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Taxonomy of the corticolous, shrubby, esorediate, neotropical species of *Usnea* Adans. (*Parmeliaceae*) with an emphasis on southern Brazil

Alice da Cruz Lima GERLACH, Philippe CLERC and Rosa Mara BORGES DA SILVEIRA

Abstract: Seventeen corticolous shrubby apotheciate *Usnea* species without vegetative propagules are reported from Brazil, including five species that are new to science: *Usnea aurantiaca-parvula* A. Gerlach & P. Clerc (characterized by an orange medulla and lageniform spinulose fibrils), *U. cirrosa* Motyka, *U. cladocarpa* Fée (syn. nov.: *U. ramillosa* Motyka), *U. concinna* Stirton (lectotype designated here, syn. nov. *U. radiata* Stirton, *U. florida* var. *scabrosa* Zahlbr.), *U. cristatula* Motyka, *U. erinacea* Vain., *U. fleigiae* A. Gerlach & P. Clerc (characterized by large spores and a thin, lax medulla), *U. grandispora* A. Gerlach & P. Clerc (characterized by large spores, a black base and protocetraric or salazinic acids in the medulla), *U. kalbiana* P. Clerc & A. Gerlach (characterized by a vitreous cortex and annular cracks in the basal part), *U. lunaria* Motyka, *U. meridionalis* Zahlbr. (syn. nov.: *U. michauxii* I. I. Tav.), *Usnea* cf. *moreliana* Motyka, *U. parvula* Motyka, *U. steineri* Zahlbr, *U. subelegans* (Vain.) B. de Lesd. (lectotype designated here), *U. subparvula* A. Gerlach & P. Clerc (characterized by spinulose fibrils and protocetraric acid in the medulla) and one as yet unidentified species (named *Usnea* sp. 1). *Usnea cirrosa*, *U. cristatula* and *U. erinacea* are new records for Brazil. A full description with morphological, anatomical (CMA and ascospores) and chemical features (TLC), as well as geographical distribution, is provided for each species along with an identification key to all species reported. Molecular data from the ITS rDNA, *RPB1* and *Mcm7* markers are present for most taxa, except for *U. concinna*, *U. cristatula*, *U. kalbiana*, *U. lunaria*, *U.* cf. *moreliana* and *U. subelegans*.

Key words: anatomy, ascospores, lichens, morphology, phylogenetics, thin-layer chromatography

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Introduction

Usnea is a hyperdiverse lichen-forming fungal genus, with more than 350 species distributed worldwide, that forms a strongly supported monophyletic lineage within the *Parmeliaceae* (Crespo *et al*. 2007; Divakar *et al*. 2015). The combination of traditional characters (e.g. the shape of the branches, thickness of the cortex, medulla and central axis, presence/absence of pigments, chemistry) used in earlier taxonomic studies of the genus (Clerc 1998; Ohmura 2001) proved to be a good predictor of species delimitation (Kelly *et al*. 2011; Truong *et al*. 2013*a*). However, due to the presence of homoplasious features, species with a similar morphology, anatomy or chemistry might not be closely related (Truong & Clerc 2016) and so traditional taxonomy seems to be unsuccessful in indicating species relationships within the genus. Thus, integrative taxonomy will prove to be a very important approach to circumscribe species and understand their relationships, helping to uncover the still poorly known diversity in tropical areas.

Recent investigations of *Usnea* in South America indicate that species diversity is high. New species have been described in several groups, as for instance in the saxicolous species (Rodriguez *et al*. 2011), the pigmented species (Truong *et al*. 2011; Truong & Clerc 2012), the pendulous

A. da C. L. Gerlach and R. M. Borges da Silveira: Universidade Federal do Rio Grande do Sul, Instituto de Biociências, Departamento de Botânica, Laboratório de Micologia, Av. Bento Gonçalves, 9500 Campus do Vale, CEP: 91501-970, Porto Alegre/RS, Brazil. Email: alice_gerlach@yahoo.com.br
A. da C. L. Gerlach and P. Clerc: Conservatoire et Jardin botaniques de la Ville de Genève, 1 ch. de l'Impératrice, 1292 Chambésy/GE, Switzerland.

species (Truong *et al.* 2013*b*), the eumitrioid species (with hollow axis) (Truong & Clerc 2013) and the shrubby sorediate species (Truong & Clerc 2016).

The species investigated herein share a short shrubby-erect thallus (i.e. the branches remain erect and divergent to the apices), usually numerous apothecia and an absence of vegetative propagules. Some of the species can occasionally exhibit a subpendulous thallus, especially under optimal conditions of humidity (Truong *et al.* 2013*b*). They mostly grow on a variety of corticolous substrata (bark and the twigs of trees or bushes) or on fence posts and occasionally on rocks.

In the sexually reproducing *Usnea* species it is striking to see that, with a few exceptions such as the European-North American species of *U. florida* (L.) F. H. Wigg. and *U. intermedia* (A. Massal.) Jatta (Clerc 1984*a*; Halonen *et al.* 1998), they tend to have a restricted geographical distribution range with most species occurring in subtropical and tropical areas (Swinscow & Krog 1979; Awasthi 1986; Ohmura 2001; Stevens 2004; Clerc 2007). This observation agrees with the hypothesis that sorediate species have broader distribution ranges than most of the esorediate species (Hale 1983; Herrera-Campos *et al.* 1998). With more than 30 species recorded so far (Motyka 1936, 1938), South America holds the highest diversity of esorediate species. Despite this diversity there is no modern revision for this group within the Neotropics.

The aim of the present study, based on an integrative taxonomic approach and including morphological, anatomical, chemical, and ecological features, as well as molecular data, is to provide information on the 17 shrubby, esorediate species recognized from southern Brazil. It is the first step towards the taxonomic revision of the whole genus in Brazil.

Materials and Methods

Morphological, anatomical and chemical studies

The following account is based on field studies and on herbarium specimens deposited in the following herbaria: BHCB, BM, CESJ, CGMS, DUKE, FH, FI, G, H, HAS, ICN, JPB, LBL, M, MBM, PC, RB, S, SP, TUR, UFP, UPCB, W, WU and Z. Type material of all species discussed in this paper was studied. All voucher specimens collected during field trips are deposited in the Federal University of Rio Grande do Sul (ICN) and some duplicates in G. The morphology of specimens was examined using a Leica MS5 stereomicroscope, with measurements taken using a Leica DM2000 microscope. The species concept used in this study follows Clerc (1998).

Density of fibrils is given as the number of fibrils mm^{-2} on a branch where the density was estimated to be the highest. For each specimen, three measurements were made. Microscopic examination of spores was carried out with a Leica DM2000 microscope at high magnification (×1000). The length and width of 10–30 mature ascospores per specimen were measured. Measurements for ascospores are given as *mean* $(\bar{x}) \pm 1$SD with extremes in parentheses. Normality of the data was tested with Shapiro tests in the software R 3.2.4 (R Development Core Team 2016) at the species level. To take into account non-normal distributions, Mann-Whitney-Wilcoxon with the Benjamini-Yekutieli correction (Benjamini & Yekutieli 2001) for non-parametric variables was carried out on groups of two species. Anatomical measurements of cortex, medulla and central axis were carried out in longitudinal sections of branches at ×40 magnification. The percentage thickness of cortex/medulla/axis of the total branch diameter (CMA) and the ratio of axis/medulla (A/M) of all the cited specimens were calculated according to Clerc (1984*a*, 1987). Measurements for CMA values are given as the *mean* $(\bar{x}) \pm 1$SD with extremes in parentheses.and follow the categories described by Clerc (2011*b*).

Analyses of the anatomical structure of the cortex were made according to Ohmura (2001), on thin hand-cut sections and observed at ×400 magnification with a Leica DM2000 microscope.

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

Fieldwork was carried out between January 2013 and December 2014 in the states of Paraná (PR), Santa Catarina (SC) and Rio Grande do Sul (RS), between 22°30'–33°45'S and 48°02'–57°40'W. Approximately 800 specimens were collected. Southern Brazil comprises 573·41 km^2 and the climate is humid subtropical with hot to temperate summers (Alvares *et al.* 2013). Field trips were conducted in the Atlantic Forest and in the Pampa (also known as the Southern grasslands), the two main biomes of the southern Brazilian region (IBGE 2004). A relict of the Cerrado occurs in northern Paraná (2% of the area) and unfortunately this small area was not visited, but a few herbarium specimens previously collected in this biome were studied. A small number of specimens originating from other biomes in Brazil, such as the Caatinga, were also studied for comparative purposes. The Atlantic Forest is the second largest

rainforest biome of South America, and corresponds to a complex mosaic of different vegetation types (see details in Iganci *et al.* 2011; Oliveira-Filho *et al.* 2015). The following types of vegetation were visited: dense rainforest (including several hills and mountains up to 1887 m a.s.l. of the Serra do Mar), *Araucaria* forest (predominantly with *Araucaria angustifolia* (Bertol.) Kuntze), the high-altitude grasslands (also known as campos de altitude) and coastal areas known as restingas that are formed of sandstone. Localities of subtropical seasonal forests were also explored. The Pampa occurs in the southern half of Rio Grande do Sul and is a non-forest vegetation type, dominated by herbaceous, shrubby and treelet plants (Overbeck *et al.* 2007). In addition, urban parks and rural areas such as pastures with forest relicts, roadsides and deforested zones were visited. At least one specimen per locality is included in the list of selected specimens and the states are mentioned according to geographical order, from south to north and from east to west.

Phylogenetic analysis

DNA extraction, PCR amplification and sequencing

DNA was extracted following Truong *et al.* (2013*a*) using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions, with small modifications as in Crespo *et al.* (2001). PCR amplifications of the ITS rDNA and fragments of the *RPB1* and *Mcm7* genes were performed. The following primers were used: USITS3-F and USITS4-R (Truong *et al.* 2013*a*) and four newly developed primers in this study: UsRPB1-R (5'-ACG GAT AAT ATC GCC AAG CT-3'), UsRPB1-F (5'-TGG AAA CAG TCT GCC ACA AC5-3'), UsMCM7-R (5'-TGC CCG TAT ATT TCT GGA GCG A-3') and UsMCM7-F (5'-ACA CCT GTG ATC GAT GTG GA-3'). For ITS, PCR reactions were performed with 5 µl of total genomic DNA, 2·5 µl ×10 buffer with 2 µM MgCl2, 0·5 µl dNTPs (10µM of each base), 1·25 µl of each primer at 10 µM, 0·625 µl of DNA polymerase ($1 U\mu l^{-1}$) and sterile water to complete a reaction mixture of 25 µl. Thermal cycling parameters were as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 1 min, 54 °C for 1 min, 72 °C for 1·5 min and final elongation at 72 °C for 10 min. For *Mcm7* and *RPB1*, PCR amplifications were conducted with the same proportions as with ITS, except increasing the concentration of the primers to 3 µl. For both genes, the thermal cycling parameters were as follows: initial denaturation at 94 °C for 10 min, followed by 6 cycles of 94 °C for 0·5 min, 56 °C for 0·5 min and 72 °C for 1 min, 30 cycles of 94 °C for 0·5 min, 52 °C for 0·5 min and 72 °C for 1 min, and a final elongation at 72 °C for 10 min. If the amplification failed, the PCR was repeated using PuReTaq Ready-To-Go PCR Beads (2·5 U of PuReTaqDNA Polymerase, 200 µM of each of dNTP, BSA, the buffers and stabilizers 10mM Tris-HCl pH 9·0, 50 mM KCl, 1·5 µM $MgCl_2$; GE Healthcare, Little Chalfont, UK) adding to the lyophilized bead 1·5 µl of each primer at 10 µM, and increasing the DNA template to 7 µl when PCR products were too weak or absent, made up to 25µm with sterile water. Amplification products were viewed on a 1% agarose gel stained with SYBER, and purification was performed by adding 2 µl of illustra^{TM} ExoProStar (GE Healthcare, Little Chalfont, UK) to 10 µl of PCR product, followed by a heat treatment of 15 min at 37 °C and 15 min at 80 °C. Sequencing was carried out with the same primers as for the PCR amplifications, using the sequencing kits ABI Prism™ Dye Terminator Cycle Sequencing Ready or BigDye™ (Applied Biosystems, Foster City, California, USA). Sequencing reactions underwent electrophoresis on a 3730 DNA Analyzer (Applied Biosystems) at the Unidad de Genómica (Parque Científico de Madrid).

Sequence alignment and phylogenetic reconstructions

The DNA sequences obtained were manually adjusted using SeqMan version 7.0 (DNAstar, Madison, WI, USA) and subjected to BLAST queries for an initial verification of their identities. To build the data matrix we chose specimens represented by more than one of the markers considered here (25 specimens), selecting a set of species from each clade determined by Truong *et al.* (2013*a*) except clade 1. Then we added 30 specimens analyzed in this study representing 11 *Usnea* species from the group examined here. We were unable to obtain sequences from *U. concinna*, *U. cristatula*, *U. kalbiana*, *U. lunaria*, *U.* cf. *moreliana* and *U. subelegans*. *Usnea densirostra* Taylor, a saxicolous shrubby esorediate species from Brazil, and *U. ghattensis* G. Awasthi, a corticolous esorediate species from India with large spores, were included. The data matrix (Table 1) contains 55 specimens representing 25 *Usnea* species.

Alignments for each locus were performed using MAFFT version 7 (Katoh & Standley 2013) with the G-INS-I alignment algorithm, a scoring matrix of 20 PAM/k = 2, 0·1 as offset value and the remaining parameters set as default. The program Gblocks v0.91b (Talavera & Castresana 2007) was used to delimit and remove ambiguous alignment nucleotide positions using the online web server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) and implementing the options for a less stringent selection of ambiguous nucleotide positions including the 'Allow smaller final blocks', 'Allow gap positions within the final blocks', and 'Allow less strict flanking positions' options. The alignments of each region and the concatenated one were analyzed using maximum likelihood (ML) and Bayesian (B/MCMC) approaches, with *Usnea aurantiaco-atra* (*Neuropogon* group) as outgroup to root the tree. Exploratory phylogenetic analyses of individual gene topologies showed no evidence of well-supported (≥70% bootstrap values) topological conflict and relationships were estimated from the concatenated, three-locus data matrices using a total-evidence approach (Wiens 1998; Divakar *et al.* 2015). For the Bayesian analysis, MrBayes v3.2.1 (Ronquist & Huelsenbeck 2003) was used. All loci were treated as separate partitions and for the protein-coding marker we used a three-partition approach using the first, second, and third codon positions as separate model partitions for the concatenated dataset. Models of DNA sequence evolution for each locus were selected

TABLE 1. *Voucher infomation, major chemotypes and GenBank Accession numbers for the* Usnea *species referred to in this study. Newly described species and newly generated sequences are in bold.*

				GenBank Accession numbers		
Species	DNA no.	Voucher	Chemotype	ITS	*Mcm7*	*RPB1*
Usnea antarctica	NW148	Tierra del Fuego	—	EF179796	—	—
U. articulata	art19	England	Protocetraric	JN943545	—	JN992591
U. articulata	59	England	Protocetraric	JN943508	—	JN992558
U. aurantiaco-atra	NW107	Falkland Islands	—	EF179797	—	EF179784
U. aurantiaco-atra	NW211	Antarctica	—	DQ767954	—	EF193048
U. aurantiaca-parvula	5246	Brazil: MS, Porto Murtinho, *V. Pott* 11873 (CGMS)	Tri-terp.	**KY021902**	**KY204412**	**KY204434**
U. cirrosa	4906	Brazil: SC, São Francisco do Sul, *E. Gumboski* 5020 (ICN)	Salazinic	**KY021903**	**KY204413**	**KY204435**
U. cirrosa	5244	Brazil: SC, Urubici, *C. Alves* (ICN)	Salazinic	—	**KY204414**	**KY204436**
U. cladocarpa	5242	Costa Rica: *P. Clerc* PC2015/664 (G)	Protocetraric	**KY021904**	**KY204415**	**KY204437**
U. cladocarpa	5243	Costa Rica: *P. Clerc* PC2015/654 (G)	Protocetraric	**KY021905**	**KY204416**	—
U. cornuta s. str.	01	Ireland	Salazinic	JN943562	—	JN992604
U. cornuta s. str.	04	England	Stictic	JN94355	—	JN992601
U. cornuta s. lat.	24	Peru	Stictic	JQ837296	JQ837339	—
U. cornuta s. lat.	27	Equador	Norstictic	JQ837297	JQ837340	—
U. densirostra	4935	Brazil: RS, Viamão, *A. Gerlach* 1494 (ICN)	Norstictic	**KY021906**	**KY204417**	**KY204438**
U. densirostra	4936	Brazil: SC, Florianópolis, *A. Gerlach* 988 (ICN)	Norstictic	**KY021907**	—	—
U. erinacea s. lat.	70	Brazil	Protocetraric	JQ837322	—	—
U. erinacea s. lat.	4804	Brazil: SC, Florianópolis, *A. Gerlach* 1211(ICN)	Protocetraric	**KY021908**	—	**KY204439**
U. erinacea s. lat.	4894	Brazil: RS, Caraá, *A. Gerlach* 1498(ICN)	Protocetraric	**KY021910**	**KY204419**	**KY204440**
U. erinacea s. lat.	4913	Brazil: SC, Urubici, *A. Gerlach* 1320 (ICN)	Protocetraric	**KY021909**	**KY204418**	—
U. flavocardia	42	Ireland	Psoromic	JN94352		—
U. fleigiae	4934	Brazil: PR, Campina Grande do Sul, *M. Engels* (ICN)	Norstictic	**KY021911**	**KY204420**	**KY204441**
U. fleigiae	5226	Brazil: SC, Campo Alegre, *A. Charnei* 563 (ICN)	Norstictic	**KY021912**	**KY204421**	—
U. fleigiae	5231	Brazil: PR, Campina Grande do Sul, *M. Engels* (ICN)	Norstictic	**KY021913**	**KY204422**	—
U. florida	26	England	Thamnolic	JN943538	—	JN992584
U. florida	29	Wales	Thamnolic	JN943535	—	JN992581
U. ghattensis	7	India: Maharashtra, *R. Bajpai* 15-027501	Unknown	**KY021914**	**KY204423**	**KY204442**
U. glabrata	113	Switzerland	Stictic	JQ837313	JQ837356	—
U. glabrata	56	Scotland	Protocetraric	JN943512	—	JN992561
U. grandispora	4930	Brazil: PR, Guaratuba, *B. Canestraro* 691(ICN)	Salazinic	**KY021915**	**KY204424**	—
U. grandispora	4931	Brazil: RS, São Francisco de Paula, *A. Magnago* 1114 (ICN). Type.	Protocetraric	**KY021916**	**KY204425**	**KY204443**
U. grandispora	5233	Brazil: PR, Guaratuba, *B. Canestraro* (ICN)	Salazinic	**KY021917**	—	—
U. grandispora	5234	Brazil: PR, Guaratuba, *A. Gerlach* 1009 (ICN)	Salazinic	**KY021918**	—	—
U. meridionalis	4919	Brazil: RS, Rio Grande, *E. Fazolino* (ICN)	Tri-terp.	**KY021919**	—	—

TABLE 1 (*continued*).

Species	DNA no.	Voucher	Chemotype	GenBank Accession numbers		
				ITS	*Mcm7*	*RPB1*
Usnea parvula	4908	Brazil: PR, Palmeira, *M. Engels* (ICN)	Caperatic	**KY021922**	—	—
U. parvula	4922	Brazil: SC, Florianópolis, *A. Gerlach* 1199 (ICN)	Caperatic	**KY021920**	**KY204426**	—
U. parvula	4923	Brazil: RS, Rondinha, *E. Fazolino* (ICN)	Caperatic	**KY021921**	**KY204427**	**KY204444**
U. rubicunda	17	Galapagos Islands	Salazinic	JQ837315	JQ837357	—
U. rubicunda	49	Ireland	Stictic	JN943516	—	JN992566
U. rubicunda	75	Portugal: Madeira	Stictic	JQ837319	JQ837361	—
U. rubicunda	4890	Brazil: SC, Urubici, *C. Alves* (ICN)	Salazinic	**KY021923**	**KY204428**	**KY204445**
U. rubicunda	4891	Brazil: RS, Caraá, *A. Gerlach* 1497(ICN)	Stictic	**KY021924**	**KY204429**	**KY204446**
U. rubrotincta	4807	Brazil: RS, Caraá, *A. Gerlach* 1499(ICN)	Stictic	**KY021925**	**KY204430**	**KY204447**
U. steineri	111	Peru	Tri-terp. UT6	JQ837333	JQ837372	—
U. steineri	65	Peru	Tri-terp. UT6	JQ837334	JQ837373	—
U. steineri	4915	Brazil: PR, Lapa, *M. Engels* (ICN)	Tri-terp.	**KY021926**	—	—
U. steineri	4924	Brazil: RS, Rio Grande, *E. Fazolino* (ICN)	Tri-terp.	**KY021927**	—	**KY204448**
U. strigosa	AF112990	USA	—	AF112990	—	—
U. subfloridana	24	Scotland	Thamnolic	JN943540	—	JN992586
U. subfloridana	27	Wales	Thamnolic	JN943537	—	JN992583
U. subglabrata	25	Bolivia	Stictic	JQ837312	JQ837355	—
U. subparvula	5245	Brazil: MS, Porto Murtinho, *V. Pott* 11873 (CGMS). Type.	Protocetraric	**KY021928**	**KY204431**	**KY204449**
U. subparvula	5247	Brazil: MS, Nova Andradina, *Simal* 245 (CGMS)	Protocetraric	**KY021929**	**KY204432**	—
U. subrubicunda	76	USA	Protocetraric	JQ837332	JQ837371	—
Usnea sp. 1	4920	Brazil: SC, Urubici, *A. Gerlach* 1321(ICN)	Norstictic	**KY021930**	**KY204433**	—

Key to Brazilian states: SC: Santa Catarina; MS: Mato Grosso do Sul; PR: Paraná, RS: Rio Grande do Sul.

with the program jModeltest2.0 (Darriba *et al.* 2012), using the Akaike information criterion (Akaike 1974). The best-fit model of evolution was as follows: GTR + G for the ITS and *RPB1* partitions and K80 + G for the *Mcm7* partition. We conducted two independent runs of 3 million generations, starting from a random tree and employing 12 simultaneous chains each, in which one in every 200 trees was sampled. Convergence among runs was visualized in Tracer v.1.5 (Rambaut & Drummond 2007) by plotting log likelihood per generation for each run and identifying the effective sample size (ESS > 200). The 50% majority-rule consensus tree was constructed by pooling trees sampled from all runs and after discarding the first 25% as burn-in, with posterior probabilities (PP) as branch support. For maximum likelihood (ML) tree reconstruction, the program RAxML v7.2.8 (Stamatakis 2006) implemented in the Cipres Science Gateway (Miller *et al.* 2010) was used, with the GTRGAMMA model. The concatenated three-loci dataset was partitioned as described in the Bayesian analysis. Support values were assessed using the 'rapid bootstrapping' option with 1000 replicates. The final phylogenetic tree was drawn using the program FigTree v1.4 (Rambaut 2009).

Results and Discussion

Morphology-anatomy

The habit of shrubby esorediate species depends mainly on characters that display broad phenotypic variability. This is the case for the density of ramification, the ramification type, the number of apothecia, the size of the apothecia, and the colour of the thallus. As a consequence, individuals within the same species might sometimes look very different in aspect. Clerc (1998), Herrera-Campos *et al.* (1998), Ohmura (2001) and Truong *et al.* (2011) discussed the characters that are diagnostic in delimiting *Usnea* species. Some important characters that were found here to be useful for delimiting shrubby apotheciate species are discussed below.

Fibrils. The shape, density and arrangement of these short branch-like appendages with a central axis that is not attached to the central axis of the mother branch (Clerc 1998) were found to be important in the systematics of this group of *Usnea* species. We define fibrils here as being spinulose when they are 2–5× taller than wide (Figs 3B, 6F, 9A & B), and slender when they are 6–15× longer than wide (Fig. 5F). *Usnea aurantiaca-parvula*, *U. parvula*, *U. subelegans* and *U. subparvula* are characterized by the presence of a majority of spinulose fibrils. Lageniform spinulose fibrils (swollen at the base, narrowed at the top) (Fig. 3C) are a special feature of *U. aurantiaca-parvula*. Fibril-like structures growing on the margin of apothecia usually share the same morphology as fibrils growing on branches.

Cortex and CMA values. From a morphological point of view, on a longitudinal section, the cortex can be matt, shiny or vitreous like broken glass. Anatomical studies of the cortical tissue have been carried out by Awasthi (1986) and Ohmura (2001). These authors described different types of plectenchyma that were, however, rarely used as diagnostic characters to separate the species. Some of these types appear to us to be variable and we believe that further studies are necessary to establish their exact taxonomic value. Differences in the relative thickness of cortex, medulla and axis (%CMA) proved to be diagnostic characters in this group. Truong *et al.* (2011) defined a CMA of the *cornuta*-type with a thin (5–8%) shiny cortex in cross-section, a moderately thick to thick medulla (28–36%), a thin axis (18–32%) and low A/M (0·5–1·3). We define here a *brasiliensis*-type CMA with a thinner shiny cortex (2–5%), a thicker medulla (35–45%), a much thinner axis (7–14%) and a very low A/M (0·2–0·4).

Apothecia and ascospores. Disposition of the apothecia on the branches was described by Herrera-Campos *et al.* (1998). This seems, however, to be a very variable character and only *Usnea subelegans* has a majority of lateral apothecia among the specimens studied. Apothecia might be scarce or even absent, and then pycnidia are usually present as small nodules on terminal branches. The two following characters are variable and thus not considered diagnostic for the Brazilian taxa: the shape of apothecia that varies from flat to mostly cup-shaped and the appearance of the disc which is usually pruinose and whitish, sometimes brownish when the pruina is absent. The density of marginal fibrils is

variable (1–3 fibrils mm^{-1}) in all species except in *U. aurantiaca-parvula* which has 8–12 fibrils mm^{-1}. Ascospores are simple, ellipsoid to broadly ellipsoid, and hyaline. The size of ascospore in the genus *Usnea* has traditionally received little attention, as is the case for most of the *Parmeliaceae* (reviewed by Crespo *et al.* 2011). However, Clerc (1984*a*) found small but significant differences in the spore size of *U. florida* and *U. intermedia*, two European apotheciate taxa. Tavares & Sanders (1998) separated *U. florida* from other taxa mainly on the basis of spore size. Kirika *et al.* (2016) also found the spore size to be an important character for delimiting species in the genus *Parmelinella* Elix & Hale. Likewise, among the species studied here we found two species with distinctly larger spores: *U. fleigiae* and *U. grandispora*. In accordance with this we propose two classes of ascospore length: class I (spores $< 13\,\mu m$) and class II (spores $\geq 13\,\mu m$) (Fig. 1). The depth of the hymenium seems to be proportional to the depth of the spores: 80–100 µm in *U. fleigiae* and *U. grandispora*, and 40–85 µm in all other species.

Chemistry

Table 2 shows the main secondary metabolites for the 17 species treated in this study. All *Usnea* species contain usnic acid in the cortex. When correlated with other morphological or anatomical characters, secondary metabolites present in the medulla in *Usnea* have a strong taxonomic value (Clerc 1998). Variations in secondary metabolites without correlation with other characters are considered as chemotypes of the same species. Most of the species of the group studied here have two chemotypes. Three species (*U. cladocarpa*, *U. kalbiana* and *U. lunaria*) have only one chemotype and one species, *U. erinacea*, has five chemotypes. As already stated by Truong *et al.* (2011, 2013*b*), the presence of triterpenoids is relatively common in *Usnea* in the neotropical region. For example, we found the same unidentified triterpenoids, UT6, referred to by Truong *et al.* (2011) in *U. erinacea* and *U. steineri*. Barbatolic and alectorialic acids were found only in the apothecia of a small number of specimens of *U. meridionalis*. Unknown substances are relatively common in *Usnea* from Brazil. Some of them seem to be of special taxonomic importance: 1) an unknown yellow spot (Rf classes A/B/C: 6/1–2/5) found in *U. parvula* (Us1 in Table 2) and 2) an unknown substance with a blue-green fluorescence after charring (Rf class A: 4–5, B: 5–6) (Us2 in Table 2). Cortical, subcortical and medullary pigmentation is a significant character in the taxonomy of *Usnea* (Swinscow & Krog 1979; Clerc 1984*b*, 2007; Ohmura 2001, 2012; Truong *et al.* 2011; Truong & Clerc 2012). It was observed in seven esorediate species from South America: *U. aurantiaca-parvula*, *U. cristatula*, *U. erinacea*, *U. meridionalis*, *U.* cf. *moreliana*, *U. steineri* and *Usnea* sp. 1.

Phylogenetic studies

In the present study, we generated a total of 68 new sequences, including 29 nuclear ITS, 16 *RPB*1 and 23 *Mcm7* from 20 samples of *Usnea* from Brazil, two from Costa Rica and one from India (Table 1). These were deposited in GenBank under Accession numbers KY021902–KY021930 and KY204412–KY204449. The ITS PCR product obtained ranged between 600 and 800 base pairs (bp). Differences in size were due to the presence or absence of insertions of *c.* 200 bp identified as group I introns (Ohmura 2002; Gutierrez *et al.* 2007) at the 3' end of the SSU rDNA. Testing for topological incongruence showed no supported conflicts (results not shown here). The partitioned ML analysis of the concatenated data matrix yielded the optimal tree with Ln likelihood value = −5630·32. The effective sample sizes (ESS) of all estimated parameters were well above 200 in the Bayesian analysis, indicating that convergence among parallel runs was reached. The best ML tree inferred from the multi-locus dataset is illustrated in Fig. 2. It contains 26 highly supported nodes (bootstrap support BS ≥ 70). The B/MCMC majority-rule consensus tree (LnL = −5713·45) with 30 highly supported nodes (PP ≥ 0·95) was almost identical to the ML tree, except for the low resolution of some of

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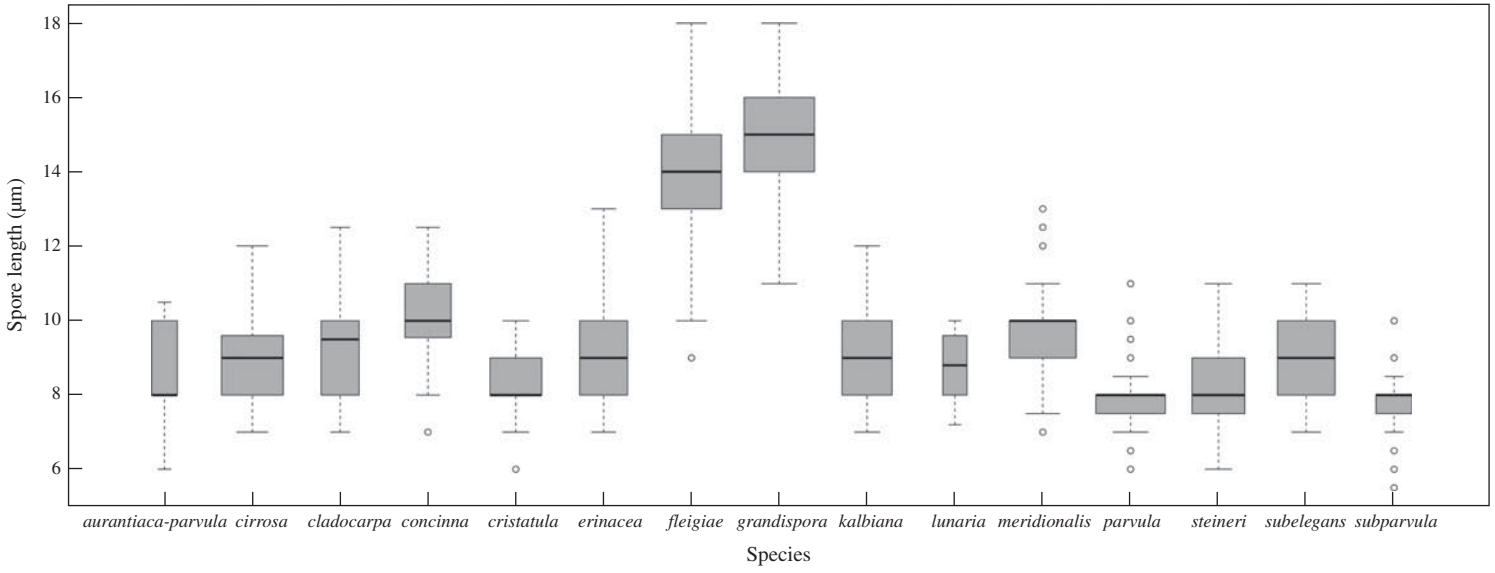

FIG. 1. Boxplots of spore length for each species of *Usnea* referred to in this study. Each boxplot shows the median (thick line) and standard deviation, and box width is proportional to the value of *n*. Dashed vertical lines correspond to the range. Outliers are represented by open circles.

TABLE 2. *Major secondary metabolites and chemotypes of Brazilian* Usnea *species.*

		Secondary Metabolites																			
Species	*n*	SAL	STI	CST	CRY	ME	NOR	GAL	DIF	BAR	PRO	FUM	PSO	CAP	TER	FA	EU	Us1	Us2	Ch0	Medulla colour test
aurantiaca-parvula	7															± rare			+		K−, P−
	5																			+	K−, P−
	1														+						K−, P−
cirrosa	27	+	± tr							± rare	± tr										K+ y → r
	14	+	±	+							± tr										K+ y → r
cladocarpa	9										+										K−, P+ or
concinna	12		+	+	+	±	± tr														K+ br. y
	2			±	+	±	+				tr										K+ y sl. → r
cristatula	13								+	±											C + y
	2									+											C + y
erinacea	17										+				±						K−, P+ or.
	10														+						K−, P−
	7						+								± t						K+ y sl. → r
	5	+					±				± tr										K+ y → r
	4		+	+	+	+	tr								+						K+ br y
fleigiae	9						+								± tr						K+ y sl. → r
	5	+					±														K+ y → r
grandispora	15	+																			K+ y → r
	8										+	+									K−, P+ or.
kalbiana	25										+										K−, P+ or.
lunaria	2										+										K−, P+ or.
meridionalis	8														+	±					K−, P−
	5	+																			K+ y → r
	5	± rare	± tr				+														K+ y sl. → r
	2																			+	K−, P−
cf. *moreliana*	4														+						K−, P−
	3																			+	K−, P−
parvula	25													±	± rare		± rare	+			K−, P−
	16													+	±			±			K−, P−
	5														+	+					K−, P−
	2																			+	K−, P−
steineri	14														+						K−, P−
	7																			+	K−, P−
	5															+					K−, P−
subelegans	48	+		±			+	+							±						K+ y → r
	1		+	+	+	+	+														K+ y sl. → r
subparvula	16										+										K−, P+ or.
	4												+								K+ sl. dull y, P+ y

Key to secondary metabolites: SAL = salazinic acid, STI = stictic acid, CST = constictic acid, CRY = cryptostictic acid, ME = menegazziaic acid, NOR = norstictic acid, GAL = galbinic acid, DIF = diffractaic acid, BAR = barbatic acid, PRO = protocetraric acid, FUM = fumarprotocetraric acid, PSO = psoromic acid, CAP = caperatic acid, TER = unidentified tri-terpenoids, FA = unidentified fatty acid, EU = eumitrin, Us1 = unknown with yellow spot (Rf classes A/B/C = 6/1-2/5), Us2 = unknown with blue (Rf class A = 4–5) and green (Rf class B = 5–6), fluorescence after charring, Ch0 = usnic acid alone; n = number of specimens studied; + = presence constant within species (highlighted in grey); ± = presence variable among specimens within species; tr = present in traces; rare = only in one/two specimens.
Key to medulla colour test: y → r = yellow turning red; br. Y = bright yellow; y sl. → r = yellow slowly turning red; sl. dull y = slowly dull yellow; y = yellow; or. = orange. *n* = number of specimens examined for that chemotype

its internal nodes. Therefore, only the ML tree is shown here with posterior probabilities added adjacent to BS values.

Within the *Usnea* clade, four highly supported clades were recovered, named hereafter as *Usnea* 1 (Usnea-2 in Truong *et al*. 2013*a*), *Usnea* 2 (Usnea-3 in Truong *et al.* 2013*a*), *Usnea* 3 (Usnea-3 in Truong *et al.* 2013*a*) and *Usnea* 4 (Usnea-4 in Truong *et al.* 2013*a*) (Fig. 2), with a low degree of geographical structures. This is consistent with the results reported in Truong *et al.* (2013*a*). However, clade Usnea-3 of Truong *et al.* (2013*a*) splits here into two clades (*Usnea* 2 and *Usnea* 3). The relationships among these clades remain unresolved. Specimens from Brazil included in this study were clustered in the clades *Usnea* 2, *Usnea* 3 and *Usnea* 4 respectively. While most of the traditionally circumscribed species in *Usnea* s. str. (Truong *et al.* 2013*a*, Fig. 3) sampled for this study were found to be monophyletic, a few did not form monophyletic groups. This is not surprising as species-level polyphylies are commonly found in *Parmeliaceae* and in lichenized fungi in general (reviewed in Crespo & Lumbsch 2010; Crespo *et al*. 2011; Lumbsch & Leavitt 2011). In the present study, *U. cirrosa* is shown to be polyphyletic for the first time. The clade *Usnea* 1 is formed by the species-pair *U. florida*-*U. subfloridana* from Europe clustered together with *U. subrubicunda*, a North American species. Clade 2 is composed only of neotropical species (*U. cornuta* s. lat., *U. subglabrata*) and included samples grouped in two strongly supported monophyletic clades referred to as *U. fleigiae* and *U. grandispora*. Samples clustered in the *U. grandispora* clade are morphologically similar to *U. florida* whereas *U. florida* belongs to the clade *Usnea* 1 (Fig. 2). Despite the morphological similarities found between *U. grandispora* and *U. florida*, our results clearly show that these are phylogenetically only distantly related. Corroborating morphological and molecular data, the clades *U. fleigiae* and *U. grandispora* are described below as two new species, respectively.

The clade *Usnea* 3 is composed of the European specimens *U. glabrata* and *U. flavocardia*, together with the Brazilian specimens of *U. meridionalis* and an undescribed species *Usnea* sp. 1, which corresponds to the possible fertile counterpart of *U. flavocardia* (see comments under *Usnea* sp. 1). Recent phylogenetic studies show that species differing only in the presence or absence of soralia (defined as "species-pairs" by Poelt 1970, 1972) usually correspond to the same lineage (Articus *et al*. 2002; Truong & Clerc 2016). However, the opposite can also occur, as for example in the genera *Letharia* (Th. Fr.) Zahlbr. (Kroken & Taylor 2001) and *Heterodermia* Trevis. (Lücking *et al*. 2008). For instance, our results show that the apparent species-pair *U. meridionalis* and *U. flavocardia* (Truong *et al*. 2011) might belong to different lineages. In our study, *U. meridionalis* forms a well-supported sister group relationship with *U. glabrata* while *U. flavocardia* is grouped with *Usnea* sp. 1. Our results suggest that assumed species-pairs should be treated and tested individually. Furthermore, Truong & Clerc (2016) stated that the evolutionary significance of reproductive traits should be corroborated with molecular data for each particular case before making any taxonomic conclusions.

Usnea clade 4 includes several species with a wide distributional range and two newly recovered clades referred to as *U. subparvula* and *U. aurantiaca-parvula*, related to *U. parvula*. Particular morphological and ecological features show that these two clades correspond to as yet undescribed taxa. Both

FIG. 2. Phylogenetic relationships among corticolous, shrubby and esorediate species of *Usnea* in Brazil based on maximun likelihood (ML) inference from the multi-locus dataset of ITS rDNA, *Mcm7* and *RPB1* gene markers. Bootstrap support (BS) followed by posterior probability (PP) from the Bayesian (B/MCMC) 50% majority-rule consensus tree are reported above branches. Thick branches indicate high support (black branches = BS ≥ 70 and PP ≥ 0·95; black grading into white branches = BS ≥ 70 or PP ≥ 0·95). Key to chemotypes: CAP = caperatic acid, NOR = norstictic acid, PRO = protocetraric acid, PSO = psoromic acid, SAL = salazinic acid, STI = stictic acid, TER = unidentified triterpenoid, THA = thamnolic acid. Newly described species are in bold. *Neuropogon* clade was used as outgroup.

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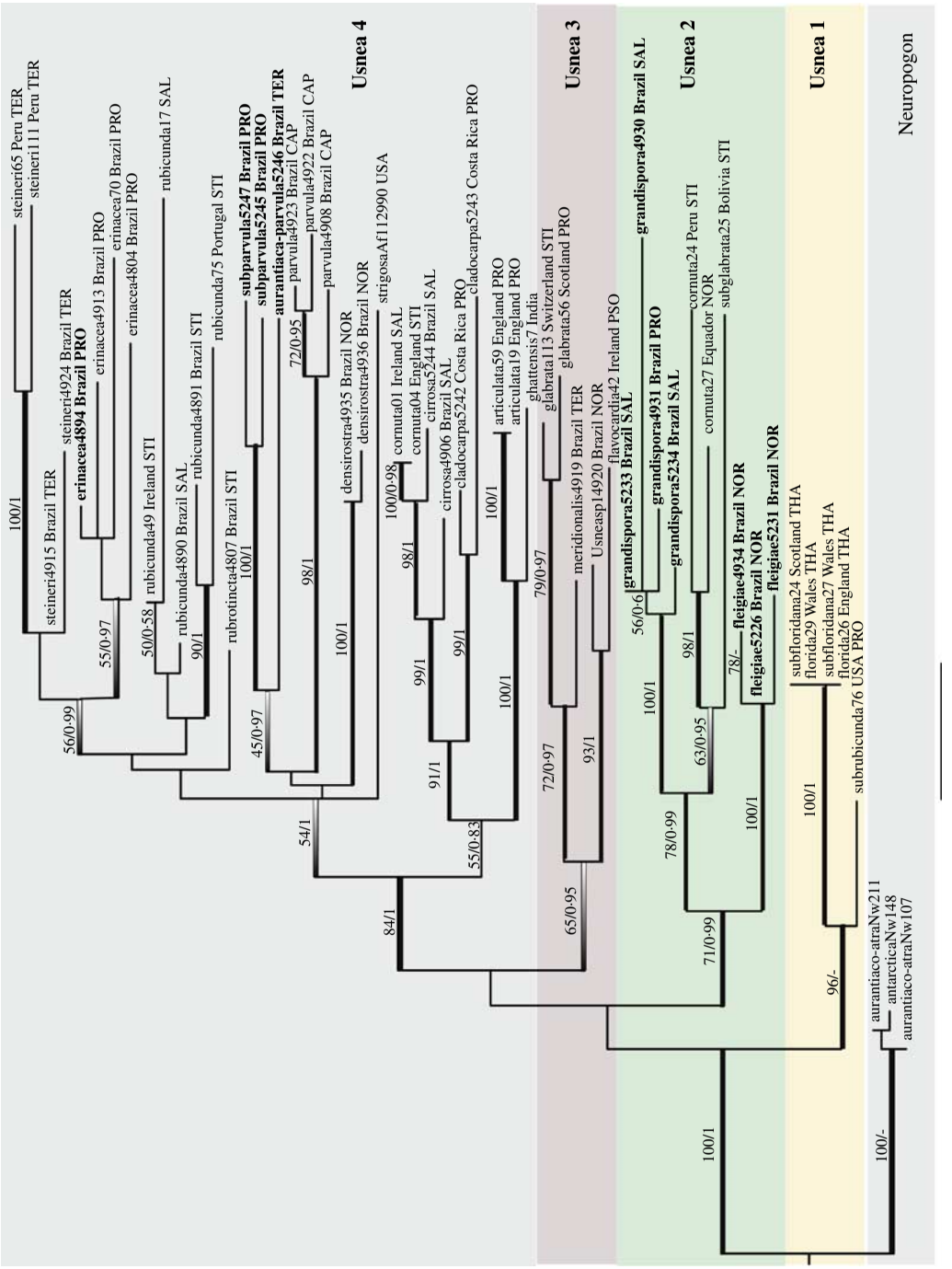

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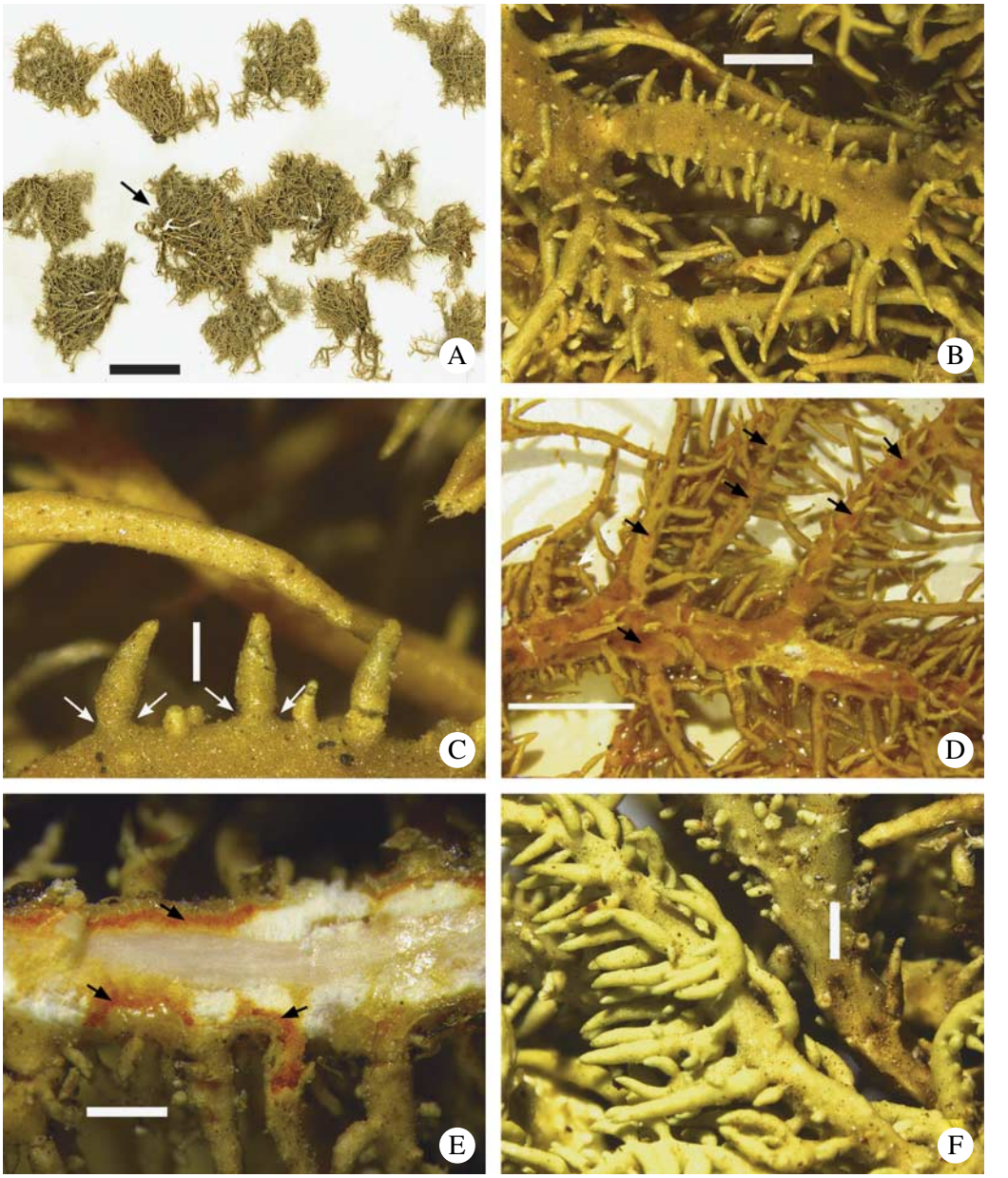

FIG. 3. *Usnea aurantiaca-parvula*. A–C, holotype: A, thallus; B, irregular branches with lageniform fibrils; C, simple lageniform fibrils constricted at the base (arrows). D, several minute foveolae (arrows) (*L. Krieger & M. Brügger* 1407b); E, Section through thallus with strong orange pigmentation occurring in patches in medulla at arrows (*M. Muryel* s. n.); F, furcate lageniform fibrils (*M. Muryel* s. n.). Scales: A = 1 cm; B = 1 mm; C = 200 µm; D = 2 mm; E & F = 500 µm. In colour online.

U. cladocarpa and *U. steineri* appear monophyletic. The position of *U. erinacea* s. lat. in our phylogeny is unresolved but a previous phylogeny of the genus *Usnea* (Truong *et al.* 2013*a*) clearly showed that this species is polyphyletic.

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Usnea cirrosa appears to be paraphyletic (clade *Usnea* 4, Fig. 2). Our results indicate that these 'morpho species' include more than one undescribed taxon. Despite our intensive taxonomic analyses we were unable to draw any conclusions about them at this time. Species with a highly variable morphology, several chemotypes and/or a wide distributional range might include more than one taxon (as is the case for *U. cornuta* and *U. erinacea*, see Truong *et al.* 2013*a*). The use of molecular tools combined with a broader sampling over the whole geographical range of the species, in parallel with traditional methods, will facilitate the re-evaluation of phenotypic characters and the understanding of species boundaries in these groups.

Taxonomy

Usnea aurantiaca-parvula A. Gerlach & P. Clerc sp. nov.

MycoBank No.: MB 819420

Similar to *U. parvula* but differs by its smaller size, orange subcortical pigment that often spreads into the whole medulla, strongly irregular branches with sometimes ± alate segments, numerous minute foveolae and ± lageniform, simple to furcate, spinulose fibrils, and a compact medulla.

Type: Brazil, Pernambuco, Buíque, Serra do Catimbau, corticolous, 1970, *L. Xavier Filho* s. n. (JPB—holotype; ICN, G—isotypes). %C/M/A: 13.5/13.5/46. Ascospores: 8–9–10 × 5·0–5·5–6·0(–7·0) µm (n = 21). Chemistry: an unknown substance with a blue (Rf class A: 4–5) and a green (Rf class B: 5–6) fluorescence after charring.

(Fig. 3A–F)

Thallus (n = 10) erect-shrubby, yellow-green, small, up to 3 cm long, with isotomic-dichotomous ramifications; *trunk* often very short, concolorous with branches, not annulated; *main branches* 0·7–1·1 mm thick, irregular, distinctly segmented, with acute-angled to almost alate segments in cross-section, sometimes deformed by the presence of deep foveolae; *lateral branches* constricted or not at ramification point; *foveolae* usually numerous on the whole thallus; *maculae*, *pseudocyphellae*, *papillae* and *tubercles* absent; *fibrils* lageniform, short and spinulose (0·7–1·2(–5·0) mm), simple to sometimes bifurcate, numerous (10–15 mm^{-2}), ± regularly distributed on the whole thallus; *fibercles* absent to rare; *cortex* ± shiny, moderately thin to moderately thick, with *ceratina*-type plectenchyma; *medulla* dense to lax, moderately thin to thick, strongly orange pigmented, pigment at first subcortical, then spreading into the inner medulla, sometimes forming irregular patches; *axis* moderately thick to thick, remaining unpigmented. CMA (n = 6): %C = (5·0–)6·0–*8·0*–10·5(–13·5); %M = (13·5–)19·5–*24·5*–29·5(–36·0); %A = (20·0–)25·5–*35·0*–44·5(–56·0). A/M = (0·4–)0·6–*1·6*–2·6(–3·3).

Apothecia numerous, lateral to terminal, often very small, 1 (-5)mm diam.; *ascospores*: length = (6·0–) *8·8* ± 1·0(–10·5) µm, width = (5·0–)*5·6* ± 0·5(–7·0) µm, n = 4.

Pycnidia not seen.

Chemistry. Medulla: K−, P−. TLC: 1) unknown Us2 with blue-green fluorescence after charring (Rf class A = 4–5, B = 5–6), ± fatty acids (Rf classes A/B/C = 2/3/4 and 3–4/4–5/5–6) (n = 7); 2) usnic acid alone (n = 5); 3) triterpenoid spot, grey-violet with orange fluorescence after charring (Rf classes A/B/C = 4—5/4/4—5) (n = 1).

Etymology. Named after the orange colour of the medulla and the resemblance to *U. parvula*.

Habitat and distribution. Corticolous or lignicolous, mainly in the Caatinga and Cerrado biomes in the north-eastern and south-eastern parts of Brazil. So far known only from Brazil (Mato Grosso do Sul, Minas Gerais, Bahia, Pernambuco and Ceará). It has not been found as yet in southern Brazil.

Taxonomic remarks. The subcortical orange pigmentation, the irregular branches with numerous foveolae and ± alate segments, the numerous lageniform spinulous fibrils and the K−, P− medulla are the main characteristics of this taxon. Sometimes the pigmentation is very weak (as observed in old herbarium specimens) and the typical fibrils might be present only on some parts of the branches. *Usnea steineri* is another fertile species with a K−, pigmented medulla. It differs from *U. aurantiaca-parvula*

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by its slenderer, not spinulose and lageniform fibrils. Furthermore, the pigment in *U. steineri* is reddish, forming a usually thin subcortical layer, often spreading into the cortex but not into the medulla. Bayesian qanalysis (Fig. 2) shows that *U. aurantiaca-parvula* constitutes a distinct lineage related to *U. parvula*.

Specimens examined. **Brazil:** *Mato Grosso do Sul*: Porto Murtinho, Fazenda São Fernando, 21°34'26·57"S, 57°45'04·81"W, 94 m, pasture field near edge of deciduous forest, 2015, *V. Pott* 11873 (CGMS). *Minas Gerais*: Diamantina, Cerrado, 1976, *L. Krieger* 14076 (JPB); Entre Rios, Fazenda da Pedra Branca, 1977, *L. Krieger* 14430 (CESJ). *Bahia*: Morro do Chapéu, proche du centre ville (1–2 km) sur une route de terre vers des affleurements rocheux, 11°33'S, 41°09'W, 1000 m, 1989, *S. Vermont-Grundlehner* s. n. (G). *Pernambuco*: Buíque, Parque Nacional do Catimbau, Trilha das Pinturas, 2013, *E. L. Nascimento* 1801, 1804 (URM); Serra do Bituri, 1968, *E. Carrazzani* s. n. (JPB). *Ceará*: Crato, Chapada do Araripe, Malhada Bonita, 2013, *M. Alves* s. n. (ICN).

Usnea cirrosa Motyka s. lat.

Lich. Gen. Usnea Stud. Monogr., Pars Syst. **2:** 526 (1937); type: Mexico, Morelia, Corrindapaz, alt. 2200 m, 1909, *Brouard* s. n. (LBL—holotype; G!—isotype). %C/M/A: 3/39/16 (isotype, specimen 57), 2/40.5/15 (isotype, specimen 58). Ascospores: 8·5–9·5–10·5 × 5·0–5·5–6·3 (–7·0) µm (n = 20). Chemistry: usnic, salazinic and norstictic acids (Herrera-Campos *et al*. 2001).

(Fig. 4A–D)

Thallus and apothecia (n = 97). For a detailed description, see Herrera-Campos *et al*. (2001) and Clerc (2007). However, we were not able to see the reddish pigment on the apothecial margin in our specimens mentioned by Herrera-Campos *et al*. (2001), neither was norstictic acid present. CMA (n = 17): %C = (3·0–)4·5–*6·5*–8·5(–11·0); %M = (22·5–)27·5–*32·5*–37·5(–40·5); %A = (11–)22–*22*–30(–40); A/M = 0·3–*0·7*–1·3(–1·8). *Cortex* with plectenchyma intermediate between *ceratina* and *merrillii*-type. *Ascospores*: length = (7·0–)*9·0* ± 0·9(–12·0) µm, width = (4·8–)*6·0* ± 0·5(–8·0) µm, n = 12.

Chemistry. K+ yellow → red. TLC: salazinic and ± protocetraric (trace) acids.

Habitat and distribution. USA (Tavares & Sanders 1998; Clerc 2007), Colombia (Motyka 1938) and Mexico (Herrera-Campos *et al*. 2001). In southern Brazil, *Usnea cirrosa* is frequent in montane areas, and less abundant in coastal areas. Specimens from coastal areas seem to be smaller, more compact and have more fibercles than specimens from montane areas where they are often well developed with larger thalli (≥ 8 cm). *Usnea cirrosa* occurs on a variety of corticolous (twigs and trunk) or lignicolous substrata. This species is recorded here for the first time in Brazil.

Taxonomic remarks. As circumscribed here, this taxon can be identified easily by the distinctly to slightly constricted lateral branches at attachment points, the swollen branch segments, the usually thin and glossy cortex and the medulla reacting K+ yellow → red due to the presence of salazinic acid as the major chemical substance. However, the CMA varies from the *cornuta*- to the *brasiliensis*-type. Detailed molecular studies might show that there could be more than one species here. *Usnea cirrosa* is paraphyletic with European samples of *U. cornuta* and additional study is needed in order to critically examine species boundaries. *Usnea cirrosa* and *U. cladocarpa* are morphologically closely related but they are readily separated by their secondary metabolites: *U. cirrosa* with salazinic acid (K+ yellow → red, P+ yellow) and *U. cladocarpa* with protocetraric acid (K−, P+ orange). Clerc (2007) disagreed with Herrera-Campos *et al*. (2001) and considered *U. cirrosa* and *U. cladocarpa* (as *U. ramillosa*) to belong to the same species. Our study (Fig. 2) shows, however, that both species are distinct at the molecular level and hence should not be considered as one species. *Usnea subelegans* has numerous spinulous fibrils and a different chemistry. *Usnea meridionalis* is another species with a *cornuta*-type CMA and salazinic or norstictic acid chemotypes. However, this species always has minute red dots on the cortex surface, especially on terminal branches.

Selected specimens examined. **Brazil:** *Rio Grande do Sul*: Cambará do Sul, Parque Nacional dos Aparados da Serra, Cânion Itaimbezinho, 2014, *A. Gerlach* 1416 (ICN); Esmeralda, Estação Ecológica Aracuri, 1984, *M. Fleig* 2453 (ICN); São Francisco de Paula, Floresta

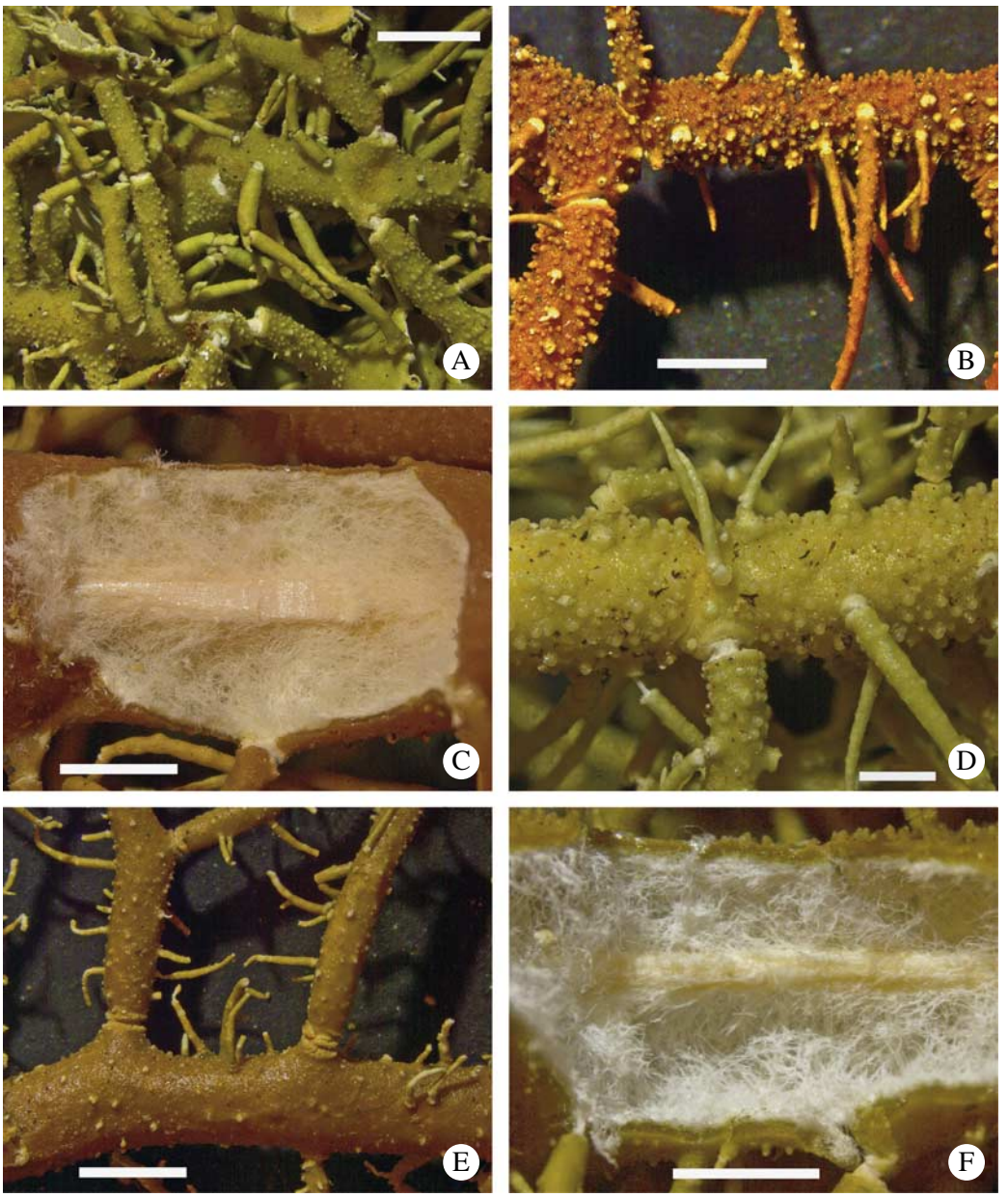

FIG. 4. A–D, *Usnea cirrosa*: A, branches constricted and inflated at ramification and foveolae (*E. Gumboski* 5020); B, branches slightly constricted and inflated at ramification and fibercles (*L. Canêz* 480); C, section through branch (*S. Grundlehner* s. n.); D, verrucose papillae (*A. Gerlach* 1510). E & F, *Usnea cladocarpa*: E, branches strongly constricted and inflated at ramification (*Schäfer–Verwimp* L9580); F, section through branch (*B. Canestraro* 485). Scales: A & E = 2 mm; B, C & F = 1 mm; D = 500 µm. In colour online.

Nacional, 2014, *A. Gerlach* 1509 (ICN); *ibid.*, Lago São Bernardo, 29°27'34"S, 50°34'16"W, 1000 m, 1989, *S. Grundlehner* s. n. (G); Vacaria, Localidade de Fazenda da Estrela, campo com *Araucaria angustifolia*, 28°04'56"S, 50°58'32·6"W, 980 m, 2003, *L. Canêz* 518 (CGMS). *Santa Catarina*: Campo Alegre, Serra do

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Quiriri, on twigs, 2012, *A. Charnei* 562 (ICN); Florianópolis, Parque Municipal da Lagoa do Peri, 2014, *A. Gerlach* 1214 (ICN); Garuva, rural area, 2013, *A. Gerlach* 1159 (ICN); São Francisco do Sul, Capri, on *Syagrus romanzoffiana*, 2013, *A. Gerlach* 980 (ICN); Urubici, Parque Nacional de São Joaquim, 2014, *A. Gerlach* 1318 (ICN). *Paraná*: Balsa Nova, Serra S'Ana, cloud forest, 1969, *G. Hatschbach* 21365 (MBM); Campina Grande do Sul, Serra Ibitiraquire, Morro Tucum, saxicolous, 1739 m, *J. Cordeiro* 1784 (MBM); Guaraqueçaba, Ilha de Superagui, 1988, *S. Eliasaro* 605 (BHCB); Guaratuba, Morro dos Perdidos, *A. Gerlach* 1032 (ICN); Lapa, Gruta do Monge, on twigs, 1996, *S. Eliasaro* s. n. (UPCB); Paranaguá, Ilha do Mel, 2012, *A. Gerlach* 785 (ICN). *São Paulo*: São Luis do Paraitinga, Parque Estadual da Serra do Mar, 23°18'48"S, 45°07'13·7"W, 930 m, 2007, *L. Canêz* 2233 (CGMS); Serra da Bocaina, 22°47'S, 44°38"W, 1550 m, 1988, *Schäfer-Verwimp & Verwimp* L-9580 (G). *Minas Gerais*: Catas Altas, Parque Natural do Caraça, 20°06'S, 43°29'W, 1275 m, 2006, *M. Benatti* 1923 (SP); Lima Duarte, Parque Estadual do Ibitipoca, 1994, *C. H. Ribeiro* 221 (CESJ). *Rio de Janeiro*: Parque Nacional do Itatiaia, 1750 m, 1966, *G. Eiten & L. Eiten* 7443 (G); *ibid.*, estrada para o Pico das Agulhas Negras, 1900 m, 2010, *A. Cervi* 9627 (MBM); Marica, restinga, on twigs of *Erythroxylum ovalifolium*, 1985, *M. A. A. Santos* s. n. (RB).

Usnea cladocarpa Fée

Essai Crypt. Ecorc. Officin. **1:** 101 (1824); type: Brazil, ad arborum truncos et ramos, misit *D. de Gestas* s. n. (G!—holotype). %C/M/A: 4.5/39/13 (thallus 12), 5.5/41/7 (thallus 13). Ascospores (apothecia absent). Chemistry: usnic and protocetraric acids (TLC by Clerc in 2008).

Usnea ramillosa Motyka *syn. nov. Lich. Gen. Usnea Stud. Monogr. Pars Syst.* **2:** 527 (1938); type: Insula Cuba, *Wright* s. n. (H-NYL!—holotype). %C/M/A: 4/40/12. Ascospores: (8·8–)9·1–*9·6*–10·0 × (6·4–)6·7–*7·0*–7·2 µm (n = 10). Chemistry: usnic and protocetraric acids (% CMA, ascospores and chemistry by Clerc in 1995).

(Fig. 4E & F)

Thallus and *apothecia* (n = 20). For a detailed description, see Herrera-Campos *et al.* (2001). CMA (n = 9): %C = 2–*3*–4(–5); %M = (36–)38–*40*–42(–43); %A = (8·0–)8·5–*13·0*–18·0(–23·0). A/M = 0·2–*0·3*–0·4(–0·6). *Cortex* with *ceratina*-type plectenchyma. *Ascospores*: length = (7·0–)*9·0* ± 1·1(–12·5) µm, width = (5·0–)*6·0* ± 0·6 (–7·5) µm, n = 7.

Distribution and habitat. Commonly found in Cuba, rarely in Jamaica and Texas (Motyka 1938, as *U. ramillosa*). This species also occurs in Ecuador (Nöske & Sipman 2004) and Mexico (Herrera-Campos *et al.* 2001). Its presence in Chile is doubtful (Motyka 1938). For Brazil, it has been reported from Santa Catarina (Motyka 1938), Rio de Janeiro (Motyka 1938; Rizzini 1952), Minas Gerais and São Paulo (Motyka 1938). *Usnea cladocarpa* is less frequent in southern Brazil compared to *U. cirrosa*, a closely related species. Based on unpublished observations of *Usnea* material from Costa Rica by the second author, the opposite situation pertains in Costa Rica, where *U. cladocarpa* is more common than *U. cirrosa*. Moreover, *U. cladocarpa* has not, so far, been found in coastal areas.

Taxonomic remarks. *Usnea cladocarpa* is recognized by its fusiform branches that are constricted at the attachment point, conspicuous foveolae, *brasiliensis*-type CMA, the A/M ratio ≤0·6 and the occurrence of protocetraric acid as the main secondary medullary substance. For differences with *U. cirrosa*, see under this latter taxon. With *Usnea meridionalis* it shares the constricted and swollen branches with the *brasiliensis*-type CMA, but differs in its chemistry (see under *U. meridionalis* for more details).

Usnea cladocarpa and *U. ramillosa* share the same swollen branches that are constricted at the attachment points, the *brasiliensis*-type CMA, as well as protocetraric acid in the medulla. Therefore they are considered here to belong to the same species and have been newly placed in synonymy.

Selected specimens examined. **Brazil:** *Paraná*: Campina Grande do Sul, 2012, *V. Ariati* 295 (ICN); Curitiba, en allant vers Vila Velha, 25°21'S, 49°34'W, 1989, *S. Grundlehner* s. n. (G); Piraí do Sul, 2012, *B. Canestraro* 485 (ICN); Tijucas do Sul, Ambrósios, on *Araucaria angustifolia*, 1991, *R. Kumrow* 3262 (MBM). *São Paulo*: Campos do Jordão, Parque Estadual de Campos de Jordão, 1996, *C. Ribeiro* 1003 (CESJ); Mogi-Guaçu, interior do Cerrado, próximo ao riacho, 22°15'20·8"S, 47°09'56"W, 650 m, 2007, *A. Spielmann* 7088 (CGMS); *ibid.*, Martinho Prado Jr., Reserva Biológica e estação experimental, Cerrado e mata ciliar do córrego, 22°16'S, 47°09'W, 630 m, *M. Benatti* 2782 (SP); Serra da Bocaina bei Sao José do Barreiro, an Sträuchern in einer Weide bei "Shangrila", 22°47'S, 44°38'W, 1550 m, 1988, *Schäfer-Verwimp & Verwimp* L 9580 (G). *Rio de Janeiro*: Rio de Janeiro, 1878, *Glaziou* s. n. (G); Tijucas, 1983, *Schwacke* 4825 (RB). *Minas Gerais*: Catas Altas, Parque

Natural do Caraça, 20°06'S, 43°29'W, 1275 m, *M. Benatti* 1923 (SP); Serra da Mantiqueira, Fazenda São Mateus, östlich von Camanducaia, 1800 m, 1980, *K. Kalb* s. n. (G).

Usnea concinna Stirt.

Scott. Naturalist (*Perth*) **6:** 103 (1881); type: Brazil, s. loc., *Mr. Weir* s. n. (BM 97192!—lectotype designated here; BM 97193!—isolectotype). %C/M/A: 9/19.5/43. Ascospores (lectotype): 8·0–10·5(–12·5) × 5·0–7·5(–8·0) µm (n = 20). Chemistry (lectotype): usnic, stictic, constictic, menegazziaic, cryptostictic and (trace) norstictic acids (TLC by Clerc in 1996).

Usnea radiata Stirt. syn. nov., *Scott. Naturalist* (*Perth*) **6:** 103 (1881); type: Brazil, statione exactius non-indicata, *Mr. Weir* s. n. (BM 97191!—lectotype designated here; BM 97190!—isolectotype). %C/M/A: 8/27/30 (lectotype). Ascospores (lectotype: 10·0–11·0 (–12·5) × 7·5–8·0 µm (n = 6). Chemistry (lectotype): usnic, stictic, constictic, menegazziaic, cryptostictic, norstictic acids and an unknown with Rf classes A/B/C 5/3/5 and green fluorescence after charring.

Usnea florida var. *scabrosa* Zahlbr. syn. nov., *Expedition der kaiserlichen Akademie der Wissenschaften nach Südbrasilien* **83:** 103 (1909); type: Brazil, São Paulo, in silvaticis prope urbem Iguape, 20–100 m, 1901, *V. Schiffner* s. n. (BM 733848!—holotype). %C/M/A: 11.5/26.5/24. Ascospores: 10·0–*10·2*–10·5(–11·0) × (5·5–)6·5–*7·2*–8·0 µm (n = 10). Chemistry: stictic, constictic, menegazziaic, cryptostictic and norstictic acids and an unknown substance with green fluorescence after charring and Rf classes: A/B/C: 5/3/5.

(Fig. 5A–C)

Thallus (n = 20) erect-shrubby, yellowish green, up to 8 cm long; *trunk* often short, 0·2–1·0 cm, rarely up to 2 cm long, concolorous with branches, always with thin annulations; *ramifications* mostly isotomic- to rarely anisotomic-dichotomous; *main branches* 0·9–2·4 mm thick, often slightly irregular, cylindrical, little segmented towards the terminal branches (1 annular crack/0·5 cm) to more segmented towards the base (3–6 annular cracks/0·5 cm) usually exposing the medulla, often with slightly swollen segments; *lateral branches* not to usually slightly constricted at the ramification point, distinctly segmented; *foveolae*, *maculae* and *pseudocyphellae* absent; *papillae* absent to rare; *tubercles* numerous, small (0·7 mm), verrucose to cylindrical, often with paler apices and sometimes eroded, regularly distributed on the whole thallus; *fibrils* present, usually numerous, slender (1–7 mm long), regularly distributed; *fibercles* often present mostly in the basal main branches, scarce to numerous; *cortex* matt to rarely ± shiny, never vitreous, moderately thick to thick, often with many irregular cracks, with *merrillii*-type plectenchyma; *medulla* white, often pale orange periaxially pigmented (probably due to the oxidation of secondary compounds), dense to compact, thin to moderately thick; *axis* ±thin to moderately thick. CMA (n = 11): %C = (8·0–)8·5–*10·3*–12·0; %M = (14·0–)18·5–*22·5*–27·0(–28·0); %A = (30·0–)27·0–*35·0*–42·5(–48·0). A/M = 1·0–*1·5*–2·5(–3·5).

Apothecia numerous, often terminal, up to 10 mm diam.; *ascospores*: length = (7·0–)*10·0* ± 1·1(–12·5) µm, width = (5·0–)*6·0* ± 0·8 (–8·5) µm, n = 9.

Chemistry Medulla: 1) K+ bright yellow, TLC = stictic, constictic, cryptostictic, ± menegazziaic and ± norstictic (trace) acids (n = 12); 2) K+ yellow slowly → red, TLC = cryptostictic, norstictic, ± constictic, ± menegazziaic and protocetraric (trace) acids (n = 2).

Habitat and distribution. *Usnea concinna* is known only from Central and South America where it seems to be widespread and found in Argentina, Bolivia, Cuba, Mexico, Paraguay, Peru and Venezuela (Motyka 1938). In Brazil, it has been recorded from Rio Grande do Sul (Fleig & Grüninger 2008), Santa Catarina, Minas Gerais and Rio de Janeiro (Motyka 1938). This species usually occurs in mountainous areas, above 900 m, mainly in the states of São Paulo and Minas Gerais.

Taxonomic remarks. *Usnea concinna* can be identified by the very slightly constricted and swollen branches covered with minute whitish verrucose to cylindrical tubercles, the matt and thick cortex (8·5–*10·3*–12·0%) and the dense to compact medulla, reacting K+ yellow (stictic acids group). Although the majority of specimens have a matt cortex, sometimes it can be somewhat shiny. The density of fibrils, fibercles and tubercles as well as the degree of constriction of the branches are also variable.

For differences from *U. kalbiana* see under the latter species. *Usnea cirrosa* differs from

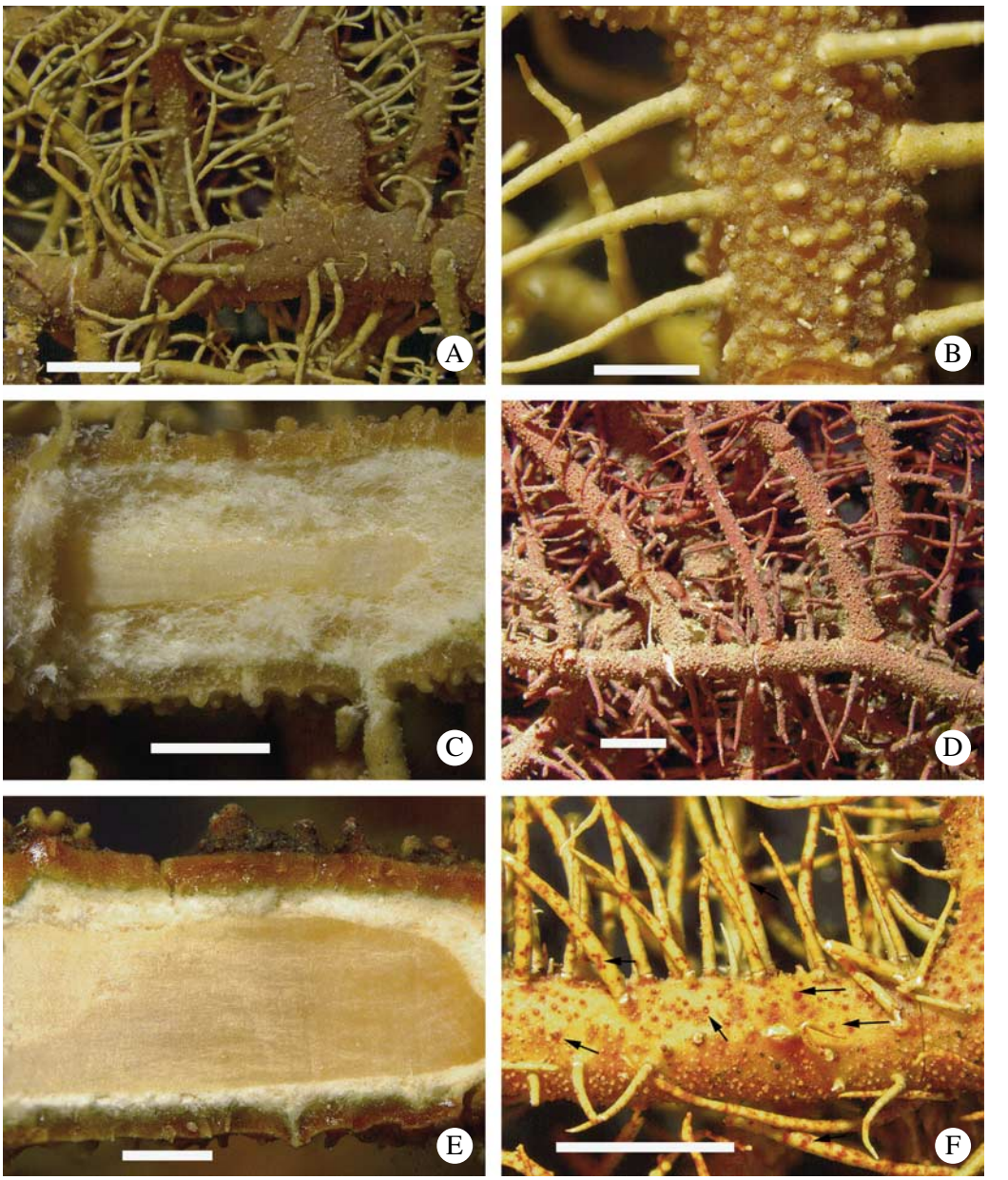

FIG. 5. A–C, *Usnea concinna* (*K. Kalb* s. n.): A, branches slightly constricted and inflated at ramification; B, verrucose tubercles; C, section through branch. D–F, *Usnea erinacea*: D, terete and tapering branches, the cortex is diffusely pigmented red on whole branches (*A. Gerlach* 1112); E, section through branch (*Schäfer–Verwimp* L9118); F, detail of thallus surface with darker spots containing red cortical pigmentation (arrows) (*A. Gerlach* 1211). Scales: A = 2 mm; B & E = 500 µm; C = 1 mm; D & F = 2 mm. In colour online.

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U. concinna by its branches that are distinctly constricted at the attachment point, the swollen branch segments, the *cornuta*-type CMA and the K+ yellow → red medulla (salazinic acid). We were unable to obtain freshly collected material for sequencing and hence the phylogenetic position of *U. concinna* remains unclear.

Usnea radiata corresponds to a smaller and more branched form of *U. concinna* that otherwise shares all the characteristics of the latter species, and the holotype of *Usnea florida* var. *scabrosa* is similar morphologically, anatomically and chemically to the original material of *U. concinna*. Therefore, both *Usnea radiata* and *U. florida* var. *scabrosa* are considered as synonyms of *U. concinna*.

Selected specimens examined. **Brazil:** *Rio Grande do Sul*: São Francisco de Paula, Centro de Pesquisa e Conservação da Natureza, Pró-Mata, on bark of *Araucaria angustifolia*, 918 m, 1998, *M. Fleig & Grüninger* 983136 (ICN). *São Paulo*: Campos do Jordão, 1991, *M. Fleig* 4455 (ICN); *ibid.*, Serra da Mantiqueira, Nebelwald am Pico do Itapeva, 2000 m, 1987, *Schäfer-Verwimp* L/8493 (G); *ibid.*, 150 km nordöstlich von São Paulo in einem hellen, feuchten Urwald, 1700 m, *K. Kalb & G. Plöbst* s. n. (G-260927). *Rio de Janeiro*: Itatiaia, Regenwald oberhalb des Museums, an Ästen auf dem Weg zum Fernsehturm, 1350 m, 1987, *Schäfer-Verwimp* L/9264 (G). *Minas Gerais*: Fazenda São Mateus, östlich von Camanducaia, 1800 m, 1980, *K. Kalb* s. n. (G-260940); Serra de Ibitipoca, 1400 m, 1975, *L. Krieger* 13464 (CESJ).

Usnea cristatula Motyka

Lich. Gen. Usnea Stud. Monogr. Pars Syst. **2**(2): 641 (1938); type: Mexico, Michoacan, Morelia, Cerro Azul, *Brouard* s. n. (LBL—holotype; LBL, S, G!—isotypes). %C/M/A: 11/17.5/42.5. Ascospores (isotype): (7·5–)8·0–*9·0*–9·5(–10·0) × 5·0–*5·5*–6·0 µm (n = 22). Chemistry: usnic, diffractaic and squamatic (trace) acids (Herrera-Campos *et al.* 1998).

Thallus and *apothecia* (n = 15). For a detailed description and illustrations see Herrera-Campos *et al.* (1998), Clerc (2007) and Truong & Clerc (2012). CMA (n = 14): %C = 8·5–*11·0*–13·5(–18·0); %M = (19·0–)20·0–*23·0*–26·60(–27·5); %A = (25·0–)26·5–*32·0*–37·5(–43·0). A/M = 1·0–*1·4*–1·8(–2·2). *Cortex* with *baileyi*-type plectenchyma. *Ascospores*: length = (6·0–)*8·3* ± 0·8(–10·0) µm, width = (4·5–)*5·4* ± 0·4(–6·0) µm, n = 10.

Chemistry. Medulla C+ yellow. TLC: 1) diffractaic, ± barbatic acids (n = 13); 2) barbatic acid (n = 2).

Habitat and distribution. Previously reported for the USA (Knudsen & Lendemer 2006), Mexico (Herrera-Campos *et al.* 1998), Bolivia, Colombia, Peru and Venezuela (Truong & Clerc 2012). Also known in Europe from Portugal (Clerc 2011*a*). Newly reported here for Brazil. Despite extensive sampling conducted in southern Brazil, *U. cristatula* could not be found and only herbarium specimens were examined.

Taxonomic remarks. *Usnea cristatula* is characterized by its pink/reddish medulla containing diffractaic and/or barbatic acids, the presence of numerous fibercles and ± slender fibrils as well as a thick and glossy cortex. The localization of the pigment can vary from subcortical to almost subaxial, rarely over the whole width of the medulla, sometimes with a periaxial yellow pigment. *Usnea strigosa* (Ach.) Eaton is a North American species with a pigmented medulla and diffractaic acid, amongst other chemotypes (Hale 1979), but the pigment is dusky red and usually fills the whole medulla, fibercles are lacking and numerous spinulose fibrils are present (Clerc 2007). For differences between this species and *Usnea flavorubescens* Truong & P. Clerc, see Truong & Clerc (2012). We were unable to acquire freshly collected material to obtain good quality DNA, hence the phylogenetic position of *U. cristatula* remains unclear.

Selected specimens examined: **Brazil:** *Rio Grande do Sul*: Santa Maria, 150 m, 1980, *M. Fleig* 1207 (ICN); Novo Cabrais, near Santa Maria, 1999, *A. Spielmann* 11884 (CGMS). *Santa Catarina*: Nova Teutonia, 1944, *F. Plaumann* s. n. (RB). *Paraná*: Vila Velha, 25°21'S, 49°34'W, 1989, *S. Grundlehner* s. n. (G); Pinhão, on fences of *Phoebe porosa*, 1975, *L. Krieger* s. n. (JPB); Ponta Grossa, Uvaia, 1976, *L. Krieger* 15374 (CESJ). *Minas Gerais*: Grão Mogol, Trilha dos garimpeiros, campo rupestre dos afloramentos rochosos, 1100 m, 1991, *M. Hatschbach* 55090 (MBM). *Distrito Federal*: Brasilia, Fazenda Água Limpa, on trunk of embaúba *Cecropia* sp., mata ciliar, 1980, *E. Sato* 3 (JPB). *Bahia*: Carrentina, 1967, *D. Vital* s. n. (JPB).

Usnea erinacea Vain. s. lat.

Dansk Botan. Arkiv. **4:** 3 (1926); type: Mexico, Chimantla, 1841, *Liebmann* s. n. (TUR-V!—holotype). %C/M/A: 7.5/17.5/50 (Clerc 2011*a*). Ascospores: (7·5–)8·0–*8·5*–9·0 (–10·0) × 5·0–*5·5*–6·0(–7·0) µm. Chemistry: usnic, salazinic and norstictic acids (Clerc 2011*a*).

(Fig. 5D–F)

Thallus ($n = 130$). For a detailed description, see Clerc (2004, 2007). CMA ($n = 20$): %C = (4·5–)6·5–*10·0*–14·0(–16·0); %M =(7·0–)15·5–*24·5*–33·5(–36·0); %A = (14–)18–*31*–44(–60). A/M = 0·4–*1·5*–3·0(–6·5). *Cortex* with *baileyi*-type plectenchyma.

Apothecia numerous, lateral, terminal to subterminal, up to 25 mm diam.; *ascospores*: length = (7·0−)*9·2* ± 1·2(−13·0) µm, width = (5·0−)*5·7* ± 0·5(−7·0) µm, $n = 7$.

Chemistry. Medulla: 1) K−, P+ orange, TLC = protocetraric acid, ± undetermined triterpenoids ($n = 17$); 2) K−, P−, TLC = undetermined triterpenoids ($n = 10$); 3) K+ yellow slowly → red, TLC = norstictic, ± undetermined triterpenoids ($n = 7$); 4) K+ yellow → red, TLC = salazinic, ± norstictic, ± protocetraric (trace) acids ($n = 5$); 5) K+ bright yellow, TLC = stictic, constictic, cryptostictic, menegazziaic, norstictic (trace), undetermined triterpenoids ($n = 4$).

Habitat and distribution. *Usnea erinacea* has a wide ecological range, from sea level to 1800 m elevation. This species is frequently found growing on the bark of *Araucaria angustifolia* in mountainous areas and on fences in rural areas. It is known from North and South America, Europe and Africa (Clerc 2004, 2007, 2011*a*). In South America, this species is so far known from Bolivia, Colombia, Equador, Peru and Venezuela (Truong *et al*. 2011). *Usnea erinacea* is probably the most abundant fertile species in Brazil but interestingly it has not been cited previously for this country. It is newly recorded here for Brazil.

Taxonomic remarks. The reddish orange pigmentation of the cortex, the tapering and terete branches that are not constricted at the attachment point, the thick (≥10%) and vitreous cortex, the compact medulla and the ratio A/M ≥ 1·5 are diagnostic for *U. erinacea* s. str. In Brazil, however, we consider *U. erinacea* s. lat. to be a very polymorphic species that shows a high level of variability in the following important characters: 1) the pattern of cortical pigmentation, 2) the shape of branches, 3) the CMA values, and 4) the chemistry (see Table 2).

Three main patterns of cortical pigmentation were found among the specimens studied: a diffuse pigmentation throughout the whole cortex (*Usnea erinacea* s. str.) (Fig. 5D); a superficial pigmentation in the upper part of the cortex; and a spot-like, irregular or punctiform cortical pigmentation that often also coloured the papillae (Fig. 5F). The branches may vary from tapering to irregular in longitudinal section and terete to obtuse-angled in cross-section. The A/M ratio may vary from ≤1 to ≥2. Intermediate forms were common and the pattern of pigmentation could not be clearly correlated with any other morphological or chemical characters. This group is weakly supported and unresolved (Truong *et al*. 2013*a*, Fig. 4) and a large-scale morphological and molecular study is needed.

Selected specimens examined. **Brazil:** *Rio Grande do Sul*: Caxias do Sul, Distrito de Santa Lucia do Piai, 29°11'48·6"S, 50°59'21·6"W, 735 m, 2010, *A. Spielmann* 8641 (CGMS); Gramado, surroundings of Lago Negro, *Araucaria* moist forest, 29°22'44"S, 50°52'26"W, 800 m, 2013, *M. Dal Forno* 2108 (ICN); Mariana Pimentel, beira de estrada, em poste, 1989, *S. Grundlehner* s. n. (ICN); São Francisco de Paula, Centro de Pesquisa e Conservação da natureza Pró-Mata, 1998, *M. Fleig* 983010 (ICN); Vacaria, Fazenda da Estrela, 28°01'58"S, 50°58'17·5"W, 900 m, 2003, *L. Canêz* 442 (CGMS). *Santa Catarina*: Alfredo Wagner, RPPN Rio das Furnas, 2014, *A. Gerlach* 1228 (ICN); Florianópolis, Parque Municipal da Lagoa do Peri, 2013, *A. Gerlach* 1211 (ICN); Bergland bei Fraiburgo, Regenwald im Park des Hotels Renar, 1070 m, 1987, *Schäfer-Verwimp* L9118 (G); Joinville, rural area, on fences, 2013, *A. Gerlach* 1112 (ICN); Rio Negrinho, Fazenda Velha, 2007, *E. Gumboski* 1020 (ICN); São Joaquim, Fazenda Santa Rita, campo de pastagem, 1400 m, 1992, *M. Fleig* 4705 (ICN); Urubici, Parque Nacional de São Joaquim, *A. Gerlach* 1363 (ICN). *Paraná*: Carambeí, Catanduva de Fora, 2013, *M. Engels* s. n. (ICN); Castro, Cânion Guartela, 2013, *L. Rocha* s. n. (ICN); Curitiba, en allant vers Vila Velha, 25°21'S, 49°34'W, saxicolous, 1989,

S. Grundlehner s. n. (G); Guarapuava, 2013, *M. Engels* s. n. (ICN); Tijucas do Sul, Vossoroca, on *Arecastrum* sp., 1973, *R. Kumrow* 150 (MBM); estrada antiga da Graciosa, on fences, 1999, *W. Sanders* 99801.1 (UFP). *São Paulo*: Campos de Jordão, Sekundärwald bei Minalba, epiphytisch, 1420 m, 1989, *Schäfer-Verwimp* L/11022 (G); Piquete, Pico dos Marins, 22°30'30·8"S, 45°07'46·4"W, 1900 m, 2007, *L. Canêz* 2438 (CGMS). *Rio de Janeiro*: Itatiaia, Parque Nacional do Itatiaia, em direçao ao Pico das Agulhas Negras, 22°23'07·5"S, 44°40'48·1"W, 2355 m, 2012, *A. Spielmann* 10153 (CGMS). *Minas Gerais*: Catas Altas, Parque Natural do Caraça, 20°06'52·7"S, 43°29'29·4"W, 1265 m, 2006, *L. Canêz* 1789 (CGMS); Itamonte, Parque Nacional do Itatiaia, Estrada das Prateleiras, 22°21'41·8"S, 44°44'08·3"W, 2134 m, 2009, *A. Spielmann* 7641 (CGMS); Lima Duarte, Parque Estadual do Ibitipoca, 1993, *C. Ribeiro* 134 (CESJ); National Park Serra de Caparo, Regenwald, epiphytisch am Rande der Erdstraβe bei 1870 m, 1987, *Schäfer-Verwimp* L8908 (G).

Usnea fleigiae A. Gerlach & P. Clerc sp. nov.

MycoBank No.: MB 819421

Similar to *Usnea florida* but differs in its concolorous with branches or paler basal part, the lax medulla, and the presence of norstictic and/or salazinic acids.

Type: Brazil, Rio Grande do Sul, Cambará do Sul, Parque Nacional da Serra Geral, Cânion Fortaleza, on *Drimys winteri*, 16 December 1986, *M. Fleig* 2877 (ICN—holotype; G—isotype). %C/M/A: 9.5/13/55 (holotype), 14/5/62 (isotype). Ascospores: (9·0–)9·5–*10·5*–11·5(–13·0) × (5·0–)6·0–*7·3*–8·0 µm ($n = 20$). Chemistry: usnic, salazinic and norstictic acids.

(Fig. 6A–C)

Thallus ($n = 16$) erect-shrubby to rarely almost subpendulous, yellow-green, up to 8 cm long, with anisotomic-dichotomous, often very dense ramifications; *trunk* usually short, up to 3 mm long, usually concolorous, with branches rarely black pigmented, with thin annulation; *main branches* tapering, terete in cross-section, distinctly segmented with *c.* 7 annular cracks/0·5 cm, with cylindrical to somewhat swollen segments; *lateral branches* not to slightly constricted at the ramification point; *foveolae*, *maculae* and *pseudocyphellae* absent; *papillae* and *tubercles* often numerous (>10 mm^{-2}), ± verrucose, ± regularly and densely distributed on the whole thallus, except sometimes close to the basal part; *fibrils* often numerous (>20/3 mm^{-2}), slender (1–7 mm long), ± regularly distributed on the whole thallus; *fibercles* few to absent; *cortex* shiny, moderately thin to thick, with plectenchyma intermediate between *ceratina*- and *florida*-type; *medulla* white, dense (near the base) to lax (in lateral branches), thin; *axis* thick. CMA ($n = 15$): %C = (6·0–)7·5–*10*–12·5(–14·5); %M = 5·0–*9·0*–13·0(–17·5); %A = 47–*62*–70 (–76). A/M = (3·0–)4·5–*8·0*–11·5(–15·0).

Apothecia numerous, mainly terminal, up to 8 mm diam.; *ascospores*: length = (9·0–) *13·9* ± 1·8(–18·0) µm, width = (5·0–)*9·5* ± 1·1 (–12·0) µm, $n = 14$.

Chemistry. Medulla: 1) K+ yellow slowly → red, TLC = norstictic acid and ± undetermined triterpenoid ($n = 9$); 2) K+ yellow → red, TLC = salazinic and ± norstictic acids ($n = 5$).

Etymology. This species is named in honour of the Brazilian lichenologist Mariana Fleig. Her rich *Usnea* collections that are housed in the ICN herbarium allowed the first author to begin her studies on *Usnea* in Brazil.

Habitat and distribution. *Usnea fleigiae* is known only from southern Brazil where it occurs in mountainous areas (above 900 m) in the Serra Geral and Serra do Mar, in three types of vegetation: dense rainforest, *Araucaria* forest and high elevation grasslands. It is found mainly on twigs of shrubby trees and is quite rare.

Taxonomic remarks. The blackish pigmentation that is sometimes seen in the basal part of the thallus might be owing to the presence of a lichenicolous fungus. Among the shrubby and esorediate *Usnea* species known from Brazil, *U. fleigiae* might be confused with *U. grandispora*. See under this taxon for differences between the two.

Selected specimens examined. **Brazil:** *Rio Grande do Sul*: Cambará do Sul, Parque Nacional da Serra Geral, Cânion Fortaleza, 1983, *M. Fleig* 2197 (ICN); *ibid.*, on *Drimys winteri*, 1986, *M. Fleig* 2878 (ICN); *ibid.*, 1030 m, 2012, *A. Spielmann* 10200, 10208 (CGMS); São Francisco de Paula, Área de Preservaçao Ambiental Rota do Sol, 2002, *S. Martins* s. n. (HAS). *Santa Catarina*: Campo Alegre, Serra do Quiriri, 1200 m, 2012, *A. Charnei* 562, 563, 566 (ICN); Urubici, 1650 m, 2004, *A. Cervi* 8712 (UPCB).

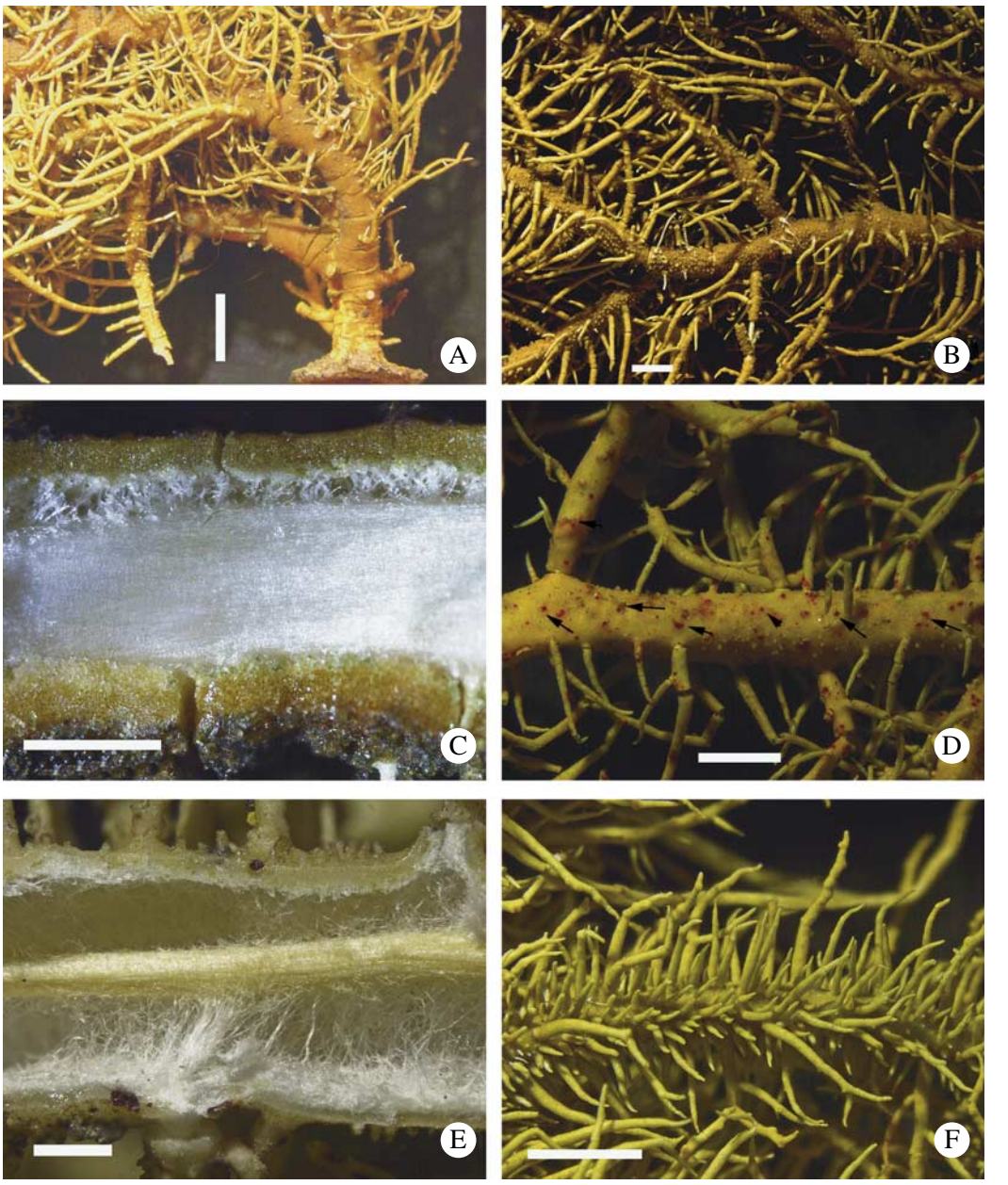

FIG. 6. A–C, *Usnea fleigiae*: A, trunk annulated, concolorous (holotype); B, branches annulated, slightly constricted and inflated at ramification (holotype); C, section through branch (isotype). D & E, *Usnea meridionalis*: D, fusiform branches with dark, red-pigmented dots on the cortex surface (arrows) (*M. Engels* s.n.); E, section through branch, the periaxial tissue is pigmented yellow (*E. Fazolino* s.n.); F, *Usnea subelegans*, branches densely covered with spinulose fibrils (*E. Fazolino* s. n.). Scales: A & B = 2 mm; C & E = 500 μm; D & F = 1 mm. In colour online.

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Paraná: Campina Grande do Sul, Serra do Ibitiraquire, cume do Morro Itapiroca, 1800 m, 2014, *M. Engels* s. n. (ICN).

Usnea grandispora A. Gerlach & P. Clerc sp. nov.

MycoBank No.: MB 819422

Similar to *U. florida* but differs in its production of protocetraric or salazinic acids in the medulla and the larger spore size.

Type: Brazil, Rio Grande do Sul, São Francisco de Paula, Floresta Nacional de São Francisco de Paula, on bark of *Araucaria angustifolia*, near the lodging, 29 November 2014, *A. Magnago* 1114 (ICN—holotype; G—isotype). %C/M/A: 14/14/44 (holotype); 12/19.5/37 (isotype). Ascospores (holotype): (13–)14–*15·5*–17(–18) × 9–*10*–11(–12) μm (n = 12). Chemistry: usnic, protocetraric and fumarprotocetraric (trace) acids (holotype).

(Fig. 7A–F)

Thallus (n = 25) erect-shrubby, up to 8 cm long, yellow-green, isotomic- to anisotomic-dichotomously branched; *trunk* often short, up to 1 cm long, pigmented jet black at least for the first 1 mm, always with thin annulations; *main branches* 0·7–1·8 mm thick, tapering to slightly irregular, terete in cross-section, distinctly segmented (3–10 annular cracks/0·5 cm) often exposing the medulla, with cylindrical to somewhat swollen segments; *lateral branches* not to rarely slightly constricted at the ramification point; *foveolae*, *maculae* and *pseudocyphellae* absent; *papillae* and *tubercles* numerous (10–30 mm^{-2}), thin and ± cylindrical to thick and ± verrucose, ± regularly distributed on the whole thallus, except sometimes close to the basal part; *fibrils* present, usually numerous, slender (1–7 mm long) to spinulous (1–2 mm long), irregularly to regularly and then densely distributed; *fibercles* absent (or rare); *cortex* matt, thick with few irregular cracks, with plectenchyma intermediate between *ceratina*- and *florida*-type; *medulla* white, dense to compact, thin; *axis* moderately thick to thick. CMA (n = 20): %C = (11·0–)13·0–*14·5*–16·0(–19·0); %M = (5·0–)9·0–*13·0*–17·0(–19·5); %A = (33·0–)37·5–*45·5*–53·5(–59·0). A/M = 1·5–*4·0*–6·5(–11·0).

Apothecia numerous, lateral, terminal to subterminal, up to 10 mm diam.; *ascospores*: length = (11·0–)*14·8* ± 1·3(–18·0) μm, width = (6·0–)*9·9* ± 0·9(–13·0) μm, n = 18.

Chemistry. Medulla: 1) K+ yellow → red, TLC = salazinic acid (n = 15); 2) K−, P+ orange, TLC = protocetraric and fumarprotocetraric acids (n = 8).

Etymology. Named after the notably large spore size.

Habitat and distribution. *Usnea grandispora* has the same ecological range as *U. fleigiae*, occurring in montane areas. This is a corticolous species, occasionally saxicolous (only two specimens). It has been found only in the southern part of Brazil.

Taxonomic remarks. Two chemotypes were found: 1) salazinic acid chemotype, usually associated with large and conspicuous tubercles/papillae and a more branched thallus (Figs 7B & F) and 2) protocetraric acid chemotype, usually associated with smaller and thinner tubercles/papillae and a less branched thallus (Fig. 7A & E). These chemotypes seem to have a distinct geographical distribution. However, they belong to the same clade (Fig. 2). Further collecting and subsequent studies are needed to evaluate both chemotypes. *Usnea grandispora* is morphologically very similar to *U. florida*. The latter species has smaller spores (8·5–11·0 μm) and a different chemistry (Clerc 1984*a*). In addition, our molecular phylogenetic analyses show that the species are not conspecific. *Usnea fleigiae* shares its annulated branches, the large spores and the salazinic acid chemotype with *U. grandispora*, but differs from the latter species mainly by the distinctly lax medulla and the CMA values (the cortex and medulla are thinner and the axis thicker in *U. fleigiae*). Moreover, the basal part of *U. fleigiae* is often concolorous with the branches and protocetraric acid is absent. These two species are only distantly related (Fig. 2). *Usnea subfusca* Stirt. is a similar north-eastern American species (Clerc & Herrera-Campos 1997) but with smaller

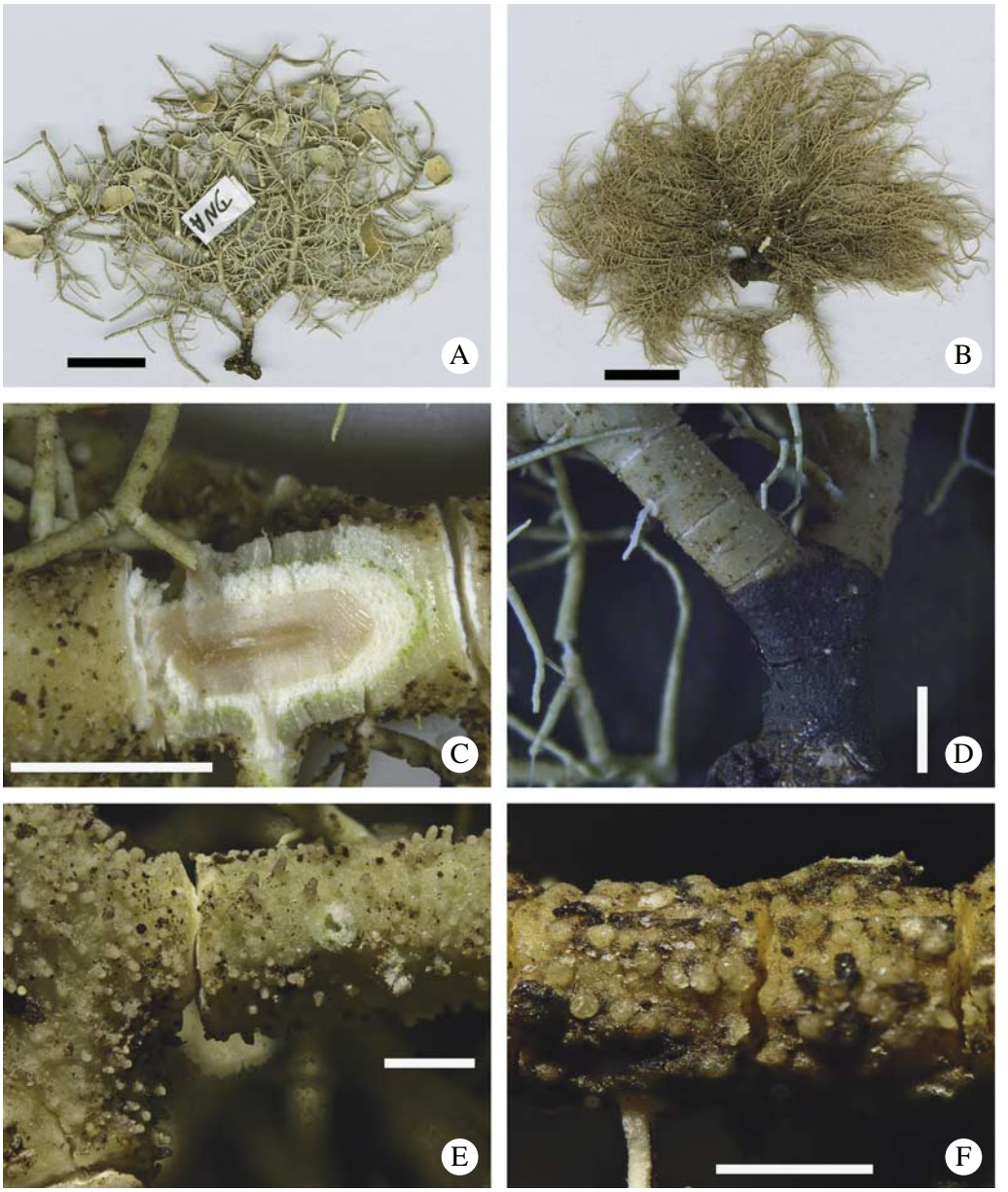

FIG. 7. *Usnea grandispora*. A, thallus (holotype); B, thallus with dense ramifications (*A. Gerlach* 1009); C, section through branch (holotype); D, trunk annulated, jet black (isotype); E, cylindrical papillae (holotype); F, verrucose tubercles, often not eroded at the apex. Scales: A & B = 1 cm; C & D = 1 mm; E & F = 500 µm. In colour online.

ascospores (<10 µm long) and never with protocetraric acid in the medulla. Three Indian apotheciate and esorediate species, *U. ghattensis*, *U. norkettii* G. Awasthi (BM!—holotype) and *U. spinosula* Stirt. (BM!—type), also have large ascospores (≥10 µm).

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Usnea ghattensis has a very stiff thallus without identified medullary substances, a thinner cortex and axis as well as a larger medulla. Furthermore, *U. ghattensis* is grouped in the *Usnea* 4 clade (Fig. 2). *Usnea norkettii* and *U. spinulosa* have strongly constricted lateral branches, a CMA of the *brasiliensis*-type and different medullary substances.

Selected specimens examined. **Brazil:** *Rio Grande do Sul*: Cambará do Sul, Parque Nacional dos Aparados da Serra, 1000 m, 1986, *M. Fleig* 2837 (ICN); *ibid.*, Cânion Itaimbezinho, on *Araucaria angustifolia*, 2014, *A. Gerlach* 1406 (ICN); São Francisco de Paula, Paulinas de São Francisco, 29°27'S, 50°34'W, 900–1000 m, 1989, *S. Grundlehner* s. n. (G). *Santa Catarina*: Serra Geral, in silva Araucariarum, 1891, *E. Ule* 120 (G); Campo Alegre, Serra do Quiriri, 1200 m, 2012, *A. Charnei* 562 (ICN); Urubici, Parque Nacional de São Joaquim, 2014, *A. Gerlach* 1354, 1360 (ICN). *Paraná*: Campina Grande do Sul, Serra do Ibitiraquire, cume do Morro Itapiroca, *c.* 1800 m, 2014, *M. Engels* s. n. (ICN); Guaratuba, Morro dos Perdidos, 2011, *S. Eliasaro* 5019 (UPCB); *ibid.*, 2014, *B. Canestraro* 691 (ICN); *ibid.*, 1260 m, 2013, *A. Gerlach* 1015 (ICN); *ibid.*, saxicolous, 2013, *E. Gumboski* 4489 (ICN).

Usnea kalbiana P. Clerc & A. Gerlach sp. nov.

MycoBank No.: MB 819423

Similar to *U. lunaria* but differs in its matt instead of vitreous cortex and in the presence of annular instead of irregular cracks in the basal part of the thallus.

Type: Brazil, Minas Gerais, Serra da Mantiqueira, Fazenda São Mateus, östlich von Camanducaia, 1800 m, 30 November 1980, *K. Kalb* s. n. (G—holotype; ICN, UPS, TNS—isotypes). %C/M/A: 13.5/11.5/50 (holotype). Ascospores (holotype): (7·5–)8·0–*8·5*–9·0 µm (n = 11). Chemistry: usnic and protocetraric acids (holotype).

(Fig. 8A–D)

Thallus (n = 33) erect-shrubby, yellowish green, up to 12 cm long, mostly isotomic-dichotomously branched; *trunk* often short, up to 3 mm long, concolorous with main branches, with annular cracks; *main branches* up to 1·5 mm thick, tapering, terete in cross-section, distinctly segmented; *segments* cylindrical and terete; *lateral branches* not constricted at the ramification point; *foveolae*, *maculae* and *pseudocyphellae* unknown; *papillae* scarce to none; *tubercles* (*young fibrils?*) often numerous, evenly distributed, cone-shaped, often eroded and whitish at summit; *fibrils* slender, up to 4 mm, few and unevenly distributed to numerous and in fishbone-like pattern; *fibercles* few to none; *cortex* matt in cross-section, sometimes slightly shiny, rarely with irregular cracks, moderately to usually thick, with *florida*-type plectenchyma; *medulla* white, dense to compact, thin; *axis* moderately thick to thick. CMA (n = 15): %C = (8·5–) 10·0–*12·5*–15·0(–16·0); %M = (8·0–)10·5–*14·0*–17·5(–18·0); %A = (33–)37–*47*–57(–67). A/M = 2–*4*–6(–8).

Apothecia numerous, terminal and lateral, up to 10 mm diam.; *ascospores*: length = (7·0–)*8·8* ± 9·7(–10·0) µm, width = (5·5–)*6·0* ± 0·4(–7·0) µm, n = 10.

Chemistry. Medulla K−, P+ orange. TLC: protocetraric acid (n = 25).

Etymology. Named after the distinguished lichenologist Klaus Kalb who has contributed so much to the current knowledge of the South American lichen flora, including numerous collections of *Usnea* from Brazil.

Habitat and distribution. *Usnea kalbiana* is a corticolous and lignicolous species. It is known only from Brazil, mainly in mountainous areas (above 1200 m) in the Serra da Mantiqueira of Minas Gerais.

Taxonomic remarks. *Usnea kalbiana* ressembles *U. lunaria* and both taxa are characterized by the presence of protocetraric acid in the medulla. However, the cortex in cross-section is matt in *U. kalbiana* (Fig. 8D) and vitreous in *U. lunaria* (Fig. 8E). Furthermore, *U. lunaria* has conspicuous irregular cortical cracks (Fig. 8F) whereas *U. kalbiana* produces annular cracks (Fig. 8B). *Usnea subparvula* is another species of the group with protocetraric acid. However, it differs from *U. kalbiana* by the absence of annulation in the basal thallus, the absence of tubercles and the presence of numerous spinulous fibrils evenly and densely distributed on the branches, a thinner cortex, a thicker medulla and a thinner axis. *Usnea*

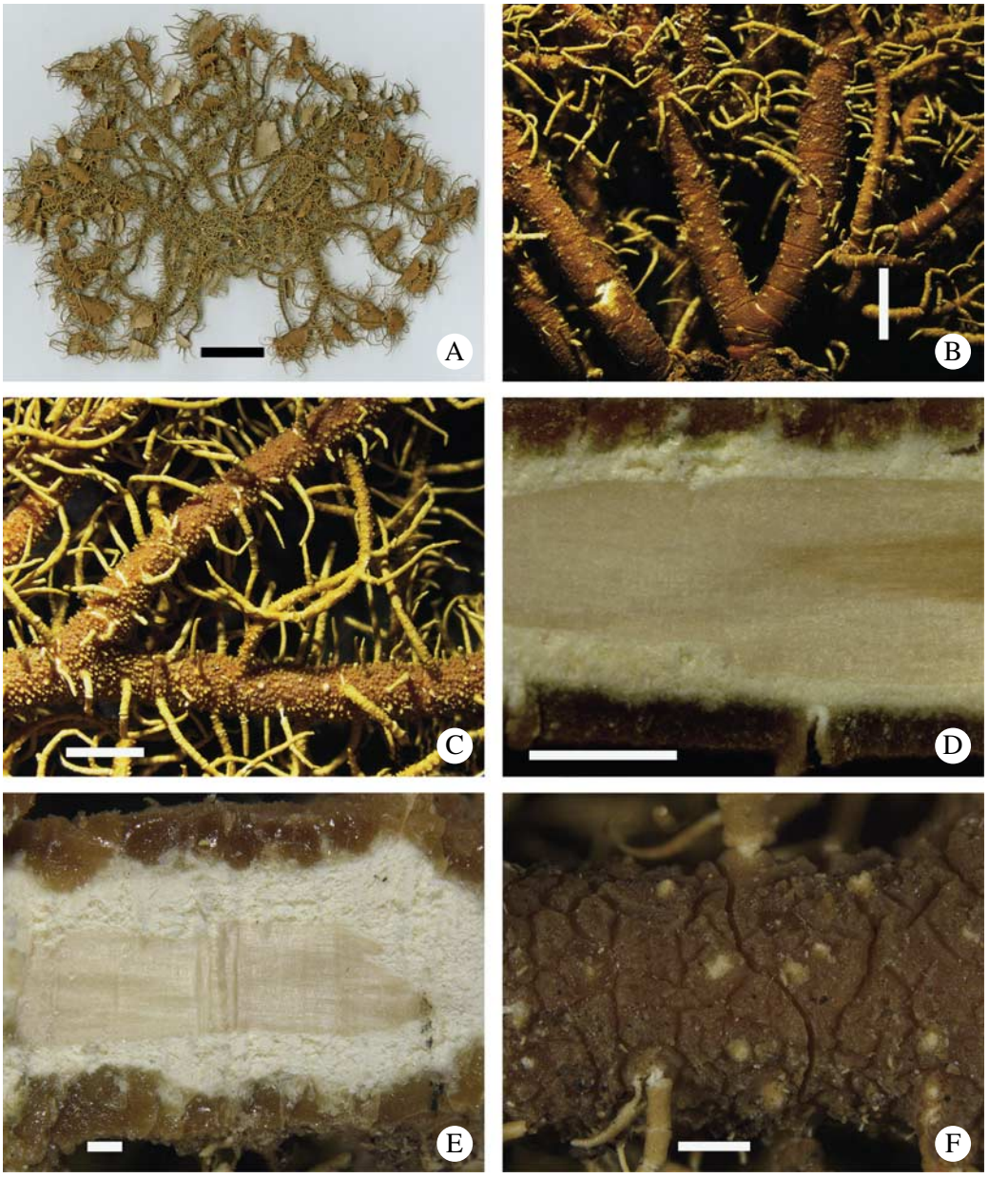

FIG. 8. A–D, *Usnea kalbiana* (holotype): A, thallus; B, trunk concolorous with branches with conspicuous annular cracks; C, branches annulated, terete and cylindrical, tubercles cone-shaped; D, section through branch with matt cortex. E & F, *Usnea lunaria* (holotype): E, section through branch with vitreous cortex; F, irregular cracks on the cortex surface. Scales: A = 4 cm; B & C = 2 mm; D & E = 500 µm; F = 1 mm. In colour online.

concinna has slightly constricted lateral branches, a thinner cortex and axis and a wider medulla, as well as a different chemistry (stictic acid group) to *U. kalbiana*. We were unable to get freshly collected material to obtain good quality DNA and hence the

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phylogenetic position of *U. kalbiana* remains unclear.

Specimens examined. **Brazil:** *Paraná*: Balsa Nova, Serra S'Ana, matinha nebular, epifita, 1969, *G. Hatschbach* 21365 (MBM). *Santa Catarina*: Caçador, Rodovia SC 451, on fences, 2013, *E. Gumboski* 4718, 4719 (ICN). *São Paulo*: Serra da Mantiqueira, Campos do Jordão, 150 km Nordöstlich von São Paulo in einem hellen, feuchten Urwald, 1700 m, 1978, *K. Kalb & G. Plöbst* s. n. (G-260935); Piquete, próximo ao Pico dos Marins, corticicola, 1200 m, 2012, *A. Spielmann* 10023 (CGMS). *Minas Gerais*: Serra da Mantiqueira, Vila Monte Verde, etwa 30 km östlich von Camanducaia, 1978, *K. Kalb & G. Plöbst* s. n. (G—260938). *Rio de Janeiro*: Itatiaia, zwischen Registro do Picú und Agulhas Negras, 1978, *K. Kalb & G. Plöbst* s. n. (G-260936).

Usnea lunaria Motyka

Lich. Gen. Usnea Stud. Monogr. Pars Syst. **2:** 328 (1938); type: Brazil, Minas Gerais, Plateau d'Itacolumi, ad saxa, *Damazio* s. n. (W!—holotype). %C/M/A: 13.5/18/36. Ascospores: (7·0–)8·0–*8·8*–9·5(–10·0) × 6·0–*6·5*–7·0 µm (*n* = 20). Chemistry: usnic and protocetraric acids.

(Fig. 8E & F)

Thallus (*n* = 2) erect-shrubby, up to 9 cm long, mostly anisotomic-dichotomously branched; *trunk* up to 7 mm long, concolorous with main branches, with annular cracks; *main branches* up to 1·9 mm thick, tapering, terete in cross-section, distinctly segmented; *segments* cylindrical and terete; *lateral branches* not constricted at the ramification point; *foveolae*, *maculae* and *pseudocyphellae* unknown; *papillae* numerous, cylindrical, ± evenly distributed; *tubercles* (*young fibrils?*) often numerous, ± evenly distributed, cylindrical, rarely eroded; *fibrils* slender, up to 3 mm, few and unevenly distributed; *fibercles* scattered; *cortex* vitreous in cross-section, with many irregular cracks on main branches, thick, with plectenchyma intermediate between *ceratina*- and *merrillii*-type; *medulla* white, compact, thin; *axis* moderately thick to thick. CMA (*n* = 2): %C = 12·5–*14·5*–16·5; %M = 12·5–*14·5*–18; %A = 30·0–*40·5*–51·0. A/M = 2–*3*–4.

Apothecia numerous, terminal and lateral, up to 18 mm diam.; *ascospores*: length = (7·0−)*8·7* ± 1·1(−12·0) µm, width = (5·0−) *5·7* ± 0·5(−7·5) µm, *n* = 2.

Chemistry. Medulla K−, P+ orange. TLC: protocetraric acid (*n* = 2).

Habitat and distribution. The holotype was collected on rocks (Motyka 1938) but the specimen collected by Schenck that was seen for this study grew on trees. Thus *U. lunaria* is both saxicolous and corticolous. In Brazil, it is known from Mato Grosso (Motyka 1938), Minas Gerais and Rio de de Janeiro.

Taxonomic remarks. *Usnea lunaria* is characterized by its thick tapering and terete branches that have a thick, vitreous and irregularly cracked cortex (Fig. 8E & F), the numerous apothecia and the presence of protocetraric acid in the medulla. For differences with *U. kalbiana*, see under this species. We were unable to get freshly collected material to obtain good quality DNA and hence the phylogenetic position of *U. lunaria* remains unclear.

Specimen examined. **Brazil:** *Rio de Janeiro*: Corcovado, an Bäumen, 1887, *H. Schenck* 4458 (G—260937).

Usnea meridionalis Zahlbr.

Denkschr. Kaiserl. Akad. Wiss., Wien. Math.-Naturwiss. Kl., **83:** 187 (1909); type: Brazil, Rio Grande do Sul, Neu-Württemberg, prope Elsenau, ad ramos Accaciarum, *A. Bornmüller* s. n. (W!—holotype; FH!—isotype). %C/M/A: 2/44/8 (measurements by Clerc in 1999). Ascospores (28 spores measured): (9·0–)10·0–*10·5*–11·0 × (6·0–)6·6–*7·0*–7·5(–8·0) µm (measurements by Herrera-Campos in 1997). Chemistry: usnic acid and an unknown substance with white fluorescence after charring with Rf classes A/B/C: 1–2/2/2.

Usnea michauxii I. I. Tav., syn. nov., *Mycotaxon* **30:** 54 (1987); type: USA, Carolina (PC!—lectotype). %C/M/A: 6/34.5/19. Ascospores: (7·5–)8·0–*9·0*–10·0 × 5·0–*5·5*–6·0 µm (*n* = 7). Chemistry: usnic acid and an unknown substance with blue fluorescence after charring with Rf classes A/B/C = 2–3/4/4.

(Fig. 6D & E)

Thallus and *apothecia* (*n* = 55). For a detailed description, see Truong *et al.* (2011). CMA (*n* = 23): %C = (2–)3–*4*–5 (–8); %M = (24·5–)31·0–*35·0*–39·0(–44·0); %A = (8–)15–*21*–27(–41). A/M = (0·3–)0·4–*0·6*– 0·8(–1·2). *Cortex* with *ceratina*-type

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plectenchyma. *Ascospores*: length = (7·0–)*9·6* ± 1·0(–13·0) µm, width = (4·5–)*5·9* ± 0·4 (–7·5) µm, *n* = 12.

Chemistry. Medulla: 1) K−, P−, TLC = undetermined triterpenoids, ± fatty acids (*n* = 8); 2) K+ yellow → red, TLC = salazinic acid (*n* = 5); 3) K+ yellow slowly → red, TLC = norstictic, ± salazinic, ± stictic (trace) acids (*n* = 5); 4) K−, P−, TLC = no medullary substances detected (*n* = 2).

Habitat and distribution. *Usnea meridionalis* is a species that is frequent in southern Brazil, occurring in a wide range of habitats: *Araucaria* forest, Pampa, high elevation tropical grasslands, restinga, open pastures and urban parks. It seems to be frequent in humid areas along river banks. This species occurs on a variety of corticolous (twigs and trunk) or lignicolous substrata and often grows together with *U. steineri* and/or *U. erinacea*.

Taxonomic remarks. *Usnea meridionalis* is characterized by the minute, ± numerous red dots (sometimes appearing as black dots on old herbarium specimens) on the cortex surface, the fusiform branches that are constricted at the attachment points and a *cornuta*- or *brasiliensis*-type of CMA with a lax medulla. The holotype of *U. meridionalis* represents an extremely well-developed thallus with main branches that are distinctly swollen, numerous foveoles and a *brasiliensis*-type of CMA. All transitional forms seem to exist between this form and the lectotype of the North American *U. michauxii* that is characterized by less swollen branches and a *cornuta*-type of CMA. The occasional presence of a yellow medullary periaxial pigment and the red dots on the cortex surface indicate that this species might be closely related to *U. flavocardia* Räsänen. Both species would benefit from revision, including molecular phylogenetic analysis. For differences between *U. meridionalis*, *U. cirrosa* and *U. cladocarpa*, see under these taxa. In our phylogenetic analysis, *U. meridionalis* was closely related to *U. glabrata* (Fig. 2).

Selected specimens examined. **Brazil:** *Rio Grande do Sul*: Bagé, Casa de Pedra, on shrubby tree, near river, 1989, *M. Fleig* 4082 (ICN); Caçapava do Sul, on shrubby tree, riverside of Rio Camacuã, 1988, *M. Fleig* 3349 (ICN); Cachoeira do Sul, on twigs, riverside Capanezinho, 1993, *M. Fleig* 5600 (ICN); Cambará do Sul, Parque Nacional da Serra Geral, Cânion Itaimbezinho, 2014, *A. Gerlach* 1410 (ICN); Caxias do Sul, Santa Lucia do Piai, Água Azul, 735 m, 2010, *A. Spielmann* 8666 (CGMS); Esmeralda, Estação Ecológica de Aracuri, 1982, *M. Fleig* 1469 (ICN); Mariana Pimentel, 100 m, 1989, *S. Grundlehner* s. n. (G); Lagoão, on shrubby tree, borda de mata, 2000, *A. Spielmann* 11902 (CGMS); Piratini, Pampa, 2015, *R. Jeeval* s. n. (ICN); Rio Grande, Estação Ecológica do Taim, on twigs, 2015, *E. Fazolino* s. n. (ICN); São Francisco de Paula, Colinas de São Francisco, on *Araucaria angustifolia*, 1000 m, 1989, *S. Grundlehner* 4273 (ICN); Triunfo, Copesul, beira de rio, 1992, *N. Cardoso* s. n. (HAS). *Santa Catarina*: Bom Jardim da Serra, near Parque Nacional de São Joaquim, on trunk of *Mimosa scabrela*, 1994, *M. Fleig* 6569 (ICN); Urubici, Parque Nacional de São Joaquim, on twigs, 2014, *A. Gerlach* 1348 (ICN); São Joaquim, Serra do Rio do Rastro, an alten Araukarien in einer Weide am Ortsrand, 1420 m, 1988, *Schäfer-Verwimp* L/10566 (G). *Paraná*: Carambeí, Catanduva de Fora, Rio Jotuba, 2013, *M. Engels* s. n. (ICN); Curitiba, Parque Tanguá, 2012, *A. Gerlach* 838 (ICN); *ibid.*, Universidade Federal do Paraná, Centro Politécnico, 1993, *S. Eliasaro* 1064 (UPCB); Palmeira, margens Rio Cariú, 2013, *M. Engels* s. n. (ICN); Ponta Grossa, Pinhão, on fences of *Phoebe porosa*, 1975, *L. Krieger* 13824 (JPB); Quatro Barras, Parque Estadual da Serra do Baitaca, Morro do Anhangava, 1200 m, 2014, *E. Santos* 101 (UPCB). *São Paulo*: Serra do Mar bei Paranapiacaba an der Eisenbahnlinie zwischen SP und Santos, Regenwald, 1000 m, 1986, *Schäfer-Verwimp* L7616 (G). *Minas Gerais*: Serra de Ibitipoca, 1975, *L. Krieger* s. n. (JPB). *Rio de Janeiro*: Itatiaia, Brejo da Lapa, 2160 m, 1984, *M. Guerra* s. n. (RB); Serra do Picu, *Schenck* 4448 (G).

Usnea cf. moreliana Motyka

Lich. Gen. Usnea Stud. Monogr. Pars Syst. **2:** 584 (1938); type: Mexico, Morelia, Cerro San Miguel, 1910, *Brouard* 137 (LBL!—neotype, isoneotype). %C/M/A: 6/32/24.5. Chemistry: usnic acid, unidentified triterpenoids UT6 (Truong & Clerc 2016).

Thallus (*n* = 10). For a detailed description and illustrations see Truong *et al.* (2011) (as *U. rubricornuta*) and Truong & Clerc (2016). *Cortex* with *ceratina*-type plectenchyma.

Chemistry. Medulla K−, P−. TLC: 1) triterpenoid UT6 (*n* = 4); 2) no medullary substances detected (*n* = 3).

Habitat and distribution. *Usnea* cf. *moreliana* is an uncommon species occurring in

southern Brazil in São Paulo and Rio de Janeiro. This taxon sometimes grows together with *U. erinacea*.

Taxonomic remarks. *Usnea moreliana* s. str. is a reddish-pigmented, sorediate taxon characterized by distinctly constricted branches at the attachment point, a *cornuta*-type CMA and a K−, P− medulla (triterpenoids UT6) (Truong & Clerc 2016). The specimens studied here most probably correspond to the fertile counterpart of *U. moreliana* s. str. and to *Usnea* sp. 2 mentioned by Truong *et al.* (2011: 65). More material and molecular assessment are necessary before any taxonomic decisions can be taken.

Selected specimens examined. **Brazil:** *Rio Grande do Sul*: Esmeralda, Estação Ecológica de Aracuri, on fences, *M. Fleig* 1815a (ICN). *Paraná*: Ponta Grossa, Parque Estadual de Vila Velha, em ramos, *Sanders* s. n. (UFP). *Santa Catarina*: Campo Alegre, Campos do Quiriri, campo de altitude, 2012, *E. Gumboski* 3584 (ICN); Prudentópolis, rural area, 2012, *A. Charnei* 551 (ICN); Rio Negrinho, Fazenda Velha, *Araucaria* forest, 2007, *E. Gumboski* 1020 (ICN). *São Paulo*: Mogi-Guaçu, Martinho Prado Jr., Reserva Biológica e Estação Experimental de Mogi Guaçu, 2007, 22°16'S, 47°09'W, *c.* 630 m, *M. Benatti et al.* s. n. (SP); Pindamonhangaba, Pico de Itapeva, 1966, *D. Vital* s. n. (JPB). *Rio de Janeiro*: Santa Maria Madalena, Parque Estadual do Desengano, Pedra do Desengano, 1500 m, campo de altitude, 1986, *G. Martinelli et al.* 11995 (RB).

Usnea parvula Motyka

Lich. Gen. Usnea Stud. Monogr., Pars Syst. **2:** 599 (1938); type: Argentina, Cordoba, Sierra Achala, 1876, *Hieronymus* s. n. (G!—isotype). %C/M/A: 6/25/38. Chemistry: usnic acid, an unknown yellow spot with Rf classes A/B/C = 6/2/5–6 and a fatty acid with Rf classes = 4/2/5–6. Ascospores: 8·0–*8·5*–9·0(–9·5) × 4·5–*5·0*–5·5(–6·0) µm (n = 20) (TLC and measurements by Clerc in 1995).

(Fig. 9A)

Thallus and *apothecia* (n = 82). For a detailed description, see Clerc (2007). CMA (n = 20): %C = (3·0–)4·5–*6·0*–7·5(–8·5); %M = (11·0–)21·0–*27·0*–33·0(–37·5); %A = (19–)24–*34*–44(–62). A/M = 0·5–*1·5*–2·5(–5·5). *Cortex* with *ceratina*-type plectenchyma. *Ascospores*: length = (6·0−)*7·8* ± 0·8(−11·0) µm, width = (4·0−)*5·2* ± 0·5(−6·0) µm, n = 17.

Chemistry. Medulla K−, P−. TLC: 1) unknown yellow spot with Rf classes A/B/C = 6/1-2/5 (Us1), ± caperatic acid, ± triterpenoid (rare), and ± eumitrin (rare) (n = 25); 2) caperatic acid, ± Us1, and ± triterpenoid (n = 6); 3) triterpenoid and fatty acids (n = 5); 4) no medullary substances detected (n = 2).

Habitat and distribution. *Usnea parvula* is known only from the American continent: USA, Mexico (Clerc 2007), Argentina, Colombia, Paraguay, Uruguay and Brazil (where it was previously mentioned only from Minas Gerais) (Motyka 1938) and Venezuela (Vareschi 2001). In southern Brazil, *U. parvula* occurs mainly in coastal habitats close to the seashore where it grows on shrubby trees on sandbanks or at the edge of lagoons. It also occurs in rural areas on trees in pastures.

Taxonomic remarks. *Usnea parvula* is characterized by the numerous spinulose fibrils that densely cover the branches, the irregular branches that are ± obtuse- to acute-angled and with ± swollen segments with foveoles and transverse furrows, the shiny cortex, the dense medulla and the presence of fatty acids (K−, P−) in the medulla. Unlike Clerc (2007), we found that the lateral branches might be slightly to distinctly constricted, reflecting the variability in the shape of the branches in this species (Fig. 9A). For differences between *U. parvula* and *U. subparvula* or *U. complanata* (Müll. Arg.) Motyka, see under *U. subparvula*. *Usnea subelegans* shares the numerous spinulose fibrils with *U. parvula*, but the former species has galbinic acid in the medulla (K+ yellow → red) as well as less irregular and more cylindrical branches that usually have terete segments in cross-section. *Usnea steineri* also has a K−, P− medulla but its fibrils are usually not spinulose and it has a thin red subcortical pigmentation. The three specimens included in the molecular study form a strongly supported group in Fig. 2 but the phylogenetic affinities of this group remain unresolved.

Selected specimens examined. **Brazil:** *Rio Grande do Sul*: Caraá, Área de Preservação Ambiental, 2014, *A. Gerlach* 1501 (ICN); Mariana Pimentel, 100 m, 1989, *S. Grundlehner* s. n. (G); Pelotas, in kleiner Baumpflanzung, 100 m, 1986, *Schäfer-Verwimp* L/7884

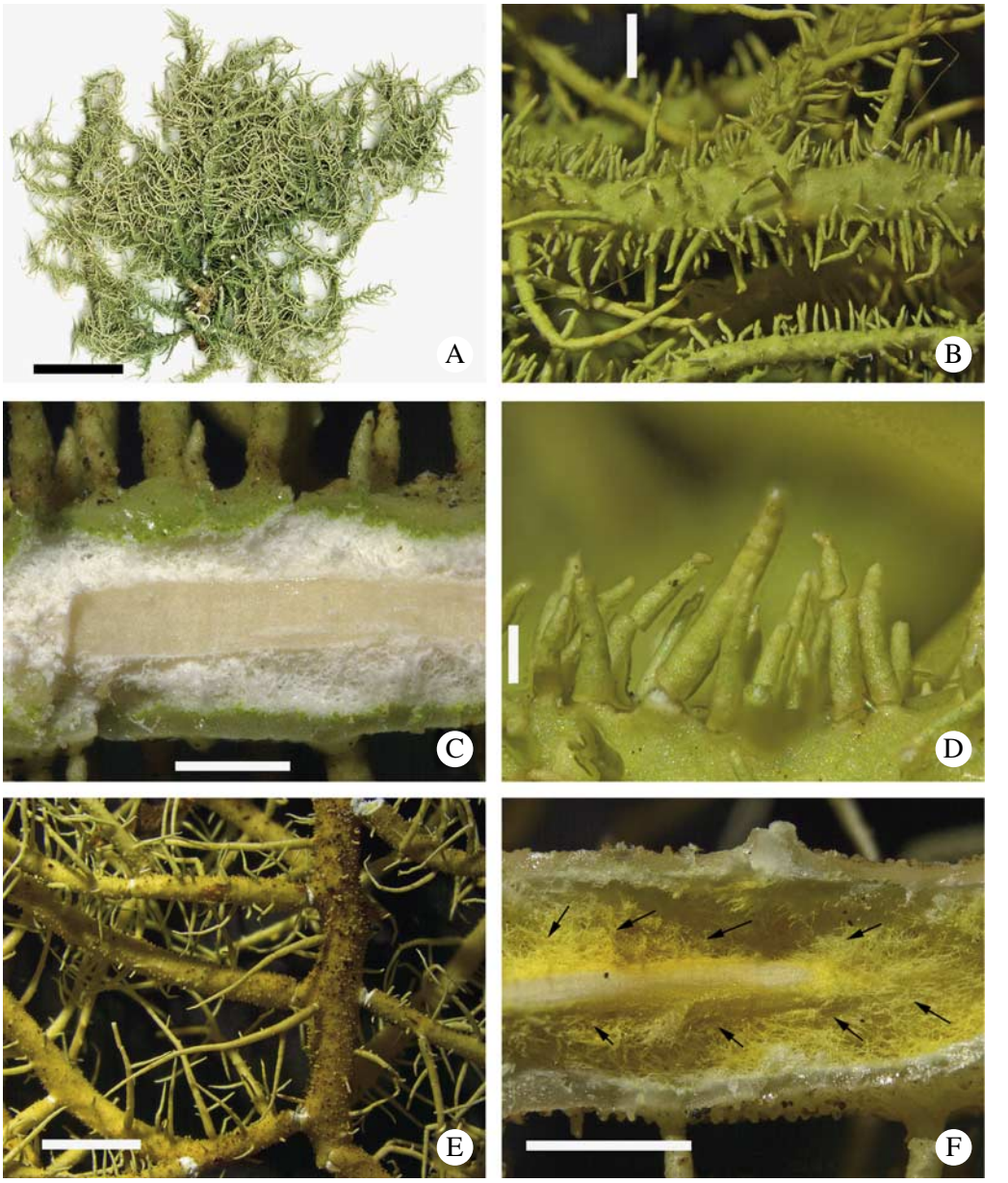

FIG. 9. A, *Usnea parvula*. A, branches densely covered with fibrils (*A. Gerlach* 870). B–D, *Usnea subparvula* (holotype): B, branches wider at ramification point, covered with spinulose fibrils; C, section through branch; D, details of conical spinulose fibrils. E & F, *Usnea* sp. 1 (*A. Gerlach* 1321): E, fusiform branches, constricted at attachment point; F, section through branch, the periaxial tissue is strongly pigmented yellow (area indicated by arrows). Scales: A & E = 2 mm; B & F = 1 mm; C = 500 µm; D = 200 µm. In colour online.

(G); Porto Alegre, Morro Santana, 2014, *A. Gerlach* 1085 (ICN); *ibid.*, Botanical Garden, 2009, *F. Lucheta* s. n. (HAS); Rio Grande, Estação Ecológica do Taim, on twigs, 2015, *E. Fazolino* s. n. (ICN); Rondinha, Arroio do Sal, on twigs in sandbank, 2014, *E. Fazolino* s. n. (ICN); Triunfo, Fazenda Santa Maria, 2013,

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F. Lucheta s. n. (ICN); Vale do Sol, 15 de Novembro, on *Arecastrum* sp., 1993, *M. Fleig* 5285 (ICN); Uruguaina, Parque Estadual do Espinilho, 1991, *T. Burdulis* s. n. (ICN); Viamão, Morro da Grota, on *Dodonea viscosa*, 1980, *L. Aguiar* 490 (HAS); *ibid.*, Itapuã, 200 m, 1989, *T. Ahti* 21 (ICN). *Santa Catarina*: Concórdia, Presidente Kennedy, 1986, *C. Grabauska* 430 (ICN); Florianópolis, Parque Municipal da Lagoa do Peri, on *Schizolobium parahyba*, 2013, *A. Gerlach* 1203 (ICN); praia da Armação, em galhos, restinga, 2013, *A. Gerlach* 871 (ICN); Joaquina beach, on shrub on sandstone, 1988, *S. Eliasaro* 632 (BHCB). *Paraná*: Curitiba, Universidade Federal do Paraná, Centro Politécnico, 1994, *C. Morales* 12 (UPCB); Ponta Grossa, riverside, 1978, *L. Krieger* 15798 (CESJ). *São Paulo*: Pardinho, Fazenda Águas de Janeiro, 800 m, 2011, *P. Jungbluth* 2860 (ICN); São Bento do Sapucaí, Serra da Mantiqueira, Westanstieg zum Pedra do Baú, epiphytisch in einer Weide bei 1350 m, 1989, *Schäfer-Verwimp* L/11816 (G).

Usnea steineri Zahlbr.

Denkschrift. Math. Naturw. Classe Kais. Akad. Wiss. Wien **83:** 183, 186 (1909); type: Brazil, São Paulo, ad Sta. Anna propre Lapa in distr. urbis S. Paulo, 1901, *Schiffner* s. n. (W!—holotype; G!—isotype). %C/M/A: 7.5/35/15. Ascospores: 7·5–10·0 × 6–7 µm. Chemistry: unidentified triterpenoids UT6 (CMA, chemistry, ascospores *fide* Truong *et al.* 2011).

(see Fig. 8 in Truong *et al.* (2011: 497))

Thallus ($n = 73$): for a detailed description, illustrations and synonyms see Truong *et al.* (2011). CMA ($n = 4$): %C = (7·0–)7·5–*8·0*–9·5; %M = (21–)22–*28*–34(–35); %A = (15–)17–*29*–41(–44). A/M = 0·5–*1·5*–2·5. *Cortex* with *baileyi*-type plectenchyma.

Apothecia numerous, lateral, terminal to subterminal, up to 25 mm diam.; *ascospores*: length = (6·0−)*8·3* ± 0·8(−11·0) µm, width = (4·0−)*5·4* ± 0·5(−6·0) µm, $n = 11$.

Chemistry. Medulla K−, P−. TLC: 1) unidentified triterpenoids ($n = 14$); 2) fatty acids ($n = 5$); 3) no medullary substances detected ($n = 7$).

Habitat and distribution. Argentina, Bolivia, Peru (Truong *et al.* 2011), Brazil, Colombia, Uruguay (Motyka 1938) and Venezuela (Vareschi 2001). Also known from tropical Africa (Swinscow & Krog 1979). For Brazil, this species has been recorded in Rio Grande do Sul, Santa Catarina, Paraná, São Paulo and Minas Gerais (Motyka 1938). It is rare in the neotropical Andes (Truong *et al.* 2011). *Usnea steineri* is common in southern Brazil, where it can grow side by side with *U. erinacea* on a variety of corticolous or lignicolous substrata.

Taxonomic remarks. *Usnea steineri* can be recognized by its red subcortical pigmentation that is found just below the cortex (the pigmentation might also spread into the lower cortex) and by the K−, P− medulla. However, *U. steineri* is a morphologically polymorphic species. According to Truong *et al.* (2011) there are three morphotypes differing in the shape of branches, the axis/medulla ratio and the type of fibrils. Most of the Brazilian specimens belong to the *steineri*-morphotype with inflated and constricted branches, a dense to often lax medulla and long fibrils scattered all along the thallus. Specimens of the *subdasaea*-morphotype (with short spinulose fibrils) and of the *krempelhuberi*-morphotype (with non-inflated, non-constricted branches) are less frequent in southern Brazil. In the phylogenetic analyses, *U. steineri* is a sister group to *U. erinacea* (Fig. 2). In Fig. 2, both specimens from Peru appear to belong to the *subdasaea*-morphotype whereas the two specimens from Brazil belong to the *steineri*-morphotype. These results, combined with the discovery in Brazil (outside the southern area) of several specimens with a different chemistry (galbinic, salazinic or stictic acids), indicate that this species might form a complex of several so far undescribed species. *Usnea aurantiaca-parvula*, *U. erinacea* and *U. meridionalis* also have an orange-reddish pigmentation. In *U. aurantiaca-parvula* the pigmentation is diffuse in the whole medulla, there are numerous foveoles and the spinules are constricted at the base. The pigmentation of *Usnea erinacea* and *U. meridionalis* is exclusively cortical.

Selected specimens examined. **Brazil:** *Rio Grande do Sul*: Caçapava do Sul, arroio Seival, mata de galeria junto a campo de pastagem, 1993, *M. Fleig* 5681 (ICN); Cachoeira do Sul, arroio Capanezinho, riparian forest, 1993, *M. Fleig* 5599 (ICN); Camaquã, margens do Arroio Velhaco, 1985, *C. Grabauska* 8 (ICN); Esmeralda, Estação Ecológica de Aracuri, on *Araucaria angustifolia*, 1984, *M. Fleig* 2441 (ICN); Itaqui,

Fazenda Bola de Ouro, on twigs of shrub, mata de galeria, 1994, *M. Fleig* 6546 (ICN); Mariana Pimentel, près de Barra do Ribeiro, 30°21'S, 51°35'W, 100 m, 1989, *S. Grundlehner* s. n. (G); Passo dos Freire, São Sepé, on shrubby tree, 1985, *M. Fleig* 2533 (ICN); Rio Grande, Estação Ecológica do Taim, 2014, *E. Fazolino* s. n. (ICN); Santana do Livramento, APA do Ibirapuitã, Fazenda Lolita, 2012, *M. Käffer* 867 (HAS); São Gabriel, mata de galeria junto a campo de pastagem, 1993, *M. Fleig* 5455 (ICN). *Santa Catarina*: Alfredo Wagner, RPPN Rio das Furnas, 2013, *A. Gerlach* 1263 (ICN); São Bento do Sul, APA do Rio Vermelho, 2012, *E. Gumboski* 3822 (ICN); São Joaquim, Serra do Rio do Rastro, an alten Araukarien in einer Weide am Ortsrand, 1420 m, 1988, *Schäfer-Verwimp* L/10567 (G); Urubici, Morro da Igreja, 1650 m, 2004, *A. Cervi* 8715 (UPCB). *Paraná*: Campo Largo, on fences, 2012, *A. Gerlach* 771 (ICN); Carambeí, Catanduva de Fora, on fences, 2013, *M. Engels* s. n. (ICN); Castro, Cânion Guartelá, 2013, *L. Rocha* s. n. (ICN); Curitiba, margem do rio Iguaçú, on *Sebastiana commersoniana*, 1985, *M. Fleig* 2639 (ICN); Guarapuava, 2013, *M. Engels* s. n. (ICN); Piraí do Sul, Fazenda Nova Era, 2012, *B. Canestraro* 483 (ICN); Ponta Grossa, Pinhão, on fences of *Phoebe porosa*, *L. Krieger* 13831 (JPB); Prudentópolis, 2012, *A. Charnei* 547 (ICN); São José dos Pinhais, Rio Iguaçú, on *Prunus sellowii*, 1985, *M. Fleig* 2651 (ICN). *São Paulo*: Mogi-Guaçu, Reserva Biológica de Mogi-Guaçu, 22°15'06·1"S, 47°09·28'6"W, 620 m, *A. Spielmann* 7145 (CGMS); Piquete, 22°31'30·1"S, 45°08'59"W, 1200 m, 2012, *A. Spielmann* 10023 (CGMS). *Rio de Janeiro*: Serra dos Órgãos, Paßstraße zwischen Teresopolis und Petropolis, in Bergregenwald epiphytisch bei 1330 m, 1986, *Schäfer-Verwimp* L/7401 (G).

Usnea subelegans (Vain.) B. de Lesd.

*Ann. Cryptog. Exot.***6:** 112 (1933).—*Usnea barbata* var. *subelegans* Vain., *Étud. Lich. Brésil* **1:** 6 (1890); type: Brazil, Minas Gerais, Sitio, 1885, *Vainio* (TUR-V 639!—lectotype designated here). %C/M/A: 6.5/26.5/34. Ascospores: 9–9·5–10 × 6–6·5–7(–8) µm (measurements by Herrera-Campos in 1997). Chemistry: usnic, galbinic, norstictic and salazinic acids (TLC by Clerc in 1996).

(Fig. 6F)

Nomenclatural note. Five syntypes of *U. subelegans* were found in TUR-V. TUR-V 638 corresponds to a spinulose sorediate thallus with galbinic acid (= *U. dasaea* Stirt.). The four remaining packets contain specimens with apothecia but without soralia: TUR-V 753 and 754 with salazinic acid, strongly inflated and irregular primary branches and a *brasiliensis*-type of %CMA (2·5–4/40–45/6–13); TUR-V 639 and 661 with galbinic, norstictic and salazinic acids, and with ± cylindrical, not inflated primary branches and a *cornuta*-type %CMA (5–6/28–35/20–32). It is possible that two different species might be present here (see under taxonomic remarks below). In the protologue, the taxon was described as reacting K+ yellow, then orange-red. Only the galbinic acid specimens were found to show such a reaction (the specimens with only salazinic acid showed almost no reaction to K). We thus decided to lectotypify this name using one of the galbinic acid-containing specimens.

Thallus ($n = 82$). For a detailed description see Clerc (2007). C/M/A ($n = 18$): %C = (3·5–)4·5–*6·0*–7·5(–9·5); %M = (24·0–)25·5–*29·5*–33·5(–35·0); %A = (20–)22–*28*–32(–39). A/M = (0·6–)0·7–*1·0*–1·3(–1·6). *Cortex* with *merrillii*-type plectenchyma.

Apothecia mainly lateral, up to 10 mm diam.; *ascospores*: length = (7·0–)*8·9* ± 0·8(–11·0) µm, width = (4·0–)*5·7* ± 0·7(–8·0) µm, $n = 10$.

Chemistry. Medulla: 1) K+ yellow → red, TLC = salazinic, norstictic, galbinic and ± constictic acids ($n = 48$); 2) K+ yellow → slowly red, TLC = stictic, constictic, cryptostictic, menegazziaic and norstictic acids ($n = 1$).

Habitat and distribution. *Usnea subelegans* usually grows in the same habitat as *U. parvula*, mainly in coastal and rural areas. It occurs in Mexico (Clerc 2007), Panama (Motyka 1938) and in South America where it is widespread: Argentina, Colombia, Paraguay, Peru, Uruguay (Motyka 1938) and Venezuela (Marcano *et al.* 1996). In Brazil, it was previously recorded from Rio Grande do Sul, Santa Catarina and Paraná (Motyka 1938), São Paulo (Zahlbruckner 1909), Minas Gerais (Vainio 1890), Rio de Janeiro (Rizzini 1952), Mato Grosso do Sul (Osório 1992) and Mato Grosso (Motyka 1938).

Taxonomic remarks. *Usnea subelegans* is the only erect-shrubby apotheciate species with galbinic acid found in southern Brazil. The densely spinulose branches (Fig. 6F), the mainly lateral apothecia, the thin and ± shiny cortex, the dense to somewhat lax medulla and the usually orange medullary pigmentation (probably due to the oxidation of secondary metabolites) around the axis make this species

easy to recognize. This taxon seems to be quite variable with ± swollen main branches (diameter varying from 0·8 to 1·8 mm), ± numerous spinulose fibrils (20 to 40 fibrils mm^{-2}) and with a variable %CMA (see above). A few specimens were found to be subpendulous to pendulous. The chemistry (salazinic, norstictic and galbinic acids) seems to be constant, although we found one specimen with only stictic acid. The syntypes containing only salazinic acid (TUR753 and TUR754), with irregular and very swollen main branches and an extremely low A/M (0·1–0·4), correspond well to *U. tincta* (Zahlbr.) Motyka (W!—holotype (chemistry: norstictic, salazinic and galbinic acids; %CMA = 4/39.5/14, A/M = 0·3; ascospores: (8·5–)9·0–*9·5*–10·0 × (5·5–)6·0–*6·5*–7·0 µm); BM!—isotype (chemistry: salazinic acid; %CMA = 2.5/41.5/12, A/M = 0·3)). More material and molecular studies are needed in order to decide whether these taxa are conspecific. The specimens mentioned by Motyka (1938: 522) for Paraná and Rio Grande do Sul under *U. tincta* correspond to the *U. subelegans* morphotype. *Usnea leioclada* (Zalhbr.) Motyka is another esorediate, apotheciate species with galbinic acid described from Brazil, but not found as yet in the south. It differs from *U. subelegans* mainly by the absence of spinulose fibrils and by the CMA values (7/17.5/51, BM!—holotype). For differences between *U. subelegans*, *U. parvula* and *U. subparvula* see under the latter two species. We were unable to obtain good quality DNA and hence the phylogenetic position of *U. subelegans* remains unclear.

Selected specimens examined. **Brazil:** *Rio Grande do Sul*: Arambaré, restinga, 2014, *F. Lucheta* s. n. (ICN); Camaquã, riverside, 1985, *C. Grabauska* 5 (ICN); Cachoeira do Sul, on twigs of *Sebastiana commersoniana*, riparian forest, 50 m, *A. Spielmann* 6387 (CGMS); Caraá, 2013, *N. Koch* s. n. (ICN); Caxias do Sul, 735 m, 2010, *A. Spielmann* 8666 (CGMS); Mariana Pimentel, 1989, *S. Grundlehner* (G); Passo dos Freire, São Sepé, 1985, *M. Fleig* 2603 (ICN); Porto Alegre, Morro Santana, 2014, *A. Gerlach* 1084 (ICN); Rio Grande, Estação Ecológica do Taim, 2014, *E. Fazolino* s. n. (ICN); Rondinha, Arroio do Sal, on sandstone, 2014, *E. Fazolino* s. n. (ICN); Santa Maria, on shrub along the road, 150 m, 1980, *M. Fleig* 1208 (ICN); Santana do Livramento, Fazenda Lolita, 2011, *M. Käffer* 445 (HAS); São Francisco de Paula, Parque Nacional da Ronda, *N. Koch* 65R (ICN); Torres, on fences, 2012, *E. Gumboski* 4063 (ICN); Viamão, Parque St. Hilaire, 1989, *S. Grundlehner* 363 (ICN). *Santa Catarina*: Fraiburgo, 2013, *E. Gumboski* 4744 (ICN); Joinville, Alto da Serra Dona Francisca, on fences, 2013, *E. Gumboski* 4664 (ICN); Major Vieira, 2012, *E. Gumboski* 4040 (ICN); Rio Negrinho, Fazenda Velha, 2009, *E. Gumboski* 937 (ICN); São Francisco do Sul, Capri, on *Syagrus romanzoffiana*, 2014, *A. Gerlach* 972 (ICN). *Paraná*: Campina Grande do Sul, Sitio do Belizario, 1000 m, 1967, *G. Hatschbach* 16437 (MBM); Carambeí, Catanduva de Fora, 2013, *M. Engels* (ICN); Guarapuava, Colônia São judas Tadeu, on shrub, 850 m, 1991, *G. Hatschbach* 55407 (MBM); Paranaguá, Ilha do Mel, 2012, *A. Gerlach* 784 (ICN); Paula Freitas, riparian forest, 2013, *M. Engels* s. n. (ICN); Piraquara, Mananciais da Serra, 2004, *R. Reis* 112 (UPCB); Ponta Grossa, 1978, *L. Krieger* 15805 (CESJ); Pontal do Paraná, Pontal do Sul, restinga, 2006, anon. (UPCB). *Minas Gerais*: Serra da Mantiqueira, Fazenda São Mateus, östlich von Camanducaia, 1800 m, 1980, *K. Kalb* s. n. (G); Antonio Carlos, *L. Krieger* 15942 (CESJ). *Mato Grosso do Sul*: Bonito, Fazenda América, deciduous forest, 2009, *V. Pott* 10682 (CGMS).

Usnea subparvula A. Gerlach & P. Clerc sp. nov.

MycoBank No.: MB 819424

Similar to *U. parvula*, but differs in the less numerous spinulose fibrils, with lateral branches that are often somewhat wider at the ramification point, a thicker cortex (8–10%), and the production of protocetraric acid in the medulla.

Type: Brazil, Mato Grosso do Sul, Porto Murtinho, Fazenda Sao Fernando, 21°34'26·57"S, 57°45'04·81"W, 94 m, pasture field near edge of deciduous forest, on fence posts, 13 September 2015, *V. J. Pott* & *A. Pott* 11873 (CGMS—holotype; G, ICN, UPS—isotypes). % C/M/A: 10/24.5/31. Ascospores: (6·0–)6·5–*7·0*–7·5(–8·0) × 4·5–5·0 µm ($n = 22$). Chemistry: usnic and protocetraric acids, an unknown protocetraric acid group with grey spot (Rf classes A/B/C = 4/4–5/5–6) and an unknown triterpenoid (?) with yellow fluorescence after charring (Rf class A = 6).

(Fig. 9B–D)

Thallus ($n = 64$) erect-shrubby, up to 8 cm long, yellow-green, with anisotomic-dichotomous ramifications; *trunk* usually short, concolorous or paler than branches, rarely reddish, not annulated, smooth but occasionally wrinkled; *main branches* 1·0–1·8 mm thick, irregular to cylindrical, terete to flattened or often obtuse- to acute-angled in cross-section; *lateral branches* not constricted (rarely slightly constricted), often

wider at the ramification point; *foveolae* usually present on main branches, not abundant; *maculae* and *pseudocyphellae* absent; *papillae* and *tubercles* absent; *fibrils* numerous (*c.* 12/mm^2), spinulose, short and thick, 0·5–1·4(–3·0) × 0·1–0·3 mm, regularly distributed on the whole thallus; *fibercles* present; *cortex* shiny, moderately thin to moderately thick, with *ceratina*-type plectenchyma; *medulla* white, compact, moderately thin to moderately thick; *axis* moderately thin to moderately thick. CMA ($n = 20$): %C = (5·0–)6·0–*8·0*–10·0(–11·5); %M = (11·5–)15·0–*20·5*–26·0(–29·0); %A = (30·0–)34·5–*43·0*–51·5 (–58·0). A/M = (1·0–)1·3–*2·4*–3·5(–5·0).

Apothecia numerous, mainly terminal, up to 5 mm diam.; *ascospores*: length = (5·5–)*7·8* ± 0·8(–10·0) µm, width = (4·0–)*5·2* ± 0·5 (–6·0) µm, $n = 6$.

Chemistry. Medulla: 1) K−, P+ orange, TLC = protocetraric acid, ± an unknown acid with grey spot (Rf classes A/B/C = 4/4–5/5–6) ($n = 16$); 2) K+ slowly dull yellow, P+ yellow, TLC = psoromic and con-psoromic acids ($n = 4$).

Etymology. Named after the strong morphological similarity to *U. parvula*.

Habitat and distribution. Corticolous on twigs of shrubby trees or lignicolous on fence posts. It occurs in relatively open sites around farms, along roads, in Cerrado, Chaco, Pampa, riparian forest and occasionally in subtropical seasonal forest. This species is so far known from Argentina, Brazil and Paraguay. There are only two herbarium specimens of *U. subparvula* from southern Brazil. *Usnea subparvula* seems to occur inland whereas *U. parvula* is more of a coastal species occuring in the Atlantic forest.

Taxonomic remarks. *Usnea parvula* has a similar morphology to this new species, with its numerous spinulose fibrils and irregular branches in cross-section that have obtuse- to acute-angled segments. It differs mainly by the K−, P− reacting medulla and the density of spinulose fibrils (*U. parvula*: 16–24 mm^{-2}, *U. subparvula*: 10–15 mm^{-2}). Both taxa seem to belong to distinct lineages within the *Usnea* clade 4; however, their phylogenetic relationship lacks support (Fig. 2). *Usnea subelegans* has a K+ reacting medulla, a higher density of fibrils (18–30 mm^{-2}), less irregular and more cylindrical branches usually with terete segments in cross-section and a much lower A/M. *Usnea complanata* is a small apotheciate African species (Swinscow & Krog 1979) which also has spinulose fibrils and psoromic acid in the medulla. However, it has a *brasiliensis*-type CMA with a sinuose axis and more lageniform fibrils.

Selected specimens examined. **Argentina:** Cordoba, Cerro Colorado, bosque serrano, on *Acacia praecox*, 2004, *J. Rodríguez* 1788 (G).—**Brazil:** *Rio Grande do Sul*: Uruguaiana, Parque Espinilho, 1991, *T. Burdulis* s. n. (ICN). *Paraná*: Guaíra, Regenwald am Rio Paraná, 200 m, 1980, *K. Kalb* s. n. (G). *São Paulo*: Pindamonhangaba, Reserva Ecologica Municipal do Trabiju, 22°48'S, 44°32'W, 1100 m, 2010, *M. Benatti* 3193 (SP). *Mato Grosso do Sul*: Aquidauana, Piraputanga, Cerrado and Caatinga, 1987, *I. Riquelme* s. n. (ICN); Bodoquena, Fazenda Marambaia, campo rodeado por capim-navalha, 669 m, 2012, *E. Souza* 121 (CGMS); Bonito, Fazenda América, cerradão com afloramento rochoso, 21°10'12·90"S, 56°35'59·40"W, 414 m, 2010, *V. Pott* 11321 (CGMS); Campo Grande, on fences, 1989, *Helio* s. n. (ICN); Corguinho, Distrito de Taboco, 19°44'37·27"S, 55°15'52·86"W, 400 m, Cerrado, 2013, *T. Sinani* 18 (CGMS); Corumbá, sub-região Pantanal do Paraguai, margen da baia do Taquaral, 18°02'42·3"S, 57°30'15·2"W, 83 m, 2010, *A. Spielmann* 8784 (CGMS); Jaraguari, Furnas do Dionisio, 20°08'34·9"S, 54°34'21·2"W, 450 m, 2015, *A. Spielmann* 11885 (CGMS); Nova Andradina, Fazenda Laranjal, RPPN Cachoeira do Mimoso, cerradão, 22°2'44·8"S, 53°23'66·5"W, 359 m, 2014, *A. L. Simal* 245 (CGMS); Poconé, 36 km ao sul, pantanal, on fence beira estrada, Cerrado inundado, 100 m, 1989, *M. Marcelli* 4444 (ICN); Porto Murtinho, Fazenda Retiro Conceição, on fences on chaco vegetation, 21°40'57·60"S, 57°45'43·70"W, 91 m, 2010, *L. Canéz* 3689 (CGMS); Rio Negro, pantanal da Nhecolândia, on fences, Cerrado, 19°17'55·83"S, 55°06'1·04"W, 165 m, 2013, *A. P. de Souza* 51 (CGMS); Terenos, Fazenda Modelo da Embrapa, on *Heteropterys coriacea*, campo úmido de Cerrado, 20°33'33·8"S, 54°47'33·6"W, 2010, *A. Spielmann* 8103 (CGMS). *Goiás*: Água Fria, Estação Repetidora da Telebrasilia de Roncador, on twigs of *Clusia* sp., campo rupestre, 1992, *G. Hatschbach* 58931 (MBM).—**Paraguay:** Gran Chaco, zwischen B. Aceval und Algarrobo, on *Eucalyptus* sp., 150 m, 1980, *K. Kalb* s. n. (G).

Usnea sp. 1

(Fig. 9E & F)

This species is characterized by the fusiform branches that are constricted at the point of

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attachment, the *brasiliensis*-type CMA (%C = 4·5–6·0, %M = 35–40, %A = 11–18, A/M = 0·4, *n* = 2) and the strongly yellow-pigmented medulla. *Cortex* with *ceratina*-type plectenchyma. The ascospores belong to class 2: (8–)9–*10*–11 (–13) × (5·0–)5.5–*6.5*–7·0(–8·0) µm (*n* = 2). The medulla reacts K+ yellow → slowly red (norstictic acid, *n* = 1).

Taxonomic remarks. This morphologically distinctive species clusters together with a specimen of *U. flavocardia* Räsänen from Europe with psoromic acid in the medulla (Fig. 2). Norstictic acid is another chemotype of *U. flavocardia* in Europe (Clerc 1984*b*, as *U. wirthii*). Furthermore, in the phylogenetic analyses *Usnea* sp. 1 forms a strongly supported group with *U. flavocardia* from Ireland (Fig. 2) and it could be the fertile counterpart of *U. flavocardia*. However, since what is called *U. flavocardia* in Europe might not be the same species as the South American *U. flavocardia* and as we found only one specimen corresponding to *Usnea* sp. 1, more material is needed before any taxonomic decisions can be taken.

Specimen examined. **Brazil:** *Santa Catarina*: Urubici, Parque Nacional de São Joaquim, on *Araucaria angustifolia*, near the lodging, 2014, *A. Gerlach* 1321 (ICN).

Uncertain or excluded species

Usnea comosa (Ach.) Vain., nom. invalid.

This species is a synonym of *U. subfloridana* Stirt. (Laundon 1965), the sorediate form of *U. florida*. The specimens from Brazil that were previously identified as *U. barbata* var. *comosa* Ach. belong in fact to several different species, such as *U. cirrosa*, *U. erinacea* or *U. subelegans*, and might even include one as yet undescribed species from Minas Gerais.

Usnea florida (L.) Wigg.

Usnea florida is the type species of the genus. It is a European shrubby apotheciate species with thamnolic acid that does not occur in Brazil. The apotheciate specimens from Brazil that were previously identified as *U. florida* or *U. barbata* var. *florida* Fr. belong to *U. erinacea*, *U. cladocarpa*, *U. meridionalis*, *Usnea* cf. *moreliana* or *U. subelegans*.

Usnea ludicra Rizz.

Usnea ludicra is an apotheciate species described by Rizzini (1952) from material collected around Rio de Janeiro. Unfortunately the type specimen(s) could not be found in Jardim Botânico do Rio de Janeiro (RB), the Museu Nacional (R), or in the Universidade Federal do Rio de Janeiro (RFA).

Usnea strigosa (Ach.) A. Eaton

Usnea strigosa is a North American shrubby apotheciate species that does not occur in Brazil. The specimens collected in Brazil that were named *U. barbata* var. *strigosa* Ach. belong to *U. cirrosa*.

Key to corticolous and shrubby-sorediate *Usnea* species in southern Brazil

Note: it is not always possible to accurately identify *Usnea* specimens, especially when the specimens are poorly developed (juvenile states) or damaged (infected by lichenicolous fungi or when they have been collected from the ground). When such specimens are to be identified, chemistry should be investigated with TLC and, where possible, specialists should be consulted.

Eumitrioid species are not included. Species in parentheses have not yet been found in southern Brazil.

*The ± pale orange pigmentation of the inner medulla around the axis often found in species with salazinic and/or norstictic and/or galbinic acids is not taken into account here. This pigmentation is most probably due to coloration by oxidation of these depsidones while the thallus is ageing.

1 Yellow, pink, orange* to reddish cortical or medullary pigmentation present . . . 2
Such cortical or medullary pigmentation absent 8

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2(1) Medulla C+ yellow (diffractaic and/or barbatic acids), pink or yellow pigment often present, tubercles present . . . . . **U. cristatula**
Medulla C− (diffractaic and barbatic acids absent), pink or yellow pigment absent or present, tubercles absent 3

3(2) Yellow medullary pigment present . . . . . **Usnea** sp. 1
Yellow medullary pigment absent 4

4(3) Pigment located only in the cortex 5
Pigment located below the cortex (subcortical) or in the medulla 7

5(4) Lateral branches distinctly constricted at attachment point, CMA of the *cornuta*-type 6
Lateral branches not distinctly constricted at attachment point, CMA otherwise . . . . . **U. erinacea** s. lat.

6(5) Pigmentation organized into well-delimited and minute red dots (sometimes blackish) on the cortex surface, medulla K−, P− (± triterpenoids) or K+ (salazinic and/or norstictic acids) . . . . . **U. meridionalis**
Pigmentation diffuse in the cortex, not organized into dots, medulla K−, P− (±triterpenoids) . . . . . **Usnea cf. moreliana**

7(4) Lageniform spinulous fibrils numerous, densely arranged on the branches, papillae absent, orange pigmentation often diffusing into the inner medulla . . . . . (**U. aurantiaca-parvula**)
Lageniform spinulous fibrils absent, papillae present, red pigment forming a thin subcortical layer, sometimes diffusing into the cortex, never in the inner medulla . . . . . **U. steineri**

8(1) Lateral branches distinctly to slightly constricted at attachment point (sometimes only a few branches are constricted) 9
Lateral branches not constricted at attachment point 15

9(8) Spinulous fibrils numerous, densely arranged on the branches, tubercles or papillae absent. 10
Fibrils not spinulous but slender, not densely arranged, tubercles or papillae present or absent 12

10(9) Medulla K−, P− (fatty acids, triterpenoids) . . . . . **U. parvula**
Medulla often K− and P+ or K+ 11

11(10) Medulla K−, P+ (protocetraric acid) or K+ slowly yellow (psoromic acid) . . . . . **U. subparvula**
Medulla K+ yellow → red (galbinic acid) or K+ quickly bright yellow (stictic acid) . . . . . **U. subelegans**

12(9) CMA of the *cornuta*-type with a rather thin axis and a thick medulla, cortex shiny. 13
CMA not of the *cornuta*- or *brasiliensis*-types with a thick axis and a rather thin medulla, cortex matt. 14

13(12) CMA often of the *cornuta*-type, medulla K+ yellow → red (salazinic acid) . . . . . **U. cirrosa** s. lat.
CMA of the *brasiliensis*-type, medulla K−, P+ orange (protocetraric acid) . . . . . **U. cladocarpa**

14(12) Medulla lax (best seen in longitudinal sections of lateral branches) and thin; K+yellow → red (norstictic and/or salazinic acids); ascospores longer than 13 µm . . . . . **U. fleigiae**
Medulla dense to compact, often K+ bright yellow (stictic acid) or K+ slowly reddish (norstictic acid); ascospores shorter than 13 µm . . . . . **U. concinna**

15(8) Spinulous fibrils numerous, densely arranged on the branches, tubercles or papillae absent. 16
Fibrils when present not spinulous but slender, tubercles or papillae present or absent. 18

16(15) Medulla K+ yellow → red (galbinic acid), rarely K+ at once bright yellow (stictic acid), branches and segments ± cylindrical in longitudinal section, segments terete in cross-section . . . . . **U. subelegans**
Medulla K− or K+ slowly dull yellow (psoromic acid), branches irregular in longitudinal section, segments ± obtuse- to acute-angled and ± swollen in cross-section. 17

17(16) Medulla K−, P+ red (protocetraric acid) or K+ slowly dull yellow (psoromic acid), lateral branches often somewhat wider at attachment point. . . . **U. subparvula**
Medulla K−, P− (fatty acids, triterpenoids), lateral branches not wider at attachment point . . . . . **U. parvula**

18(15) Basal part pigmented jet black, conspicuously annulated, ascospores on average longer than 13 µm. 19
Basal part concolorous with main branches, annulated or not, ascospores on average longer or shorter than 13 µm 20

19(18) Medulla lax (best seen in longitudinal sections of lateral branches) and thin; K+ yellow → red (norstictic and/or salazinic acid) . . . . . **U. fleigiae**
Medulla dense to compact, K−, P+ orange (protocetraric acid) or K+ yellow → red (salazinic acid) . . . . . **U. grandispora**

20(18) Medulla K−, P+ orange (protocetraric acid), cortex in cross-section matt or vitreous 21
Medulla K+, cortex in cross-section matt to slightly glossy, never vitreous 22

21(20) Cortex in cross-section vitreous and irregularly cracked close to the basal part . . . . . **U. lunaria**
Cortex in cross-section matt to slightly glossy, never vitreous, without irregular cracks (only with annular cracks) . . . . . **U. kalbiana**

22(20) Medulla lax (best seen in longitudinal sections of lateral branches) and thin; K+yellow → red (norstictic and/or salazinic acid); ascospores longer than 13 µm . . . . . **U. fleigiae**
Medulla dense to compact, often K+ bright yellow (stictic acid) or K+ slowly reddish (norstictic acid); ascospores shorter than 13 µm . . . . . **U. concinna**

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