Bull. Natl. Mus. Nat. Sci., Ser. B, 47(1), pp. 13–20, February 22, 2021

Usnea esperantiana (Parmeliaceae, lichenized Ascomycota) New to Asia

Yoshihito Ohmura[1, *] and Philippe Clerc[2]

[1] Department of Botany, National Museum of Nature and Science,
4–1–1 Amakubo, Tsukuba, Ibaraki, 305–0005, Japan
[2] Conservatoire et Jardin botaniques de la Ville de Genève, Geneva, Switzerland
*E-mail: ohmura-y@kahaku.go.jp

(Received 26 November 2020; accepted 23 December 2020)

Abstract *Usnea esperantiana* is reported as new to Asia. It was collected from Taiwan where it grew on coniferous and broad-leaf trees at elevations between 1716 and 2580 m. The ITS rDNA sequences of Taiwanese and European materials of *U. esperantiana* form a monophyletic clade within the already reported clade consisting of *U. cornuta* and the related taxa. Although two distinct clades were formed in the *U. esperantiana* clade, no morphological and chemical differences were found between them. All Taiwanese specimens contain usnic, salazinic and bourgeanic acids. The description is given based on the Taiwanese specimens.

Keywords: chemistry, distribution, ITS rDNA, lichenized fungi, morphology, phylogeny, soralia, Taiwan, taxonomy.

Introduction

The genus *Usnea* (Parmeliaceae, lichenized Ascomycota) in Taiwan was primarily revised by the first author and 41 accepted taxa were reported before the present study (Ohmura, 2001, 2012, 2014; Ohmura *et al.*, 2010).

During the course of taxonomic study of the genus *Usnea* in Taiwan, *U. esperantiana* P.Clerc was newly revealed to occur in Taiwan based on morphological, anatomical, chemical and molecular phylogenetic examinations.

The aim of this study is to provide a description based on the Taiwanese materials and to show the phylogenetic position based on the ITS rDNA sequences compared to those of European materials that identifications were confirmed by the second author.

Materials and Methods

This study is based on the examinations of herbarium specimens housed in the herbarium of the National Museum of Nature and Science (TNS), Tsukuba, Japan.

Morphological observations for identification were made using a dissecting microscope and a bright field microscope. The ratios of thickness of the cortex, medulla, and axis for the branch were measured following the method of Clerc (1984, 1987). The measurements are given as (minimum–) range including mean ± standard deviation (–maximum) (n = number of measurements). Cross sections of thallus to observe the cortex type (see Ohmura, 2001) were cut by hand with a razor blade, and observed after mounting in water.

Chemical compounds of the herbarium specimens were examined by means of Thin Layer Chromatography (TLC) (Culberson and Kristinsson, 1970). Solvent systems A (toluene : 1,4-dioxane : acetic acid = 180 : 45 : 5) (Culberson and

Ammann, 1979), B′ (hexane: methyl tert-butyl ether: formic acid, 140 : 72 : 18) (Culberson and Johnson, 1982), and C (toluene: acetic acid = 170 : 30) (Mietzsch *et al.*, 1994) were 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 partial 18S rDNA, ITS1, 5.8S rDNA, ITS2, and partial 28S rDNA) was performed using the primer set of ITS1F (Gardes and Bruns, 1993) as the 5′ primer and LR1 (Vilgalys and Hester, 1990) as the 3′ primer. In some cases, when no PCR product was produced when using the ITS1F/LR1 primer pair, USITS1-F as the 5′ primer and USITS2-R as the 3′ primer (Ohmura, 2008) were used. 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.*, 2019) using the default settings. After removing sites with gaps, missing data and ambiguous data, the resulting alignment of 474

Table 1. Vouchers and their GenBank accession numbers for ITS rDNA. New sequences are in bold.

Species	Voucher	Chemistry*	GenBank accession no.	Reference
Usnea articulata	England; 19 (E)	PRO	JN943545	Kelly *et al.* (2011)
U. clerciana	Galapagos; 125 (G)	SAL	JQ837311	Truong *et al.* (2013)
U. cornuta	USA; 32 (G)	SAL	JQ837300	Truong *et al.* (2013)
	France; 42 (G)	STI	JQ837301	Truong *et al.* (2013)
U. crocata	Peru; 35 (G)	PRO	JQ837303	Truong *et al.* (2013)
U. esperantiana	England; N. Sanderson & A. Cross 1319 (E)	SAL, BOU	FR799089	Kelly *et al.* (2011)
	England; B. Benfield s.n. (E)	SAL, BOU	FR799090	Kelly *et al.* (2011)
	Scotland; B. J. Coppins & A. M. Coppins 22424 (E)	SAL, BOU	FR799091	Kelly *et al.* (2011)
	Ireland; P. Lambley *et al.* s.n. (E)	SAL, BOU	FR799092	Kelly *et al.* (2011)
	England; J. A. Norton L (E)	SAL, BOU	FR799093	Kelly *et al.* (2011)
	Scotland; 12 (E)	SAL, BOU	JN943511	Truong *et al.* (2013)
	England; 13 (E)	SAL, BOU	JN943551	Truong *et al.* (2013)
	Taiwan; Y. Ohmura 6036 (TNS)	SAL, BOU	**LC597851**	This study
	Taiwan; Y. Ohmura 6037 (TNS)	SAL, BOU	**LC597852**	This study
	Taiwan; Y. Ohmura 7262 (TNS)	SAL, BOU	**LC597853**	This study
	Taiwan; Y. Ohmura 8719 (TNS)	SAL, BOU	**LC597854**	This study
	Taiwan; Y. Ohmura 10368 (TNS)	SAL, BOU	**LC597855**	This study
	Taiwan; Y. Ohmura 10373 (TNS)	SAL, BOU	**LC597856**	This study
	Taiwan; Y. Ohmura 10376 (TNS)	SAL, BOU	**LC597857**	This study
	Taiwan; Y. Ohmura 10377A (TNS)	SAL, BOU	**LC597858**	This study
	Taiwan; Y. Ohmura 10377B (TNS)	SAL, BOU	**LC597859**	This study
U. glabrata (most probably *U. esperantiana*)	Taiwan; Li351 (TNM)	SAL**	FJ494932	Shen (2008)
U. rubicunda	Scotland; 47 (E)	STI	JN943518	Kelly *et al.* (2011)
U. subcornuta	France; 130 (G)	STI	JQ837325	Truong *et al.* (2013)
U. tenuicorticata	Madeira; 44 (G)	PRO	JQ837294	Truong *et al.* (2013), as "*U. brasiliensis*"

*Major chemical compounds except usnic acid are mentioned. Abbreviations for the lichen products: BOU, bourgeanic; PRO, protocetraric; SAL, salazinic; STI, stictic acid.

**Chemistry was examined by HPLC.

sites was used for the molecular phylogenetic analyses.

The maximum likelihood (ML) (Felsenstein, 1981) and neighbor-joining (NJ) (Saitou and 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 10.1.8 (Kumar *et al.*, 2018).

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

Results and Discussion

Species

Usnea esperantiana P.Clerc, Candollea 47(2): 514. 1992.

Type: SPAIN. Iles Canaries, Tenerife, Tanque, Los Partidos de Franquis, petite colline à l'ouest d'un village abandonné, flan NE de la colline, 1200 m, sur les branches de *Pinus canariensis*, 8 Septembre 1986, P. Clerc (G–holotype!, G, TFMC–isotypes!). %C/%M%A = 4.5/32/27 (holotype). Chemistry: usnic, salazinic (major) and bourgeanic (major) acids (holotype) (Clerc, 1992, 2006).

[Fig. 1]

Thallus fruticose, shrubby, erect, up to 5.0 cm long, grayish green when fresh, straw-yellow to brown in herbarium specimens, concolor to dark brown at the base; branching anisotomic-dichotomous; branches matt to slightly glossy on the surface, lacking pseudocyphellae, maculae and foveoles, terete, inflated, gradually tapering, with many fibrils and lateral branches, 0.7–0.9 mm in diameter at the well-developed main branch; lateral branches slightly to distinctly constricted at the base, twisted and recurved when soralia are well-developed and dense; papillae sparse to numerous, verrucose to cylindrical; soralia common, formed mainly on lateral branches, developed from cortex, more or less discrete, rounded in shape or confluent each other to form irregular mass of soredia, larger than branch diameter, with raised to excurved cortical margin, concave to convex at the top with granular soredia. **Cortex** thin, (5.0–)5.2–6.0% of the radius ($n = 7$), *merrillii*-type plectenchymatous; hyphae pachydermatous, lacking red pigment, with oblong or turbinate lumina. **Medulla** dense, (27.5–)29.2–33.6(–34.0)% of the radius ($n = 7$), white. **Axis** solid, thin, (21.0–)21.1–30.9(–35.0)% of the diameter ($n = 7$), I–. **Apothecia** not seen.

Chemistry. Usnic, bourgeanic, salazinic, and ± consalazinic acids.

The diagnostic features of *U. esperantiana* in the materials collected in Taiwan are (1) the erect thallus with anisotomic-dichotomous branching and twisted and recurved terminal branches (Fig. 1A, B), (2) the concolor to dark brown base (Fig. 1C), (3) the inflated branches with slightly to distinctly constricted at the attachment point (Fig. 1E), (4) the white medulla which is moderate to dense (Fig. 1D), (5) the concave to convex soralia (rarely being excavate) with granular soredia and without isidiomorphs, which remain at the surface of the branches and that are often larger than branch diameter, with raised to excurved cortex margins (Fig. 1F), (6) the *merrillii*-type cortex (Fig. 1G), and (7) the presence of salazinic and bourgeanic acids.

The cortex type can be identified as *merrillii*-type because the cortical hyphae are loosely conglutinated each other and the interspaces between the hyphae are observed (see arrows in Fig. 1G) although the cortex somewhat looks like *ceratina*-type due to the slightly enlarged lumina in the cortical hyphae compared to those of medullary hyphae.

Taiwanese materials agree well with the protologue in morphology and chemistry except the ratio of cortex and the presence of foveoles or depressions on the thallus surface. The ratio of the cortex for the Taiwanese materials is slightly thicker [(5.0–)5.2–6.0% ($n = 7$)] than that of European materials [(3.0–)3.5–5.7(–8.0)%

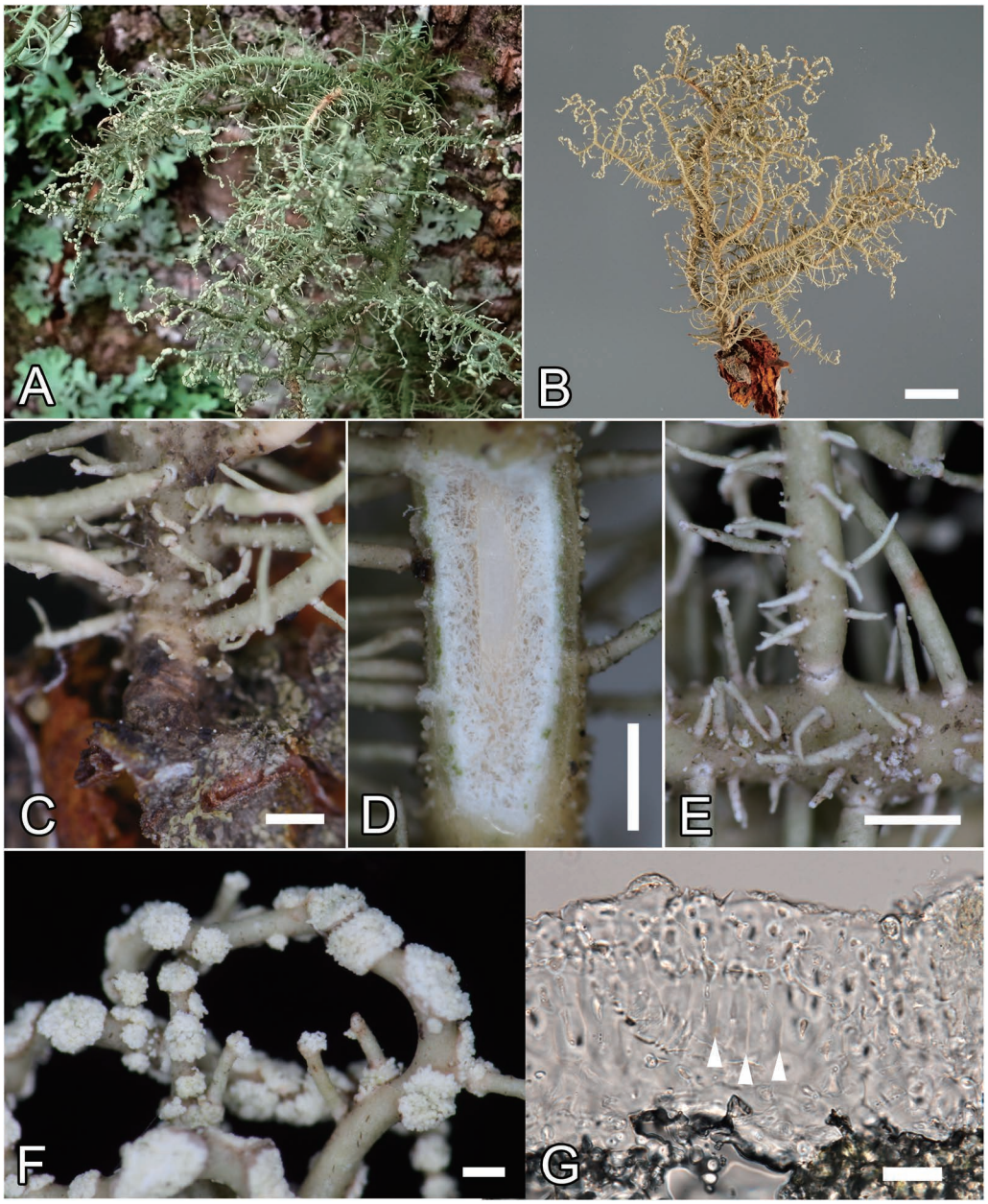

Fig. 1. *Usnea esperantiana* collected in Taiwan. A. Fresh material in the field (Y. Ohmura 10373, TNS). B. Herbarium material showing thallus (Y. Ohmura 10373, TNS). C. Base (Y. Ohmura 10373, TNS). D. Cortex, medulla and axis in a well-developed branch (Y. Ohmura 10377B, TNS). E. Lateral branch constricted at the attachment point (Y. Ohmura 10373, TNS). F. Soralia on twisted and recurved terminal branches (Y. Ohmura 10373, TNS). G. *Merrillii*-type cortex (Y. Ohmura 10373, TNS). Arrows showing the interspaces between cortical hyphae. Scales: B = 5 mm, C–E = 0.5 mm, F = 0.2 mm, G = 20 µm.

(*n* = 33), (Clerc, 1992)]. Taiwanese materials do not have foveoles or depressions, while they are often present in European material (Clerc, 1992). However, as mentioned below, the molecular phylogenetic analysis confirms that these differences are regarded as variation within this spe-

cies which might be caused by environmental parameters.

Some morphotypes of *U. esperantiana* may be confused with *U. cornuta* Körb., *U. dasaea* Stirt., *U. fragilescens* Hav. ex Lynge, *U. fulvoreagens* (Räsänen) Räsänen, *U. glabrata* (Ach.) Vain., *U. nipparensis* Asahina, *U. perplexans* Stirt., *U. pygmoidea* (Asahina) Y.Ohmura, and *U. wasmuthii* Räsänen in having shrubby thallus with inflated branches and soralia. However, *U. esperantiana* can be morphologically distinguished from *U. cornuta*, *U. dasaea*, *U. fragilescens*, *U. nipparensis*, *U. pygmoidea* by the larger soralia with excurved cortical margin, never producing isidiomorphs and isidiofibrils; from *U. fulvoreagens*, *U. perplexans* by the constricted lateral branches and the soralia that are rarely excavate; from *U. wasmuthii* by constricted lateral branches and the never jet-black pigmented basal part of the thallus. The eastern Asian materials of *U. esperantiana* and *U. glabrata* are sometimes difficult to separate only by morphology. The latter species has usually excavate soralia, a somewhat thinner cortex and never produces bourgeanic acid which is diagnostic for *U. esperantiana*. The TLC spots of bourgeanic acid and other *Usnea* substances in different solvent systems are illustrated in Fos and Clerc (2000). Because bourgeanic acid is a fatty acid, it may be easily overlooked on TLC aluminium plate but better seen on TLC glass plate. It should be very carefully checked after wetting with water or a spray of 10% sulphuric acid and before heating.

According to our knowledge, *U. esperantiana* is the only species with bourgeanic acid being a major diagnostic substance. However, it can be sometimes missing or undetected with TLC in this taxon. Furthermore, bourgeanic acid is sometimes detected as an accessory substance in e.g., *U. baileyi* (Stirt.) Zahlbr., *U. diffracta* Vain., *U. rubrotincta* Stirt., and *U. trichodeoides* Vain. (Ohmura, 2001).

This species is known to occur in Europe (Bulgaria, France, Ireland, Italy, the Netherlands, Portugal, Spain, Switzerland, and U.K.) (Clerc, 1992; Vust *et al.*, 2015; Fos and Clerc, 2000; Spier *et al.*, 2008; Saag *et al.* 2011; Aptroot and van Dort, 2016), Africa (Algeria, the Canary Islands, Morocco, and Tunisia) (Clerc, 1992; Monia *et al.*, 2018; El Mokni and Clerc, 2020), and North America (Canada, Mexico, and U.S.A.) (Halonen *et al.*, 1998; Herrera-Campos *et al.*, 2001). The distribution is now extended to Asia (Taiwan). The total number of taxa in the genus *Usnea* in Taiwan is now 42 species.

Specimens examined. **TAIWAN**. Taichung Co.: Wulin Guest House, Heping Township (N24°21′43.7″, E121°18′39.3″), on bark of *Prunus* sp., 1716 m elev., 29 September 2010, Y. Ohmura 7262 (TNS). Nantou Co.: around Chui-Feng Parking, along the Ren-He Road, Ren-ai Township (N24°05′51.2″, E121°11′31.0″), on bark of *Cryptomeria japonica*, 2340 m elev., 27 August 2008, Y. Ohmura 6036 (TNS); Mt. Dasheue (N24°15′25.6″, E121°00′32.4″), on bark of *Metasequoia glyptostroboides*, 2260 m elev., 2 September 2008, Y. Ohmura 6037 (TNS). Miaoli Co.: Xuejian Recreation Area, Taian Township (N24°25′36.2″, E121°00′53.1″), on twig of broad-leaf tree, 1886 m elev., 8 October 2013, Y. Ohmura, 10376 (TNS); *ditto*, on branch of broad-leaf tree, Y. Ohmura 10377A, 10377B (TNS); *ditto*, on trunk of broad-leaf tree, Y. Ohmura 10368, 10373 (TNS). Chiayi Co.: Mt. Alishan, Alishan Township (N23°32′, E120°48′), on twig of *Salix* sp., 2580 m elev., 4 October 2011, Y. Ohmura 8719 (TNS).

Molecular analyses

A total of nine sequences of ITS rDNA (including partial 18S rDNA, ITS1, 5.8S rDNA, ITS2, and partial 28S rDNA) of *U. esperantiana* collected in Taiwan was obtained in this study. At least two haplotypes were recognized among them.

The phylogenetic position for *U. esperantiana* in the subgenus *Usnea* based on multi-locus molecular tree (using ITS rDNA, nu LSU and parts of RPB1 and MCM7) was analyzed by Truong *et al.* (2013). The topology of phylogenetic tree in this study based on ITS rDNA sequences of *U. esperantiana* and selected taxa (Fig. 2) was

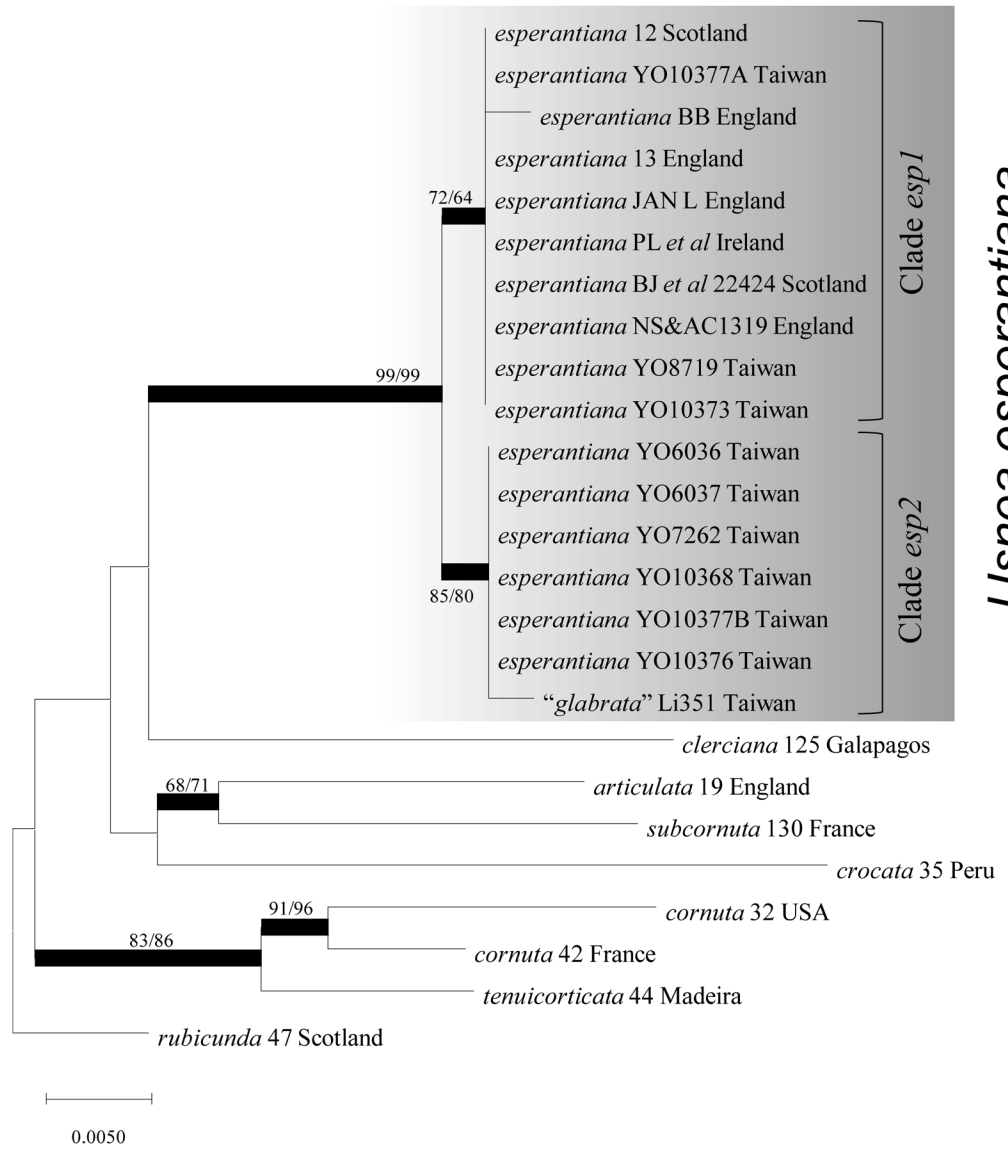

Fig. 2. Molecular phylogenetic tree of *Usnea esperantiana* based on ITS rDNA sequences. The tree was constructed by ML method, and the reliability of each branch was tested by ML and NJ methods. The bootstrap values for ML/NJ were generated from 1,000 replicates and shown on the thick branches only when both are ≥50% simultaneously. All positions containing gaps and missing data were eliminated. There was a total of 474 positions in the final dataset. The OTU names indicated the taxon epithet, the voucher number, and location (see Table 1). The collector names for the vouchers, if present, are shown only the initials (more than three collectors: with "*et al*").

not in conflict with the tree shown in Truong *et al.* (2013).

Based on the molecular phylogenetic analyses made in this study, two distinct clades ("*esp1*" and "*esp2*") were found (bootstrap values for ML/NJ = 72/64 and 85/80 respectively) differing only by two informative sites in the alignment after removing sites with gaps. We were not able to find any morphological or chemical differences in the materials between the two clades.

These two clades form together a monophyletic clade with high support bootstrap value (99/99) (Fig. 2). The Clade *esp1* contain both Taiwanese and European materials but the Clade *esp2* contain only Taiwanese materials. Although it is possible that the Clade *esp2* would be present only in Asian population, further data are needed to study and discuss the genetic diversification in different geographic regions. In consideration of morphological, chemical and genetic data obtained in this study, the genetic differences detected in these two clades can be treated as variations within a single species.

Shen (2008) registered an ITS rDNA sequence of "*Usnea glabrata*" collected in Taiwan into GenBank (accession no.: FJ494932). However, it most probably belongs to *U. esperantiana*, since it was included into the Clade *esp2* in this study (Fig. 2). Shen (2008) did not detect bourgeanic acid. But it might be due to the chemical examination method using High Performance Liquid Chromatography (HPLC). The detection of fatty acid lacking benzene ring in the structure with HPLC is generally problematic (Huneck *et al.*, 1994). The presence of bourgeanic acid in the voucher specimen in Shen (2008) should be carefully tested by means of TLC in order to confirm the identification.

Acknowledgments

We wish to express our gratitude to S.-H. Lin, J.-D. Yang, K.-Y. Yao, E.-L. Chu, S.-C. Chen, T.-Y. Hsiao, Z.-Y. Zheng, Y.-W. Chen, Y.-C. Chen, and M.-H. Kao for their kind arrangements and helps of field excursions in Taiwan; an anonymous reviewer for critical reading of this manuscript.

References

Aptroot, A. and van Dort, K. 2016. Three corticolous cetrarioid lichens found on a wooden sluice. Buxbaumiella 105: 1–3.

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. 1987. Systematics of the *Usnea fragilescens* aggregate, and its distribution in Scandinavia. Nordic Journal of Botany 7: 479–495.

Clerc, P. 1992. Some new or interesting species of the genus *Usnea* (lichenised Ascomycetes) in the British Isles. *Candollea* 47: 513–526.

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

Culberson, C. F. and Ammann, K. 1979. Standardmethode zur Dunnschichtchromatographie von Flechtensubstanzen. Herzogia 5: 1–24.

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.

Culberson, C. F. and Kristinsson, H. D. 1970. A standardized method for the identification of lichen products. Journal of Chromatography 46: 85–93.

El Mokni, R. and Clerc, P. 2020. Two new N-African records in the genus *Usnea* (Parmeliaceae, lichenized Ascomycota) from Kroumiria, NW Tunisia. Herzogia 33: 257–261.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. Journal of Molecular Evolution 17: 368–376.

Felsenstein, J. 1985. Confidence limits on phylogenies an approach using the bootstrap. Evolution 39: 783–791.

Fos, S. and Clerc, P. 2000. The lichen genus *Usnea* on *Quercus suber* in Iberian cork-oak forests. The Lichenologist 32: 67–88.

Gardes, M. and Bruns, T. D. 1993. ITS primers with enhanced specificity for Basidiomycetes—application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118.

Halonen, P., Clerc, P., Goward, T., Brodo, I. M. and Wulff, K. 1998. Synopsis of the genus *Usnea* (lichenized Ascomycetes) in British Columbia, Canada. The Bryologist 101: 36–60.

Herrera-Campos, M. A., Nash, T. H., III and Zambrano Garcia, A. 2001. Preliminary study of the *Usnea fragilescens* aggregate in Mexico. The Bryologist 104: 235–259.

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.

Huneck, S., Feige, G. B. and Lumbsch, H. T. 1994. High performance liquid chromatographic analysis of aliphatic lichen acids. Phytochemical Analysis 5: 57–60.

Katoh, K., Rozwicki, J. and Yamada, K. D. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioin-

formatics 20: 1160–1166.

Kelly, L. J., Hollingsworth, P. M., Coppins, B. J., Ellis, C. J., Harrold, P., Tosh, J. and 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.

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35: 1547–1549.

Mietzsch, E., Lumbsch, H. T. and Elix, J. A. 1994. *Wintabolites* (*Mactabolites for Windows*) Users Manual. 2nd ed. Universität Essen, Essen.

Monia, A. A., Rafika, B. and Tarek, H. 2018. Lichen diversity in the Edough Peninsula, north east of Algeria. *Botanica Complutensis* 42: 9–18.

Ohmura, Y. 2001. Taxonomic study of the genus *Usnea* (lichenized Ascomycetes) in Japan and Taiwan. Journal of the Hattori Botanical Laboratory 90: 1–96.

Ohmura, Y. 2008. Taxonomy and molecular phylogeny of *Usnea rubicunda* and *U. rubrotincta* (Parmeliaceae, lichenized Ascomycotina). Journal of Japanese Botany 83: 347–355.

Ohmura, Y. 2012. A synopsis of the lichen genus *Usnea* (Parmeliaceae, Ascomycota) in Taiwan. Memoirs of the National Museum of Nature and Science 48: 91–137.

Ohmura, Y. 2014. *Usnea flavocardia* (Parmeliaceae, lichenized Ascomycota) new to Asia. Bulletin of the National Museum of Nature and Science, Series B 40: 69–72.

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

Saag, L., Tõrra, T., Saag, A., Del-Prado, R. and Randlane, T. 2011. Phylogenetic relations of European shrubby taxa of the genus *Usnea*. The Lichenologist 43(5): 427–444.

Saitou, N. and Nei, M. 1987. The Neighbor-Joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406–425.

Shen, Y.-M. 2008. Taxonomy of the lichen genus *Usnea* in Taiwan. Master Thesis submitted to National Taiwan University, Taiwan.

Spier, L., van Dort, K. and Fritz, Ö. 2008. A contribution to the lichen mycota of old beech forests in Bulgaria. Mycologia Balcanica 5: 141–146.

Truong, C., Divakar, P. K., Yahr, R., Crespo, A. and 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.

Vilgalys, R. and Hester, M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172: 4238–4246.

Vust, M., Clerc, P., Habashi, C. and Mermilliod, J. -C. 2015. Lichen Inventory of the Canton of Geneva, Switzerland—Large Biodiversity for a Small Canton. Herzogia 28: 153–184.