

Standard Paper

Notes on the genus *Usnea* Adans. (lichenized *Ascomycota*, *Parmeliaceae*) from the islands of São Tomé and Príncipe in tropical West Africa

Miko R. A. Nadel¹  and Philippe Clerc² 

¹Department of Biology, San Francisco State University, 1600 Holloway Ave., San Francisco, California 94132, USA and ²Conservatoire et Jardin botaniques de la Ville de Genève, 1 ch. de l'Impératrice, CH-1292 Chambésy/GE, Switzerland

Abstract

An investigation of the genus *Usnea*, in the biodiversity hotspot of the Republic of São Tomé and Príncipe in tropical West Africa, is presented here. Fifteen species, or species aggregates, were recorded for the islands: *Usnea articulata* aggr., *Usnea baileyi* (Stirt.) Zahlbr., *Usnea beckeri* P. Clerc & Nadel, *Usnea bicolorata* Motyka, *Usnea erinacea* aggr., *Usnea exasperata* (Müll. Arg.) Motyka, *Usnea firmula* (Stirt.) Motyka, *Usnea krogiana* P. Clerc, *Usnea longiciliata* P. Clerc & Nadel, *Usnea nodulosa* Swinscow & Krog, *Usnea pectinata* aggr., *Usnea sorediosula* Motyka, *Usnea submollis* J. Steiner, and two undetermined species. Two species of lichen are described as new to science: *U. beckeri* and *U. longiciliata*. Both species are characterized by a dense and brittle, dark green thallus, the presence of apothecia surrounded by long cilia-like fibrils, a lack of soredia, and the presence of two unknown substances; however, whereas *U. beckeri* has a pendant growth form and can reach 25 cm in length, *U. longiciliata* is differentiated by a shrubby growth form of less than 6 cm. These two species also have different ascospore dimensions. A molecular phylogenetic analysis is presented that lends support to their description as new species. Additionally, *Usnea krogiana* is noted as new to continental Africa and nine *Usnea* species or aggregates are noted as new to the Islands of São Tomé and Príncipe in the Gulf of Guinea.

Key words: biodiversity, lichens, phylogeny, *U. beckeri*, *U. longiciliata*

(Accepted 6 June 2022)

Introduction

Usnea is one of the most speciose genera in the family *Parmeliaceae*, with estimates ranging from *c.* 350 taxa (Thell *et al.* 2012; Lücking *et al.* 2017) to over 400 (Lücking *et al.* 2020). This hyperdiverse genus has historically been difficult to delineate into species due to a high degree of variability and the lack of characters to draw from within the genus (Clerc 1998; Ohmura 2001). The integration of molecular techniques to delineate species has been a beneficial resource but on its own is not adequate to distinguish species (Lücking *et al.* 2021). For the purposes of this article, the fruticose thallus type with usnic acid in the cortex and the presence of a central cartilaginous axis are accepted as delineating a single genus *Usnea*, with internal groups *Eumitria*, *Dolichousnea* and *Usnea* s. str. accepted at the infrageneric rank.

Geographically there is an under-representation of *Usnea* research in Africa and an over-representation in Europe and North America, as is the case with lichenology in general. Dodge (1956, 1957) reported *Usnea* species primarily from southern Africa based entirely on morphological data, while Swinscow &

Krog (1974, 1975, 1976a, b, 1978, 1979, 1986, 1988) and Krog (1994) provided detailed studies of Eastern African species based on both morphological and chemical analyses.

This work provides descriptions of the *Usnea* species found at a geographical hotspot in West Africa, the republic of São Tomé and Príncipe, an island pair in the Gulf of Guinea region located *c.* 250–300 km west of the country of Gabon, Africa, very near 0° latitude and 0° longitude (Fig. 1). The islands are oceanic in origin, arising from a volcanic hotspot (the Cameroon volcanic mountain line). Due to this origin type and the relatively old geological age (São Tomé at 15.7 my, Príncipe at 30.4 my) the islands show very high levels of endemism. The islands were not populated by humans until the early 16th century and, although there have been many non-native species introduced at lower elevations of the islands, higher elevations (≥ 800 m São Tomé, ≥ 400 m Príncipe) have remained as largely untouched primary forest.

Historically, this island group has been mostly ignored from a lichenological point of view, except for collections made in the late 19th century that were primarily sent to William Nylander for determination, and the expeditions by the first author in 2012 and 2013 (Nadel 2016). Only five species of *Usnea* were reported by Nylander (1889), viz. *U. articulata* (L.) Hoffm., *U. ceratina* Ach., *U. florida* (L.) F. H. Wigg., *U. longissima* Ach. and *U. trichodea* Ach. All were collected from the larger island of São Tomé and determinations were made without modern tools of thin-layer chromatography and genetic sequencing.

Author for correspondence: Miko R. A. Nadel. E-mail: nadelmo@gmail.com

Cite this article: Nadel MRA and Clerc P (2022) Notes on the genus *Usnea* Adans. (lichenized *Ascomycota*, *Parmeliaceae*) from the islands of São Tomé and Príncipe in tropical West Africa. *Lichenologist* 54, 271–289. <https://doi.org/10.1017/S0024282922000238>

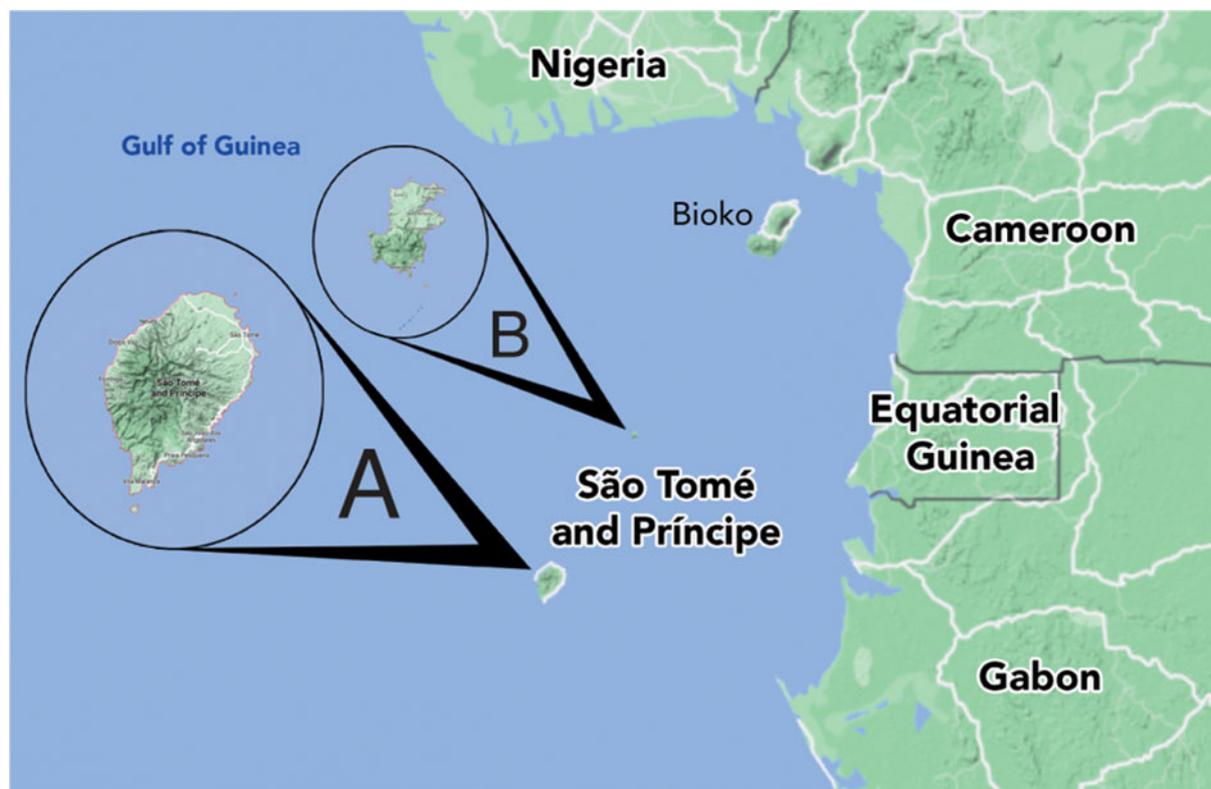


Fig. 1. Map of the Republic of São Tomé and Príncipe showing the islands relative location to continental west Africa and Bioko. A, São Tomé. B, Príncipe. In colour online.

A sixth species, *U. speciosa* Motyka, was added to the list of *Usnea* from the islands when Motyka (1936–1938) described it as a new species based on a specimen collected on São Tomé by Adolfo Möller and determined by Nylander (1889) as *U. articulata*. The neighbouring island of Bioko (historically Fernando Po) is a part of the same volcanic chain but is of a continental island landform. *Usnea* collections from Bioko have been used to describe two eumitrioid species, *U. baileyi* Stirt. and *U. firmula* (Stirt.) Motyka.

Specimens collected in 2012–2013 show 15 distinct taxonomic units from within *Usnea*, all within the subgenera of *Eumitria* (Stirt.) Zahlbr. or *Usnea* s. str. Morphological, chemical and molecular characteristics were used to determine the different species or species groups. Only *U. articulata* was re-observed within the modern collection. While *U. florida*, *U. longissima* and *U. trichodea* were not observed, it should be noted that, according to our modern concept of these species, they are found only in the Northern Hemisphere. The similar appearance of the species that do occur in São Tomé surely led to these incorrect determinations. Originally, the specimens collected in 2012–2013 were determined to represent 11 species (Nadel 2016) and were later published in Lücking *et al.* (2020) based solely on molecular data, using the original names or slight variations of them, from the unpublished thesis of the first author. This study, for the first time, combines molecular, morphological and chemical data to publish species determinations from the São Tomé and Príncipe collection. There are, therefore, 14 species or species aggregates that are reported as new to São Tomé and Príncipe, at least one new to Africa, and two species new to science presented here. This paper presents the 15 species of *Usnea* found during the 2012 and 2013 collecting expeditions.

Materials and Methods

Molecular sequencing and phylogenetic analyses

DNA extractions were obtained from all specimens included in the phylogeny and Sanger sequencing was performed between 2014 and 2015 (Nadel 2016). Approximately 10–15 mg of tissue was cut from the terminal branches and placed in a 1.5 ml microcentrifuge tube with 1000 µl of purified water and centrifuged for 60 s at 1500 rpm. After centrifugation, the samples were transferred to a new microcentrifuge tube where two 1 mm glass beads were added, and mechanical disruption of the sample was performed using a Retsch TissueLyser II Ball Mill Homogenizer for two cycles of 30 s each. Extractions were performed using the Omega E.Z.N.A. HP Plant DNA Mini Kit according to the manufacturer's specifications. To obtain sequences of the internal transcribed spacer (ITS) gene region, polymerase chain reaction (PCR) was performed with the primers USITS4-R (Truong *et al.* 2013a) and one newly designed for this study using Geneious v. 7.1.9 (USITS3-F INT: 5'-TGC GGA AGG ATC ATT ACC GAG-3'). AccuPower® PyroHotStart Taq PCR PreMix (Bioneer Corporation) tubes were used with 2 µl of DNA template. Thermal cycling settings followed those of Truong *et al.* (2013a). PCR products were visualized using a 1.8% agarose gel stained with ethidium bromide and UV transillumination. Successfully amplified product was purified using 2 µl EXOsap-IT (Applied Biosystems) diluted at 1:5 mixed with 3 µl of template. The same primers used for PCR were used for the cycle sequencing reaction. Following precipitation and re-suspension in Hi-Di Formamide (Applied Biosystems), the samples were loaded into an ABI Prism® 3100 Genetic Analyzer (Applied Biosystems) for sequencing.

Resulting sequences were edited and assembled into contigs in Geneious v. 7.1.9 (Biomatters Ltd). Sequences were then manually inspected to determine correct assembly and direction. From the original 87 sequences (Nadel 2016; Lücking *et al.* 2020), 56 were selected for this study by choosing only specimens examined and removing sequences that were deemed of low quality based on ambiguous characters. These 56 sequences were combined with 82 sequences gathered from the National Center for Biotechnology Information (NCBI) website (<http://www.ncbi.nlm.nih.gov>) (see Supplementary Material Table S1, available online). These NCBI sequences were evaluated on the length, and the quality of sequences as determined by the number of ambiguous nucleotides, and the requirement for publication in a peer-reviewed journal. Final sequences were aligned using MUSCLE v. 3 with default settings and the resultant alignment was inspected and corrected manually where necessary. The resulting alignment consisted of 673 nucleotides for 138 taxa but was trimmed to 500 nucleotides after excluding end regions where many taxa had missing character data due to varying individual sequence lengths. We used jModelTest v. 2.1.10 to determine GTR + I + G as the best fit model of nucleotide substitution. Maximum likelihood analyses (ML) were run in RAxML v. 8.2.12 (Stamatakis 2014) and consisted of 1000 replicates using the model GTRGAMMAIX with a starting seed of 12345. Bayesian MCMCMC analyses were performed as implemented in MrBayes v. 3.2.7a (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) using the CIPRES Science Gateway v. 3.3 (<http://Phylo.org>) (Miller *et al.* 2010). The Bayesian analyses consisted of two chains run for 16 million generations with default parameters, uninformative priors and a burn-in of 0.25. BEAGLE v. 3.1.2 (Ayres *et al.* 2012) was used to produce a tree based on the Bayesian analysis and the resultant trees were visualized using FigTree v. 1.4.4.

Field collection

Specimen collection took place over two field collecting trips during April–May 2012 and April–May 2013. Over the course of the two years, approximately four weeks were spent on the main island of São Tomé and five weeks on the smaller island of Príncipe. On São Tomé, *Usnea* were collected primarily from elevations between 800 m and the highest peak at 2009 m. The collections from Príncipe were primarily collected at elevations between 400 and 715 m. Specimens were collected along with relevant ecology and substratum data, when possible, as well as precise geolocation and altitude measurements using a Garmin Oregon 450 GPS. Attempts to separate species were made in the field with placement into separate ‘Rite in the Rain’ packets, with further separation of mixed collections occurring later. Packets containing *Usnea* were dried without heat by taking advantage of the dehumidifier effect of the hotel air conditioning. A subset of 69 specimens was included in this study.

Morphological, anatomical and chemical studies

The following account is based on field studies and on herbarium specimens deposited in BM, CAS, G, LBL, M, S, TUR-V and W. Type material of most of the species discussed in this paper was studied.

The morphology of specimens was examined using a Leica MS5 stereomicroscope, with measurements taken using a Leica DM2000 microscope. The species concept and morphological terms used in this study follow Clerc (1998, 2011), Herrera-Campos *et al.* (1998) and Ohmura (2001). Anatomical measurements of cortex, medulla

and central axis were carried out in longitudinal sections of branches at $\times 40$ magnification. The percentage thickness of cortex/medulla/axis of the total branch diameter (CMA) and the ratios of axis/medulla (A/M), axis/cortex (A/C) and medulla/cortex (M/C) of all the cited specimens were calculated according to Clerc (1984, 1987), Gerlach *et al.* (2019, 2020) and Clerc & Naciri (2021). Measurements for CMA values follow the categories described by Clerc (2011). These values are presented with extreme values in parentheses, standard deviations in plain text and the mean value in italics. Analyses of the anatomical structure of the cortex were made following the methods of Ohmura (2001), on thin hand-cut sections and observed at $\times 1000$ magnification with a Leica DM2000 microscope.

Chemical analyses were performed on all cited specimens using thin-layer chromatography (TLC) following Culberson & Ammann (1979), with solvent B modified according to Culberson & Johnson (1982). K, C and Pd spot tests, according to Hale (1979), were directly applied to the medulla in longitudinal sections of the branches.

Results

ITS sequence data for the study were generated by the first author in 2014 and 2015 for his thesis (Nadel 2016) and later included as part of a larger phylogenetic study (Lücking *et al.* 2020). Fifty-six sequences from that initial work are included in this study. Of these, 33 represent species that had not been sequenced prior to 2015 according to a search of GenBank. This includes *U. beckeri* (7), *U. exasperata* (3), *U. firmula* (5), *U. krogiana* (1), *U. longiciliata* (6), *U. nodulosa* (6), *U. sorediosula* (1), *U. submollis* (2), as well as *Usnea* sp. 1 (MN0523) and *Usnea* sp. 2 (MN0526).

The best ML tree (LNL -5257.88) inferred from the analysis of the ITS rDNA region was compared with the Bayesian probability percent and evaluated for strength of tree branches. At the subgenus level, both *Dolichousnea* (Y. Ohmura) Articus and *Eumitria* are highly supported by the Bayesian analysis (posterior probability (PP) = 1) and supported, although moderately in the case of *Dolichousnea* (ML = 79), by ML. *Usnea* s. str. is not supported by ML but has strong support using Bayesian inference with a PP of 0.99 (Fig. 2). There are 31 moderately to highly supported nodes with ML bootstrap values ≥ 70 and Bayesian PP ≥ 0.90 (Figs 2–5). *Usnea* subgenus *Usnea* s. str. encompasses most of the clades. This includes the species where single collections were made: *U. erinacea* aggregate, *U. krogiana* and *U. sorediosula*, as well as the *U. articulata* aggregate (Fig. 3) and the *U. beckeri* and *U. longiciliata* clades (Fig. 4). There is strong support for the monophyly of both new species, *U. beckeri* and *U. longiciliata* (ML = 97–100, PP = 1). Most of the highly supported regions fall within the clades containing the *Eumitria* subgenus (Fig. 5) and the two new species presented here, *Usnea beckeri* and *U. longiciliata* (Fig. 4). The *Eumitria* tree (Fig. 5) shows that monophyly of *U. firmula* is supported on a node with a 100/1 ML/PP value. These sequences are the first to have been isolated from this species. The *U. baileyi* and *U. pectinata* aggregates are also moderately to highly supported clades within *Eumitria* alongside *U. firmula*.

Taxonomy

Usnea articulata aggr.

Incl.: *Usnea articulata* (L.) Hoffm., *Deutschl. Fl.* (Erlangen) 2, 133 (1796) [1795].—*Lichen articulatus* L., *Sp. Pl.* 2, 1156 (1753); type:

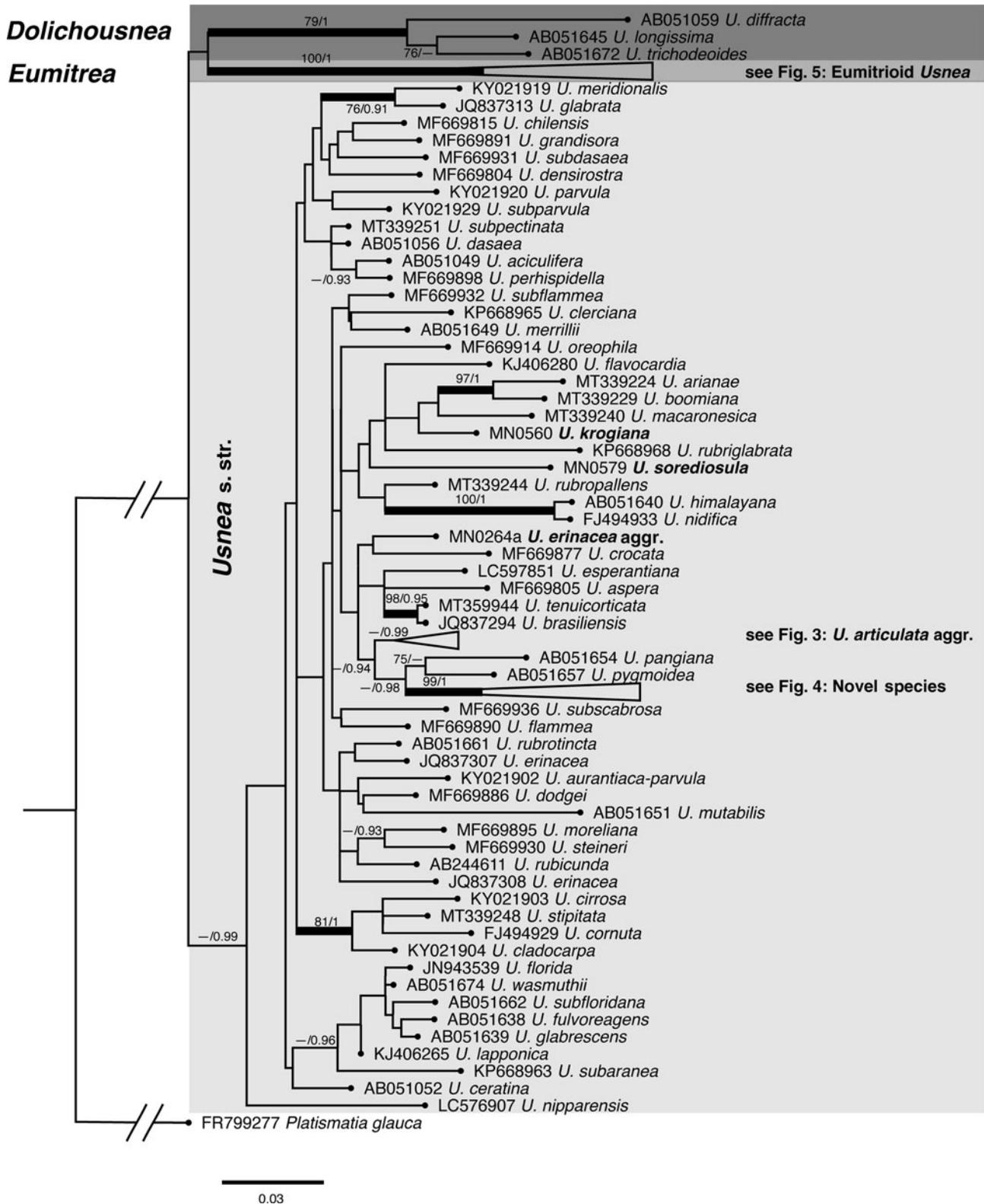


Fig. 2. Molecular phylogeny of *Usnea* including the three subgenera, *Dolichousnea*, *Eumitrea* and *Usnea* s. str., based on the ITS rDNA sequences. Bold branches represent support from both maximum likelihood (ML) and Bayesian posterior probability (PP) inference (ML ≥ 70 and PP ≥ 0.90). See Supplementary Material Table S1 (available online) for voucher, locality and chemistry information.

sine loco, (lectotype—Dillenius, *Hist. Musc.*, 1741, Tab. 11, fig. 4, *fide* Jørgensen, James & Jarvis, *Bot. J. Linn. Soc.* **115**, 372 (1994)); Burnley [England], s. n. et d., *Sherard* s. n. (epitype—OXF, *fide*

Jørgensen, James & Jarvis, *Bot. J. Linn. Soc.* **115**, 372 (1994)). Chemistry (epitype): usnic, protocetraric and fumarprotocetraric acids (trace) (TLC: P. W. James).

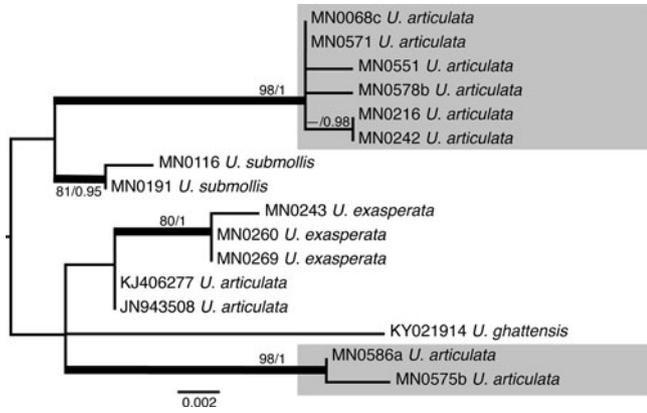


Fig. 3. Molecular phylogeny of the *Usnea articulata* aggregate based on the ITS rDNA sequences. Bold branches represent maximum likelihood (ML) ≥ 70 and Bayesian posterior probability (PP) ≥ 0.90 . The phylogeny shows the polyphyletic nature of the group. *Usnea submollis* and *U. exasperata* are included along with *U. ghattensis*, but the branch lacks support at the base.

Usnea pseudocyphellata Motyka, *Lich. Gen. Usnea Monogr.*, 125 (1936–1938); type: Kamerun, Geb. Bula, vorzüglich an alten Bäumen, häufig, 02.1910, *Deister* (holotype—LBL!). Chemistry: usnic and protocetraric acids. %CMA: 2/41.5/13, A/M: 0.3, A/C: 6, M/C: 19.5.

Usnea speciosa Motyka, *Lich. Gen. Usnea Monogr.*, 112, 124 (1936–1938); type: [São Tomé] Insula Stis Thomae (in Sinu Guineensi), 1300–2100 m, 1885, *Moller* (holotype—W!). Chemistry: diffractaic and barbatic (tr.) acids. %CMA = 2.5/42/11; A/M = 0.3; A/C = 4.4; M/C = 17.

Complete descriptions and further synonyms can be found in Swinscow & Krog (1976a, 1978, 1988).

CMA. (Specimens collected in São Tomé only, $n = 6$). %C = (1.5–) 2.0–2.5–3.0; %M = (38–)38.7–40.9–43.1(–43.5); %A = 10–13.2–17.1(–19); A/M = (0.2)0.3–0.4–0.5; A/C = (4.0–)4.3–5.3–6.3(–6.6); M/C = 12.6–17–21.9(–26.4).

Chemistry. (Specimens collected in São Tomé only, $n = 6$). 1) Medulla K–, Pd+ red orange; protocetraric and fumarprotocetraric

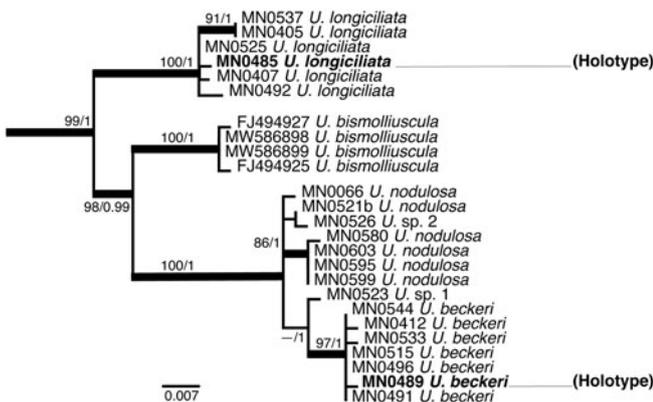


Fig. 4. Molecular phylogeny of the lineage within *Usnea* s. str. which includes the two novel species, *Usnea longiciliata* and *U. beckeri* and the associated holotypes. The phylogeny is based on ITS rDNA sequence data and analyzed using maximum likelihood (ML) and Bayesian posterior probability (PP) inference. Bold branches are supported with both ML (≥ 70) and PP (≥ 0.90).

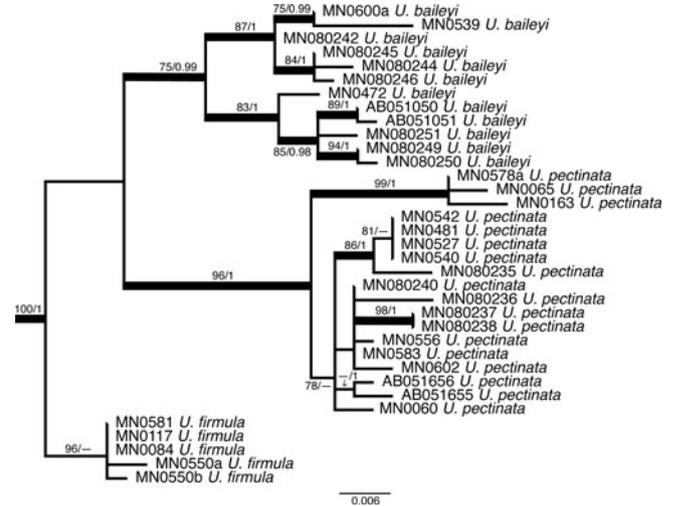


Fig. 5. Molecular phylogeny of the subgenus *Eumitria* with bold branches showing support (maximum likelihood (ML) ≥ 70 and Bayesian posterior probability (PP) ≥ 0.90) for the monophyly of *Usnea firmula* while *U. baileyi* and *U. pectinata* show varying levels of support and require further study.

acids, unknown 4–5/6/6 greyish spot ($n = 2$). 2) Medulla K–, Pd–; diffractaic and barbatic (trace) acids ($n = 4$).

Diagnostic characters. *Usnea articulata* is morphologically and anatomically well characterized by a pendulous thallus with strongly swollen segments with pseudocyphellae (Fig. 6C) of various shapes, the absence of papillae, a very thin cortex (1–3%), a large and lax medulla (38–45%), a thin central axis (10–20%) and thus a *tenuicorticata*-type of CMA (Gerlach *et al.* 2020) (Fig. 6A) with a fairly high M/C.

Variability. Whereas European specimens seem to correspond exclusively to the protocetraric acid chemotype, the African specimens show quite a variable chemistry with the protocetraric acid, salazinic acid, psoromic acid and diffractaic acid chemotypes (Swinscow & Krog 1988). No correlations between morphology and these chemotypes could be found (Swinscow & Krog 1976b). The degree to which segments are swollen is also variable. Some specimens have branches that are not swollen at all (Fig. 6E). In these cases, the presence of pseudocyphellae, the absence of papillae, the anatomy and the chemistry are diagnostic. Pseudocyphellae might be of various shapes from rounded to elongated, thin to large, or even almost absent in some specimens. Rarely, pseudocyphellae enlarge and produce soredia; such morphotypes correspond exactly to the type specimen of *U. pseudocyphellata* Motyka.

Distribution and ecology. *Usnea articulata* occurs in south-western Europe, the British Isles, Macaronesia, Africa, South America and Australia (Truong *et al.* 2013b). It is a frequent and abundant species in tropical Africa (Dodge 1956; Swinscow & Krog 1976b). In São Tomé and Príncipe, it occurs in primary, *Rubiaceae*-dominated forest growing mainly on twigs and branches of tropical hardwood and fallen into leaf litter, between elevations of 1162 and 1863 m on the island of São Tomé only. It is found associated with *U. bicolorata*, *U. exasperata*, *U. firmula*, *U. pectinata* Taylor and *U. submollis*.

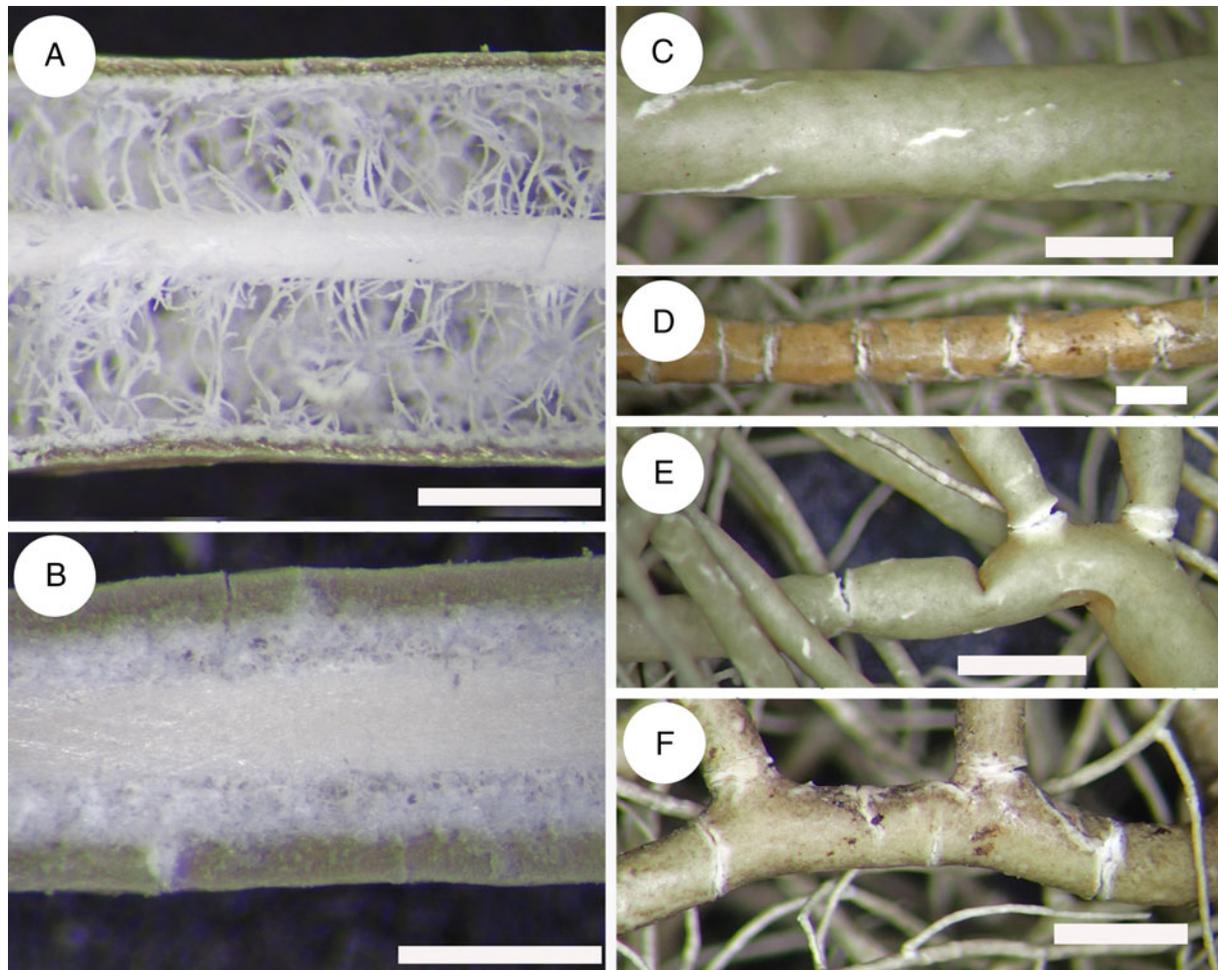


Fig. 6. *Usnea articulata* MN0586a. A, transversal section of a main branch. C, thin and elongated pseudocyphellae. E, main, irregular branch with constricted lateral branches and transversal furrows. *Usnea exasperata* MN0269. B, transversal section of a main branch. D, main, almost cylindrical branch with numerous irregular annulations. F, main branch with non-constricted lateral branches. Scales: A & B = 0.5 mm; C–F = 1 mm. In colour online.

Discussion. In Africa, *Usnea articulata* seems to be a highly variable species. Motyka (1936–1938) considered this species to be restricted to Europe and North Africa and he described many ‘*articulata*-morphotypes’ occurring in Central Africa as new species or new varieties (Motyka 1936–1938, 1956). Today, most of these taxa are considered synonyms of *Usnea articulata* (Swinscow & Krog 1976b, 1978). *Usnea speciosa* is a robust morphotype with elongated pseudocyphellae and diffractaic acid whose description was based on a specimen collected in São Tomé. Swinscow & Krog (1976b) considered this taxon to be a different species characterized by the effigurate-linear pseudocyphellae and the diffractaic acid chemotype. However, firstly the specimen MN586a collected in São Tomé has numerous effigurate-linear pseudocyphellae (Fig. 6C) as well as protocetraric acid in the medulla and, secondly, there are specimens with both protocetraric and diffractaic acids, such as the type of *U. flavescens* Motyka (Swinscow & Krog 1976b). One specimen (MN0068c) has the typical enlarged and \pm circular pseudocyphellae that become sorediate and are diagnostic for *U. pseudocyphellata*. In our phylogenetic tree, *U. articulata* belongs to the clades 3 & 4 of Truong *et al.* (2013a). Figure 3 shows *U. articulata* being paraphyletic with two highly supported clades. However, there are no correlations with the chemistry or with any morphological characters. Furthermore, the lack of support in the deeper nodes does

not allow any definitive conclusion to be drawn. For this reason, we here consider our specimens to be part of an aggregate. Studies of this cosmopolitan species on a worldwide scale with the tools of integrative taxonomy are needed to resolve this group (Lücking *et al.* 2020).

Specimens examined. **São Tomé and Príncipe:** *Island of São Tomé:* Parque Natural Obô de São Tomé, on road from Bom Sucesso to ‘Macambara’ Radio Station, 1162–1319 m, 2012, M. Nadel & J. Shevock MN0068c (CAS); trail between Pico Cálvario and mesa below Pico de São Tomé, 1647 m, 2012, M. Nadel, J. Shevock & A. Stanbridge MN0216 (CAS); trail between Pico Cálvario and mesa below Pico de São Tomé, 1863 m, 2012, M. Nadel, J. Shevock & A. Stanbridge MN0216 (CAS); primary trail from Bom Sucesso to Lagoa Amelia, 1298 m, 2013, M. Nadel, J. Shevock, T. Daniel & E. Soares MN0551 (CAS); primary trail from Bom Sucesso to Lagoa Amelia, 1440 m, 2013, M. Nadel, J. Shevock, T. Daniel & E. Soares MN0575b (CAS); overlook above Lagoa Amelia, 1470 m, 2013, M. Nadel, J. Shevock, T. Daniel & E. Soares MN0571 (CAS); secondary trail from Bom Sucesso to Lagoa Amelia, 1191 m, 2013, M. Nadel, J. Shevock, T. Daniel & E. Soares MN0578a (CAS); *ibid.*, 1196 m, 2013, M. Nadel, J. Shevock, T. Daniel & E. Soares MN0586a (CAS).

***Usnea baileyi* (Stirt.) Zahlbr.**

Denkschr. Kaiserl. Akad. Wiss. Wien, Math.-Naturwiss. Kl. **83**, 182 (1909).—*Eumitria baileyi* Stirt., *Scott. Natural.* **6**, 100 (1881) [1881–1882]; type: [Australia], Queensland, *F. M. Bailey* 16 (lectotype—BM!) (*vide* Rogers & Stevens 1988).

Complete descriptions, images and synonyms can be found in Swinscow & Krog (1974, 1988), Stevens (1999), Ohmura (2001), Clerc (2007), Truong & Clerc (2013) and Temu *et al.* (2019).

CMA. (Specimens collected in São Tomé only, $n = 5$). %C = (3.0–) 3.5–5.1–7.7(–9.5); %M = 2.5–4.3–6.2(–7.0); %A = (67–)72.9–81–87; A/M = (9.5–)12.1–21.5–30.9(–32.5); A/C = (6.8–)11.1–18.8–26.5; M/C = 0.7–0.9–1.4(–1.8); %TBA = 35–45.6–59(–63).

Chemistry. (Specimens collected in São Tomé only, $n = 2$). Medulla K+ yellow turning orange, Pd+ yellow turning red-orange, hyphae of the tubular axis K+ yellow, Pd–. Norstictic and salazinic (trace) acids, unknowns 4.5–5/5–5/5–6 pale brownish spots, fatty acid?/3/4 ($n = 2$).

Diagnostic characters. *Usnea baileyi* is characterized by a stiff, shrubby to subpendant thallus (Fig. 8D), a pale basal part concolorous with main branches (Fig. 8G), tapering branches with cylindrical segments, long and slender fibrils (Fig. 8C), the number of which is inversely proportional to the number of tubercles (juvenile fibrils) or fiberclcs (scars of broken fibrils) or soralia (enlarged fiberclcs producing soredia and isidiomorphs), a thin, reddish pigmented medulla and a broad tubular central axis (Fig. 8H).

Variability. The morphology of *Usnea baileyi* is highly variable depending on the growth of fibrils and whether they are shed or not. Its chemistry is also very variable with numerous chemotypes (see Rogers & Stevens 1988).

Distribution and ecology. *Usnea baileyi* is known to occur on every continent except Antarctica and Europe (Swinscow & Krog 1974; Stevens 1999; Ohmura 2001; Truong & Clerc 2013; Esslinger 2019). It is primarily a corticolous species growing on a wide variety of trees and shrubs, occasionally also lignicolous (fencepost) or saxicolous. It is a species with a rather wide ecological range, from humid to arid places in subtropical and tropical areas of the world (Rogers & Stevens 1988). It occurs on both São Tomé and Príncipe in various forest types, from 200–1400 m elevation on São Tomé and 500–600 m on Príncipe. The species was found both in undisturbed forest and more disturbed areas alongside roads and trails. The growth form was always corticolous, sometimes fallen from the canopy onto the forest floor and boulders. It was found associated with *U. pectinata*. *Usnea baileyi* is newly reported for São Tomé and Príncipe.

Discussion. *Usnea baileyi* with its tubular central axis and phylogenetic position belongs to the subgenus *Eumitria*. (Ohmura 2001, 2002; Temu *et al.* 2019). In the phylogenetic tree (Figs 2 & 5), our specimens cluster in the *Eumitria* subgenus clade and are nested in the *U. baileyi* clade, sister of the *U. pectinata* clade. As Temu *et al.* (2019) and Lücking *et al.* (2020) have already shown, *U. baileyi* seems to be quite heterogenous and more detailed studies are necessary to understand the heterogeneity of this pantropical species (Clerc 2007). For differences from *U. formula*, see under that species.

Specimens examined. **São Tomé and Príncipe:** *Island of São Tomé:* trail past Ponta Furada at the end of the road on west coast, 215 m, 2012, *M. Nadel, J. Shevock & A. Stanbridge* MN0472 (CAS); Parque Natural Obô de São Tomé, on the primary trail from Bom Sucesso to Lagoa Amelia, 1246 m, 2013, *M. Nadel, J. Shevock, T. Daniel & E. Soares* MN0549a (CAS); unimproved road between Bemposta and Chamico, 862 m, 2013, *M. Nadel, J. Shevock, T. Daniel & Q. Quade* MN0600a (CAS). *Island of Príncipe:* trail from Terreiro Velho to Morro de Leste, 581 m, 2013, *M. Nadel & O. Rocha* MN0535 (CAS); trail from Terreiro Velho to Morro de Leste, 596 m, 2013, *M. Nadel & O. Rocha* MN0539 (CAS).

***Usnea beckeri* P. Clerc & Nadel sp. nov.**

Mycobank No.: MB 843679

Thallus pendulous, stiff and brittle, up to 25 cm long, dark green-coloured, with apothecia and without soralia. Main branches cylindrical to slightly irregular, smooth. Lateral branches not constricted. Axis moderately thin to moderately thick (30–50%). Medulla dense, containing the stictic acid gr., US1 and US2 *sensu* Ohmura (2001). Apothecia mainly lateral, with non-eroded cortical rim and long (2–15 mm) cilia. Ascospores 8–9 × 5–6 µm.

Type: Republic of São Tomé and Príncipe, Gulf of Guinea, Island of Príncipe, trail up to the summit of Pico Papagaio from Santa Trindade, 536 m elevation, 15 April 2013, *M. Nadel, J. R. Shevock, T. Daniel & O. Rocha* 489 (holotype—CAL-1318542).

(Fig. 7)

Thallus pendulous and brittle, up to 25 cm long, dark green-coloured, with filamentous ramifications (Fig. 7A); *basal part* short, pale to concolorous with branches; *main branches* 0.6–0.9 mm thick, usually smooth (Fig. 7D), cylindrical to slightly irregular, tapering only at the extremities, often partly blackened especially close to the basal part; *segments* cylindrical or slightly to distinctly swollen in cross-section, sometimes very short, especially on main branches, with distinct annulations, sometimes with regenerated cortex between segments giving the impression of a double annulation; *lateral branches* not constricted at attachment point; *terminal branches* thin, smooth, capillaceous, with few ramifications; *foveolae* and *transverse furrows* absent; *maculae* and *pseudocyphellae* absent; *papillae* absent; *tubercles* (young fibrils?) small (50 µm), few, scattered on main branches only; *fibrils* absent; *fiberclcs* small (50 µm), few, scattered on main branches only; *soralia* absent.

Apothecia never numerous, sometimes absent, 1–5 mm wide, mainly lateral and subterminal, flat to slightly cupular, with pruinose discs surrounded by (sometimes few) long cilia (2–15 mm long) and a non-eroded cortical rim (Fig. 7B); *ascospores* ellipsoid, 8–11 × 5–7 µm.

Cortex slightly shiny in section, of the *merrillii*-type plectenchyma, often with incomplete circular cracks, thin to moderately thin, %C = (4.5–)4.8–5.7–6.6(–7.0) ($n = 9$); *medulla* dense, moderately thin to moderately thick, %M = (18.5–)19.9–23.7–27.5(–29); *axis* moderately thin to moderately thick, %A = (29–)32.8–41.3–49.8(–53); A/M = (1.0–)1.2–1.9–2.6(–2.8); A/C = (4.5–)5.1–7.6–10.1(–12.1); M/C = 3.3–4.2–5.1(–6.3) (Fig. 7C).

Chemistry. ($n = 4$). Medulla K+ yellow turning slowly orange, Pd+ orange. Stictic, constictic, cryptostictic, menegazziaic and

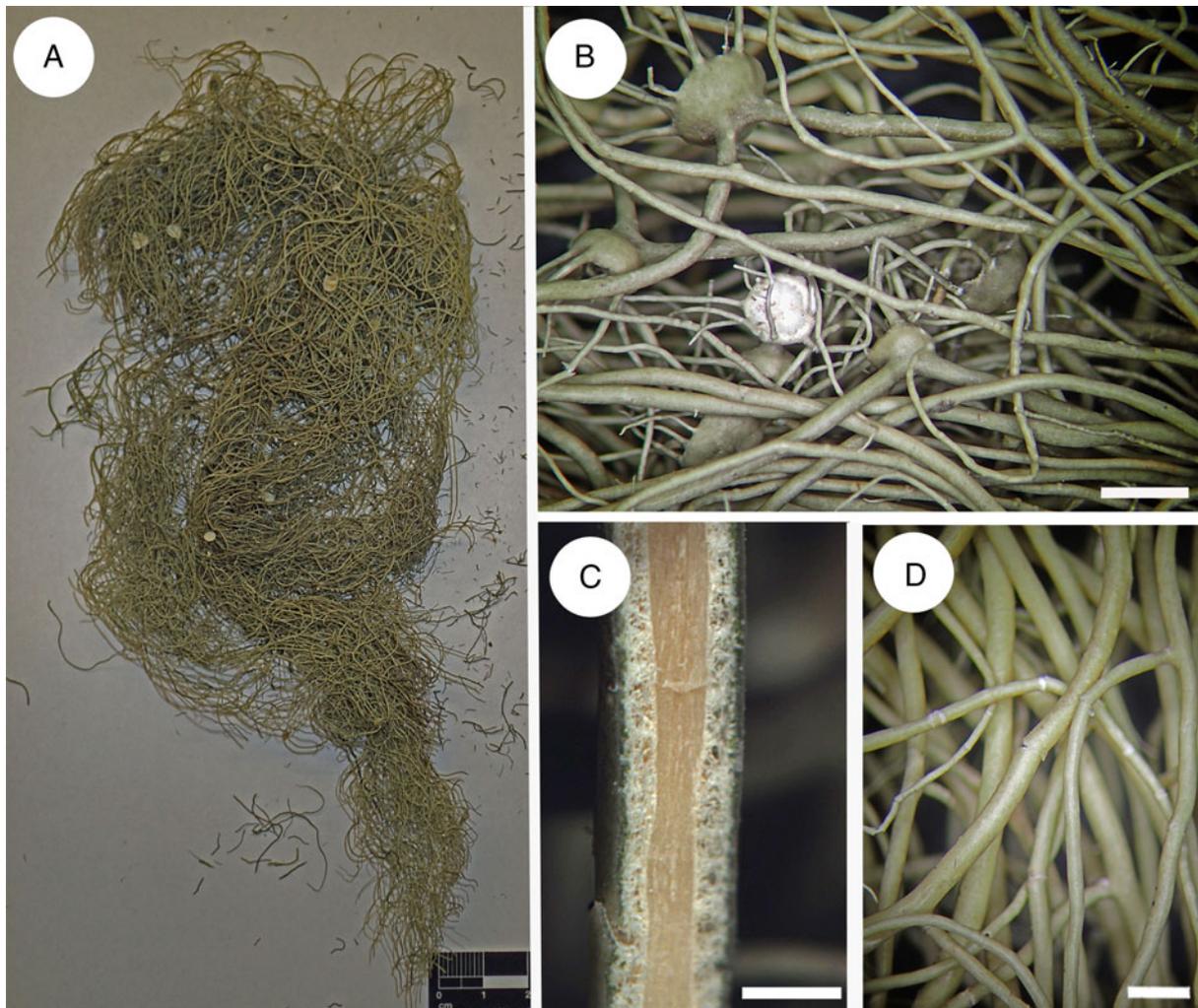


Fig. 7. *Usnea beckeri* holotype. A, thallus; note the small, detached branch fragments illustrating how brittle the branches are. B, apothecia, serial and subterminal. C, anatomy of a main branch. D, smooth branches. Scales: B = 2 mm; C = 0.5 mm; D = 1 mm. In colour online.

norstictic (tr.) acids, and the unknown substances US1 and US2 *sensu* Ohmura (2001).

Etymology. The first author of this taxon names it in honour of his good friend Uwe Becker (Cologne, Germany), who did his Ph.D. on the lichens of the inselbergs in Zimbabwe, promoting the knowledge of African lichens; in shared memory of the good times we spent together, collecting lichens on these extraordinary inselbergs. Uwe is now a distinguished and successful children's author.

Diagnostic characters. The main diagnostic characters of *Usnea beckeri* are the dark green and brittle pendulous-filamentous thallus, with apothecia, without soralia, the overall smooth, cylindrical to slightly irregular branches with distinct annulations and circular cracks, the non-constricted lateral branches, the moderately thick and shiny cortex, the dense medulla, the moderately thick axis and the presence of the stictic acid gr. with the unknown US1 and US2 in the medulla.

Variability. Some specimens consist of a network of thick main branches of approximately the same diameter, without smaller

branches ending in thin capillaceous apices. In this case, branches are distinctly irregular. Apothecia can be totally absent. Some thalli are almost shrubby or subpendulous.

Distribution and ecology. Known only from the island of Príncipe, observed in primary, *Rubiaceae*-dominated hardwood tropical forests between 501 and 715 m elevation. It is associated with the other novel species, *U. longiciliata*, and has a corticolous growth habit, although many specimens were collected from the ground, including on rocky areas and leaf litter where it had fallen from the canopy.

Discussion. A search among the type specimens and protologues of all the pendulous non-sorediate and apotheciate species occurring in Africa and South America to find an already published name was unsuccessful. *Usnea beckeri* strongly resembles the other new species *U. longiciliata*. Both are fertile, non-sorediate taxa that share the peculiar dark green coloration of the thallus, the long cilia on the apothecia, the dense medulla and the presence of the unknowns US1 and US2 in the medulla. They differ essentially in their life form, pendulous in *U. beckeri* (Fig. 7A) and shrubby in *U. longiciliata* (Fig. 8A), in the cortex type, of

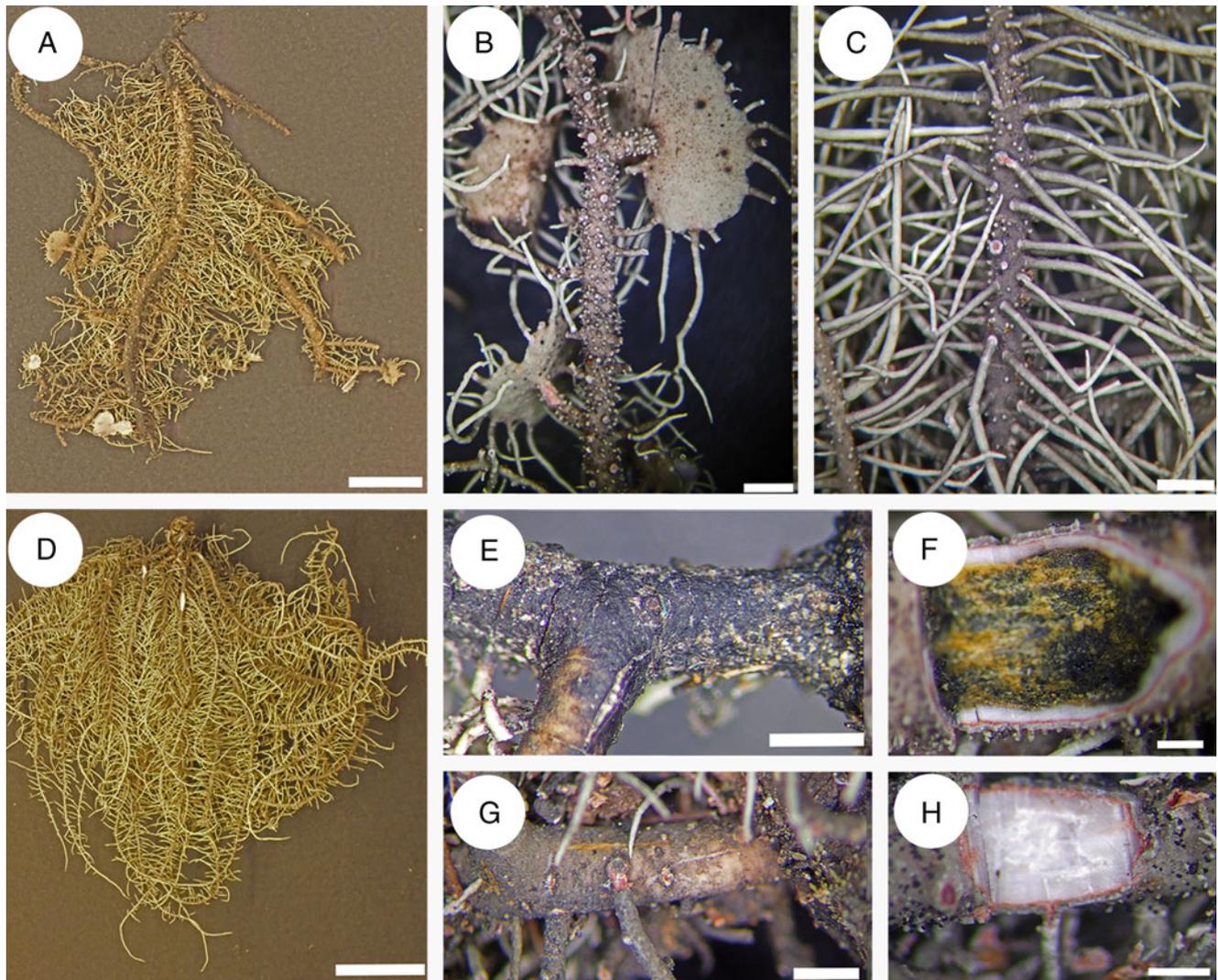


Fig. 8. *Usnea firmula* MN0084. A, thallus with irregular main branches. B, lateral apothecia. E, jet black-pigmented basal part. F, anatomy of a main branch with wide tubular axis. *Usnea baileyi* MN0539. C, main 'fish bone' like branches with long and slender fibrils. D, thallus with tapering branches. G, concolorous basal part. H, anatomy of a main branch with tubular axis. Scales: A & D = 1 cm; B, C, F & H = 0.5 mm; E & G = 1 mm. In colour online.

the *merrillii*-type in *U. beckeri* and of the *ceratina*-type in *U. longiciliata*, in the presence of numerous fiberclcs, the larger ascospores and in the absence of the stictic acid gr. in *U. longiciliata*. *Usnea himalayana* C. Bab. looks superficially like *U. beckeri*. However, the former taxon has pseudocyphellae, a much thinner central axis and a lax medulla producing salazinic acid. For differences with *U. nodulosa* and *U. submollis*, see under these taxa. In the phylogenetic tree (Fig. 2), *U. beckeri* belongs to the clades 3 & 4 of Truong *et al.* (2013a). It is monophyletic and belongs to a highly supported but unresolved clade containing *U. nodulosa*, *Usnea* sp. 1 and *Usnea* sp. 2 (Fig. 4).

Paratypes. **São Tomé and Príncipe:** Island of Príncipe: trail from Santa Trindade to Pico Papagaio, 501 m, near rope ladder, 2012, M. Nadel & J. Shevock MN0412 (CAS); trail from Santa Trindade to Pico Papagaio, 536 m, 2013, M. Nadel, J. Shevock, T. Daniel & O. Rocha MN0489 (CAS); trail from Santa Trindade to Pico Papagaio, 674 m, 2013, M. Nadel, J. Shevock, T. Daniel & O. Rocha MN0496 (CAS); ridge leading to Pico Príncipe, 715 m, 2013, M. Nadel & O. Rocha MN0515 (CAS); trail from Terreiro

Velho to Morro de Leste, 565 m, 2013, M. Nadel & O. Rocha MN0532, MN0533 (CAS); trail from Terreiro Velho to Morro de Leste, 606 m, 2013, J. Shevock, T. Daniel, O. Rocha & M. Nadel MN0543, MN0544 (CAS).

Usnea bicolorata Motyka

Lich. Gen. Usnea Monogr., 336 (1936–1938); type: Africa centralis, in montibus Kivu, de Witte s. n. (holotype—LBL-920!). Chemistry: usnic, protocetraric and barbatic acids. %CMA = 4.5/35/21; A/M = 0.6; A/C = 4.4; M/C = 7.4.

Usnea griseola Motyka, *Annls Univ. Mariae Curie-Skłodowska, Sect. C, Biol.* 11, 120 (1959) [1956]; type: Nkanda, im Hang gegen dem Satel, an *Hypericum* und *Ericaceen*, 2700 m, 26.xi.1954, Stauffer 3398/984 (holotype—G60597!). Chemistry: usnic, protocetraric (main), salazinic and alectorialic acids. %CMA = 6/38/12; A/M = 0.3; A/C = 2; M/C = 6.4.

Usnea bicolorata var. *pseudorubescens* Motyka, *Annls Univ. Mariae Curie-Skłodowska, Sect. C, Biol.* 11, 122 (1959) [1956]; type: Muhavura, auf schräg aufsteigendem Stamm eines

Rhodblattbaumes 862, Oberseite, 3300 m, 19.xi.1954, *Stauffer* 3370/872 (holotype—G66569!). Chemistry: usnic, protocetraric, alectorialic and barbatic acids. %CMA = 4.5/33.5/24; A/M = 0.7; A/C = 5; M/C = 7.2.

Detailed description and images of *U. bicolorata* are given in Swinscow & Krog (1979, 1988) and Ohmura *et al.* (2010). However, none of these authors were successful in tracing the type specimen. In the type specimen of *U. bicolorata*, as in the types of *U. griseola* and *U. bicolorata* var. *pseudorubescens*, branches are not tapering but irregular (i.e. the largest part of the main branches is not close to the basal part). Furthermore, lateral branches are slightly but distinctly constricted and the CMA-type is close to the *tenuicorticata*-type (M/C > 7).

CMA. (Specimens collected in São Tomé only, $n = 3$). %C = (4.5–) 4.7–6.2–7.5; %M = 32–34.5–37.6(–38); %A = 15–21.3–27.8(–28); A/M = 0.4–0.6–0.7; A/C = 3.0–3.1–3.2; M/C = 4.4–5.9–7.8(–8.1).

Chemistry. (Specimens collected in São Tomé only, $n = 1$). Medulla K–, Pd+ red-orange; protocetraric acid. The two other specimens collected are K–, Pd+ red-orange (no TLC performed).

Diagnostic characters. The main diagnostic characters of *U. bicolorata* are the shrubby sorediate thallus, the jet black-pigmented basal part, the irregular branches, the constricted lateral branches, the orange-red subcortical pigment, the large (> 1/2 branch diameter) and irregular soralia that can encircle the branches, the *cornuta-tenuicorticata* type of CMA and the presence of protocetraric acid in the medulla.

Variability. The development of soralia, from punctiform and minute to large, ±convex and encircling the branches, varies greatly depending on the maturity of the thallus as well as on environmental conditions. The chemistry is variable with different chemotypes (Ohmura *et al.* 2010).

Distribution and ecology. *Usnea bicolorata* is known to occur in tropical Africa (Motyka 1936–1938, 1956; Dodge 1957; Swinscow & Krog 1975, 1988) and in Taiwan (Ohmura *et al.* 2010). In Taiwan, *U. bicolorata* was found on the bark of *Pinus* at an elevation of *c.* 2600 m. It is a common species on shrubs in the montane forests and ericaceous zone at 1900–3500 m elev. in East Africa (Swinscow & Krog 1988). In São Tomé and Príncipe, it was found in primary *Rubiaceae*-dominated forests on the island of São Tomé between 1230 and 1298 m, growing on hardwood branches with *U. articulata*, *U. firmula* and *U. submollis*. *Usnea bicolorata* is newly reported for São Tomé and Príncipe.

Discussion. Both specimens collected in São Tomé are juvenile and small with punctiform and minute underdeveloped soralia. However, all other characters fit well with the definition of *U. bicolorata*. Several other sorediate *Usnea* species are known to have an orange-reddish subcortical pigmentation, such as *U. crocata* Truong & P. Clerc, *U. dorogawensis* Asahina, *U. grandisora* Truong & P. Clerc, *U. poliothrix* Kremp., *U. subcornuta* Stirt. and *U. subdasaea* Truong & P. Clerc. However, *U. bicolorata* is the only species among them with a distinct jet black-pigmented basal part. For differences with *U. sorediosula* Motyka, the other African species with an orange-reddish subcortical pigmentation, see under that species. The var. *australiensis* G. N. Stevens with

galbinic acid, ‘pale or black’ trunk, ‘soralia sparse or quite dense in pseudocypheallae’ [soralia] should be further studied since it might not correspond to *U. bicolorata*. *Usnea bicolorata* could not be sequenced since it was separated out from mixed collections after the sequencing had already been completed in 2014–2015.

Specimens examined. **São Tomé and Príncipe:** *Island of São Tomé:* Parque Natural Obô de São Tomé on the primary trail from Bom Sucesso to Lagoa Amelia, 1298 m, 2013, M. Nadel, J. Shevock, T. Daniel & E. Soares MN0552c (CAS); secondary trail from Bom Sucesso to Lagoa Amelia, 1230 m, 2013, M. Nadel, J. Shevock, T. Daniel & E. Soares MN0579c (CAS).

Usnea erinacea aggr.

Incl.: *Usnea erinacea* Vain. *Dansk Bot. Ark.* 4 (no. 11), 3 (1926); type: Mexico, Chimantla, 1846, Liebmann (holotype—TUR!). Chemistry: salazinic and norstictic acids. %CMA = 7.5/17.5/50; A/M = 2.9; A/C = 6.7; M/C = 2.3.

Usnea sanguinea Swinscow & Krog, *Lichenologist* 11, 243 (1979); type: Tanzania, Northern Province, Arusha National Park, Juniper Hill, Swinscow & Krog T3/16 (holotype—BM!). Chemistry: salazinic and norstictic acids. %CMA: 10.5/15/49, A/M: 3.3, A/C: 4.7, M/C: 1.4

A detailed description is provided by Clerc (2004, 2007).

CMA. (Specimens collected in São Tomé only, $n = 1$). %C = 8; %M = 28; %A = 29; A/M = 1; A/C = 3.6; M/C = 3.5.

Chemistry. (Specimen collected in São Tomé only, $n = 1$). Medulla K–, Pd+ red-orange; protocetraric acid, unknown 3/6/5 greyish green.

Diagnostic characters. The main diagnostic characters are the shrubby apotheciate, non-sorediate thallus with a cortical red pigmentation, the tapering branches and the non-constricted lateral branches.

Distribution and ecology. The *Usnea erinacea* aggregate occurs on a wide variety of trees, on rocks, and rarely on dead trunks or fences and scrubs in the subtropical/tropical areas over a wide altitudinal range (200–3000 m), mainly in South America (Truong *et al.* 2011; Gerlach *et al.* 2017), Africa (Swinscow & Krog 1988) and Australia (Stevens 1999). In São Tomé and Príncipe, it was found on the island of São Tomé only, at 2009 m on the summit of the highest peak on the island. The specimen was blown by wind onto the concrete monument on the peak but had a clearly corticolous growth habit. The surrounding vegetation consisted of small trees and shrubs covered in large amounts of lichen and liverworts including *U. exasperata*. The *Usnea erinacea* aggregate is newly reported for São Tomé and Príncipe.

Discussion. *Usnea erinacea* as currently understood is polyphyletic (Truong *et al.* 2013a; Gerlach *et al.* 2017; Lücking *et al.* 2020) and requires a thorough study with the tools of integral taxonomy. It contains several species differing mainly in the type of reddish pigmentation, in the pigmentation of the basal part, in the anatomical characters (cortex structure and thickness) and in the chemistry. The small specimen (2 cm long) without apothecia collected on São Tomé Island (MN0264a) has a variegated reddish

cortical pigmentation, a jet black-pigmented basal part, a matt cortex and protocetraric acid in the medulla. All these characters do not fit with the concept of either *U. erinacea* s. str. or *U. sanguinea*. In our phylogenetic tree (Fig. 2), MN0264a belongs to the clades 3 & 4 of Truong *et al.* (2013a). It appears as the sister species of *U. crocata*, another pigmented taxon, but this relationship is not supported. It is most probably an undescribed species. More specimens from Africa and South America are needed to formally describe this species.

Specimens examined. São Tomé and Príncipe: *Island of São Tomé:* Parque Natural Obô de São Tomé, on the summit of Pico de São Tomé, 2009 m, 2012, M. Nadel & O. Rocha MN0264a (CAS).

Usnea exasperata (Müll. Arg.) Motyka

In Zahlbruckner & Hauman, *Nat. Hist. Danish Lich.* 5, 24 (1936).—*Usnea dasygoides* var. *exasperata* Müll. Arg., *Flora (Regensburg)* 73, 336 (1890); type: Kilimandjaro [Kilimanjaro], b. 3000 m, x.1889, Hans Meyer (holotype—G00066257!)

A detailed description, synonyms and images of *U. exasperata* can be found in Swinscow & Krog (1978, 1988).

CMA. (Specimens collected in São Tomé only, $n = 3$). %C = 9.0–10.5–12; %M = 24.5–25.7–27.3(–27.5); %A = 27–28.3–29.8(–30); A/M = 1.0–1.1–1.2; A/C = (2.2–)2.3–2.7–3.0; M/C = 2.1–2.5–3.0.

Chemistry. (Specimens collected in São Tomé only, $n = 3$). Medulla K⁺ slowly yellowish, Pd⁺ citrine yellow; psoromic and 2'-O-demethylpsoromic acids.

Diagnostic characters. (Specimens collected in São Tomé only, $n = 3$). The main diagnostic characters of this form of *U. exasperata* are the pendulous thallus, the irregular branches, the cylindrical to slightly acute-angled segments in cross-section (Fig. 6D), the lateral branches widened at ramification points (Fig. 6F), the smooth cortex with linear cracks developing into pseudocyphellae, ±circular and ±stipitate soralia that might aggregate but not fuse together, the matt and relatively thick cortex, the dense to compact medulla with, on average, the same thickness as the central axis in cross-section (Fig. 6B), and the presence of psoromic acid in the medulla.

Variability. Swinscow & Krog (1978) discussed in detail the variability of *U. exasperata*. They accepted a broad concept of the species, with the inclusion of a total of 17 taxa described by Motyka (1956) into synonymy with *U. exasperata*. The morphotype encountered on São Tomé corresponds to the 'smooth form' described by Swinscow & Krog (1978: figs 11 & 12) in East Africa.

Distribution and ecology. *Usnea exasperata* was so far known to occur only in East Africa (Motyka 1936–1938; Swinscow & Krog 1978). The localities in São Tomé extend the distribution of this species to West Africa; it was found on the island of São Tomé at high elevation (1800–2009 m) in the primary, Rubiaceae-dominated forest, corticolous on the shrubs and hardwood trees and fallen on leaf litter. It was associated with *U. articulata*, *U. erinacea* aggr. and *U. pectinata* aggr. *Usnea exasperata* is newly reported for São Tomé and Príncipe.

Discussion. Among the material collected in São Tomé, *U. exasperata* could only be superficially confused with *U. articulata*. However, the latter species has a much thinner cortex, a larger and laxer medulla and thinner central axis, and protocetraric and/or diffractaic acids in the medulla. Only the chemotype of *U. exasperata* with psoromic acid was found in São Tomé. The type specimen of *U. exasperata* has protocetraric and fumarprotocetraric acids in the medulla. Among the 17 taxa put into synonymy by Swinscow & Krog (1978), only *U. elegantissima* Motyka shares this chemotype with *U. exasperata* and would be the available name for this 'smooth morphotype' in case future molecular studies would split this taxon into distinct species corresponding to the chemotypes. In the phylogenetic tree (Fig. 2), *U. exasperata* belongs to the clades 3 & 4 of Truong *et al.* (2013a). It appears monophyletic, although not strongly supported, and is nested into a weakly supported clade containing *U. articulata*, *U. submollis* and *U. ghattensis* G. Awasthi (Fig. 3). This would confirm the close relationship with *U. articulata* as already mentioned by Swinscow & Krog (1976b).

Specimens examined. São Tomé and Príncipe: *Island of São Tomé:* Parque Natural Obô de São Tomé, trail between Pico Cálvario and Pico Mesa below Pico de São Tomé, 1863 m, 2012, M. Nadel, J. Shevock & A. Stanbridge MN0243 (CAS); on the summit of Pico de São Tomé, 2009 m, 2012, M. Nadel & O. Rocha MN0260 (CAS); trail between Pico Mesa and the Rio Cantador Valley, 1837 m, 2012, M. Nadel, J. Shevock & A. Stanbridge MN0269 (CAS).

Usnea firmula (Stirt.) Motyka

Lich. Gen. Usnea Monogr., 51 (1936–1938).—*Eumitria firmula* Stirt., *Scott. Natural.* 6(3), 100 (1881) [1881–1882]; type: [Bioko] Fernando Po, lava beds, G. Thomson s. n., undated [19th century] (holotype—BM). Chemistry: protocetraric acid.

Syn. nov.: *Usnea baileyi* var. *pinnatifida* Swinscow & Krog, *Norw. J. Bot.* 21(2), 172 (1974); type: Tanzania, Central Province, Mpwapwa District, Kiboriani Mountains, highest peak N of Mpwapwa, 1800 m, evergreen bush on quartzite rocks of the SSE slope, 11.v.1972, T. Pocs & L. Mezosi (holotype—BM-6566/I!). Chemistry: protocetraric acid.

Complete descriptions, images and synonyms can be found in Swinscow & Krog (1974, 1988).

CMA. (Specimens collected in São Tomé only, $n = 4$). %C = (2.0–)2.5–3.4–4.0; %M = (1.0–)1.2–2.1–3.0; %A = (86–)86.5–89.3–92; A/M = 27.7–48–71.3(–81.3); A/C = 20.9–28.9–39.7(–44.7); M/C = (0.3–)0.4–0.7–1.0; %TBA = (79–)79.3–83–86.7(–87).

Chemistry. (Specimens collected in São Tomé only, $n = 2$). Medulla K⁺ yellow turning orange, Pd⁺ red-orange, hyphae of the tubular axis K⁺ pale greenish yellow, Pd[–]. Protocetraric acid, unknowns 4.5–5–5/3–3/5–5.5 pale brownish spots.

Diagnostic characters. *Usnea firmula* is characterized by a stiff shrubby to subpendant thallus (Fig. 8A), a jet black-pigmented basal part with black pigmentation often spreading on the main branches (Fig. 8E), irregular branches with cylindrical segments (Fig. 8A), long and slender fibrils the number of which is inversely proportional to the number of tubercles (juvenile fibrils), the absence of soralia, the frequent occurrence of

apothecia mainly growing laterally on short branches (Fig. 8B), a thin, reddish pigmented medulla and a broad tubular central axis (Fig. 8F).

Variability. The morphology of *Usnea firmula* is highly variable depending on the growth of fibrils and whether they are shed or not. The presence of apothecia is also variable, from absent to numerous.

Distribution and ecology. *Usnea firmula* was described on the basis of a specimen collected on the island of Bioko (Fernando Po) situated to the NW of São Tomé, also in the Gulf of Guinea. It is so far known only from Africa (Cameroon, Kenya, Republic of Equatorial Guinea and Tanzania). In São Tomé and Príncipe it occurs on the island of São Tomé in the primary, *Rubiaceae*-dominated forest between 1162 and 1323 m, growing corticolous on hardwood branches often fallen to the forest floor and in leaf litter. It is found associated with *U. articulata*, *U. bicolorata* and *U. submollis*. This collection of *Usnea firmula* is noted as new for São Tomé and Príncipe (Lücking *et al.* 2020).

Discussion. *Usnea firmula*, with its tubular central axis and phylogenetic position (Figs 2 & 5), belongs to the subgenus *Eumitria*. It differs from *U. baileyi* mainly by its black-pigmented basal part, its irregular branches, the absence of soralia, and the frequent occurrence of apothecia. There also seems to exist differences in the %CMA and %TBA, but the number of specimens studied here is too small to draw definitive conclusions. The species also differ in their chemistry: *Usnea baileyi* contains norstictic acid whereas *U. firmula* produces protocetraric acid in the medulla. However, Swinscow & Krog (1974) described two varieties of *U. baileyi* (var. *pinnatifida* Swinscow & Krog and var. *planiuscula* Swinscow & Krog), both with protocetraric acid in the medulla. *Usnea baileyi* var. *pinnatifida* corresponds well to *U. firmula* with an extended jet black-pigmented basal part, the irregular branches, and the chemistry. It is considered here as a synonym of *U. firmula*. *Usnea baileyi* var. *planiuscula* is a different taxon that significantly differs from both *U. firmula* and *U. baileyi*. Further studies are necessary to determine its status. *Usnea firmula* and its affiliation to the *Eumitria* subclade is phylogenetically confirmed here, as in Lücking *et al.* (2020). All sequenced specimens are clustered in a partly supported monophyletic clade sister to that formed by *U. baileyi* and *U. pectinata* (Figs 2 & 5).

Specimens examined. São Tomé and Príncipe: Island of São Tomé: Parque Natural Obô de São Tomé, on road from Bom Sucesso to 'Macambara' Radio Station, 1162–1319 m, 2012, M. Nadel & J. Shevock MN0084 (CAS); trail from 'Macambara' Radio Station leading into the forest, 1323 m, 2012, M. Nadel & J. Shevock MN0117 (CAS); primary trail from Bom Sucesso to Lagoa Amelia, 1298 m, 2013, M. Nadel, J. Shevock, T. Daniel & E. Soares MN0550a, MN0550b (CAS); secondary trail from Bom Sucesso to Lagoa Amelia, 1241 m, M. Nadel, J. Shevock, T. Daniel & E. Soares MN0581 (CAS).

Usnea krogiana P. Clerc

Lichenologist 38, 199 (2006); type: Espagne [Spain], Iles Canaries, La Gomera, Alajero, Lomo de la Mulata, sur les pentes SE du Garajonay, surplombant la route menant à Lajero, 1320–1340 m, sur *Erica arborea* dans des plantations de pins en

pente SSE, avec *Teline canariensis*, 23.ix.1986, P. Clerc (holotype —G00066621). Chemistry: stictic (major), constictic, cryptostictic, menegazziaic and norstictic acids (minor). %CMA = 11/9/60; A/M = 6.6; A/C = 5.5; M/C = 0.8.

A detailed description and images of *U. krogiana* can be found in Clerc (2006).

CMA. (Specimens collected in São Tomé only, *n* = 1). %C = 12; %M = 9; %A = 57; A/M = 4.7; A/C = 4.7; M/C = 0.8.

Chemistry. (Specimens collected in São Tomé only, *n* = 1). Medulla K+ yellow turning slowly orange-red, Pd+ citrine yellow; soralia K+ yellowish, Pd+ yellow; norstictic and salazinic acids. This chemotype is new for *U. krogiana*.

Diagnostic characters. *Usnea krogiana* is an easily recognizable species with the small stiff and shrubby thallus, the basal part that is sharply delimited with a black pigment, the tapering branches with cylindrical segments, the small and minute fiber-cles/soralia, the matt and relatively thick cortex, the thin and compact medulla and the large central axis (> 50%).

Variability. *Usnea krogiana* is not a very variable species and can be easily recognized in the field. Chemistry, however, shows a little variation with three chemotypes: 1) stictic acid gr. and norstictic acid; 2) norstictic acid; 3) norstictic and salazinic acids.

Distribution and ecology. Prior to the São Tomé collection (Nadel 2016; Lücking *et al.* 2020), *Usnea krogiana* was known to occur only in Macaronesia (Azores and Canary Islands) and in the West Indies (Cuba, Dominican Republic, Haiti and Puerto Rico) (Clerc 2006). In São Tomé and Príncipe it was found only on the island of São Tomé, at Lagoa Amelia, a vegetation covered lake in a dormant volcanic crater, growing on a shrubby species of *Schefflera*.

Discussion. The specimen collected on São Tomé corresponds well morphologically and anatomically to the type. The presence of salazinic acid together with norstictic acid had not been previously recorded in this species. For differences from other shrubby sorediate taxa, see Clerc (2006). *Usnea krogiana* cannot be confused with any other taxa discussed in this article. The sequences generated (Nadel 2016) and analyzed here and in Lücking *et al.* (2020) mark the first time that *U. krogiana* has been sequenced. It belongs to clades 3 & 4 of Truong *et al.* (2013a). It is interesting to note that in our phylogenetic tree (Fig. 2), this Macaronesian species belongs to an unsupported clade mainly containing species originally described from Macaronesia (*U. macaronesica* P. Clerc and *U. boomiana* P. Clerc), together with *U. arianae* P. Clerc *et al.*

Specimen examined. São Tomé and Príncipe: Island of São Tomé: Lagoa Amelia, 1418 m, 2013, M. Nadel, J. Shevock, T. Daniel & E. Soares MN0560 (CAS).

Usnea longiciliata P. Clerc & Nadel sp. nov.

MycoBank No.: MB 843680

Thallus shrubby, stiff, dark green-coloured, with apothecia and without soralia. Lateral branches not constricted. Axis thick

(> 50%). Medulla dense, not compact, containing the unknown substances US1 and US2 *sensu* Ohmura (2001). Apothecia mainly subterminal, with long (5–15 mm) cilia. Ascospores 9–12 × 6–8.5 µm.

Type: Republic of São Tomé and Príncipe, Gulf of Guinea, Island of Príncipe, trail up to the summit of Pico Papagaio from Santa Trindade, 509 m elev., fallen from tree onto trail, 15 April 2013, M. Nadel 485 (holotype—CAS1318659). Chemistry: US1, US2 (*sensu* Ohmura 2001). %CMA = 10/10.5/59; A/M = 5.6; A/C = 5.9; M/C = 1.1. Ascospores: 9–12 × 6–8.5 µm.

(Fig. 9)

Thallus shrubby and stiff, up to 6 cm long, dark green-coloured, with ±isotomic-dichotomous ramifications (Fig. 9A); *basal part* short, brownish-blackish (not jet black) close to the holdfast; *main branches* 0.6–1 mm thick, tapering or cylindrical, rarely slightly irregular; *segments* cylindrical, terete in cross-section; *lateral branches* not constricted at attachment point; *terminal branches* moderately thin, almost unbranched; *foveolae* and *transverse furrows* absent; *maculae* and *pseudocyphellae* absent; *papillae* absent; *tubercles* (*young fibrils*) numerous (Fig. 9D); *fibrils* numerous, 3–5 mm long, slender on young thalli, almost absent on mature thalli; *fibercles* few on young thalli, numerous on mature thalli; *soralia* absent.

Apothecia numerous on mature thalli, 1–6 mm wide, mainly terminal, sometimes pseudoterminal, flat to slightly cupular, with pruinose discs surrounded by long cilia (5–15 mm long), with an eroded cortical rim (Fig. 9C); *ascospores* ellipsoid, 9–12 × 6–8.5 µm.

Cortex slightly shiny in section, of the *ceratina*-type plectenchyma, moderately thick, %C = (7.5–)8.3–9.8–11.3(–11.5) ($n = 5$); *medulla* dense, thin, %M = 10.5–13–16.2(–16.5); *axis* thick, %A = (46–)48.8–54.2–59; A/M = (2.8–)2.9–4.4–5.6; A/C = (4.3–)4.5–5.6–6.7(–6.9); M/C = 0.9–1.4–1.9(–2.2) (Fig. 9B).

Chemistry. ($n = 5$). Medulla K+ pale yellow, Pd–; unknowns US1 and US2 (*sensu* Ohmura 2001).

Etymology. The name *longiciliata* refers to the long cilia (fibrils) surrounding the apothecial discs.

Diagnostic characters. The shrubby, stiff and apotheciate thallus, the long fibrils (cilia) on the edge of the apothecia, the numerous fibercles, the non-constricted lateral branches, the cylindrical to tapering main branches, the thick central axis and thin but dense medulla and the distinctive chemistry are the main diagnostic characters of this species.

Variability. Young thalli might lack apothecia (Nadel 405). They usually have numerous fibrils and few fibercles. Mature thalli lose fibrils and therefore have numerous fibercles (type specimen).

Distribution and ecology. *Usnea longiciliata* is currently endemic to the island of Príncipe. It is a corticolous species occurring in the primary *Rubiaceae* forest between 465 and 580 m in the central region of the island, often fallen to the forest floor on small twigs and branches. It is found associated with the other new species, *U. beckeri*.

Discussion. A search among the type specimens and protologues of all the shrubby, non-sorediate and apotheciate species

occurring in Africa and South America to find a previously published name was unsuccessful. *Usnea hakonensis* Asahina and *U. beckeri* are the only other species for which the unknown substances US1 and US2 are diagnostic (Ohmura 2001). Morphologically and anatomically it does not resemble any other shrubby and apotheciate African or South American species. For differences from *U. beckeri*, *U. nodulosa* and *U. submolliis*, see under these taxa. In our phylogenetic tree (Fig. 2), *U. longiciliata* belongs to the clades 3 & 4 of Truong *et al.* (2013a). It is monophyletic and sister clade to that formed by *U. bismolliuscula*, *U. nodulosa* and *U. beckeri* (Fig. 4).

Paratypes. São Tomé and Príncipe: Island of Príncipe: trail from Santa Trindade to Pico Papagaio, 465 m, between roça and rope ladder, 2012, M. Nadel & J. Shevock MN0405 (CAS); *ibid.*, 488 m, between roça and rope ladder, 2012, M. Nadel & J. Shevock MN0407 (CAS); trail from Santa Trindade to summit Pico Papagaio, 543 m, 2013, M. Nadel, J. Shevock, T. Daniel & O. Rocha MN0492 (CAS); slope between Pico Príncipe and Ribeira Banzu, 480 m, 2013, M. Nadel & O. Rocha MN0525 (CAS); trail from Terreiro Velho to Morro de Leste, 585 m, 2013, J. Shevock, T. Daniel, O. Rocha & M. Nadel MN0537 (CAS).

Usnea nodulosa Swinscow & Krog

Lichenologist 11, 232 (1979); type: Uganda, Masaka District, Bukoto County, Jubiya forest, elev. 1100 m, on twigs of exposed shrubs, 1971, Swinscow 3U 32/5 (holotype—BM!). Chemistry: usnic, constictic and barbatic acids. %CMA = 3/39/16; A/M = 0.4; A/C = 5.7; M/C = 13.9. Ascospores: 7.5–10 × 5–6 µm.

A detailed description is provided in Swinscow & Krog (1979, 1988).

CMA. (Specimens collected in São Tomé only, $n = 7$). %C = (3.0–)3.2–3.9–4.6(–5.0); %M = (33–)34.4–36.4–38.4(–39.5); %A = (14–)16.1–19.1–22; A/M = 0.4–0.5–0.6(–0.7); A/C = 4.0–5.0–6.0(–7.0); M/C = (6.8–)7.6–9.5–11.4(–11.8).

Ascospores. (Specimens collected in São Tomé only, $n = 2$). 7–9 × 5.5–7.0 µm.

Chemistry. ($n = 8$). Medulla, central axis, apothecial discs: K+ dark yellow, Pd+ red-orange. 1) Constictic and barbatic acids (type specimens, $n = 3$); 2) stictic, constictic, cryptostictic, menegazziaic and barbatic acids (São Tomé specimens, $n = 5$), new chemotype.

Diagnostic characters. The main diagnostic characters of *U. nodulosa* are the erect, bushy thallus with numerous terminal apothecia containing small ascospores, without soralia, the constricted lateral branches, the numerous fibrils and fibercles, the *tenuicorticata*-type of CMA with glossy cortex and lax medulla, and the presence of barbatic acid with either the stictic acid group (including constictic acid) or with constictic acid alone in the medulla.

Variability. The position and dimension of the apothecia are variable. Some thalli have a majority of terminal apothecia while in others, apothecia are mainly lateral. The density of fibrils/fibercles is also variable, as is the size of the thallus.

Distribution and ecology. *Usnea nodulosa* was so far known only from Uganda, Kenya and Tanzania (Swinscow & Krog 1978,

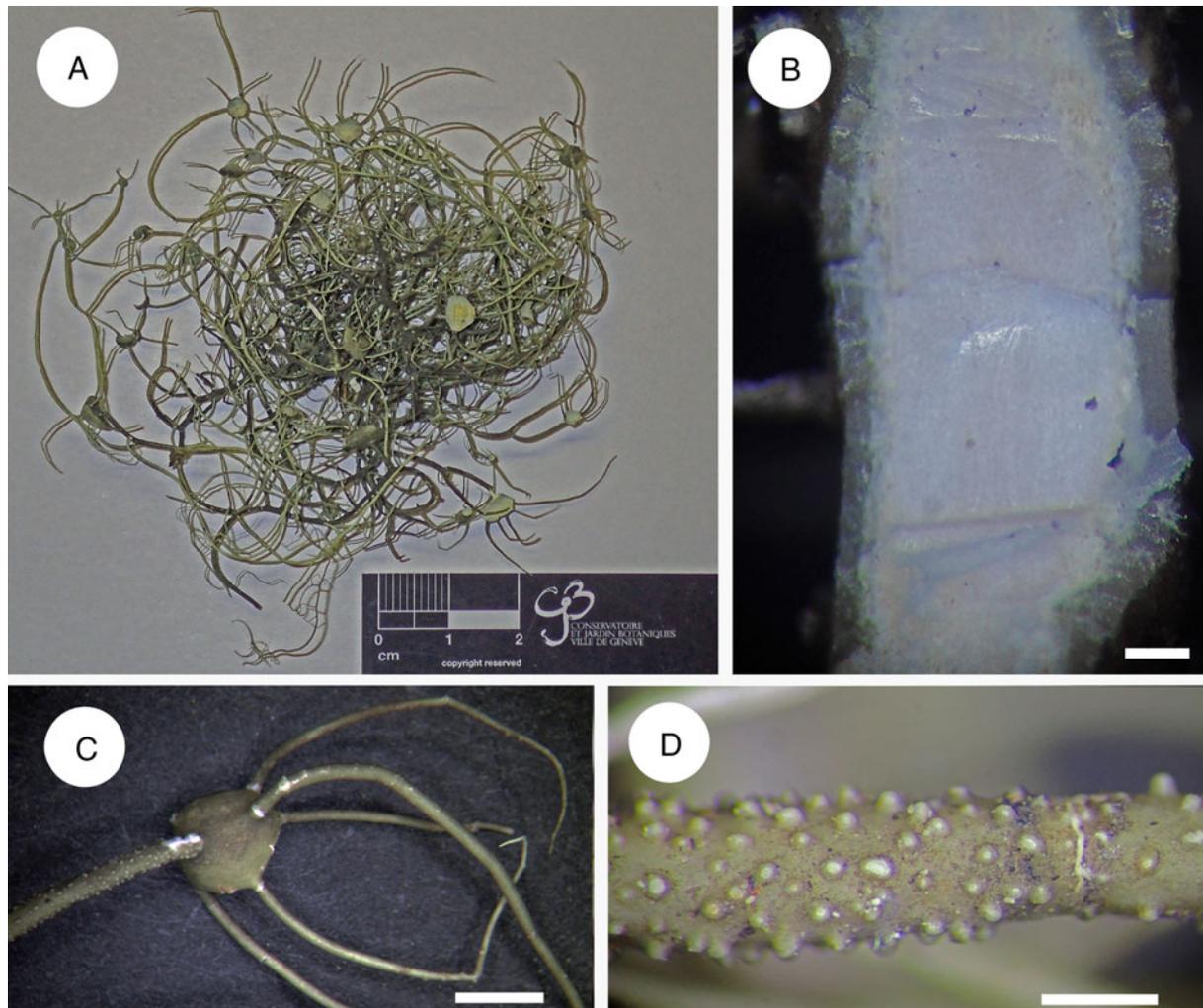


Fig. 9. *Usnea longiciliata* holotype. A, thallus. B, transversal section of a main branch. C, subterminal apothecia with long fibrils (cilia). D, branch with tubercles (young fibrils) and fiberclones. Scales: B = 0.2 mm; C = 2 mm; D = 0.5 mm. In colour online.

1988). In Eastern Africa, *U. nodulosa* is a corticolous species occurring mainly on twigs of exposed shrubs in hot, dry bushland at 1000 to 1500 m elev. The occurrence in São Tomé and Príncipe is an important extension of its distribution area to the western part of Africa. It was found in the primary *Rubiaceae*-dominated forest on both São Tomé and Príncipe islands. On São Tomé it was found at mid elevations between 862 and 1229 m and on Príncipe one collection was made at 598 m. It is found associated with *U. pectinata*. *Usnea nodulosa* is new for São Tomé and Príncipe.

Discussion. The tubercles (or nodules) of *U. nodulosa* with pseudocypbellae at their apices described by Swinscow & Krog (1978) are reinterpreted here as fiberclones (scars left after fibrils are shed). In comparison, *U. submollis* has no or few fiberclones, but numerous small verrucose true papillae, larger ascospores and a different chemistry (norstictic and salazinic acids). *Usnea beckeri* has a smooth cortex without papillae or tubercles and a different chemistry (stictic acid group, US1 and US2). *Usnea longiciliata* has a different chemistry (US1 and US2), non-constricted lateral branches and a thicker axis. *Usnea albomaculata* Motyka is a similar species with fewer fiberclones, conspicuous medullary extrusions at the level of the axils and fiberclones, and a different chemistry (psoromic acid).

Usnea picta (J. Steiner) Motyka has lateral branches that are not constricted, another CMA-type (isotype (G): %CMA = 7/24.5/37; A/M = 1.5; A/C = 5.3; M/C = 3.5), a dense to compact medulla and a different chemistry (salazinic acid). *Usnea nodulosa* was sequenced here for the first time. In our phylogenetic tree (Fig. 2), it belongs to the clades 3 & 4 of Truong *et al.* (2013a) and is the sister species of *U. beckeri* (Fig. 4).

Specimens examined. São Tomé and Príncipe: *Island of São Tomé:* Parque Natural Obô de São Tomé, on road from Bom Sucesso to 'Macambara' Radio Station, 1162–1319 m, 2012, M. Nadel & J. Shevock MN0066 (CAS); secondary trail from Bom Sucesso to Lagoa Amelia, 1229 m, 2013, M. Nadel, J. Shevock, T. Daniel & E. Soares MN0580 (CAS); unimproved road between Bemposta and Chamico near São Luís, 885 m, 2013, M. Nadel, J. Shevock, T. Daniel & Q. Quade MN0595 (CAS); unimproved road between São Luís and Chamico, 898 m, 2013, M. Nadel, J. Shevock, T. Daniel & Q. Quade MN0599 (CAS); unimproved road between Bemposta and Chamico, near Bemposta, 862 m, 2013, M. Nadel, J. Shevock, T. Daniel & Q. Quade MN0600b, MN0603 (CAS). *Island of Príncipe:* slope between Pico Príncipe and Ribeira Banzu, 598 m, 2013, M. Nadel & O. Rocha MN0521b (CAS).

Usnea pectinata aggr.

Incl.: *Usnea pectinata* Taylor, *London J. Bot.* **6**, 191 (1847); type: Himalaya, Sylhet [Bangladesh], s. d., Wallich s. n. (isotype—M!). Chemistry: stictic, constictic, cryptostictic, menegazziaic and norstictic acids. %CMA = 4/7.5/77; A/M = 10.1; A/C = 20.3; M/C = 2.

Usnea gigas Motyka, *Lich. Gen. Usnea Monogr.*, 388, 400 (1936–1938); type: Congo Belge, Banana, Penge Irumu, forêt vierge de l'Ituri, épiphyte sur les branches dans la couronne des arbres, 24.xi.1914, Becquaert (isotype—S!). Chemistry: protocetraric acid (TLC by H. Krog in 1977). %CMA = 5.5/10/68; A/M = 6.7; A/C = 12; M/C = 1.8.

Usnea himantodes Stirt., *Scott. Natural.*, N. S. **1**(7), 75 (1883) [1883–1884]; type: Australia, New South Wales, Illawarra, 1882, Kirton s. n. (holotype—BM!). Chemistry: stictic, constictic, cryptostictic, menegazziaic and norstictic acids (tr.). %CMA = 8.5/13.5/56; A/M = 4.1; A/C = 6.6; M/C = 1.6.

Usnea mexicana Vain., *Dansk Bot. Ark.* **4**, 3 (1926); type: Mexico, Paso de Doña Juana, in arboribus, ii.1841, Liebman 7703 (lectotype—TUR-V!). Chemistry: diffractaic and constictic acids. %CMA = 5.5/6.5/76; A/M = 11.7; A/C = 13.8; M/C = 1.2.

A detailed description and images of the different morphologies and chemistry of the *U. pectinata* aggregate are given in Ohmura (2001), Swinscow & Krog (1978, 1988; as *U. gigas*), Herrera-Campos *et al.* (1998; as *U. mexicana*), Stevens (1999; as *U. himantodes*), Clerc (2007; as *U. mexicana*) and Truong *et al.* (2013b; as *U. mexicana*).

CMA. (Specimens collected in São Tomé only, $n = 10$). %C = (3.5–)3.9–5.6–7.3(–8.0); %M = (8.0)10.1–14.5–18.9(–21.5); %A = (43–)49.2–59.8–70.4(–75); A/M = (2.0–)2.2–4.8–7.4(–9.5); A/C = (6.6–)7.1–11.8–16.5(–18.3); M/C = 1.6–2.8–4.0(–5.1).

Chemistry. (Specimens collected in São Tomé only, $n = 11$). 1) Medulla K–, Pd+ red-orange; protocetraric acid ($n = 3$). 2) Medulla K+ yellow turning dark red, Pd+ yellow; diffractaic, barbatic (tr.) and \pm protocetraric (tr.) acids ($n = 1$). 3) Medulla K+ yellow turning dark red, Pd+ yellow; salazinic and constictic acids ($n = 1$). 4) Medulla K+ yellow, Pd+ orange; constictic acid ($n = 1$). 5) Medulla K+ dirty yellow, Pd+ orange; constictic, diffractaic and barbatic acids (tr.). ($n = 1$). 6) Medulla K+ dirty yellow, Pd+ orange; salazinic, protocetraric and fatty acids 5/3/4 ($n = 1$). 7) Medulla K+ dirty yellow, Pd+ orange; salazinic, diffractaic and \pm protocetraric (tr.) acids ($n = 3$).

Diagnostic characters. The main diagnostic characters of the *U. pectinata* aggregate are the pendulous, stiff and brittle thallus with long fibrils in an almost fishbone-like arrangement (Fig. 10A), the irregular branches that are circular to almost alate in cross-section (Fig. 10C–H) (see Swinscow & Krog 1978: fig. 7), the thick (< 50%) and often yellowish brown to dark brown pigmented axis (Fig. 10B) that is often somewhat fistulose (only in the thickest branches), and the frequent thick and whitish zones of cortex regeneration between the branch segments.

Variability. This aggregate displays a huge morphological and chemical variation (see the discussion in Swinscow & Krog (1978)).

Distribution and ecology. The *Usnea pectinata* aggr. is found on all continents with a tropical/subtropical climate. It grows on tree

bark or commonly on rocks as, for example, in Japan (Ohmura 2001). In Eastern Africa, it is a widespread species locally common on trunks and large branches of trees in open woodland and by waysides at 1000–2000 m elev. (Swinscow & Krog 1988). It was found on both São Tomé (825–1863 m elev.) and Príncipe (443–596 m) in open primary forests of rubiaceous tropical hardwoods, as well as roadside in disturbed secondary forests. It was often found fallen from the canopy into leaf litter but also found growing on shrubs, tree branches and tree trunks. It was associated with *U. articulata*, *U. baileyi*, *U. exasperata* and *U. nodulosa*. This collection of the *Usnea pectinata* aggregate is noted as newly reported for São Tomé and Príncipe (Lücking *et al.* 2020).

Discussion. As a consequence of the huge morphological and chemical variation, more than 20 species have been described in this aggregate. Figure 10C–H illustrates part of the important main branch morphological variation in this aggregate. In our phylogenetic tree (Fig. 2), *Usnea pectinata* s. str., corresponding to the stictic acid chemotype with an unpigmented central axis (AB051655 & AB051656), is nested inside a group of specimens with a pigmented central axis and various chemotypes (Fig. 5) corresponding to some of the other species described in the aggregate. A thorough and worldwide study using the tools of integrative taxonomy seems to be necessary to clarify whether there is one very variable species or many small well-defined taxa (Temu *et al.* 2019; Lücking *et al.* 2020). *Usnea gigas* is the sexually reproducing taxon of this aggregate.

Specimens examined. **São Tomé and Príncipe:** *Island of São Tomé:* road to São Nicolau, 825 m, 2012, M. Nadel & J. Shevock MN0060 (CAS); Parque Natural Obô de São Tomé, on road from Bom Sucesso to 'Macambara' Radio Station, 1162–1319 m, 2012, M. Nadel & J. Shevock MN0065, MN0068a (CAS); trail from Bom Sucesso to Pico Cálvario, 1192 m, 2012, M. Nadel, J. Shevock & A. Stanbridge MN0163 (CAS); trail between Pico Cálvario and Pico Mesa, 1863 m, 2012, M. Nadel, J. Shevock & A. Stanbridge MN0241 (CAS); primary trail from Bom Sucesso to Lagoa Amelia, 1381 m, 2013, M. Nadel, J. Shevock, T. Daniel & E. Soares MN0556 (CAS); secondary trail from Bom Sucesso to Lagoa Amelia, 1191 m, 2013, M. Nadel, J. Shevock, T. Daniel & E. Soares MN0578a (CAS); *ibid.*, 1216 m, 2013, M. Nadel, J. Shevock, T. Daniel & E. Soares MN0583 (CAS); road between Bemposta and Chamico, near Bemposta, 862 m, 2013, M. Nadel, J. Shevock, T. Daniel & Q. Quade MN0602 (CAS). *Island of Príncipe:* trail up to summit of Pico Papagaio from Santa Trindade, 443 m, 2013, M. Nadel, J. Shevock, T. Daniel & O. Rocha MN0481 (CAS); trail from Terreiro Velho to Morro de Leste, 522 m, 2013, M. Nadel & O. Rocha MN0527 (CAS); *ibid.*, 596 m, 2013, M. Nadel & O. Rocha MN0540 (CAS); *ibid.*, 585 m, 2013, J. Shevock, T. Daniel, O. Rocha & M. Nadel MN0542 (CAS).

Usnea sorediosula Motyka

Lich. Gen. Usnea Monogr., 330 (1936–1938); type: Usambara [Tanzania], Waldsteppe am Kumba Sumpf, 1894, Holst 3526 (holotype—G0066557!). Chemistry: protocetraric acid, unknown substance (TLC by H. Krog in 1973). %CMA = 13/14/46; A/M = 3.3; A/C = 3.5; M/C = 1.0.

Nomenclatural note: *Usnea barbata* var. *sorediosula* (Müll. Arg.) Müll. Arg., *Bot. Jb.* **20**, 245 (1894) is based on *U. dasypogoides* var. *sorediosula* Müll. Arg. *Flora (Regensburg)* **68**, 499–

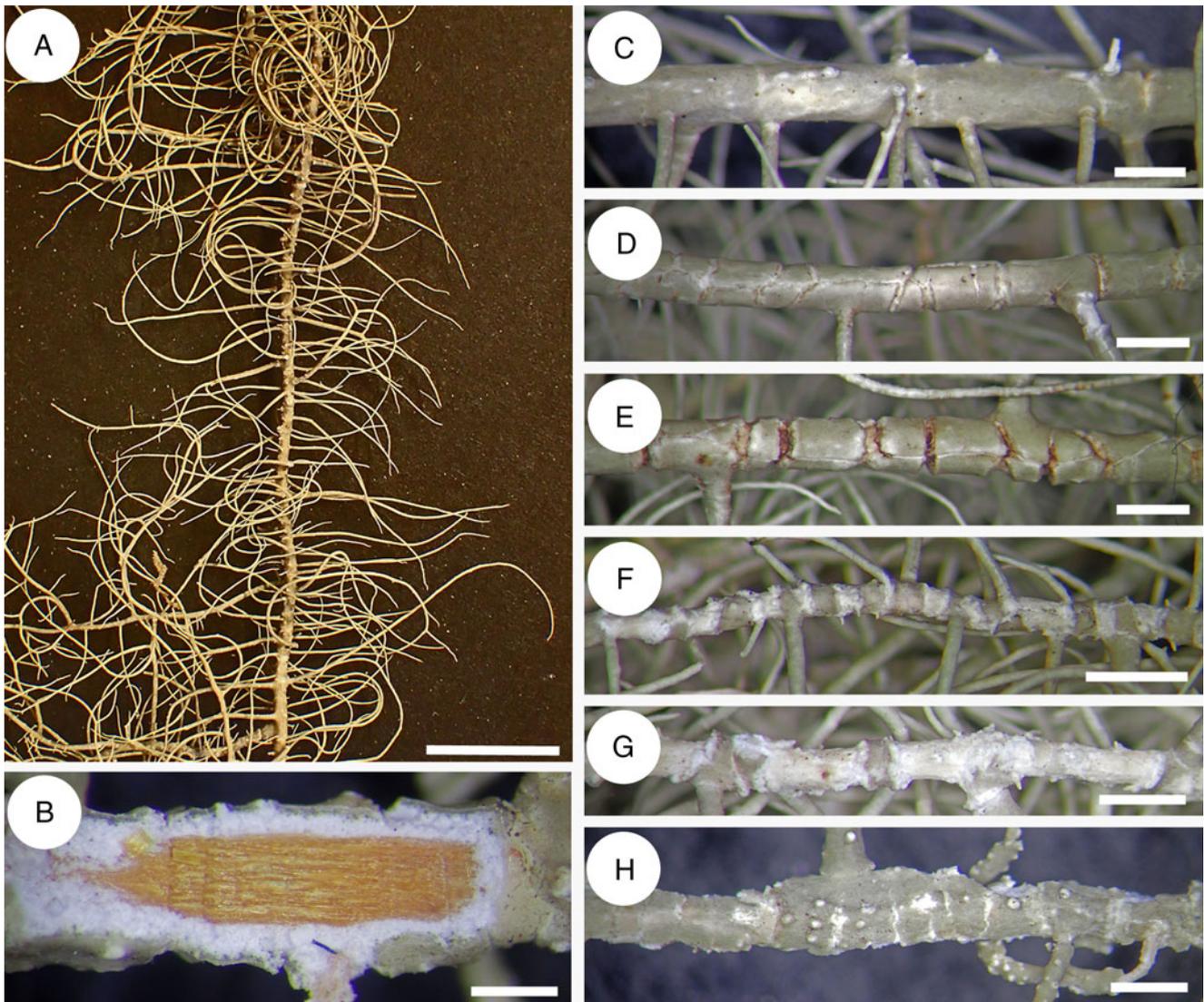


Fig. 10. *Usnea pectinata*. A, typical fish bone-like arrangement of fibrils and branches (MN0241). B, transversal section of a main branch, large and pigmented central axis (MN0583). C, main branches almost smooth, slightly ridged (MN0556). D, main branches with thin annular cracks (MN0527). E, main branches with thick annular cracks (MN0527). F, main branches partly decorticated (MN0163). G, main branches almost fully decorticated (MN0163). H, main branches ridged or acute-angled (MN0583). Scales: A = 1 cm; B = 0.5 cm; C–H = 1 mm. In colour online.

500 (1885); type: Prope Andrangolovaka in Madagascaria centrali, *Hidlebrandt* (holotype—G66446!). Motyka wrote ‘*Usnea sorediosula* (Müll. Arg.) Mot., nova species. *U. barbata* v. *sorediosula* Müll. Arg. pro parte in herb. Typus in Mus. Botan. Univ. Geneva. Locus classicus: Usambara, silva stepposa ad paludem Kumba, 1883 C. Holst.’. Clearly he was basing his taxon *U. sorediosula* on a specimen in G identified as *U. barbata* var. *sorediosula*, but not the type species of this taxon, so he was describing a new species and not making a new combination. The type specimen of *U. dasopogoides* var. *sorediosula* Müll. Arg. does not have a red-orange subcortical pigment.

A detailed description and images of *U. sorediosula* are provided in Swinscow & Krog (1975, 1988). However, what Swinscow & Krog (1975, 1988) called pseudocyphellae are interpreted here as soralia.

CMA. (Specimen collected in São Tomé). %C = 9; %M = 19; %A = 43; A/M = 2.3; A/C = 4.7; M/C = 2.1.

Chemistry. (Specimen collected in São Tomé). Medulla K–, Pd+ orange-red; protocetraric acid.

Diagnostic characters. The main diagnostic characters for *U. sorediosula* are the shrubby sorediate thallus with a red-orange subcortical pigment, the concolorous basal part, the tapering to cylindrical branches, the lateral branches that are not constricted, the punctiform, strongly irregular soralia aggregating into consoralia, with numerous isidiomorphs, the moderately thick cortex (8–10%) and central axis (40–50%), and the presence of protocetraric acid in the medulla.

Variability. See under ‘Discussion’ below.

Distribution and ecology. *Usnea sorediosula* is known from tropical Africa (Motyka 1936–1938; Swinscow & Krog 1975, 1988). The report from the Philippines (Galinato *et al.* 2018) is highly dubious and should be checked. In Eastern Africa, *U. sorediosula*

is said to be widespread but uncommon on twigs of shrubs in open woodland and bush scrub at 1000–2000 m elevation. In São Tomé and Príncipe, it was found only on the island of São Tomé, fallen from the canopy into the leaf litter of primary *Rubiaceae*-dominated hardwood forests at 1230 m elevation.

Discussion. *Usnea solediosula* differs from *U. bicolorata*, the other species in São Tomé with red-orange subcortical pigmentation and protocetraric acid in the medulla, by the concolorous basal part, the tapering or cylindrical main branches, the non-constricted lateral branches, the thicker cortex and central axis, and by the much lower M/C. *Usnea solediosula* was sequenced here for the first time. In our phylogenetic tree (Fig. 2), it belongs to the clades 3 & 4 of Truong *et al.* (2013a).

Specimens examined. São Tomé and Príncipe: Island of São Tomé: Parque Natural Obô de São Tomé, secondary trail from Bom Sucesso to Lagoa Amelia, 1230 m, 2013, *M. Nadel, J. Shevock, T. Daniel & E. Soares* MN0579 (CAS).

Usnea submollis J. Steiner

Verh. zool.-bot. Ges. Wien 53, 229 (1903); type: [Kenya], British Ost-Africa, Machakos, 1896, *Liechtenstein & Pospichill* (holotype —W!). Chemistry: alectorialic acid (trace in apothecium) (TLC by Swinscow & Krog (1978)). %CMA = 3.5/39.5/14; A/M = 0.4; A/C = 4; M/C = 11.3. Ascospores: 11–14.5 × 5–9 µm.

A detailed description and images of *U. submollis* are given in Swinscow & Krog (1978, 1988).

CMA. (Specimens collected in São Tomé only, *n* = 4). %C = 3.5–4.0–4.7(–5.0); %M = (35–)35.4–37.4–39; %A = (14–)14.1–17.3–20; A/M = 0.4–0.5–0.6; A/C = 3.2–4.3–5.5(–6.0); M/C = (7.0–)7.6–9.4–11.

Ascospores. (Specimens collected in São Tomé only, *n* = 3). 11–14 × 6.5–9 µm.

Chemistry. (Specimens collected in São Tomé only, *n* = 3). Medulla and apothecial disc K+ yellow turning red, Pd+ yellow turning orange-red; norstictic, salazinic, protocetraric (weak) acids.

Diagnostic characters. The main diagnostic characters of *U. submollis* are the erect, bushy thallus with numerous terminal or lateral apothecia containing large ascospores, without soralia, the constricted lateral branches, the numerous small, verrucose and true papillae, the *tenuicorticata*-type of CMA with glossy cortex and lax medulla, and the presence of norstictic and salazinic acids in the medulla.

Variability. The general aspect of the thallus is very variable, with small thalli that are more compact and large, almost subpendulous, thalli with large and distinct primary branches. Apothecia vary from lateral to terminal dispositions.

Distribution and ecology. *Usnea submollis* is known to occur only in tropical Africa (Motyka 1936–1938, 1956; Dodge 1957; Swinscow & Krog 1978, 1988). Motyka (1936–1938) mentions a specimen collected on lava beds on Fernando Po. In Eastern Africa, this taxon is commonly found mainly on branches and

twigs in montane forest and the ericaceous zone at elevations of 1500–3500 m. (Swinscow & Krog 1978). In São Tomé and Príncipe, it was found only on the island of São Tomé, in the primary tropical *Rubiaceae*-dominated forest on small branches fallen into the leaf litter at elevations between 1298 m and 1354 m. It is associated with *U. articulata*, *U. bicolorata* and *U. firmula*. *Usnea submollis* is newly reported for São Tomé and Príncipe.

Discussion. *Usnea submollis* differs from all other species discussed here by the larger ascospores. Moreover, it differs from *U. beckeri* and *U. longiciliata* by its constricted lateral branches and different chemistry. *Usnea submollis* was sequenced here for the first time. In our phylogenetic tree (Fig. 2), both specimens form a monophyletic clade (although not strongly supported) nested inside the *U. articulata*-*U. exasperata* clade (Fig. 3). For differences with *U. nodulosa*, see under that species.

Specimens examined. São Tomé and Príncipe: Island of São Tomé: Parque Natural Obô de São Tomé, trail from 'Macambara' leading into the forest, 1323 m, 2012, *M. Nadel & J. Shevock* MN0116 (CAS); trail from Bom Sucesso to Pico Cálvario, 1354 m, 2012, *M. Nadel, J. Shevock & A. Stanbridge* MN0191 (CAS); primary trail from Bom Sucesso to Lagoa Amelia, 1298 m, 2013, *M. Nadel, J. Shevock, T. Daniel & E. Soares* MN0552 (CAS), MN0553 (CAS).

Usnea sp. 1

This specimen (MN0523) is very similar to *U. beckeri*. However, its position in the phylogenetic tree (Fig. 4) casts some doubts on its identity. This is a very large specimen (thallus 40–50 cm long) that was collected on the ridge leading to Pico Príncipe, the highest ridge on the smaller island. This was the most difficult site to visit on either island due to logistical access issues, rugged terrain and heavy rainfall. It differs from *U. beckeri* by the much thicker branches, strong and thick annulations on primary branches and fistulose axis. It has a much thinner cortex (2%), a thinner medulla (15.5%), a much thicker axis (65%) and as a consequence higher A/C (29.3) and M/C (6.9). More specimens are needed to recognize this morphotype as a new species or as an extreme morphotype of *U. beckeri*.

Specimen examined. São Tomé and Príncipe: Island of Príncipe: on slope between Ribeira Banzu and Pico Príncipe, 497 m, *M. Nadel & O. Rocha* MN0523 (CAS).

Usnea sp. 2

Through its shrubby to subpendant thallus (Fig. 11A), the inflated branches (Fig. 11B), the presence of perforations in the cortex (Fig. 11C), the thin and shiny cortex, and the large and lax medulla with conglutinated hyphae (Fig. 11E), this specimen (MN0526) is similar to *U. bismolliuscula* (for a description of this species see Ohmura (2001)). It differs, however, by the absence of the typical irregular and confluent soralia, the presence of the numerous papillae (young fibrils) and fiberclcs (Fig. 11D), and the presence of barbatic acid together with the stictic acid group. In the phylogenetic tree (Fig. 4) it appears to be nested inside the *U. nodulosa*-*beckeri* clade that is highly supported and also a sister clade of *U. bismolliuscula* s. str. *Usnea bismolliuscula* is so far the only known species with perforations in the

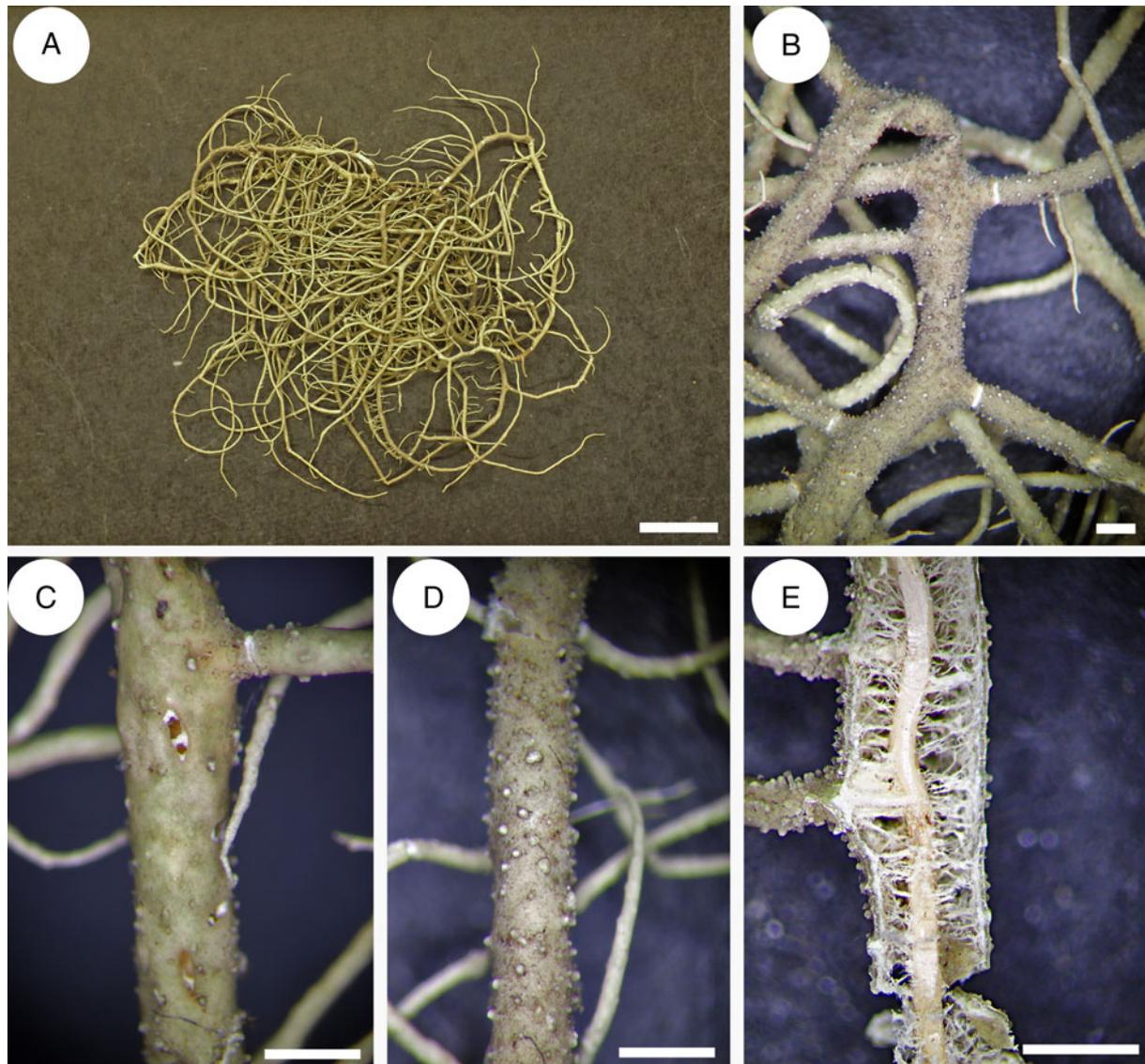


Fig. 11. *Usnea* sp. 2 (MN0526). A, thallus. B, irregular main branch with constricted lateral branches. C, perforations in the cortex. D, tubercles (young fibrils) and fiberclicles. E, transversal section of a main branch, medulla with conglutinated hyphae. Scales: A = 1 cm; B–E = 1 mm. In colour online.

cortex. However, we are reluctant to describe a new taxon based on only one specimen and will wait for more material to formally describe it.

Specimen examined. **São Tomé and Príncipe:** *Island of Príncipe:* along Ribeira Banzu between Pico Príncipe and São Carlos do Fundão, 262 m, *M. Nadel & O. Rocha* MN0526 (CAS).

Acknowledgements. We warmly thank the curators of the BM, CAS, LBL, M, S, TUR-V and W herbaria for the loan of several collections of *Usnea* type specimens. Carlos Boluda and Yamama Naciri (G) are thanked for fruitful discussions, Yoshihito Ohmura (TNS) for confirming the cortex type of the new species, Bob Drewes and the California Academy of Sciences for past funding and the amazing opportunity to collect lichens in São Tomé and Príncipe, and Dennis Desjardin for providing academic guidance. Special thanks to Brian Perry and James Shevock for many hours of mentorship and assistance throughout this project.

Author Contribution. MN conceived the study, performed PCRs, DNA isolation and sequencing and designed Figs 1–5. PC was responsible for the

taxonomic section, performed TLC and designed Figs 6–11. Both PC and MN wrote the manuscript.

Author ORCIDs.  Miko Nadel, 0000-0001-8367-4139; Philippe Clerc, 0000-0003-1453-0865.

Competing Interests. The authors declare none.

Supplementary Material. To view Supplementary Material for this article, please visit <https://doi.org/10.1017/S0024282922000238>.

References

- Ayres DL, Darling A, Zwickl DJ, Beerli P, Holder MT, Lewis PO, Huelsenbeck JP, Ronquist F, Swofford DL, Cummings MP, *et al.* (2012) BEAGLE: an application programming interface and high-performance computing library for statistical phylogenetics. *Systematic Biology* **61**, 170–173.
- Clerc P (1984) Contribution à la révision 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 fragiliscens* aggregate and its distribution in Scandinavia. *Nordic Journal of Botany* **7**, 479–495.

- Clerc P** (1998) Species concepts in the genus *Usnea* (lichenized *Ascomycetes*). *Lichenologist* **30**, 321–340.
- Clerc P** (2004) Notes on the genus *Usnea* Adanson. II. *Bibliotheca Lichenologica* **88**, 79–90.
- Clerc P** (2006) Synopsis of *Usnea* (lichenized *Ascomycetes*) from the Azores with additional information on the species in Macaronesia. *Lichenologist* **38**, 191–212.
- Clerc P** (2007) *Usnea*. In Nash TH, III, Gries C and Bungartz F (eds), *Lichen Flora of the Greater Sonoran Desert Region, Vol. III*. Tempe, Arizona: Lichens Unlimited, Arizona State University, pp. 302–335.
- Clerc P** (2011) *Usnea* Adans. *Nordic Lichen Flora* **4**, 107–127.
- Clerc P and Naciri Y** (2021) *Usnea dasopoga* (Ach.) Nyl. and *U. barbata* (L. F. H. Wigg. (*Ascomycetes*, *Parmeliaceae*)) are two different species: a plea for reliable identifications in molecular studies. *Lichenologist* **53**, 221–230.
- Culberson CF and Ammann K** (1979) Standardmethode zur Dünnschichtchromatographie von Flechtensubstanzen. *Herzogia* **5**, 1–24.
- Culberson CF 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.
- Dodge CW** (1956) Some lichens of tropical Africa. II. *Usnea*. *Annals of the Missouri Botanical Garden* **43**, 381–396.
- Dodge CW** (1957) Some lichens of tropical Africa. II. *Usnea* (Continued). *Annals of the Missouri Botanical Garden* **44**, 1–76.
- Esslinger TL** (2019) A cumulative checklist for the lichen-forming, lichenicolous and allied fungi of the continental United States and Canada, Version 23. *Opuscula Philolichenum* **18**, 102–378.
- Galinato MGM, Baguinon JRC and Santiago KAA** (2018) Review of the lichen genus *Usnea* in the Philippines. *Studies in Fungi* **3**, 39–48.
- Gerlach ACL, Clerc P and Borges da Silveira RM** (2017) Taxonomy of the corticolous, shrubby, esorediate, neotropical species of *Usnea* Adans. (*Parmeliaceae*) with an emphasis on southern Brazil. *Lichenologist* **49**, 199–238.
- Gerlach ACL, Zeynep T, Naciri Y, Araujo Caviro E, Borges da Silveira RM and 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.
- Gerlach A, Borges da Silveira RM, Rojas C and 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.
- Hale ME** (1979) *How to Know the Lichens*, 2nd edition. Dubuque, Iowa: William C. Brown.
- Herrera-Campos M, Clerc P and Nash TH, III** (1998) Pendulous species of *Usnea* from the temperate forests in Mexico. *Bryologist* **101**, 303–329.
- Huelsbeck JP and Ronquist F** (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**, 754–755.
- Krog H** (1994) New observations on *Usnea* subgenus *Eumitria* in Eastern and Central Africa. In Seyani JH and Chikuni AC (eds), *Proceedings of the XIIIth Plenary Meeting of AETFAT, Zomba, Malawi, 1–11 April 1991*. Zomba, Malawi: National Herbarium and Botanic Gardens of Malawi, pp. 813–821.
- Lücking R, Hodkinson BP and Leavitt SD** (2017) The 2016 classification of lichenized fungi in the *Ascomycota* and *Basidiomycota* – approaching one thousand genera. *Bryologist* **119**, 361–416.
- Lücking R, Nadel MRA, Araujo E and Gerlach ACL** (2020) Two decades of DNA barcoding in the genus *Usnea* (*Parmeliaceae*): how useful and reliable is the ITS? *Plant and Fungal Systematics* **65**, 303–357.
- Lücking R, Leavitt SD and Hawksworth DL** (2021) Species in lichen-forming fungi: balancing between conceptual and practical considerations, and between phenotype and phylogenomics. *Fungal Diversity* **109**, 99–154.
- Miller MA, Pfeiffer W and Schwartz T** (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In *Proceedings of the Gateway Computing Environments Workshop (GCE)*, 14 November 2010, New Orleans, Louisiana, pp. 1–8.
- Motyka J** (1936–38) *Lichenum Generis Usnea, Studium Monographicum, Pars Systematica*. Leopoli: Selbstverl.
- Motyka J** (1956) Die Flechtengattung *Usnea* Wigg. im Virung-Gebiet (Zentralafrika). *Annales Universitatis Mariae Curie Skłodowska* **11**, 103–150.
- Nadel MRA** (2016) *A monograph of Usnea from São Tomé and Príncipe*. Master's thesis, San Francisco State University.
- Nylander W** (1889) *Lichenes Insularum Guineensium (San Thomé, do Príncipe, das Cabras)*. Paris: P. Schmidt.
- 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** (2002) Phylogenetic evaluation of infrageneric groups of the genus *Usnea* based on ITS regions in rDNA. *Journal of the Hattori Botanical Laboratory* **92**, 231–243.
- 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, Tokyo* **46**, 69–76.
- Rogers RW and Stevens GN** (1988) The *Usnea baileyi* complex (*Parmeliaceae*, lichenized *Ascomycetes*) in Australia. *Australian Systematic Botany* **1**, 355–361.
- Ronquist F and Huelsenbeck JP** (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574.
- Stamatakis A** (2014) RAXML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**, 1312–1313.
- Stevens GN** (1999) A revision of the lichen family *Usneaceae* in Australia. *Bibliotheca Lichenologica* **72**, 1–128.
- Swinscow TDV and Krog H** (1974) *Usnea* subgenus *Eumitria* in East Africa. *Norwegian Journal of Botany* **21**, 165–185.
- Swinscow TDV and Krog H** (1975) The *Usnea undulata* aggregate in East Africa. *Lichenologist* **7**, 121–138.
- Swinscow TDV and Krog H** (1976a) The *Usnea bornmuelleri* aggregate in East Africa. *Norwegian Journal of Botany* **23**, 23–31.
- Swinscow TDV and Krog H** (1976b) The *Usnea articulata* aggregate in East Africa. *Norwegian Journal of Botany* **23**, 261–268.
- Swinscow TDV and Krog H** (1978) Pendulous species of *Usnea* in East Africa. *Norwegian Journal of Botany* **25**, 221–241.
- Swinscow TDV and Krog H** (1979) The fruticose species of *Usnea* subgenus *Usnea* in East Africa. *Lichenologist* **11**, 207–252.
- Swinscow TDV and Krog H** (1986) *Usnea antiqua* sp. nov. described from Tanzania. *Lichenologist* **18**, 293–295.
- Swinscow TDV and Krog H** (1988) *Macrolichens of East Africa*. London: British Museum (Natural History).
- Temu SG, Clerc P, Tibell L, Tibuhwa DD and Tibell S** (2019) Phylogeny of the subgenus *Eumitria* in Tanzania. *Mycology* **10**, 250–260.
- Thell A, Crespo A, Divakar PK, Kärnefelt I, Leavitt SD, Lumbsch HT and Seaward MRD** (2012) A review of the lichen family *Parmeliaceae* – history, phylogeny and current taxonomy. *Nordic Journal of Botany* **30**, 641–664.
- Truong C and Clerc P** (2013) Eumitrioid *Usnea* species (*Parmeliaceae*, lichenized *Ascomycota*) in tropical South America and the Galapagos. *Lichenologist* **45**, 383–395.
- Truong C, Bungartz F and Clerc P** (2011) The lichen genus *Usnea* (*Parmeliaceae*) in the tropical Andes and the Galapagos: species with a red-orange cortical or subcortical pigmentation. *Bryologist* **114**, 477–503.
- Truong C, Divakar PK, Yahr R, Crespo A and Clerc P** (2013a) 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.
- Truong C, Rodriguez JM and Clerc P** (2013b) Pendulous *Usnea* species (*Parmeliaceae*, lichenized *Ascomycota*) in tropical South America and the Galapagos. *Lichenologist* **45**, 1–39.