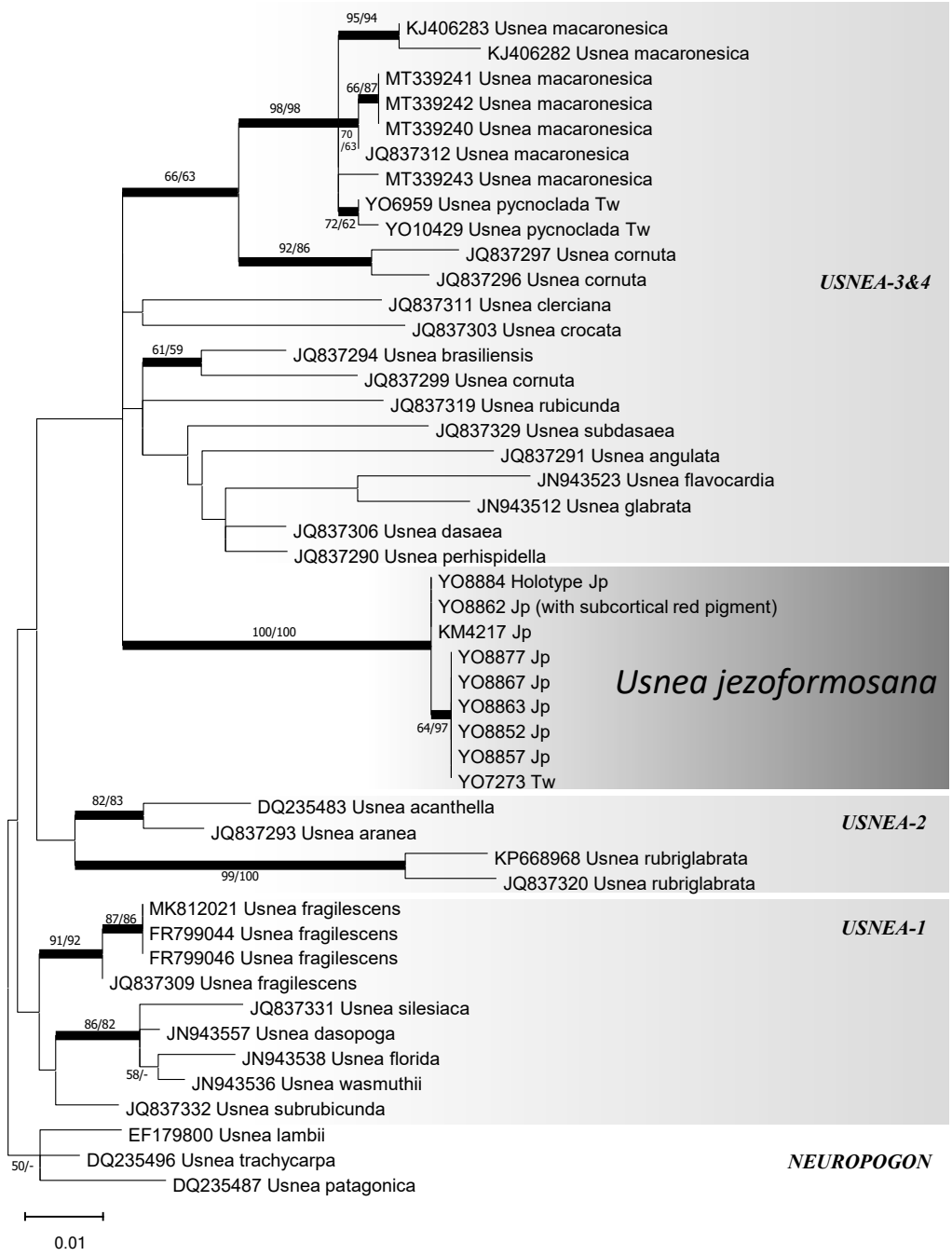

Fig. 1. Molecular phylogenetic tree of *Usnea jezoformosana* and the related taxa based on ITS rDNA sequences. The tree was constructed by maximum likelihood (ML) method, and the reliability of each branch was tested by ML and neighbor-joining (NJ) methods. The bootstrap values for ML/NJ analyses are shown only when both are ≥ 50% simultaneously on the bold branches. The detail information for samples is shown in Table 1. Jp and Tw indicate Japan and Taiwan as the collection locality. Clade names are identical with those in Truong & Clerc (2016).

Table 1. Samples used for the molecular analyses. New sequences obtained in this study are in bold.

Species	Voucher ID (Herbarium)	Locality	Chemotype	GenBank accession No. for ITS-rDNA	Reference
U. acanthella	NW169 (F)	Ecuador	—	DQ235483	Wirtz et al. 2006
U. angulata	85 (G)	Peru	norstictic	JQ837291	Truong et al. 2013
U. aranea	121 (G)	Ecuador	stictic	JQ837293	Truong et al. 2013
U. brasiliensis	44 (G)	Madeira	protocetraric	JQ837294	Truong et al. 2013
U. clerciana	125 (G)	Galapagos	salazinic	JQ837311	Truong et al. 2013
U. cornuta s.l.	27 (G)	Ecuador	norstictic	JQ837297	Truong et al. 2013
U. cornuta s.l.	24 (G)	Peru	stictic	JQ837296	Truong et al. 2013
U. cornuta	29 (G)	Peru	salazinic	JQ837299	Truong et al. 2013
U. crocata	35 (G)	Peru	protocetraric	JQ837303	Truong et al. 2013
U. dasaea	81 (G)	Ecuador	galbinic	JQ837306	Truong et al. 2013
U. dasopoga	EDNA09-01565 (E)	Scotland	salazinic	JN943557	Kelly et al. 2011
U. flavocardia	EDNA09-02348 (E)	Ireland	psoromic	JN943523	Kelly et al. 2011
U. florida	EDNA09-02127 (E)	England	thamnolic	JN943538	Kelly et al. 2011
U. fragilescens	EDNA10-00742 (E)	Scotland	—	FR799044	Kelly et al. 2011
U. fragilescens	EDNA09-02346 (E)	Scotland	stictic	FR799046	Kelly et al. 2011
U. fragilescens	119 (G)	Bolivia	salazinic	JQ837309	Truong et al. 2013
U. fragilescens	O-L-200604 (O)	Norway	—	MK812021	Marthinsen et al. 2019
U. glabrata	EDNA10-00069 (E)	Scotland	protocetraric	JN943512	Kelly et al. 2011
U. jezoformosana	**KM4217 (TNS)**	**Japan**	**barbatic, protocetraric**	**LC764859**	**This study**
U. jezoformosana	**YO7273 (TNS)**	**Taiwan**	**barbatic, protocetraric**	**LC764858**	**This study**
U. jezoformosana	**YO8852 (TNS)**	**Japan**	**barbatic, protocetraric**	**LC764856**	**This study**
U. jezoformosana	**YO8857 (TNS)**	**Japan**	**barbatic, protocetraric**	**LC764857**	**This study**
U. jezoformosana	**YO8862 (TNS)**	**Japan**	**barbatic, protocetraric**	**LC764852**	**This study**
U. jezoformosana	**YO8863 (TNS)**	**Japan**	**barbatic, protocetraric**	**LC764855**	**This study**
U. jezoformosana	**YO8867 (TNS)**	**Japan**	**barbatic, protocetraric**	**LC764854**	**This study**
U. jezoformosana	**YO8877 (TNS)**	**Japan**	**barbatic, protocetraric**	**LC764853**	**This study**
U. jezoformosana	**YO8884 Holotype (TNS)**	**Japan**	**barbatic, protocetraric**	**LC764851**	**This study**
U. lambii	NW42 (F)	Ecuador	—	EF179800	Wirtz et al. 2006
U. macaronesica	25 (G)	Bolivia	stictic	JQ837312	Truong et al. 2013
U. macaronesica	AM298 (S)	Brazil	—	KJ406282	Millanes et al. 2014
U. macaronesica	AM297 (S)	Portugal	—	KJ406283	Millanes et al. 2014
U. macaronesica	240BR (G)	Brazil	barbatic, protocetraric	MT339240	Gerlach et al. 2020
U. macaronesica	246BR (G)	Brazil	barbatic, protocetraric	MT339241	Gerlach et al. 2020
U. macaronesica	280BR (G)	Brazil	barbatic, protocetraric	MT339242	Gerlach et al. 2020
U. macaronesica	304BR (G)	Brazil	barbatic, protocetraric, stictic	MT339243	Gerlach et al. 2020
U. patagonica	NW63 (F)	Ecuador	—	DQ235487	Wirtz et al. 2006

Species	Voucher ID (Herbarium)	Locality	Chemotype	GenBank accession No. for ITS-rDNA	Reference
U. perhispidella	137 (G)	Peru	stictic	JQ837290	Truong et al. 2013
U. pycnoclada	**YO6959 (TNS)**	**Taiwan**	**barbatic, protocetraric**	**LC764860**	**This study**
U. pycnoclada	**YO10429 (TNS)**	**Taiwan**	**barbatic, protocetraric**	**LC764861**	**This study**
U. rubicunda	75 (G)	Madeira	stictic	JQ837319	Truong et al. 2013
U. rubriglabrata	135 (G)	Peru	protocetraric	KP668968	Truong & Clerc 2016
U. rubriglabrata	2 (G)	Peru	protocetraric	JQ837320	Truong et al. 2013
U. silesiaca	88 (G)	Ecuador	salazinic	JQ837331	Truong et al. 2013
U. subdasaea	22 (G)	Galapagos	galbinic	JQ837329	Truong et al. 2013
U. subrubicunda	76 (G)	USA	protocetraric	JQ837332	Truong et al. 2013
U. trachycarpa	NW173 (F)	Argentina	—	DQ235496	Wirtz et al. 2006
U. wasmuthii	EDNA09-02129 (E)	Wales	barbatic	JN943536	Kelly et al. 2011

the field, straw yellow in herbarium specimens, brown to black at the base; branching anisotomic-dichotomous; branches superficially slightly glossy, lacking pseudocyphella and maculae, terete, inflated, gradually tapering, with sparse fibrils and lateral branches especially towards the apices, 0.7–1.5 mm in diameter; lateral branches constricted at ramification points; papillae common, verrucose; soralia common, formed mainly on lateral and terminal branches, developed from top of eroded papillae, more or less discrete, of irregular shape to ± rounded, becoming as large as the branch diameter, slightly stipitate, cortical margin not reflexed, slightly concave with granular soredia, rarely with isidiomorphs or convex with abundant isidiomorphs and/or isidiofibrils. Cortex thin to moderately thin, (4.4–)4.7–6.2%–7.7(–9.1) (n = 14), lacking red pigment, of the *merrilli*-type. Medulla lax, thick, (27.2–)29.6–33.0%–36.4(–37.8), red pigment absent in most individuals or present just beneath the cortex in some individuals, sometimes forming double layers i.e. conglutinated hyphae just beneath the cortex and lax hyphae in most part. Axis solid, moderately thin to moderately thick, (15.7–)17.1–22.2%–27.3(–32.2). CMA of the *cornuta*-type. Apothecia not seen.

Color spot tests: P+ orange red, K–, C–, KC+ yellow.

Chemistry: Usnic, barbatic (major to trace), 4-O-demethylbarbatic (major to trace), psoromic (±), protocetraric (major), salazinic acids (trace), and unidentified substance (±) (Rf class 3–4, pale brown after heating, sprayed with H_2SO_4) (n = 20).

Protocetraric acid was detected at high concentration in all specimens, while barbatic and 4-O-demethylbarbatic acids were variable in concentration. It is then possible that insufficient extraction of the TLC sample may result in overlooking the presence of barbatic acid.

In recent years, molecular phylogenetic methods have increasingly emphasized the importance of chemical differences in the taxonomy of *Usnea* (Gerlach et al., 2019, 2020). Therefore, accurate detection of chemical substances becomes increasingly important.

Etymology: This epithet is derived from the old name for Hokkaido, “Jezo” (“Ezo” or “Yezo”), in Japan and the old name for Taiwan, “Formosa”.

Distribution and ecology: So far, only known from Japan and Taiwan. In Japan, this species was collected in southeastern Hokkaido, where it occurs on bark of *Abies sachalinensis* and *Picea glehnii* and on a wooden roof at elevations between 5 and 120 m. These collecting sites are located in a highly humid maritime area, one of the foggiest areas in Japan. In Taiwan,

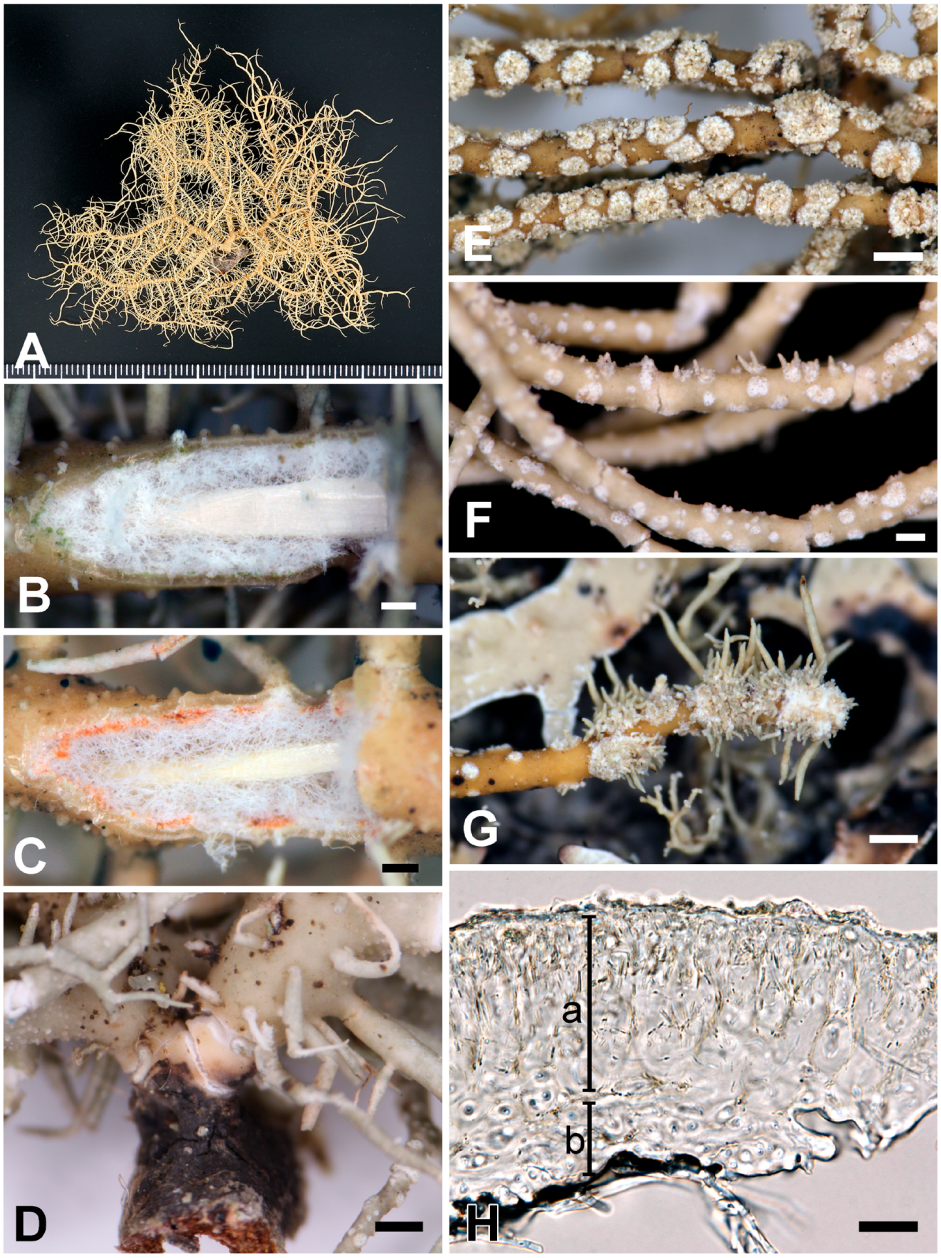

Fig. 2. *Usnea jezoformosana*. A. Thallus (Y. Ohmura 8884, K. Onimaru & Y. Takashima, TNS-holotype). B. Section through a main branch showing cortex, white medulla, and axis (Y. Ohmura 8884, K. Onimaru & Y. Takashima, TNS-holotype). C. Section through a main branch showing cortex, medulla with red pigment just beneath the cortex, and axis (Y. Ohmura 8862, TNS). D. Base of thallus (K. H. Moon 4217, TNS). E. Soralia with granular soredia (K. H. Moon 4489, TNS). F. Soralia with isidiomorphs (Y. Ohmura 8884, K. Onimaru & Y. Takashima, TNS-holotype). G. Soralia with isidiofibrils (K. H. Moon 4136, TNS). H. Section of cortex composed of *merrillii*-type cortex (a) and conglutinated medullary hyphae (b) (Y. Ohmura 8884, K. Onimaru & Y. Takashima, TNS-holotype).

this species was collected on bark of *Betula* sp., *Chamaecyparis formosensis*, *Pinus* sp. and saxicolous on cliffs at elevations between 1948 and 2540 m. These localities were also situated in foggy areas.

Diagnostic features: *Usnea jezoformosana* is characterized by the following features: 1) shrubby-erect to subpendent thallus with anisotomic-dichotomous branching (Fig. 2A), 2) brown to black at the basal part (Fig. 2D), 3) irregular or inflated branches which are constricted at the ramification points, 4) soralia of irregular shape to ± circular, becoming as large as the branch diameter and slightly concave with granular soredia, rarely with isidiomorphs (Fig. 2E–F) or convex with abundant isidiomorphs and/or isidiofibrils (Fig. 2G), 5) a CMA of the *cornuta*-type (see Truong et al., 2011), with a thin cortex and 6) the presence of protocetraric acid as the major substance together with major to trace of barbatic and 4-O-demethylbarbatic acids.

Taxonomic notes: Species of the *U. cornuta* aggr. with large soralia might resemble to *U. jezoformosana*. For instance, *U. fragilescens* has large, ± circular soralia but with farinose, not granular soralia as in *U. jezoformosana*. The soralia of *U. fragilescens* are never crowded and aggregated as it is often the case in *U. jezoformosana*. Moreover, the stictic ac. gr. is the main set of medullary substances in *U. fragilescens* (Clerc 1987a). Finally, the phylogenetic tree based on ITS rDNA sequences confirms the independence of *U. jezoformosana* from *U. fragilescens* (Fig. 1).

Usnea boomiana P. Clerc has distinctly stipitate soralia with farinose soredia and caperatic acid in the medulla (van den Boom et al., 2015). Some species of the *U. cornuta* aggr. have protocetraric and/or barbatic acids as main medullary substances. This is the case of *U. macaronesica* P. Clerc with its three chemotypes: 1) stictic ± barbatic acids (Macaronesia and South America), 2) barbatic acid (Macaronesia), and 3) protocetraric and barbatic acids (South America) (Clerc, 2006, 2011; Gerlach et al., 2020). However, *U. macaronesica* differs morphologically from *U. jezoformosana* by its excavate soralia of the *lapponica*-type (Clerc, 1987b). *Usnea pycnoclada* Vain. has barbatic and protocetraric acids in the medulla too and the same type of soralia as *U. macaronesica* (Ohmura et al., 2010). The phylogenetic tree based on ITS rDNA sequences confirms the independence of *U. jezoformosana* from both latter species (Fig. 1). A further taxon of this group, *U. tenuicorticata* P. Clerc & A. Gerlach, has protocetraric acid and large, but the soredia are farinose and the CMA is of the *brasiliensis*-type (Gerlach et al., 2020).

The presence of subcortical red pigment was confirmed in two specimens (Y. Ohmura 8862 and S. Arakawa 1693, TNS). This is usually considered to be an important character at the species level as for instance in *U. bicolorata* Motyka, *U. crocata* Truong & P. Clerc, and *U. subdasaea* Truong & P. Clerc (Swinscow & Krog, 1979; Ohmura, 2001; Clerc, 2008; Truong et al., 2011). However, the ITS rDNA sequence of Y. Ohmura 8862 (TNS) is the same with those of holotype (Y. Ohmura 8884, TNS) and K. H. Moon 4217 (TNS) that lacks the subcortical red pigment. The sequences from the specimens with red subcortical pigmentation differ only by three sites in the alignment (one variable site and two gaps) from other non-pigmented specimens. Thus, the presence or absence of subcortical red pigment in *U. jezoformosana* is here considered to be an intraspecific variation. *Usnea jezoformosana* with subcortical red pigment resembles *U. bicolorata* in the gross morphology and the chemistry. But the latter has a cortex of *florida*-type (Ohmura et al., 2010).

Specimens examined: JAPAN. Hokkaido. Kushiro Prov.: ca. 1.5 km east of Rokubansawa, Hamanaka-cho, Akkeshi-gun (43°04′N, 140°04′E), on bark of *Picea glehnii*, elevation about 40 m, July 9, 1997, S. Arakawa 1693 & 1694 (TNS); Hillside facing Kiritappu Marsh, Hamanaka-cho, Akkeshi-gun (43°04′N, 145°02′E), on bark of *Abies sachalinensis*, elevation 20 to 40 m, September 2, 1999, K. H. Moon 4217 (TNS); the same locality (43°04′N, 145°03′E), September 2, 1999, K. H. Moon 4389 (TNS); Fukushima, Biwase-mura, Hamanaka-cho, Akkeshi-gun (43°06′N, 145°00′E), on decayed wood of *Abies sachalinensis*, elevation about 75 m, September 1, 1999, K. H. Moon 4136 (TNS); ca. 6 km from Chiripu-mura to Akkeshi-cho, Akkeshi-gun (43°01′N, 145°02′E), on bark of *Abies sachalinensis*, elevation about 70 m, September 3, 1999, K. H. Moon 4484 (TNS); Aikappu, Akkeshi-gun, on bark of *Abies sachalinensis*, elevation about 70 m,

August 8, 2002, T. Shiba 229 (TNS); Suehiro, Akkeshi-gun, on bark of *Abies sachalinensis*, elevation about 30 m, August 4, 2003, T. Shiba 921 (TNS); Nenohi Park, Ponto, Akkeshi-cho, Akkeshi-gun (N43°01′43″, E144°51′41″), on twig of *Abies sachalinensis*, elevation about 5 m, May 28, 2012, Y. Ohmura 8852, K. Onimaru & Y. Takashima (TNS); Tobai, Akkeshi-cho, Akkeshi-gun (N42°59′54″, E144°53′31″), on twig of *Abies sachalinensis*, elevation about 80 m, May 28, 2012, Y. Ohmura 8857, K. Onimaru & Y. Takashima (TNS); Ayamegahara, Mabiro, Akkeshi-cho, Akkeshi-gun (N42°59′43″, E144°55′09″), on wooden roof, elevation about 120 m, May 28, 2012, Y. Ohmura 8862, 8863, 8867, K. Onimaru & Y. Takashima (TNS); Tokitai, Akkeshi-cho, Akkeshi-gun (N42°59′25″, E144°57′38″), on trunk of *Abies sachalinensis*, elevation about 100 m, May 28, 2012, Y. Ohmura 8877, K. Onimaru & Y. Takashima (TNS); Kiritappu Marsh, Yonbanzawa, Hamanaka-cho, Akkeshi-gun (N43°04′59″, E145°03′17″), on twig of *Salix caprea*, elevation about 5 m, May 28, 2012 , Y. Ohmura 8884, K. Onimaru & Y. Takashima (TNS). TAIWAN. Hsinchu Co.: at 3K of Eastline of Talulindao, Kuanwu (N 24°30′61″, E121°06′21″), on cliff of roadside, elevation 1950 m, October 18, 1994, C.-K. Lin 4208 (duplicate in TNS; original in TNM, not seen). Taichung Co.: between 0 km and 6.8 km point of mountain trail, en route from Shiyuan Yakou to Mt. Nanhu (N24°23′29″, E121°21′09″), on bark of *Betula* sp., elevation 1948 m, September 30, 2010, Y. Ohmura 7269 (TNS); the same locality (N24°23′14″, E121°21′29″), on bark of *Pinus* sp., elevation 1977 m, September 30, 2010, Y. Ohmura 7273 (TNS); the same locality (N24°22′30″, E121°21′08″), on trunk of *Pinus* sp., elevation 2249 m, September 30, 2010 Y. Ohmura 7367 (TNS). Chiayi Co.: Mt. Alishan, Alishan Township (N23°32′, E120°48′), on stump of *Chamaecyparis formosensis*, elevation 2540 m, October 4, 2011, Y. Ohmura 8701 (TNS).

ACKNOWLEDGEMENTS

We thank one anonymous reviewer and Dr. Polina Degtjarenko for their critical reading of the manuscript and their valuable constructive comments. We are honored to celebrate Dr. Tiina Randlane's 70th birthday with this publication.

REFERENCES

Clerc P. 1984. Contribution à la revision de la systématique des usnées (Ascomycotina, *Usnea*) d'Europe. I.—*Usnea florida* (L.) Wigg. emend. Clerc. *Cryptogamie Bryologie et Lichénologie* 5: 333–360.

Clerc, P. 1987a. Systematics of the *Usnea fragilescens* aggregate and its distribution in Scandinavia. *Nordic Journal of Botany* 7: 479–495. https://doi.org/10.1111/j.1756-1051.1987.tb00969.x

Clerc, P. 1987b. On the morphology of soralia in the genus *Usnea*. *Bibliotheca Lichenologica* 25: 99–102.

Clerc, P. 2006. Synopsis of *Usnea* (lichenized Ascomycetes) from the Azores with additional information on species in Macaronesia. *Lichenologist* 38: 191–212. https://doi.org/10.1017/S002428290600569X

Clerc, P. 2008. *Usnea*. In: Nash T. H. III, Gries, C. & Bungartz, F. (eds.), Lichen Flora of the Greater Sonoran Desert Region, vol. 3. Lichen Unlimited Arizona State University, Tempe. pp. 302–335.

Clerc, P. 2011. Notes on the genus *Usnea* Adanson (lichenized Ascomycota). III. *Bibliotheca Lichenologica* 106: 41–51.

Culberson, C. F. and Johnson, A. 1982. Substitution of methyl tert.-butyl ether for diethyl ether in the standardized thin-layer chromatographic method for lichen products. *Journal of Chromatography* 238: 483–487. https://doi.org/10.1016/S0021-9673(00)81336-9

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular Evolution* 17: 368–376. https://doi.org/10.1007/BF01734359

Felsenstein, J. 1985. Confidence limits on phylogenies an approach using the bootstrap. *Evolution* 39: 783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Gardes, M. & Bruns, T. D. 1993. ITS primers with enhanced specificity for Basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Gerlach, A., Clerc, P. & Borges da Silveira, R. M. 2017. Taxonomy of the corticolous, shrubby, esorediate, neotropical species of *Usnea* Adans. (*Parmeliaceae*) with an emphasis on southern Brazil. *Lichenologist* 49: 199–238. https://doi.org/10.1017/S0024282917000196

Gerlach, A., Toprak, Z., Naciri, Y., Caviró, E. A., de Silveira, R. M. B. & Clerc, P. 2019. New insights into the *Usnea cornuta* aggregate (Parmeliaceae, lichenized Ascomycota): Molecular analysis reveals high genetic diversity correlated with chemistry. *Molecular Phylogenetics and Evolution* 131: 125–137. https://doi.org/10.1016/j.ympev.2018.10.035

Gerlach, A., da Silveira, R. M. B., Rojas, C. & Clerc, P. 2020. Naming and describing the diversity in the *Usnea cornuta* aggregate (lichenized Ascomycota, Parmeliaceae) focusing on Brazilian specimens.

Plant and Fungal Systematics 65: 272–302. https://doi.org/10.35535/pfsyst-2020-0024

Herrera-Campos, M. A., Nash, T. H., III & Zambrano Garcia, A. 2001. Preliminary study of the *Usnea fragilescens* aggregate in Mexico. *The Bryologist* 104: 235–259. https://doi.org/10.1639/0007-2745(2001)104[0235:PSOTUF]2.0.CO;2

Hosaka, K. 2009. Phylogeography of the genus *Pisolithus* revisited with some additional taxa from New Caledonia and Japan. *Bulletin of the National Museum of Nature and Science, Series B* 35: 151–167.

Katoh, K., Rozwicki, J. & Yamada, K. D. 2017. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 2017 Sep 6. https://doi.org/10.1093/bib/bbx108

Kelly, L. J., Hollingsworth, P. M., Coppins, B. J., Ellis, C. J., Harrold, P., Tosh, J. & Yahr, R. 2011. DNA barcoding of lichenized fungi demonstrates high identification success in a floristic context. *New Phytologist* 191: 288–300.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111–120. https://doi.org/10.1007/BF01731581

Lücking, R., Hodkinson, B. P. & Leavitt, S. T. 2017. The 2016 classification of lichenized fungi in the Ascomycota and Basidiomycota – approaching one thousand genera. *The Bryologist* 119: 361–416. https://doi.org/10.1639/0007-2745-119.4.361

Ohmura, Y. 2001. Taxonomic study of the genus *Usnea* (lichenized Ascomycetes) in Japan and Taiwan. *Journal of the Hattori Botanical Laboratory* 90: 1–96. https://doi.org/10.18968/jhbl.90.0_1

Marthinsen, G., Rui, S. & Timdal, E. 2019. OLICH: A reference library of DNA barcodes for Nordic lichens. *Biodiversity Data Journal* 7: e36252.

Millanes, A. M, Truong, C., Westberg, M. & Diederich, P. 2014. Host switching promotes diversity in host-specialized mycoparasitic fungi: uncoupled evolution in the *Biatoropsis-Usnea* system. *Evolution* 68: 1576–1593.

Ohmura, Y., Moon, K. H. & Kashiwadani, H. 2000. *Usnea fragilescens* Lynge (Parmeliaceae, lichenized Ascomycetes) new to Japan. *Journal of Japanese Botany* 75: 303–307. https://doi.org/10.51033/jjapbot.75_5_9447

Ohmura, Y., Lin, C.-K. & Wang, P.-H. 2010. Three sorediate species of the genus *Usnea* (Parmeliaceae, Ascomycota) new to Taiwan. *Memoir of National Museum of Nature and Science* 46: 69–76.

Orange, A., James, P. W. & White, F. J. 2001. Microchemical Methods for the Identification of Lichens. British Lichen Society. 101 pp.

Saitou, N. & Nei, M. 1987. The Neighbor-Joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4: 406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Swinscow, T. & Krog, H. 1979. The fruticose species of *Usnea* subgenus *Usnea* in East Africa. *The Lichenologist* 11: 207–252. https://doi.org/10.1017/S0024282979000293

Tamura, T., Stecher, G. & Kumar, S. 2021. MEGA11: Molecular Evolutionary Genetics Analysis version 11. *Molecular Biology and Evolution* 38: 3022–3027. https://doi.org/10.1093/molbev/msab120

Truong, C. & Clerc, P. 2016. New species and new records in the genus *Usnea* (Parmeliaceae, lichenized Ascomycota) from tropical South America. *The Lichenologist* 48: 71–93. https://doi.org/10.1017/S0024282915000419

Truong, C., Bungartz, F. & Clerc, P. 2011. The lichen genus *Usnea* (Parmeliaceae) in the tropical Andes and the Galapagos: species with a red-orange cortical or subcortical pigmentation. *The Bryologist* 114: 477–503. https://doi.org/10.1639/0007-2745-114.3.477

Truong, C., Divakar, P. K., Yahr, R., Crespo, A. & Clerc, P. 2013. Testing the use of ITS rDNA and protein-coding genes in the generic and species delimitation of the lichen genus *Usnea* (Parmeliaceae, Ascomycota). *Molecular Phylogenetics and Evolution* 68: 357–372. https://doi.org/10.1016/j.ympev.2013.04.005

Van den Boom, P. P. G., Clerc, P. & Ertz, D. 2015. New records of lichens and lichenicolous fungi from La Gomera (Canary Islands), including the new species: *Usnea boomiana* P. Clerc. *Candollea* 70: 165–177. http://dx.doi.org/10.15553/c2015v702a1

Vilgalys, R. & Hester, M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172: 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Wirtz, N., Printzen, C., Sancho, L. G. & Lumbsch, H. T. 2006. The phylogeny and classification of *Neuropogon* and *Usnea* (Parmeliaceae, Ascomycota) revisited. *Taxon* 55: 367–376. https://doi.org/10.2307/25065584