UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

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PROGRAMA DE PÓS-GRADUAÇÃO EM BOTÂNICA

Ph. D. Thesis

Systematics of the lichen genus *Usnea* Adans. (Parmeliaceae, Ascomycota) in Southern Brazil / Sistemática do gênero *Usnea* Adans. (Parmeliaceae, Ascomycota liquenizados) no Sul do Brasil

Alice da Cruz Lima Gerlach

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Systematics of the lichen genus *Usnea* Adans. (Parmeliaceae, Ascomycota) in Southern Brazil

Alice da Cruz Lima Gerlach

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Botany at Universidade Federal do Rio Grande do Sul (UFRGS).

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We have never been individuals (Gilbert *et al*. 2012)

Drawing by Maud Oïhénart and photo by E. Gumboski (with permission).

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RESUMO

Sistemática do gênero *Usnea* Adans. (Parmeliaceae, Ascomycota liquenizados) no Sul do Brasil

Usnea é um gênero de *Parmeliaceae* (*Ascomycota* liquenizados) com espécies de hábito fruticoso. É um grupo altamente diverso e atualmente compreende cerca de 400 espécies distribuídas mundialmente. A excepcional plasticidade morfológica devida às condições ambientais causa dificuldade para a circunscrição das espécies. Nesta tese, a taxonomia do gênero foi investigada no Brasil, com ênfase na Região Sul. Particular atenção foi dada as espécies férteis (17 spp.), pendentes (23 spp.) e as do complexo *Usnea cornuta* (9 spp.).

Um total de 70 espécies de *Usnea* foram identificadas para o Brasil. Descrições (ou notas taxonômicas) e chaves de identificação são fornecidas para 48 espécies. Quatorze espécies são propostas como novas para a ciência; cinco espécies são novos registros para o Brasil e quatro para a Região Sul do país. Cinco espécies são ressuscitadas, uma vez que não foram mais mencionadas após a decada de 1970: *U. cladocarpa* Fée, *U. concinna* Stirt., *U. meridionalis* Zahlbr., *U. lunaria* Motyka and *U. venusta* Motyka.

Adicionalmente aos estudos taxonômicos, a delimitação de espécies do complexo cosmopolita *Usnea cornuta* foi investigada através de um marcador nuclear ribossomal (ITS rDNA) e dois codificadores de proteínas (*Mcm7* e RPB1). O numero de espécies neste grupo foi estimado através do modelo de coalescência (STACEY), o qual utiliza o método Bayesiano. Os resultados indicam a presença de pelo menos nove linhagens fortemente suportadas no agregado *U. cornuta*. Cada uma destas linhagens, baseadas em dados moleculares e morfológicos, são caracterizadas por componentes secundários específicos (químicos). Este estudo mostra que as espécies de *Usnea* devem ser caracterizadas por um número menor de quimiotipos. Concluímos que a combinação de caracteres morfológicos, anatômicos, químicos e moleculares são fundamentais para a delimitação das espécies. Mais estudos usando a teoria de coalescência serão necessários para delimitar as espécies neotropicais de *Usnea*.

Finalmente, o uso do reagente anisaldeído sulfúrico é recomendado como um solvente spray alternativo, a ser rotineiramente usado nas cromatografias em camada delgada (CCD). Sendo importante para detectar a presença de terpenos, esteróides e açúcar na medula de espécies de *Usnea*, uma vez que estas distintas classes de substâncias medulares foram frequentemente encontradas nas espécies estudadas. O valor taxonômico destes componentes permanece não compreendido completamente.

Palavras-chave: Taxonomia integrativa, macroliquens, morfologia, filogenia, Neotrópicos, genes codificadores de proteínas, análises de delimitação de espécies, STACEY, cromatografia em camada delgada.

ABSTRACT

Systematics of the lichen genus *Usnea* Adans. (*Parmeliaceae*, Ascomycota) in Southern Brazil

The lichen genus *Usnea* comprises fruticose members of the family Parmeliaceae (lichenized Ascomycota). It is highly diverse and currently includes approximately 400 species worldwide. The exceptional morphological plasticity of the species towards environmental parameters causes difficulties in the circumscription of species. In this thesis, the taxonomy of the genus *Usnea* was investigated in Brazil, focusing on Southern Region. Particular emphasis was given to the exclusively sexually reproducing species (17 spp.), the pendulous species (23 spp.) and the *Usnea cornuta* aggregate (9 spp.).

We identified a total of 70 species of *Usnea* in Brazil. Descriptions (or taxonomical notes) and identification keys were provided for 48 species. Fourteen species were described as new for science; five species were newly reported for Brazil and four for the Southern region of the country. Five species not anymore mentioned since the seventies were resurrected: *U. cladocarpa* Fée, *U. concinna* Stirt., *U. meridionalis* Zahlbr., *U. lunaria* Motyka and *U. venusta* Motyka.

In addition to the taxonomic studies, the species delimitation of the widely distributed *Usnea cornuta* aggregate was investigated using the nuclear ribosomal genes ITS rDNA, as well as two protein-coding genes *Mcm7* and RPB1. We estimated the species tree under the multispecies coalescent model in a Bayesian framework using the STACEY method. Our results indicate the presence of at least nine strongly supported lineages in the *U. cornuta aggr.* An integrative approach with an *a posteriori* searching for subtle morphological characters based on a larger sampling allowed to characterize phenotypically some of these putative species and consequently six new species were described in the *U. cornuta* aggregate. Each one of these lineages, based on molecular and morphological data, are characterized by a specific chemistry. This study shows that *Usnea* species might have a much smaller number of chemotypes than usually recognized. We conclude that the combinations of morphological, anatomical, chemical and molecular characters are fundamental for the delimitation of species. More studies using the multispecies coalescence theory will be needed to better understand species delimitation in the Neotropical species.

Finally, we propose to routinely use anisaldehyde sulfuric acid as an alternative spray reagent in thin layer chromatography (TLC) studies to detect the presence of

terpenes/steroids/sugars in the medulla of the *Usnea* species, since these distinctive classes of medullary substances were found to be quite frequent in the species studied. The taxonomical significance of these compounds remains, however, not fully understood.

Key words: Integrative taxonomy, macrolichens, morphology, phylogenetic, Neotropics, protein-coding genes, species delimitation analyses, STACEY, thin-layer chromatography.

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Unless explicitly stated in the legends, illustrations are from the author.

1. Lichens

Lichens are *Fungi* engaged in a trophic relationship with microscopic green algae and/or cyanobacteria. This symbiosis between three partners has been recently challenged by the discovery of numerous additional microorganisms that potentially occur as obligatory participants in the symbiosis, including bacteria (Grube & Wedin 2016) as well as basidiomycetes yeasts embeded in the cortical layer of the thallus (Spribille *et al.* 2016). This recent breakthrough is challenging the field of lichenology, and tools drawn from the advancing field of genomics will be needed to fully understand the relationships that make up the complex organisms that are lichens.

Lichens constitute almost one fifth (20%) of all known fungal species (Kirk *et al.* 2008). Most of lichen-forming *fungi* belong to *Ascomycota* (99%), while only less than 1% of all lichens belongs to *Basidiomycota* (Lücking *et al.* 2016). *Parmeliaceae* corresponds to the largest family of lichen-forming *fungi* (*Ascomycota*) with 2.765 species spread across 77 genera. This is one of the most well studied lichen families in terms of phylogeny, generic and species delimitations with species of almost all genera studied with molecular data (except *Davidgallowaya* Aptroot and *Parmotremopsis* Elix & Hale) (Lücking *et al.* 2016). It includes a high diversity of thallus morphology (e.g. foliose, fruticose, crustose, peltate and umbilicate) and even two lichenicolous *fungi*: *Nesolechia* A. Massal. and *Raesaenenia* D. Hawksw., Boluda & H. Lindgr., both recently reduced to synonymy with *Punctelia* Krog and *Protousnea* Motyka respectively (Divakar *et al.* 2017). The classification inside the *Parmeliaceae* is still ongoing mostly with the help of molecular characters (e.g. Blanco *et al.* 2006; Crespo *et al.* 2007, 2010; Divakar *et al.* 2015, 2017; Thell *et al.* 2012). These studies confirmed that the genus *Usnea* Adans. belongs to the *Parmeliaceae* which has been known for a long time due to the apothecial ontogeny.

Being "dual" organisms, reproduction and dispersal of lichens is challenging since both partners have to be present for the successful development of a new lichen thallus (Seymour *et al.* 2005). A successfull strategy is the production of asexual "symbiotic propagules" (e.g. soralia) that allows the propagation of both partner, the fungus and the algae without necessity of *de novo* relichenization (Büdel & Scheidegger 1996). Consequently, lichens which have developed these symbiotical propagules, have also acquired the possibility of exploring and

colonizing much larger areas than the ones of their at least partly sympatric fertile counterpart (Tehler 1982). Thallus without these propagules (i.e. with sexual structures, for example apothecia), need to reproduce sexually by their fungal spores and then encounter with a suitable photobiont in order to reestablish the symbiotic partnership.

Photobiont transmission was studied among others by Dal Grande *et al.* (2012, 2014) and seems to be a complex process. Curiously, these interactions are not always stable, i.e. more than one photobiont can interact with the fungal partner in their symbiosis relationships over the time. It is the case of *Xanthoria parietina* a widespread apotheciate species that can form temporary relationships with a non-species-specific photobiont(s) and later this photobiont could then be replaced by a species-specific partner at a later stage (see Seymour *et al.* 2005). Promiscuous host-symbiont associations in lichen symbioses may be more common than currently recognized (Dal Grande *et al.* 2014).

2. The genus *Usnea* Adans.

The genus *Usnea* forms a strong supported clade (named "usneoid") within the phylogeny of *Parmeliaceae* (Crespo *et al.* 2007) sister to *Cornicularia normoerica* (Gunnerus) Du Rietz (Divakar *et al.* 2015). *Usnea* is a fruticose lichen genus easily recognized by its thallus branches with radial symetry, the presence of a central cartilaginous axis and the production of usnic acid in the cortex. *Protousnea* (Motyka) Krog and *Lethariella* (Motyka) Krog are two similar genera, also fruticose with a central cartilaginous axis but not closely related to *Usnea* **(Fig. 1)**.

Protousnea differs mainly by the presence of divaricatic acid (absent in *Usnea*). Moreover it is characterized by the soft pendulous thallus, the brown apothecial discs efibrillate at their margin, the paucity of secondary morphological characters like papillae and fibrils and their restricted distribution in Southern South America (Chile, Patagonia, Falkland Island) (Krog 1976, Calvelo *et al.* 2005). *Lethariella* produces atranorin inside the cortex instead of usnic acid, often together with the orange pigment canarionic acid. The cortex is thin, soft and spongy; the discs of apothecia are matt brown to black, without fibrils on the margin (Krog 1976). Compared to *Usnea*, *Lethariella* has restricted distribution occuring in the mountains of central Asia (the majority of species) and in the mediterranean-macaronesian area (two species) (Krog 1976). Recently, Divakar *et al.* (2017) suggested that the monophyly of *Lethariella* will require further studies due to insufficient taxon sampling.

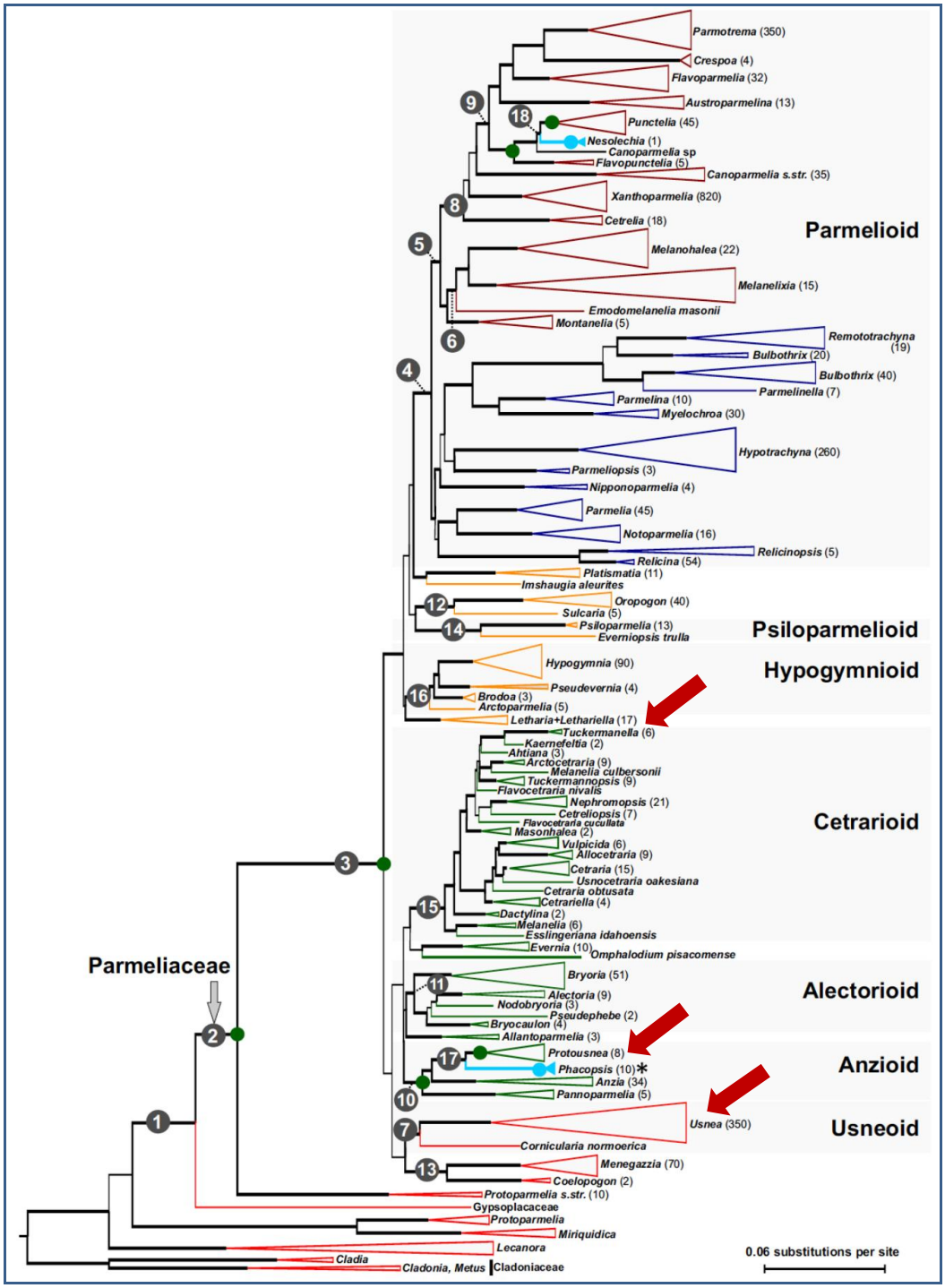


Figure 1. Tree showing phylogenetic relationships among major lineages of *Parmeliaceae*. Red arrows show the position of the genus *Usnea*, *Protousnea* and *Lethariella*. Source: Divakar *et al.* (2015).

The first major work about the genus *Usnea* was published by Josef Motyka (1936–1938), who published more than 750 names in his world monograph. However this work suffers from the following major problems: i) a strong typological species concept, where species differ only by one character; ii) species concepts based on characters that are strongly modified by environmental variables; iii) Motyka never did fieldwork outside Europe so his concepts of tropical species were based solely on herbarium material; and iv) the study of the lichen chemistry was only at its beginning at that time. This resulted in the description of too many species, of which many are now considered synonyms of well-known species (Clerc 1998).

Swinscow & Krog (1976a-b), in their study of East African species, were the first to take into account the variability of the species in the field and to routinely use TLC in their work. Later modern revisions of the genus were done in **Africa** (Swinscow & Krog 1978, 1979, 1988), **Australia** (Stevens 1992, 1999, 2004), **Europe** (Clerc 1984, 1987a-b, 1992, 1994, 2006, 2011a; Halonen *et al.* 1998, 1999; Fos & Clerc 2000; Caviró 2015), **India** (Awasthi 1986), **Japan and Taiwan** (Ohmura 2001, 2012), **New Zealand** (Galloway 2007), **North America** (Tavares & Sanders 1998, Herrera-Campos *et al.* 1998, 2001; Clerc 2007; Hinds & Hinds 2007; Herrera-Campos 2016), **Polar regions** (Walker 1985, Wirtz *et al.* 2008, 2012) and **Russia** (Ohmura *et al.* 2017). Studies on the genus in South America started recently in the framework of two PhD studies specifically in Argentina (Rodriguez 2011, Rodriguez *et al.* 2011) and in neotropical areas of the Andes and Galapagos (Truong 2012, Truong *et al.* 2011, 2013a-b; Truong & Clerc 2012, 2013, 2016). These two studies left Brazil more or less untouched and thus there are very little modern data on the genus *Usnea* from this very large country.

Usnea is one of the largest genus of lichen-forming *fungi*, within *Parmeliaceae* is the most speciose, only after the foliose genus *Xanthoparmelia* (Lücking *et al.* 2016). About 1,134 names are cited in the literature (Clerc *et al.*, unpublished world checklist). The exact number of well-defined and accepted species is still unknown, but it is most probably around 400 (Clerc 2016). The genus is found in polar, temperate and tropical regions and its center of distribution seems to be in the Neotropics (Clerc 2016).

3. Infrageneric classification

The genus *Usnea* was segregated in five subgenera by Josef Motyka in his world monograph (Motyka 1936, 1938), all of them considered as being "usneoid lichens" since they all have a

fruticose thallus with a central mechanical tissue inside: *Chlorea*, *Eumitria*, *Eu-usnea* (*Usnea* s. str.), *Lethariella*, *Neuropogon* and *Protousnea*. However the genus *Usnea* in broad sense forms a strongly supported clade and the existence of these infrageneric groups and their relationships have been a matter of debate for decades and are still controversial today (Truong *et al.* 2013a; Divakar *et al.* 2017). However, it is clear nowadays that *Protousnea* and *Lethariella* do not belong to *Usnea*.

Eumitria Stirt. is characterized by the presence of a tubular central axis. Otherwise, it shares all major morphological and anatomical characters with *Usnea s. str*. Unlike *Lethariella* and *Protousnea, Eumitria* has a wide distribution area, with taxa occurring in Africa (Swinscow & Krog 1974, Krog 1994), Australia (Stevens 1999), Asia (Ohmura 2001, 2012) and South America (Truong & Clerc 2013). Its generic status is controversial since species without central tubular axis might cluster together (within a strongly supported clade) with species having a central tubular axis (Truong *et al.* 2013a). For this reason these authors keep *Eumitria* as subgenus of *Usnea* agreeing with most authors (Ohmura 2002; Ohmura & Kanda 2004; Wirtz *et al.* 2006) but disagreeing with Articus (2004). Recently Divakar *et al.* (2017) proposed to consider *Eumitria* as a genus since the diversification of the genus *Usnea* predates the diversification of *Eumitria*. However, the number of specimens and species analyzed in this group is still limited and further analyses are needed to resolve this question.

Neuropogon Nees & Flot. is characterized by the black pigmentation in the cortex, the dark pigmented apothecial discs, and a *sphacelata*-type cortex (Ohmura & Kanda 2004). It shows a restricted distribution too as it occurs in Antarctic, Arctic and high Andean regions, exclusively on saxicolous substrata (Truong *et al.* 2013a). The production of melanoid substances in the thalline cortex was suggested to be an adaptation to harsh environmental conditions (Lumbsch & Wirtz 2011). There are several studies using molecular data in this group (Articus 2004; Ohmura & Kanda 2004; Lumbsch & Wirtz 2011; Wirtz *et al.* 2006; Truong *et al.* 2013a) but the backbone of the phylogeny is still not fully resolved and there are indications that *Neuropogon* is polyphyletic, nested within *Usnea s. str*. (Wirtz *et al.* 2006; Truong *et al.* 2013a). Therefore the black pigmentation which characterizes this group might have evolved twice independently under similar ecological conditions (Wirtz *et al.* 2006). So, based on molecular data and the absence of clear morphological characters, neuropogonoid species should be considered so far to belong to *Usnea s. str.*, (Wirtz *et al.* 2006, Truong *et al.* 2013a).

Finally, *Dolichousnea* Ohmura was segregated as a subgenus based on morphological criteria, i.e. the presence of annular pseudocyphellae, a thicker hypothecium and a positive iodine reaction of the central axis (Ohmura 2001). It includes three species occurring mainly in the Northern hemisphere (*U. diffracta* Vain., *U. longissima* Ach. and *U. trichodeoides* Vain.). These three species formed a well-supported clade based on sequences of ITS region (Ohmura 2002) and Articus (2004) elevated *Dolichousnea* at the generic level. Recently Divakar *et al.* (2017), furthered these results with estimated time of divergence compared with *Usnea s. str.* Only eumitrioid species (not treated in this thesis) and taxa belonging to *Usnea s. str*. occur in Brazil.

4. Taxonomical studies on the genus *Usnea* in Brazil

4.1 Historical context

One of the first reference to Brazilian specimens of *Usnea* is found in *Flora Brasiliensis* (Martius 1833) which is the result of the first major official scientific expedition in Brazil conducted by Martius and Spix. Lichens collected during this expedition were identified by **F.G. Eschweiler** who described two saxicolous species (*Usnea aspera* (Eschw.) Vain. and *U. laevis* (Eschw.) Nyl.).

The Swiss botanist **Müller Argoviensis** was one of the most influential lichenologist of the 19th century; he mainly worked on lichens sent to him by other botanists mostly from the tropics. Some *Usnea* species from Brazil collected by Puiggari, Fée, Schenck and Ule were studied by himself (Müller 1881, 1887, 1891a-b) including newly described taxa as for example *U. complecta* (Müll. Arg.) Motyka and *U. trachyclada* (Müll. Arg.) Zahlbr.

The Finnish lichenologist **E.A. Vainio**, the father of the Brazilian Lichenology (Marcelli 1998b), described 15 names of *Usnea* from Brazil collected mainly for the Minas Gerais State, including newly proposed taxa as for example *U. subelegans* (Vain.) Räsänen (Vainio 1890). Indeed Vainio's PhD study included the most complete treatment of *Usnea* from Brazil in the 19th century with descriptions based on careful examinations and revisions. However, of course modern revisions of all names cited are still in need.

The german botanist **A. Krempelhuber** mentions a few *Usnea* species collected by Glaziou in the state of Rio de Janeiro (Krempelhuber 1876) and described two new taxa *U. ceratina* f. *pusilla* Kremp. and *U. poliothrix* Kremp. (Krempelhuber 1868, 1873).

In the 20th century, the Austrian botanist **J.B. Zahlbruckner** studied many specimens collected in Brazil by several collectors (e.g. the Brazilian botanist L.B. Damázio) and

published some new *Usnea* species (e.g. *U. brasiliensis* Zahlbr. and *U. meridionalis* Zahlbr.) (Zalbruckner 1902, 1904, 1905, 1909). At this time the Swedish botanist **G.O.A. Malme** traveled twice to Brazil reaching to the Southern region of Brazil in the *Regenellian Expedition* (Marcelli 1998a). The *Usnea* specimens collected by him were sent to the Polish lichenologist **J. Motyka** who, in his monography of the genus (Motyka 1936, 1938), listed 64 *Usnea* species found in Brazil) among them several new species (e.g. *U. alata* Motyka, *U. malmei* Motyka, *U. papillata* Motyka, etc.).

The study of brazilian species of *Usnea* by Brazilian researchers started only in the middle of the 20th century. The presence of usnic acid (important because of its antibacterial properties) in this genus drew the attention of the Brazilian botanist **C.T. Rizzini** (1952, 1956) who seemed to be the first to study Brazilian *Usnea* species from Rio de Janeiro State (Serra dos Órgãos). He described two new taxa (*U. elongata* Motyka f. *sorediifera* Rizz. and *U. ludicra* Rizz.) and provided a key of identification for 17 species written in latin and based on Motyka's species concept. Unfortunately, the type specimens of these two taxa seem to be lost and the status of these names remains thus uncertain.

M. Fleig was the first Brazilian lichenologist to dedicate her efforts to study *Usnea* in Southern Brazil. She collected hundreds of specimens mainly in the Rio Grande do Sul state. Some of them were published in the form of species lists by herself alone or with her collaborators (Fleig 1988, 1990, 1995, Osorio & Fleig 1986, 1988–1991, 1994). More recently a field guide provided descriptions of several lichens among them three *Usnea* species (Fleig & Grüninger 2008).

The german lichenologist **K. Kalb** made numerous lichen collections in Brazil (mainly in Rio de Janeiro, São Paulo and Mnas Gerais States) and published a serie *Lichenes Neotropici* focusing mainly on the crustose lichens but a few *Usnea* species were also mentioned (Kalb 1982a-b, 1986). In the same way the dutch lichenologist **A. Aptroot** (2002) mentioned 11 species of *Usnea* based on material collected in the Minas Gerais and São Paulo states.

This short history about the *Usnea* research in Brazil showed that despite the efforts of some adventurer lichenologists to study the genus in Brazil, the data on the majority of existing brazilian data is sparse and has been published in the form of species lists without taxonomic treatments. Some of the names published this way were reviewed but there is still a long way to go before we can publish a reliable Brazilian checklist of the genus.

Very recently some Brazilian specimens were studied in the framework of a thesis on the genus *Usnea* in Neotropical South America (Truong 2012) and several species (e.g. *U. perhispidella* J. Steiner, *U. geissleriana* P. Clerc; *U. subdasaea* Truong & P. Clerc) were added to the mycota of Brazil (Truong *et al.* 2013b; Truong & Clerc 2016). This PhD thesis (Truong 2012), together with the large collection of M. Fleig (housed at ICN herbaria) were my first sources of inspiration starting the study of the genus in Brazil during the two first years of this PhD study conducted in Porto Alegre (UFRGS), Southern Brazil.

In summary, approximately 110 different taxon names were mentioned for Brazil (Motyka 1936, 1938; Spielmann 2006; Gumboski & Eliasaro 2011). Fifty four of these species were described based on material collected in Brazil **(see Appendix 1)**.

4.2 Usnea collections in Brazil

The South and Southeast regions of Brazil have the highest number of records of *Usnea* specimens collected and deposited in national herbaria (specieslink 2017) **(Fig. 2).** Most of these specimens are unidentified, suggesting that the diversity of *Usnea* species in the country is poorly known. For example, *Usnea erinacea* Vain., a very common species in Brazil, was only recently mentioned for the first time in the country (see **Chapter 1**)

On the other hand twelve States have no records of *Usnea* species (Acre, Amapá, Amazonas, Ceará, Maranhão, Paraíba, Piauí, Rio Grande do Norte, Rondônia, Roraima, Sergipe and Tocantins) (specieslink 2017), which suggests on one side that this genus is undercollected in these States, but on the other side also an heterogenous distribution of the genus throughout the country. Recently, a few specimens were collected in the Brazilian biome Amazonia (Mathias Engels, pers. com.) which may be the first *Usnea* records for this area in Brazil, since, for instance, *U. amazonica* Motyka was not described from the Brazilian but from the Peruvian Amazonia.

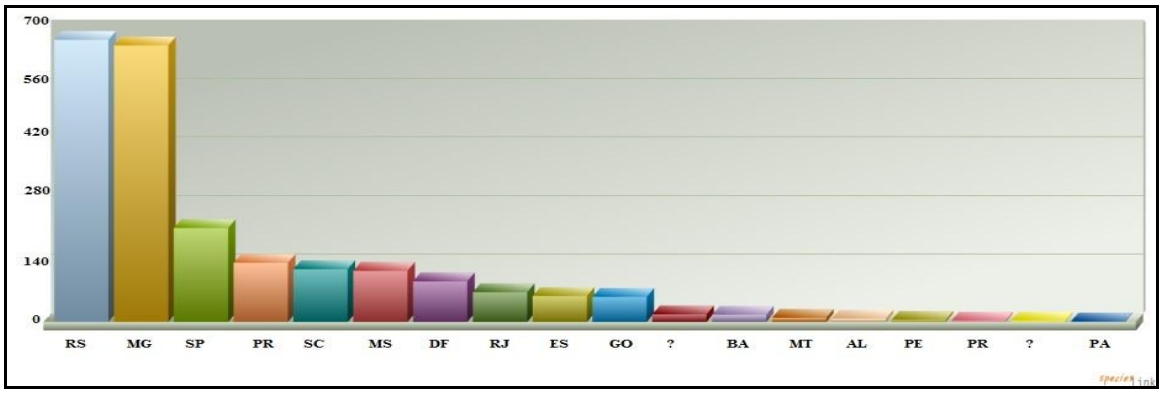


Figure 2. Number of records (Y axis) of the genus *Usnea* in the states of Brazil (X axis).. **RS:** Rio Grande do Sul, **MG**: Minas Gerais, **SP**: São Paulo, **PR**: Paraná, **SC**: Santa Catarina, **MS**: Mato Grosso do Sul, **DF**: Distrito Federal, **RJ**: Rio de Janeiro, **ES**: Espírito Santo, **GO**: Goiás, **BA**: Bahia, **MT**: Mato Grosso, **AL**: Alagoas, **PE**: Pernambuco, **PR**: Paraná, **PA:** Pará, ?: without data. Source: specieslink, 2017

5. Biomes and vegetation of Brazil

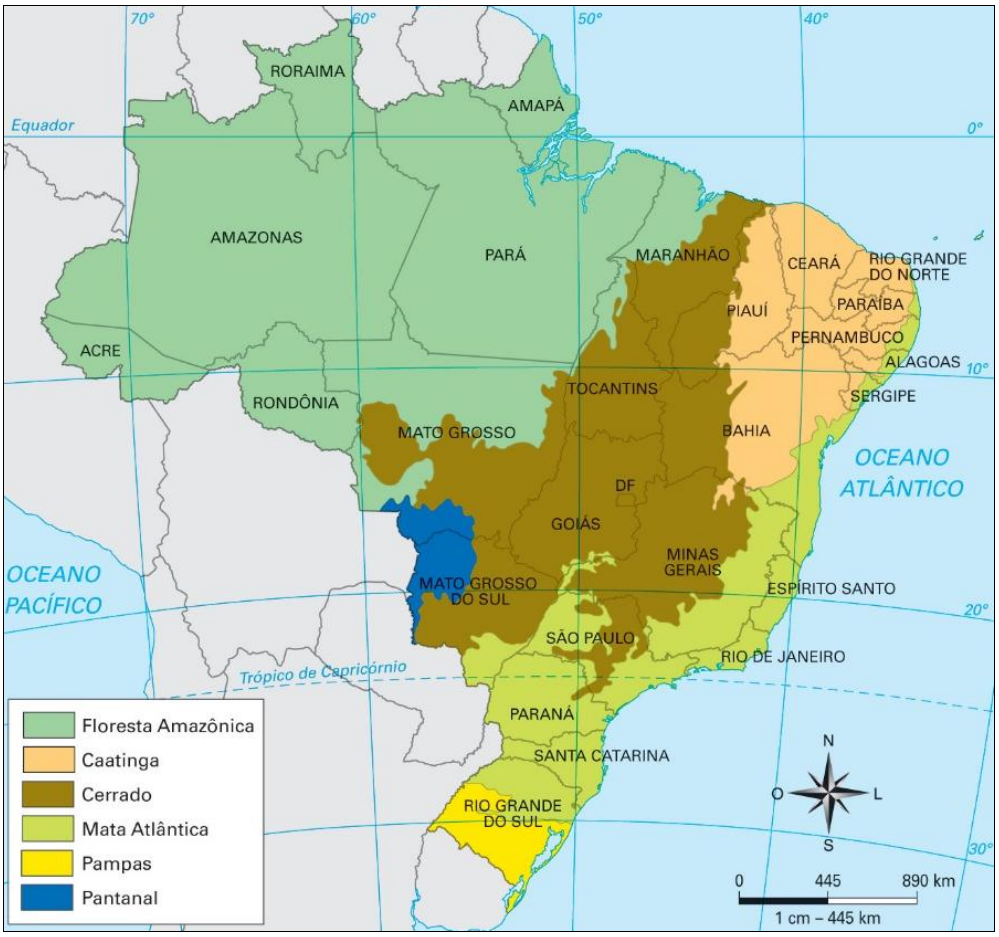
Brazil is a megadiverse country (Forzza *et al.* 2012) and according to the current official classification of vegetation in Brazil by IBGE (2004), it possesses six biomes: Amazonia, the Atlantic Forest, the Caatinga, the Cerrado (tropical savannas), the Pantanal (forested wetlands), and the Pampa (Southern grasslands) (**Fig. 3**). The Southern region, which includes the States of Paraná, Santa Catarina, and Rio Grande do Sul, contains part of the Atlantic forest biome and of the Pampa (southern half of Rio Grande do Sul) as well as some Cerrado fragments (in the northern Paraná) (**Fig. 3)**.

The Atlantic forest is one of the 25 hotspots for conservation priorities with only 7.5% of the original extent (Myers *et al.* 2000), and it is the second largest rainforest biome of South America. It corresponds to a complex mosaic of different vegetation types (e.g. dense rainforests, Araucaria forests, high-altitude grasslands, and coastal areas) (for details see Iganci *et al.* 2011; Oliveira-Filho *et al.* 2015) (**Fig. 4**).

The Pampa biome grasslands corresponds to a non-forest ecosystems, and is among the most species-rich grassland in the world (Overbeck *et al.* 2007). Like the Pampa, the Cerrado Biome is a non-forest ecosystem. The climate is hot with a pronounced dry season. Vegetation varies from open grasslands to closed-canopy forests. Forested wetlands include riparian forests, interfluvial depressions mostly fed by rain or groundwater (veredas), and hyperseasonal savannas linked to seasonal flood pulses of large rivers (Wittmann *et al.* 2017).

Usnea is a genus of hygrophilous and photophilous species that are found abundantly in moist and relatively open sites (Halonen 2000). As a consequence, they are abundant in

mountainous regions, where humidity is brought by the clouds (Truong 2012). In Brazil, a high number of specimens of *Usnea* were reported in elevated and humid areas (Rizzini 1952; Marcelli 1998; Fleig & Grüninger 2008). There is hudge amount of *Usnea* thalli growing in the *Araucaria* forests, above ca. 900 meters in the canopy of *Araucaria angustigolia* usually intermingled with species of the fruticose genus *Ramalina* and foliose species of the genus *Parmotrema* (Marcelli 1998). The genus *Usnea* in Brazil can be found from the sea level up to the highest mountains (**Figs. 5 and 6**).



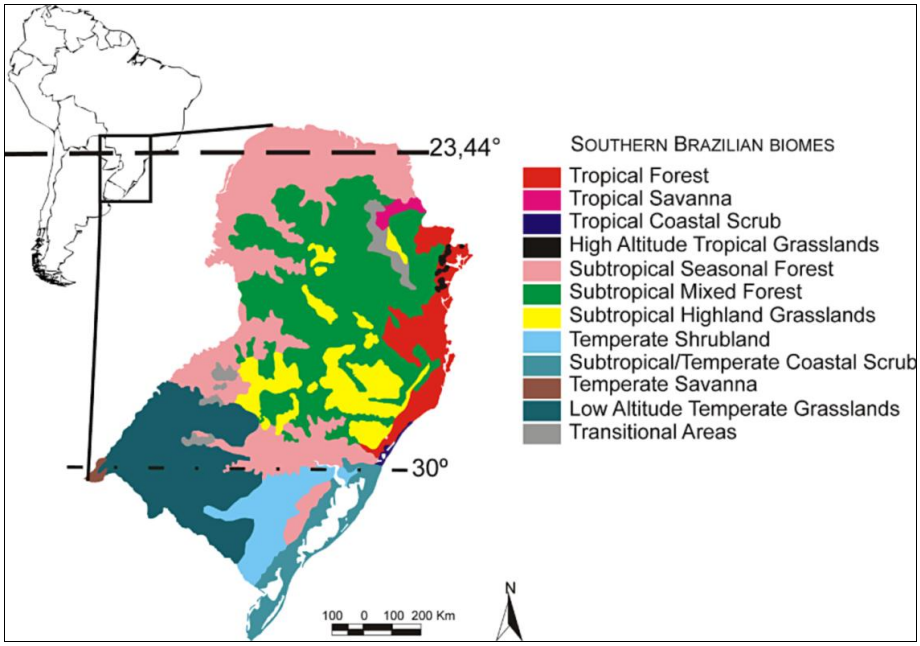


Figure 3. Biomes from Brazil (source: http://sanderlei.com.br/PT/Ensino-Fundamental/Santa-Catarina-Historia-Geografia-31). (above)

Figure 4. Vegetation of Southern Brazil (source: Iganci *et al.* 2011). (below)

Figure 5. Diversity of *Usnea* species and their habitats in Southern Brazil. **A**, *Araucaria* forest, Parque Nacional de São Joaquim; **B**, semi deciduous Forest, Parque Nacional de Foz de Iguaçu; **C**, *Araucaria* forest, numerous *Usnea* growing on twigs, Floresta Nacional de São Francisco de Paula (photo by A. Spielmann); **D**, Rainforest, *Usnea* growing on rocks in coastal area, Lagoa da Conceição, Florianópolis municipality; **E,** *Usnea* growing in rural area, Rio Negrinho, Santa Catarina State (photo by E. Gumboski).

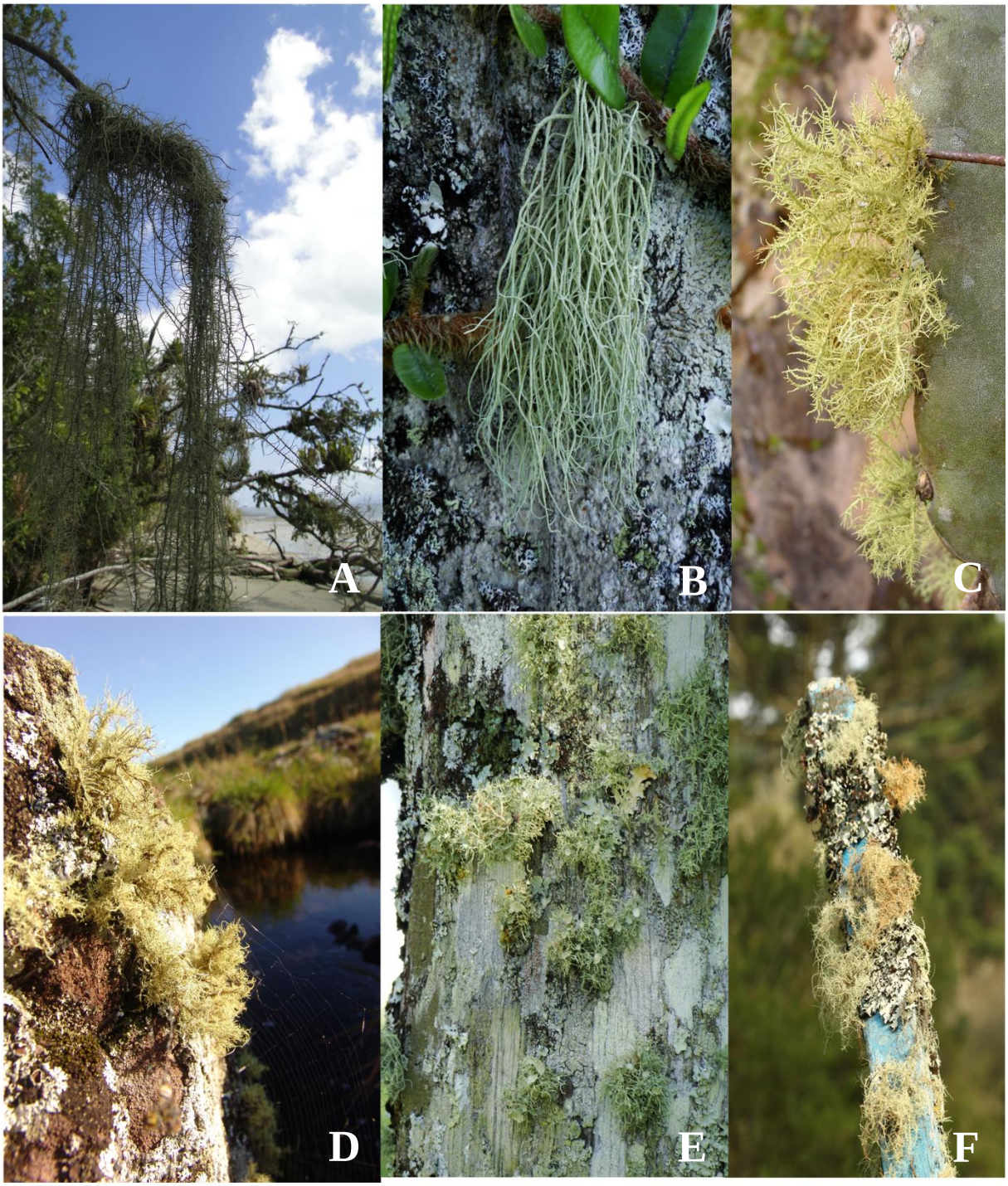


Figure 6 . Diversity of *Usnea* species and their habitats in Brazil. **A**, restinga area in front of the sea, Ilha do Mel, Paranaguá Municipality; **B,** *Usnea* growing on rocks close to the shore, São Francisco do Sul; **C,** *Usnea* growing on cacti, Parque Natural do Caraça (photo by A. Spielmann); **D,** saxicolous *Usnea*, riparian forest (photo by A. Spielmann); **E,** *Usnea* growing on bark of *Syagrus romanzzofiana*; **F,** *Usnea* growing on fence posts, Sao Francisco de Paula (photo by A. Spielmann).

6. Taxonomy of *Usnea* s. str.

6.1 Species concept

According to Wilkins (2011) there are 27 different definitions of species. Instead of asking *what* is a species (a philosophical question strongly related to what type of organism we are working with), most of the modern systematists take a pragmatic approach asking *how* to identify species: a) relying largely on morphological criteria or other observable patterns of discontinuity (**taxonomic species concept,** Kärnefelt 1979); b) relying on genetical data to estimate evolutionary lineages (**general lineage concept,** de Queiroz 2007). Modern systematists have paved the way towards an **integrative approach** that aims at delimiting the units of life's diversity from multiple and complementary perspectives (Dayrat 2005).

Clerc (1998) discussed the concept of species in the genus *Usnea*. The **typological concept** was employed by Motyka (1936, 1938) in his monography of the genus, where species are seen as invariant units based on a 'perfect type', allowing thus very little opportunity for variation (one character = one species). This concept was responsible for the inflated number of species and and the confuse nomenclature in the genus. On the other hand, stands the **populational species concept** where such phenotypical variation is allowed to occur intra-specifically. According to Clerc (1998) species vary in three dimensions: morphology, anatomy and chemistry. The intra-specifical variation form a 'cloud' of individuals characterized by the correlation of at least two of these characters (Clerc 1998 in Fig. 2). Thus, if the chemical variation (called chemotypes) is not correlated with any morphological or anatomical differences it should be considered as a variation within one species (Clerc 1998, Herrera-Campos *et al.* 2008). Indeed, the choice of the morphological characters to be analysed is crucial and the characters choosen should be less prone to variation by environmental factors (Clerc 1998). Using such a concept linked with a correct selection of important taxonomical characters brought several names to be merely considered as variation of the same species (Clerc 1998).

Nowadays most systematists agree that species are evolutionary lineages, meaning by this segments of separately evolving metapopulation lineages (de Queiroz 2007). Lineages with different evolutionary histories are different species, these lineages must be the smallest units of evolution. The analytical methods to estimate species delimitation are imperfect interpretations of the evolutionary processes in nature (Carstens *et al.,* 2013), which are full of complex artefacts (Naciri & Linder 2015, Leavitt *et al.* 2016b) and thus should be considered as hypotheses (Pante *et al.* 2015). This thesis applies the integrative concept of species (Dayrat 2005), using the current taxonomic species concept of the genus *Usnea* (Clerc 1998), associated with ecological and genetic data in order to find and delimit well defined lineages.

6.2 Characters used in the taxonomy of the genus

The taxonomy of lichenized *Fungi* is mostly based on the mycobiont, i.e. the fungal partner. Therefore aspects relative to the photobiont, the green-alga *Trebouxia* spp. in *Usnea*, were not considered in this thesis.

The genus *Usnea* has a relatively larger number of usefull morphological and anatomical characters for taxonomy (**Figs. 7 and 8**), if compared for example with *Bryoria* Brodo & D. Hawksw., another fruticose genus in the *Parmeliaceae*, which is not so diverse in morphological characters (Boluda 2017). This thesis follows the definitions of morphological and anatomical character established by Clerc & co-authors (Clerc 1987a-b; Clerc 1998; Herrera-Campos *et al.* 1998; Clerc 2011a; Rodriguez *et al.* 2011; Truong *et al.* 2011, 2013b) and Ohmura (2001).

It is important to emphasize that species of the genus *Usnea* have a fruticose shrubby to pendant thallus with a large total surface in contact with external environmental parameters (i.e. light, humidity, temperature and air pollution) compared with other lichens lifeforms (as for example the foliose and crustose lichens). Consequently, they are especially prone to morphological variation (phenotypical plasticity) through environmental influences (Clerc 1998). With this in mind, it is important, when defining and delimiting the species in the genus *Usnea*, to select those characters that are less susceptible to be affected by these environmental factors.

6.2.1 Morphological characters

A protocol of description was developed where 19 morphological characters were accurately analysed following Clerc (1998): *Thallus*, *ramification*, *basal part*, *branches*, *branch segments*, *lateral branches*, *annular cracks*, *foveoles and transverse furrows*, *maculae*, *pseudocyphellae*, *papillae*, *tubercles*, *fibrils*, *fibercles*, *soralia*, *isidiomorphs*, *isidiofibrils*, *apothecia*, *ascospores*).

Thallus (habitus): If all the branches are erect-bushy and divergent, the thallus is considered as being **erect-shrubby** (or shrubby); if the thallus start to grow erect with divergent branches and soon becomes hanging with some branches running parallel at their apices, then the thallus is considered as being **subpendent**; if all branches from the beginning growing parallel to each other, hanging downward, then the thallus is considered to be **pendent**

(Ohmura 2001, in Fig. 1). Despite its usefullness to delimit artificial groups of species for taxonomic studies, this character is somewhat influenced by environmental factors and thus should be used with caution (Clerc 1998, Truong *et al.* 2013b).

Ramification-type: there are three main types of branching: a) **anisotomic-dichotomic**, where a main branch runs through to the apex with smaller secondary branches ramifying with 90° angles; B) **isotomic-dichotomic**, where the basal part divides into two main branches of ± equal diameter, the latter dividing themselves the same way and so one (e.g. *U. grandispora*); **filamentose**, where a short and thin, almost indistinct main branch divides at once, producing secondary branches of almost equal diameter that elongate into cylindrical type branches that run parallel to each other (Ohmura 2001 in Fig. 2; Clerc 2011a in Fig. 1). There are however often intermediate types or species showing two types of ramification. This character was not very useful to recognize the species here studied.

Basal part is the region between the hodfast (point of fixation of the thallus to the substrate) and the first main ramification. This region can be concolorous to the main branches (the majority of the species), jet-black pigmented (e.g. *U. grandispora, U. subsilesiaca*) or orangish-reddish (e.g. *U. subscabrosa*). The presence of annular cracks is for example important to recognize *U. kriegeriana ad. int*. The characters related to the basal part are especially important for the saxicolous species since some of them have a special kind of holdfast (called a "proliferating holdfast") (see Rodriguez *et al.* 2011).

Branches: the shape of main branches in transverse and longitudinal sections is an important taxonomical character, especially in pendulous species (Swinscow & Krog 1978, Herrera-Campos *et al.* 1998, Truong *et al.* 2013b). According to Herrera-Campos *et al.* (1998, in Fig. 4) and Clerc (2011 in Fig. 5) the shape of branch in transverse section can be terete, flattened, irregular, foveolate or furrowed with deformed segments, ridged (e.g. *U. chilensis*), alate (e.g. *U. angulata*). According to Clerc (2011, in Fig 2) the shape in longitudinal section may be cylindrical (the diameter of the branch remains the same along most of the length of the branch) (*U. malmei*), tapering (the diameter of the branch ± gradually decreases from the basal part towards the apex) (e.g. *U. subsilesiaca ad. int.*), irregular (the diameter of the branch varies irregularly along the length of the branch) (e.g. *U. chilensis*), fusiform (swollen ± at middle part and tapering towards each end like a spindle (e.g. *U. brasiliensis*).

Branches segments may be cylindrical or swollen (sausage-like) (typically in *Usnea venusta* Chapter 3, Fig. 6).

Lateral branches may be cylindrical at base (e.g. *Usnea malmei*), broadened at base (e.g. *U. amabilis*) or constricted at base (e.g. *U. cornuta*, *U. brasiliensis*) (Clerc 1987b, 2011a in Fig. 3; Ohmura 2001 in Fig. 6). The degree of constriction can vary among species from strongly constricted (*U. subglabrata* Truong & P. Clerc) to slightly constricted (*U. dasaea* Stirt.). This character is especially important to delimit the *U. cornuta* aggregate **(Chapter 5)** but also to separate species among the sexually reproducing taxa **(Chapter 1)**. Intermediate forms were sometimes observed in Brazilian specimens, in which the branches are first broadened at base, then becoming slightly constricted.

Annular cracks: the presence of annular cracks along the branches is especially important in the pendulous species: annulation can be thin with eroded margin (*U. subgracilis*, Fig. 7C) or ±thick with irregular cortical regeneration areas (*U. malmei*, Fig. 7B). The same cortical regeneration areas can have the shape of beads (*U. merrillii*, Fig. 7A).

Foveoles and transverse furrows: foveoles, nearly circular depressions in the cortex or transverse furrows, seem to originate from mechanical disturbances of the cortex (Clerc 1998 in Figs. 5–8). According to Clerc (1998) the occurrence of foveoles is correlated with a thinner cortex. *U. disjuncta* is for instance a species found with a very thin cortex and also displaying the most pronounced foveoles found in this study (Chapter 2 in Fig. 15).

Maculae are white patches on the surface of branches where the cortex is thinner than in other areas and without subcortical algae (Ohmura 2001); it is found spotting the cortex of *U. arthroclada* (Chapter 2 in Fig. 13), *U. regia* (Chapter 2 in Fig. 25), and in the terminal branches of *U. merrillii* (Chapter 2 in Fig. 32). *Usnea venusta* has maculae covering most of its surface (best seen on fresh material).

Pseudocyphellae are whitish, usually thin and elongate, more or less fusiform breaks in the cortex that never produce soredia (Clerc & Herrera-Campos 1998). The presence of pseudocyphellae and maculae is especially important in the pendulous (Truong *et al.* 2013b) and saxicolous species (Rodriguez *et al.* 2011). *Usnea malmei*, and *U. papillata* are the only species to display conspicuous pseudocyphellae on the main branches (**Chapter 3, Fig. 26**).

Papillae and **tubercles** are short cortex outgrowths that differ by the presence of medulla in tubercles (Clerc 1998). They can be hemispherical, verrucose or cylindrical (Swinscow & Krog 1979, Ohmura 2001 in Fig. 7). Both papillae and tubercles can be present in the same species; for instance *U. grandispora* presents two mophotypes: one with cylindrical papillae and other one with verrucose tubercles (Chapter 1, Fig. 7). The presence of tubercles is diagnostic for some species (e.g. *U. subflammea*, chapter 2 in Fig. 39; *U. transitoria*, Fig. 7D). They can be sometimes difficult to tell apart though.

Fibrils are short branch-like appendages with a central axis that is not attached to the central axis of the branches on which they occur (Clerc & Herrera-Campos 1997). They are rarely absent and their size, shape and arrangement on the branches are important characters. Fibrils may be **spinulose** when they are 2–5× taller than wide and **slender** when they are 6–15× longer than wide **(Gerlach *et al.* 2017, Chapter 1)**. They are often conical, but a special kind of spinulose fibrils, swollen at the base and narrowed at the top (called lageniform), was found for instance only in *U. aurantiaca-parvula* (Fig. 8J). The density of the spinulose fibrils was found also as an important character to differentiate *U. parvula* and *U. subparvula* (Fig. 8D–E). Some pendulous species presents a special pattern of arrangement of the fibrils, the so called "fish-bone" like pattern, in which fibrils are more or less regularly distributed on both sides of the branches (Herrera-Campos *et al.* 1998, in Fig. 5 d–e). This pattern is particularly clear in *U. angulata* and *U. transitoria*.

Fibercles are more or less protuberant scars left after the breaking away of fibrils (Clerc & Herrera-Campos 1997). These authors highlighted the importance to differentiate tubercles from fibercles as some species produce only tubercles (*U. ceratina*) while other species produce only fibercles (*U. amblyoclada* and *U. nashii*). The presence of these structures seems to be especially important in the saxicolous species. We found for instance this structure to be a diagnostic character only in *U. oreophila ad. int.* (see chapter VI)

Soralia are one of the most important character in the taxonomy of *Usnea* and consequently they have been largely used in identification keys (Clerc 1998). According to Clerc (1987a) soralia can be characterized by: a) their *size*: from punctiform (smaller than half of the diameter of the branch) to enlarged. Most of the sorediated Brazilian specimens studied have small punctiform soralia, although species with large soralia were also found (e.g. *U. macarronesica* P. Clerc; **Appendice II**) and *U. pseudobrasiliensis ad. int.* (Fig. 8Lb) their *shape in side view*: raised (± stipitate), even with the cortex (Fig. 8H), depressed with respect

to the cortex (excavate or concave) or convex (efflorescent) (Truong & Clerc 2016, Fig. 4H); c) their *shape in top view*: regular or irregular, rounded to longitudinally or transverselly elliptical; d) with a sharply delimited margin or without such a margin; e) if they fusing together or not; f) their density and regularity; g) their position on the main branches, the secondary branches, the apices or the fibrils; their origin: ad initio from the cortex, from eroded tubercles, from fibercles, or at the edges of eroded segments. It is important to highlight the fact that these characters should be checked only on well developed specimens and on mature soralia that are usually found on the terminal branches of the thalli (see **Chapter 5**, *U. kriegeriana ad. int.*).

Isidiomorphs are not formed as an outgrowth of the cortex (as isidia), but from the medullary tissues of soralia mainly (Clerc 1998). Their presence or absence in soralia is an important diagnostic character in the genus *Usnea*. They are for instance always absent in some species (e.g. *U. esperantiana*, *U. lapponica* or *U. fulvoreagens* (Räsänen) Räsänen (Clerc 1998). In South America, for example, isidiomorphs are abundant in *U. aranea* whereas they are usually absent in *U. subaranae* (Truong & Clerc 2016).

Isidiofibrils are taller isidiomorphs growing and developing secondarily a central axis (Truong *et al.* 2011). They are for instance present in *U. isidiofibrillosa* sp. nov., *U. perhispidella* and *U. poliotrix*. It is an important character to recognize these three species **(see Chapters 2, 5)**.

Apothecia and **spores**: The apothecia are lecanorine, located lateral, serial, subterminal or terminal on the branches; ± cup-shaped; the margin presents few to numerous fibrils; the asci are of the *Lecanora*-type, 8-spored; the ascospores are simple, ellipsoid to broadly ellipsoid, 7–11(–18) × 5–7(–12) µm, colorless (Awasthi 1986, Clerc 2011a, Gerlach *et al.* 2017). The sizes of ascospores, as well the morphology of the apothecia, have traditionally received little attention and are rarely used as diagnostic characters in identification keys of the genus. Nevertheless, Clerc (1984) found differences in spore size to discriminate between the *U. florida* and *U. intermedia* in Europe. Awasthi (1986) used this criterion in the identification keys for *Usnea* species from India. A great variation on the size of ascospores were found among some species in Brazil (Gerlach *et al.* 2017) **(see Chapter 1)**.

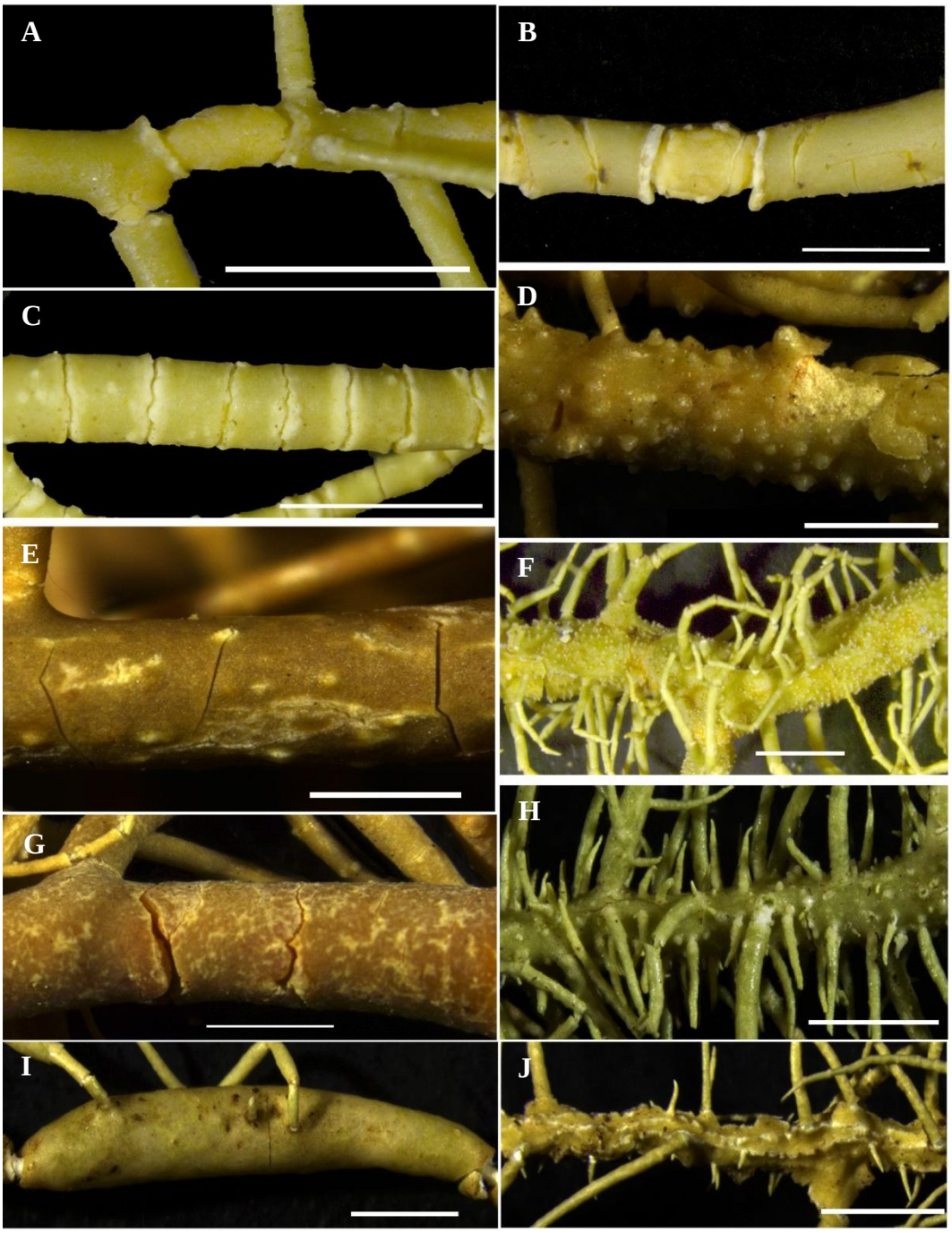


Figure 7. Morphological characters used in the taxonomy of *Usnea*. **A**, bead-like cortical annulation in *U. merrillii*; **B**, annular cracks with regeneration area *in U. malmei*; **C**, thin and eroded annular-cracks in *U. subgracilis*; **D**, large tubercles in *U. transitoria*; **E**, pseudocyphellae in *U. malmei*; **F**, ridged branches and papillae in *U. chilensis*; **G**, maculae in *U. regia*; **H**, terete branches and spinulose fibrils in *U. dasaea*; **I**, sausage-like branches in *U. venusta*; **J**, irregular branches with trapezoidal segments in *U. angulata*.

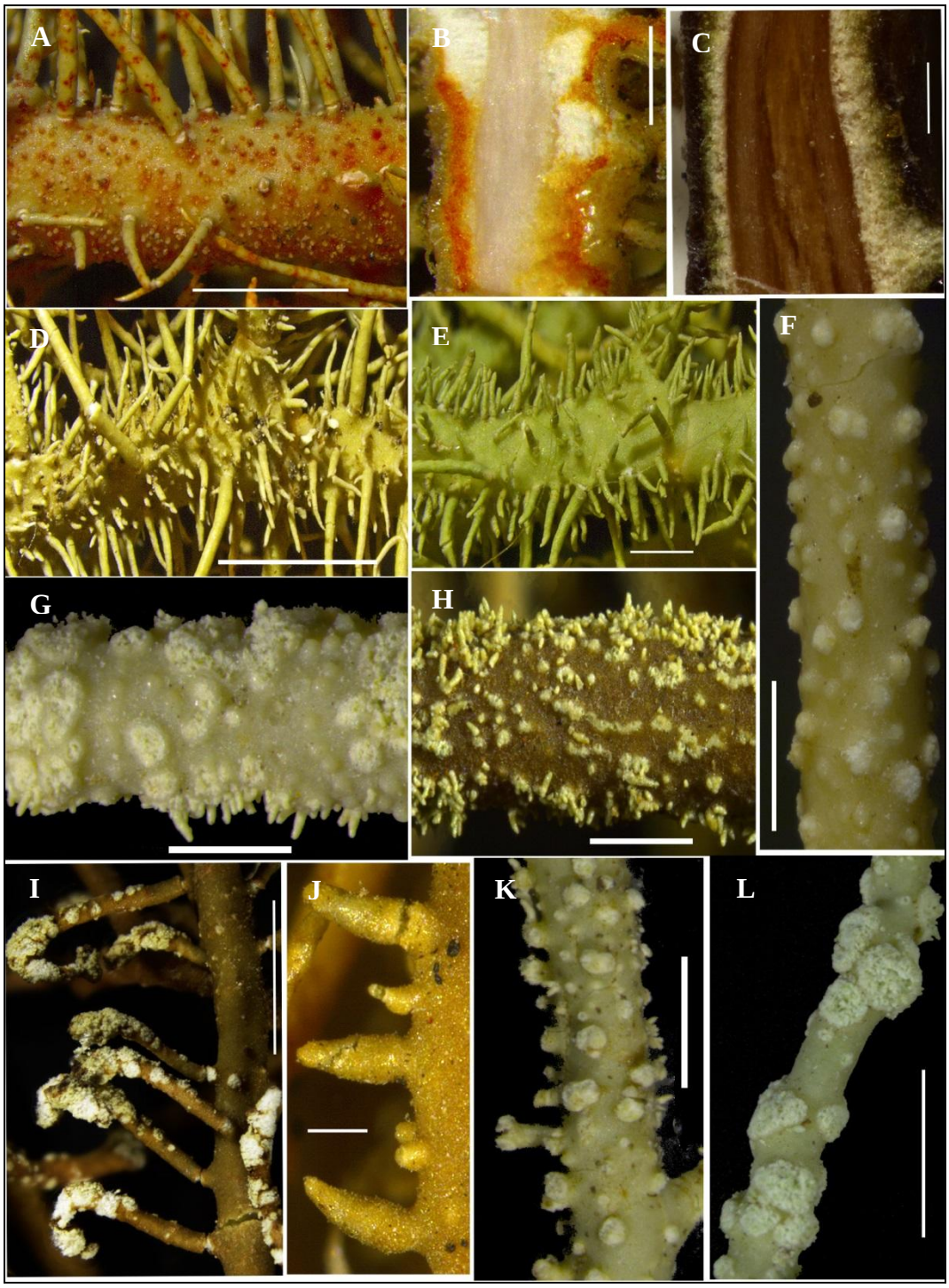


Figure 8. Morphological characters used in the taxonomy of *Usnea*. **A**, red cortical pigment in *U. erinacea*; **B**, orange medullar pigment in *U. aurantiaca-parvula*; **C**, central axis ochraceous in *U. mexicana*; **D**, branch densely covered by spinulose fibrils in *U. parvula*; **E**, branches sparsely covered by spinulose fibrils in *U. subparvula*; **F**, punctiform soralia arise from tubercles in *U. subsilesiaca*; **G**, confluent soralia in *U. cornuta*; **H**, punctiform, irregular and even soralia in *U. perhispidella*; **I**, soralia simulating skeletal fingers in *U. dodgei*; **J**,

lageniform spinulose fibrils in *U. aurantiaca-parvula*; **K**, punctiform soralia arising from fibercles in *U. oreophila*; **L**, large soralia in *U. pseudobrasiliensis*.

6.2.2 Anatomical characters

An important criteria deserving attention is the relative thickness of cortex, medulla and axis (%CMA) in longitudinal section (Clerc 1984, 1998). To simplify the descriptions of species one particular type of CMA was defined by Truong *et al.* (2011): the CMA *cornuta*-type, with a thin (< 7.5%) and shiny cortex in section, a wide (>28%), lax to dense medulla and a thin axis (<30%), with a ratio A/M ratio < 1.3. For the same reasons the CMA *brasiliensis*-type was defined: 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) **(see Chapter 1)**. These two particular types of CMA were especially important to recognize the *Usnea cornuta* aggregate **(see Chapter 3)** and even to distinguish species within this aggregate **(see Chapter 5)**.

Cortex (thickness , aspect, color, structure): Regarding the thickness cortex may vary to thin (<6%), moderately thin (6–8%), moderately thick (8–10%) to thick (>10%) (Clerc 2011a). Another important character is the superficial aspect of the cortex in a longitudinal section (Clerc 1998): shiny (e.g. *U. cornuta* Körb), vitreous (e.g. *Usnea subscabrosa*), or mat (e.g. *U. subflammea* P. Clerc). This character was especially useful to separate two similar non-sorediate species (*U. kalbiana versus U. lunaria*) **(see Chapter 1)**.

Some species can be characterized by a red coloration on the cortex surface or inside the cortex (*U. erinaceae s. lat., U. meridionalis*) (Chapter 1). Pigmentation can vary from diffuse to red-spotted in *U. erinacea* s. lat., (Fig. 8A).

Ohmura (2001) conducted detailed anatomical studies of the cortical tissues of *Usnea* species and defined four types of cortical plectenchyma (*florida-, merrillii-, ceratina-,* and *eumitria*-types) regarding the hyphal type, the degree of thickening of the cell wall and lumina, the conglutination and the growing direction of hyphae. The type of cortical tissue was considered to have taxonomic importance by Ohmura (2001). Despite its taxonomical importance this character should be carefully interpreted since, for intance , the *merrillii*-type seems to occur at some stage of the ontogeny of each species (Ohmura 2001). The cortex anatomy of some of the brazilian species treated here was investigated, but the results of this investigation were not considered as being important and decisive in the taxonomy of *Usnea* in Brazil (see **Chapter 1**).

Medulla (thickness, aspect, color): Regarding the thickness cortex may vary to thin (<18%), moderately thin (18–23%), moderately thick (23–28%) to thick (>28%) (Clerc 2011a). The medulla is compact, dense to lax according to whether the individual hyphae are still visible and densely (medulla dense) or loosely (medulla lax) interwoven or not visible anymore (compact) (Clerc 1998, Ohmura 2001). The thicknes of the medulla is often correlated with these types of medulla: for example a thin medulla is typically very compact whereas a large medulla is often lax to dense (Ohmura 2001; Truong *et al.* 2011). We found one exception where the opposite occurs, in *U. fleigiae* a species with narrow but at the same time lax medulla **(see Chapter 1).** The medulla can have a pigment located just bellow the cortex (e.g. *U. steineri*) or spreading irregularly inside the whole the medulla (*U. aurantiaca-parvula*, Fig. 8B). The pigmentation present in medulla should be analysed with caution because 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 most probably due to the oxidation of these depsidones while the thallus is ageing. For more details on the medullary pigmented species, see Truong & Clerc (2012).

Central axis (thickness, aspect, color): Regarding the thickness, the axis may vary to thin (<30%), moderately thin (30–40%), moderately thick (40–50%) to thick (>50%) (Clerc 2011a). The axis is solid, more rarely fistulose (i.e. empty in its central part) in the basal part of large branches (and in saxicolous species like *U. oreophila*). It is usually whitish, with the exception of *U. mexicana* where axis is often ochraceous-brown pigmented (Fig. 8C).

6.2.3 Chemistry

There are two main groups of lichen compounds: primary metabolites (intracellular) and secondary metabolites (extracellular) (Elix 1996). An example of primary metabolite is the carbohydrates released by the algae and supplied to the fungus (glucose if the photobiont is a cyanobacteria; polyol if the photobiont is a green-algae) (Elix 1996).

The majority of organic components found in lichens are secondary metabolites (Elix 1996) produced by the mycobiont, and accumulated in the cortex (such as atranorin, usnic acid or fungal melanins) or in the medulla (depside, depsidones, terpenes). They are deposited as extracellular tiny crystals on the outer surfaces of the hyphae (Elix 1996; Huneck 1999) and correspond to a high number of unusual secondary metabolites that are almost exclusively

produced by fungal (Stocker-Wörgötter 2015). Various potential biological roles have been identified for such metabolites, including photoprotection of the algae against intense radiation, as well as antiherbivory, antiviral, antibacterial, antitumoral and antioxidant actions (Huneck 1999; Fernández *et al.* 2006; Molnár & Farkas 2010).

Secondary metabolities have been widely used in the taxonomy of lichens (Culberson 1969; Hawksworth 1976; Lumbsch 2002). However, the significance of chemical variation within species has been widely debated and is still controversial (Brodo 1986). For example most North American, Japanese and Australian lichenologists are more willing to distinguish chemically different populations at the species level (Lumbsch 1998). Some molecular studies have shown that chemistry seems to be less variable in lichen species than thought before (Schmitt & Lumbsch 2004). On the other hand in *Bryoria fuscecens s.l.* clades defined with molecular data are not correlated with chemotypes (Boluda *et al.* 2015).

Culberson & Culberson (1976) introduced the term "chemosyndromic variation" in reference to lichens that produce a group of biosynthetically related compounds, i.e. substances produced in different steps of the same biosynthetical pathway (e.g salazinic, constictic and stictic acids) where the concentration of each compound might vary in different species.

All *Usnea* species have usnic acid in the cortex in variable amounts. This pigment was isolated for the first time by Rochleder in 1834, from *U. barbata* and other lichens (Honda & Vilegas 1998). The majority of secondary metabolites are formed via acetyl-polymalonate pathway (e.g β-orcinol-depsidones; β-orcinol-depsides) and are produced in various amounts in the medulla. In addition, compounds derived from the mevalonic acid pathway (e.g. terpenes and steroids) are sometimes detected in the medulla (Table 1). However, their identity and their value as diagnostic characters in the genus *Usnea* are yet poorly understood.

Table 1. Major class of lichens substances found in the genus *Usnea*. Source: Elix (2014).

Acetate-polymalonate pathway		
Fatty acid		e.g. caperatic acid
Phenolic compounds	depsidones	e.g. constictic, fumarprotocetraric, galbinic, norstictic, protocetraric, psoromic, salazinic, stictic acids
	depsides	e.g. barbatic, diffractaic, thamnolic, squamatic acids
	orcinol Depsidones	e.g. lobaric acid
Mevalonic acid pathway		
	steroids	
	terpenes	

Fatty acids were reported in the genus *Usnea* (Clerc 1992; Halonen *et al.* 1999; Halonen 2000; Ohmura 2001; Rodriguez 2011). Some of them were found to have an

important diagnostic value in the taxonomy of *Usnea*. For example, bourgeanic acid and the murolic acid aggregate are diagnostic for *U. esperantiana* Clerc and *U. hirta* (L.) Wigg. respectively (Clerc 1992). Recently a compilation of data focusing on phytochemistry aspects of *Usnea* revealed the presence of seven distinct fatty acids in the genus (Prateeksha *et al.* 2016). Although fatty acids seem to be relatively frequent in Brazilian specimens of the genus *Usnea*, their identity and taxonomical significance is not well understood yet. For instance, caperatic acid was the only well known fatty acid identified with some taxonomic importance **(see Chapter 1)**. Besides in *Usnea* well known secondary metabolites, we found some unknown substances which prooved to be useful diagnostic characters **(see Chapters 2 and 5).**

6.2.4 Molecular characters (DNA)

Molecular systematics studies mainly includes two different approaches: a phylogenetics one and a population genetics one. It is useful to point out here that the term *phylogeny* was originally used when studying evolutionary relationships between species, whereas *genealogy* was the preferred term to describe the shared ancestry within a population (Vandamme 2009). One difference between the two approaches comes from the fact that molecular phylogeny uses a single to a few specimens to represent a species, while population genetics relies on many specimens per species. Furthermore population genetics is, among others, interested in polymorphysms and their evolution as well as in the way populations are structured.

In summary, molecular phylogeny usually uses a few specimens to represent one species while population genetics uses many specimens per species since they are interested in mutations that are not fixed (i.e. not presents in all members of a species).

6.2.4.1 Molecular markers

Nuclear ribosomal marker or region, especially the internal transcribed spacer (ITS) are widely used for phylogenetic studies and ITS is the marker used to barcode *Fungi* (Del-Prado *et al.* 2010; Kelly *et al.* 2011, Schoch *et al.* 2012). With its high mutation rate, ITS provides a signal with a high inter- and intraspecific genetic variability. Since this spacer is of small size and present in a high number of copies within the genome, it is relatively easy to study (Schoch *et al.* 2012).

However, the use of ITS alone usually provide poorly supported phylogenies especially in deeper relationships among taxa. Single copy protein-coding genes such as the RNA polymerases (RPB1 and RPB2) and *Mcm7* were successfully used to obtain well-resolved and highly supported phylogenies (Crespo *et al.* 2007, Schmitt *et al.* 2009, Divakar *et al.* 2015). These three markers (ITS, *Mcm7*, RPB1) were prooved to be useful studying the phylogeny of *Usnea* (Truong *et al.* 2013a). Additionally, three other nuclear markers were used by Mark *et al.* (2016) in their studies of *Usnea*: the partial intergenic spacer (IGS), fragments from β-tubulin (Bt) and RPB2.

6.2.4.2 Incomplete Lineage sorting

In many cases a small number of sequenced loci are sufficient to infer phylogenetic relationships between organisms without any ambiguity. In a growing number of cases, however, different markers produce different topologies. Disagreement among tree topologies can be due to multiple factors, including incomplete lineage sorting (ILS, **Figure 9**) (Naciri & Linder 2015; Hahn & Nakhleh 2016; Leavitt *et al.* 2016b).

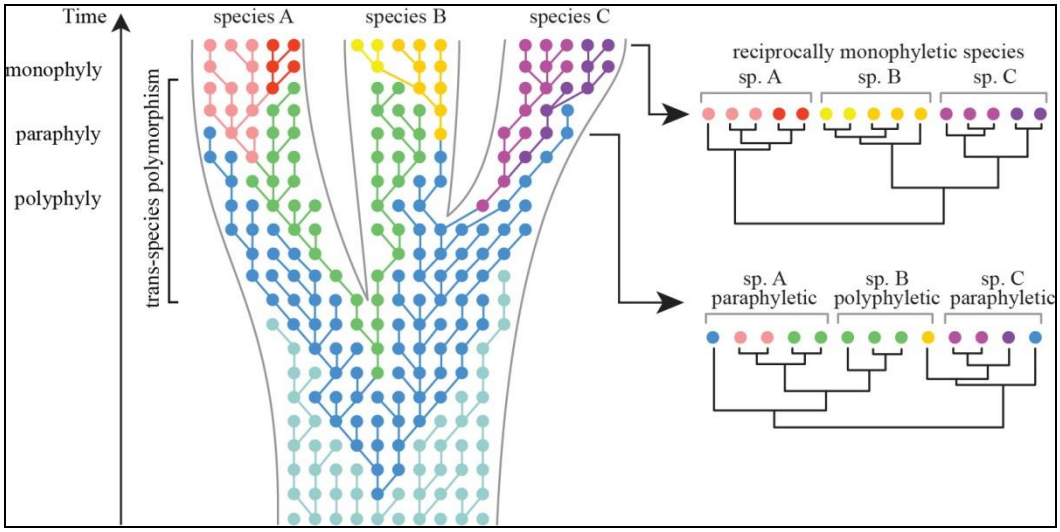


Figure 9. Time to reach reciprocal monophyly after speciation. Each dot represents an individual gene copy, each colour a different allele, and each line connects a gene copy to its ancestor in the previous generation. Within a population, selection and/or drift will result in changing allele frequencies over time. In the initial stages of lineage splitting, sister species will largely share identical alleles, which has important consequences for species delimitation. In this example, constructing a gene tree at an early stage of speciation would result in none of the three species being monophyletic. Only after sufficient time has gone by, alleles will be

completely sorted in each lineage, resulting in reciprocal monophyly for each of the three species. Source: Leliaert *et al.* (2014).

6.2.4.3 Multi species coalescent approach versus concatenation

Some phylogenetical analyses (consensus methods) assume that the most commonly observed topology represent the species tree, whereas some methods relax this assumption but assume that ILS is the only cause of discordance (so-called "coalescent" methods) (Hahn & Nakhleh 2016). The multi species coalescent based methods (MSC; Rannala & Yang 2003) accommodate topological heterogeneity among gene trees, basically with two parameters, the speciation time (Θ) and the effective population size (N) to reach coalescent time after speciation. Other factors, beside the ILS, that can influence gene tree heterogeneity in plants are: intergenomic transfers, hybridization, genome organisation, demography, selection, and phylogeographic structure (Naciri & Linder 2015) that are not taken into account in the MSC.

Before the advent of the MSC based methods, reconstructions of species phylogenies has relied mostly on concatenation methods, in which phylogenies are inferred from a single combined gene matrix (Huelsenbeck *et al.* 1996). These methods assume that all the involved genes have evolved according to the same or very similar evolutionary histories. However, studies have shown that concatenation methods can yield misleading results (Xi *et al.* 2014) and therefore the outcomes need to be carefully interpreted.

This approach is now implemented in several softwares used for species delimitation based on DNA sequences data (Leavitt *et al.* 2015) and have been largely applied on species delimitation within lichen forming-*Fungi* (Singh *et al.* 2015, Alors *et al.* 2016, Leavitt *et al.* 2016a-b, Wei *et al.* 2016) including the genus *Usnea* (Mark *et al.* 2016). To estimate species boundaries in the *Usnea cornuta* aggr. a MSC based species delimitation method was applied and an example of ILS was found **(see Chapter 3).**

7. Objectives of this study

- Describe the diversity of *Usnea* focusing on the species occuring in southern Brazil by providing detailed taxonomic treatments, including species descriptions, nomenclatural revisions, ecological and distributional informations as well as identification keys;
- Explore species boundaries and evaluate morphologically circumscribed species and chemotypes in the light of molecular data;
- Investigate the presence of terpenes and other chemical structural families on some species of the genus *Usnea* from Brazil.

8. Material and Methods

8.1 Field work and herbarium studies

Over 2177 *Usnea* specimens were analyzed in this study: 1057 specimens were collected by the author in 27 municipalities mainly in the Southern Brazil (**Table 2**), some (ca. 150) were received as donation from colleagues, and a total of 737 specimens were analyzed from the following national herbaria: BHCB, CESJ, CGMS, HAS, ICN, JPB, MBM, RB, SP, UFP, UPCB. Additionally we ask for the loan of 153 specimens (mainly types) from the following international herbaria: B, BM, BR, FH, FI, H, K, LBL, S, TUR, VAL, W, WU. The study of type specimens enabled to resolve nomenclatural questions and to stabilize the use of species names in Brazil. Some 80 specimens collected in Brazil and housed in G were analyzed. Most of them were studied or described by Müller Argoviensis, or collected by Klaus Kalb and Sybille Vermont-Grundlehner.

All voucher specimens collected during field trips are deposited in the Federal University of Rio Grande do Sul (ICN) and some duplicates in the Botanical Garden of Geneva (G).

Table 2. Municipalities from were *Usnea* specimens were collected in Brazil. Localities were mentioned under parentheses when one specifical locality was sampled for each municipality**.**

MINAS GERAIS
Catas Altas (Parque Natural do Caraça)
Gonçalves
PARANÁ
Campo Largo
Curitiba
PARANÁ
Foz de Iguaçu (Parque Nacional de Foz de Iguaçu)

Guaratuba (Morro dos Perdidos)
Palmeira (Recanto dos Papagaios)
Paranaguá (Ilha do Mel)
Rio Branco do Sul
RIO GRANDE DO SUL
Cambará do Sul (Parque Nacional Aparados da Serra-Serra Geral)
Caraá (Apa Caraá)
Porto Alegre (Morro Santana)
São Francisco de Paula (Floresta Nacional São Francisco de Paula)
SANTA CATARINA
Alfredo Wagner (Reserva Particular do Patrimônio Natural Rio das Furnas)
Campo Alegre
Dom Pedro de Alcântara (Reserva Particular do Patrimônio Natural Luiz Batista)
Florianópolis
Garuva
Itapoá
Joinville
Palhoça
Santo Amaro da Imperatriz
São Francisco do Sul
São Pedro de Alcântara
Urubici (Parque Nacional de São Joaquim)
SÃO PAULO
Apiaí
Botucatu

8.2 Species identification

Sometimes, some specimens are impossible to identify, this for several reasons: i) when the specimens are poorly developed (juvenile states), ii) when the specimen was exposed to extreme climatic conditions (shady or sun exposed localities); iii) when specimens are not correctly collected (as for example without the basal part), or damaged (when are for instance collected from the ground with some necrotic parts that might look like a reddish pigmentation); iv) when they are infected by lichenicolous *Fungi*; v) when the group to which the specimen belongs is still poorly known. When such specimens need to be identified, chemistry should be investigated with TLC and specialists be consulted. In order to identify the lichenicolous *Fungi* commonly growing on the surface of the *Usnea* thallus specially literature should be consulted (see *Parasiti Usnearum*, British Lichen Society Bulletin, winter 2014, Millanes *et al.* 2014).

In order to deal with such a large amount of material, specimens were subdivided into the following morphological groups:

• Erect-shrubby fertile species (**Chapter 1**)

• The *Usnea cornuta* aggregate (**Chapters 3 and 5**)

• Pendulous species (partially treated in this thesis **(Chapter 2**)

• Saxicolous (partially treated in this thesis) (**Chapter 6**)

• Erect-shrubby sorediate species (partially treated in this thesis) (**Chapter 5**)

• Species with a tubular central axis - *Eumitria* (not treated; see Truong & Clerc 2013)

• Species with a cortical or medullar pigmentation (not treated; see Truong *et al.* 2011; Truong & Clerc 2012)

8.3 Morphological and anatomical studies

The morphology of specimens was examined using a Leica MS5 stereo microscope, with spores measurements made using a Leica DM2000 microscope. The diameter of branches and the relative thickness of the cortex, medulla and axis (CMA%) were measurued using well-developed thicker branches at 40× magnification according to Clerc (1984).

Scratches of the hymenium (apothecia) were made using a hand-razor and montage in water to measure the ascospores. Longitudinal sections of the cortex were made and montage in GAW (glycerin / ethanol / whater: 1/1/1) follow Ohmura (2001) and in lugol in order to evaluate the types of the cortex (Chapter 1).

8.4 Chemical studies

Spot tests using potassium hydroxide (KOH, abbreviated as K), calcium hypochlorite ($Ca(ClO)_2$, abbreviated as C), or paraphenylenediamine (P) were directly applied to the medulla in longitudinal sections of the branches according to Hale (1979).

Thin-layer chromatography (TLC): Secondary compounds were extracted in acetone, boiled and spotted several times (5–10) onto Merck silica gel 60 F254 glass plates. Chemical analyses were performed in three routinely used solvent systems: A (toluene/ dioxane/ acetic acid: 180:45:5), B (n-hexane/MTBE/ formic acid–65:40:10) and C (toluene/ acetic acid–200:30) following Culberson & Ammann (1979) with solvent B modified according to Culberson & Johnson (1982). After brief drying, the plates are visualized on UV light (254 nm and 365 nm) and are sprayed with sulfuric acid 10%. until wet, and then heated at 110º until development of spots, which were visualized on visible light.

An alternative spray solvent, anisaldehyde sulfuric reagent (ANS: anisaldehyde/acetic acid/methanol/sulfuric acid–0.5:10:85:5, Le Pogam *et al.* 2015), were tested on some

specimens for which the presence of terpenes were suspected (without color tests reactions, K–, C–, P–). Then, three species were choice (*U. malmei, U. moreliana and U. papillata*) due their intensively occurrence in Brazil to proceed the TLC investigations with more solvents systems, controls and spray solvents (see Chapter 4 for details).

TLC were done for ca. 1500 specimens (ca. 70%) of the specimens analyzed from which 108 specimens were analyzed with ANS spray solvents.

8.5 Molecular studies

DNA was extracted from the central axis to avoid contamination by lichenicolous *Fungi* and following two different protocols: using the DNeasy Plant Mini Kit (Qiagen) according the manufacturer's instructions, with small modification as in Crespo *et al.* (2001) (**Chapter 1**); and used an SDS–Phenol–Chloroform protocol modified from Zolan & Pukkila (1986) (kindly provided by C. Truong) (**Chapter 3**).

PCR amplifications of the nuclear ITS rDNA and fragments of the *Mcm7* and RPB1 protein-coding genes were performed. The following specifical primers were used: UsITS3-F and UsITS4-R (Truong *et al.* 2013a) and four newly developed primers in this study. Thermal cycling parameters were indicated in Gerlach *et al.* (2017) (**Chapter 1**). Purification was performed by adding 2 µl of illustraTM ExoProStar to 10 µl of PCR product (**Chapter 1**) or using NucleoFast© plates (Macherey-Nagel) (**Chapter 3**).

The alignments of each region (ITS, *Mcm7* and RPB1) 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 (**Chapter 1**).

To infer the number of potential species in the *Usnea cornuta* aggr., we performed multispecies coalescent model (MSC) based species tree analyses using the program STACEY, version 1.2.2 implemented in BEAST 2.4.5. The input files were prepared using the STACEY template supplied by BEAUTI2 (**Chapter 3**).

CHAPTER 1

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

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Usnea subparvula A. Gerlach & P. Clerc from Brazil, Mato Grosso do Sul, Jardim.
Photo: A. Spielmann

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

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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

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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 stereo microscope, 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™ 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*).

				GenBank Accession numbers		
Species	DNA no.	Voucher	Chemotype	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 µm) and class II (spores ≥ 13 µm) (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

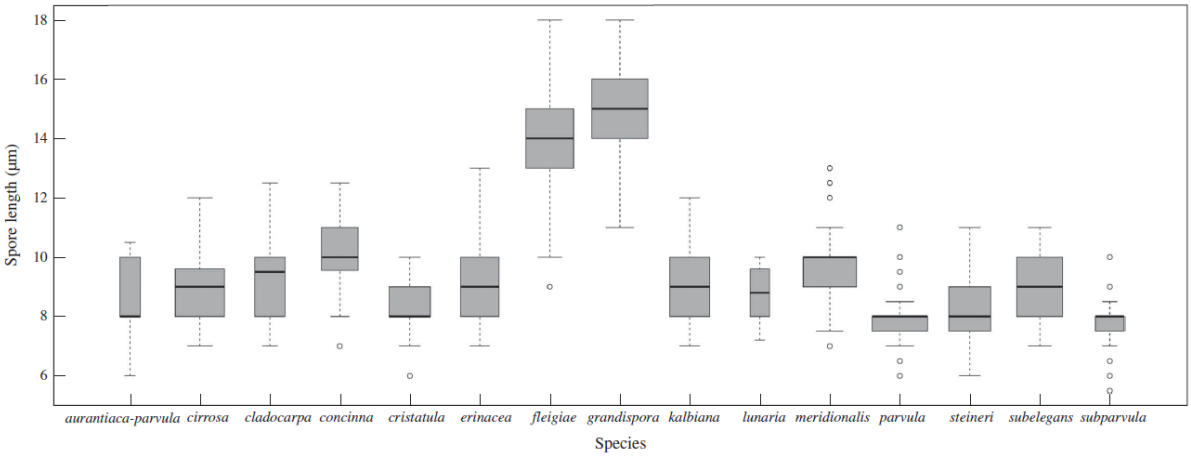


FIG. 1. Boxplots of spore length for each species of *Usnea* referred to in this study. Each boxplot shows the median (thick line) and standard deviation, and box width is proportional to the value of *n*. Dashed vertical lines correspond to the range. Outliers are represented by open circles.

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

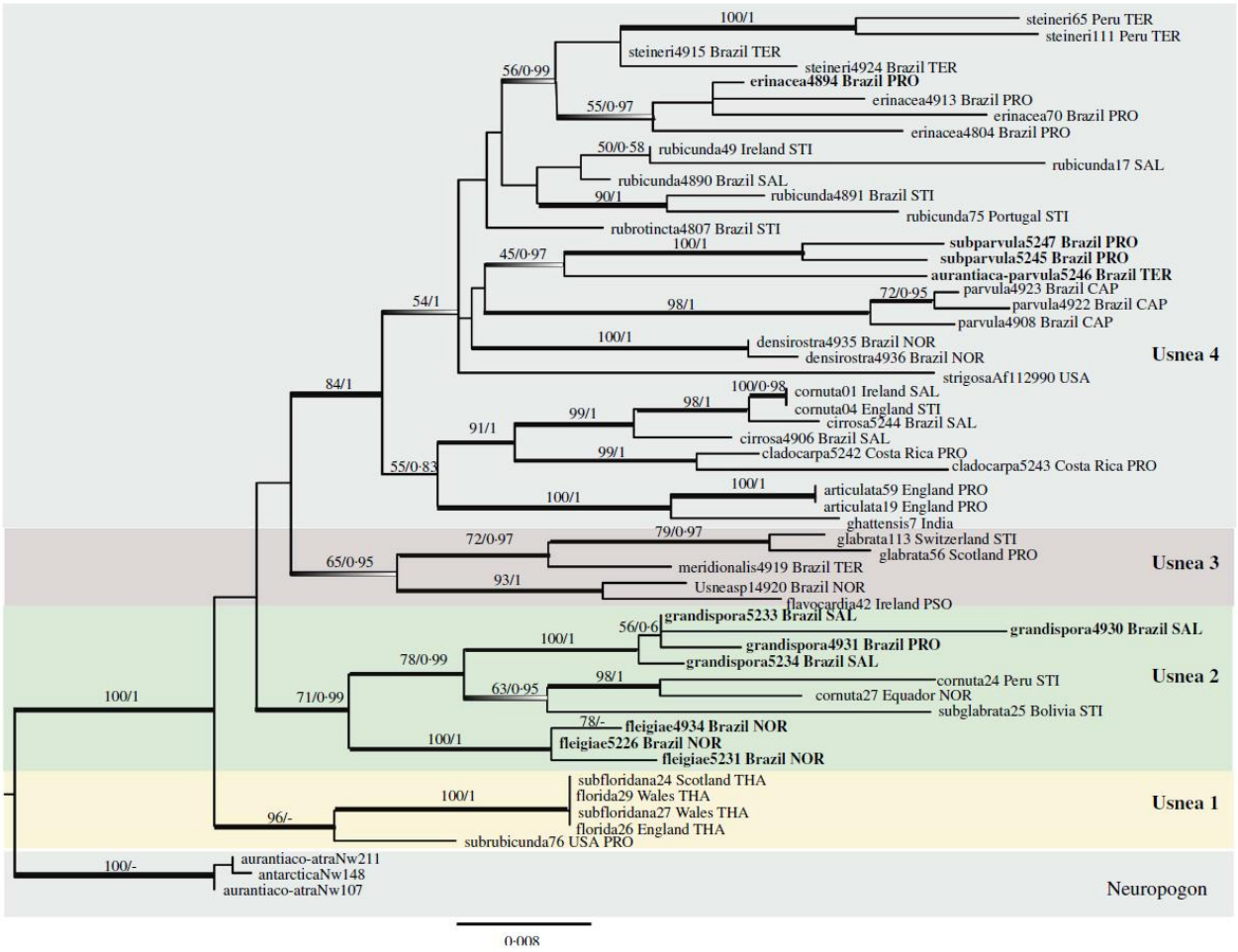
its internal nodes. Therefore, only the ML tree is shown here with posterior probabilities added adjacent to BS values.

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.


steineri65 Peru TER
steineri111 Peru TER
steineri4915 Brazil TER
steineri4924 Brazil TER
erinacea4894 Brazil PRO
erinacea4913 Brazil PRO
erinacea70 Brazil PRO
erinacea4804 Brazil PRO
rubicunda49 Ireland STI
rubicunda17 SAL
rubicunda4890 Brazil SAL
rubicunda4891 Brazil STI
rubicunda75 Portugal STI
rubrotincta4807 Brazil STI
subparvula5247 Brazil PRO
subparvula5245 Brazil PRO
aurantiaca-parvula5246 Brazil TER
parvula4923 Brazil CAP
parvula4922 Brazil CAP
parvula4908 Brazil CAP
densirostra4935 Brazil NOR
densirostra4936 Brazil NOR
strigosaAf112990 USA
cornuta01 Ireland SAL
cornuta04 England STI
cirrosa5244 Brazil SAL
cirrosa4906 Brazil SAL
cladocarpa5242 Costa Rica PRO
cladocarpa5243 Costa Rica PRO
articulata59 England PRO
articulata19 England PRO
ghattensis7 India
glabrata113 Switzerland STI
glabrata56 Scotland PRO
meridionalis4919 Brazil TER
Usneasp14920 Brazil NOR
flavocardia42 Ireland PSO
grandispora5233 Brazil SAL
grandispora4930 Brazil SAL
grandispora4931 Brazil PRO
grandispora5234 Brazil SAL
cornuta24 Peru STI
cornuta27 Equador NOR
subglabrata25 Bolivia STI
fleigiae4934 Brazil NOR
fleigiae5226 Brazil NOR
fleigiae5231 Brazil NOR
subfloridana24 Scotland THA
florida29 Wales THA
subfloridana27 Wales THA
florida26 England THA
subrubicunda76 USA PRO
aurantiaco-atraNw211
antarcticaNw148
aurantiaco-atraNw107
Usnea 4
Usnea 3
Usnea 2
Usnea 1
Neuropogon
0·008

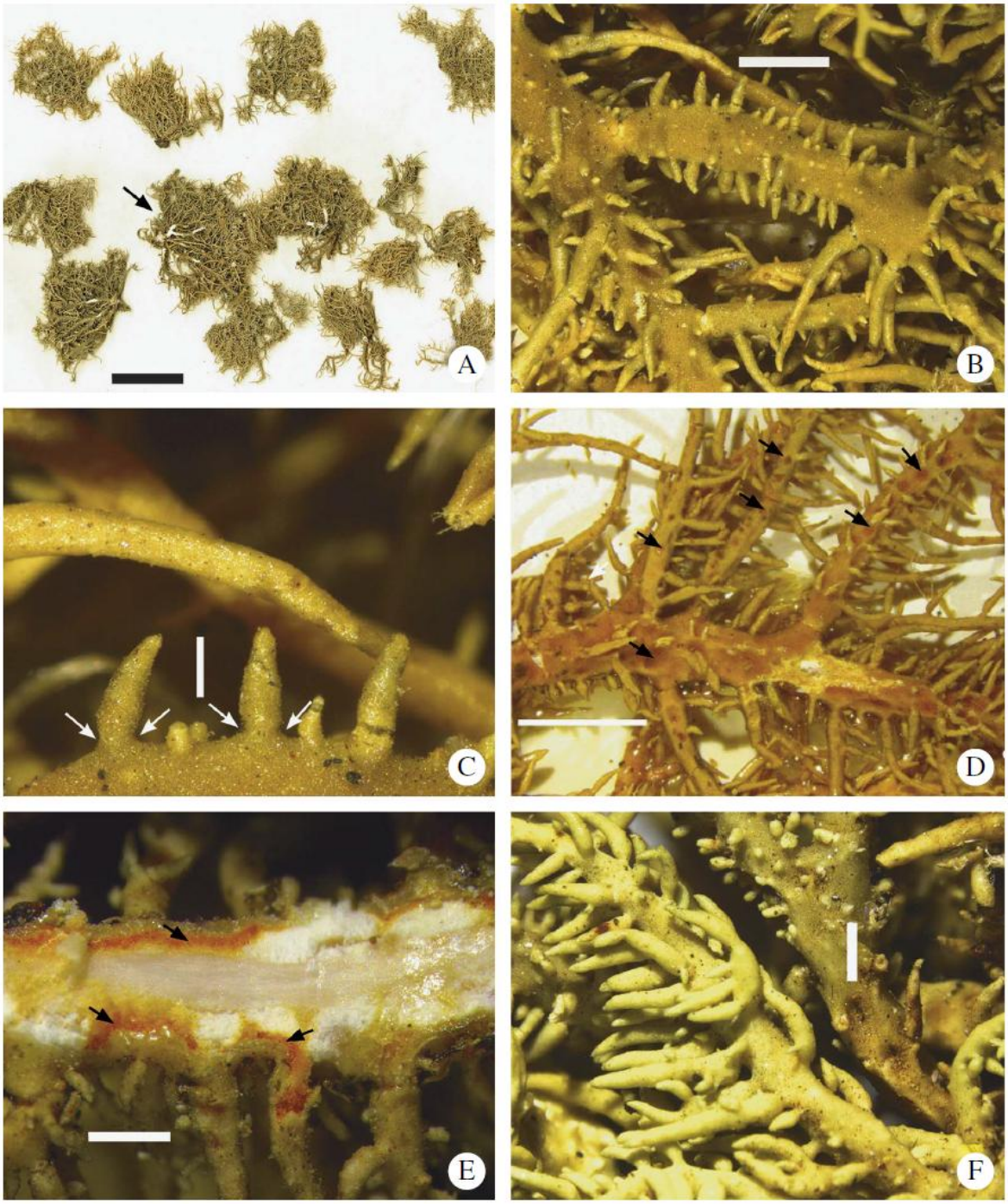


FIG. 3. *Usnea aurantiaca-parvula*. A–C, holotype: A, thallus; B, irregular branches with lageniform fibrils; C, simple lageniform fibrils constricted at the base (arrows). D, several minute foveolae (arrows) (*L. Krieger & M. Brügger* 1407b); E, Section through thallus with strong orange pigmentation 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.

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*

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

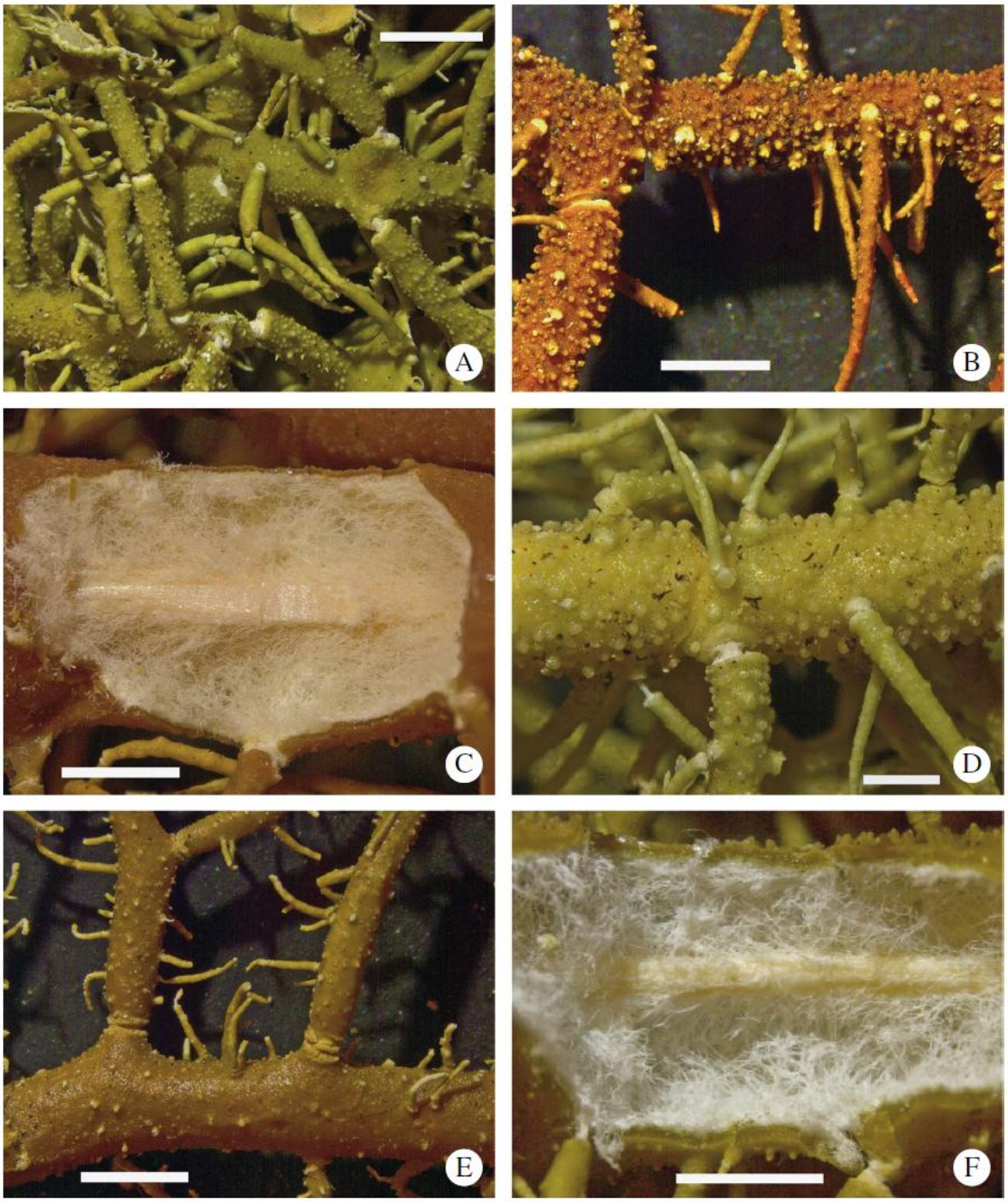


FIG. 4. A–D, *Usnea cirrosa*: A, branches constricted and inflated at ramification and foveolae (*E. Gumboski* 5020); B, branches slightly constricted and inflated at ramification and fibercles (*L. Canêz* 480); C, section through branch (*S. Grundlehner* s. n.); D, verrucose papillae (*A. Gerlach* 1510). 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

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

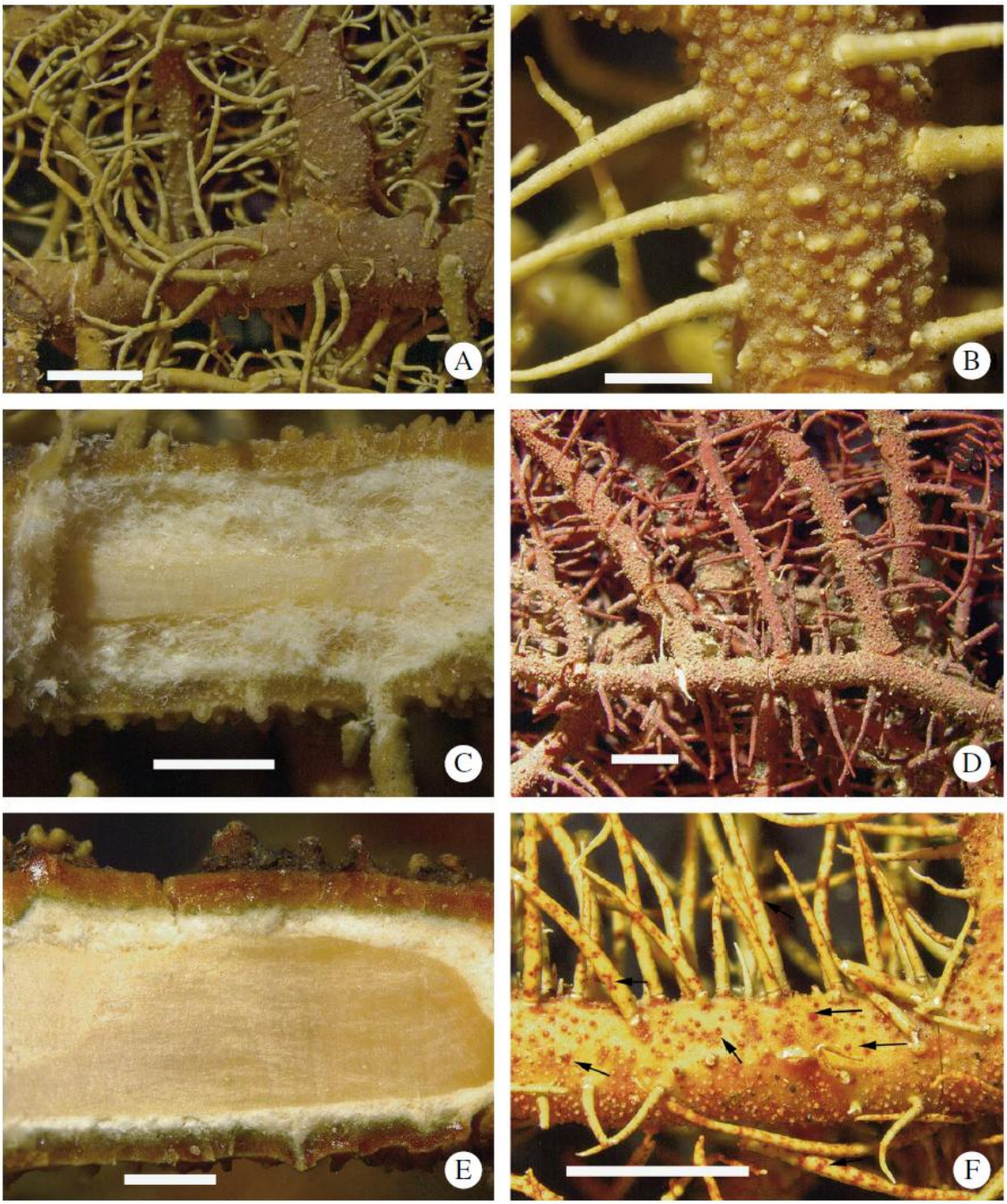


FIG. 5. A–C, *Usnea concinna* (*K. Kalb* s. n.): A, branches slightly constricted and inflated at ramification; B, verrucose tubercles; C, section through branch. D–F, *Usnea erinacea*: D, terete and tapering branches, the cortex is diffusely pigmented red on whole branches (*A. Gerlach* 1112); E, section 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.

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 Erdstraße 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).

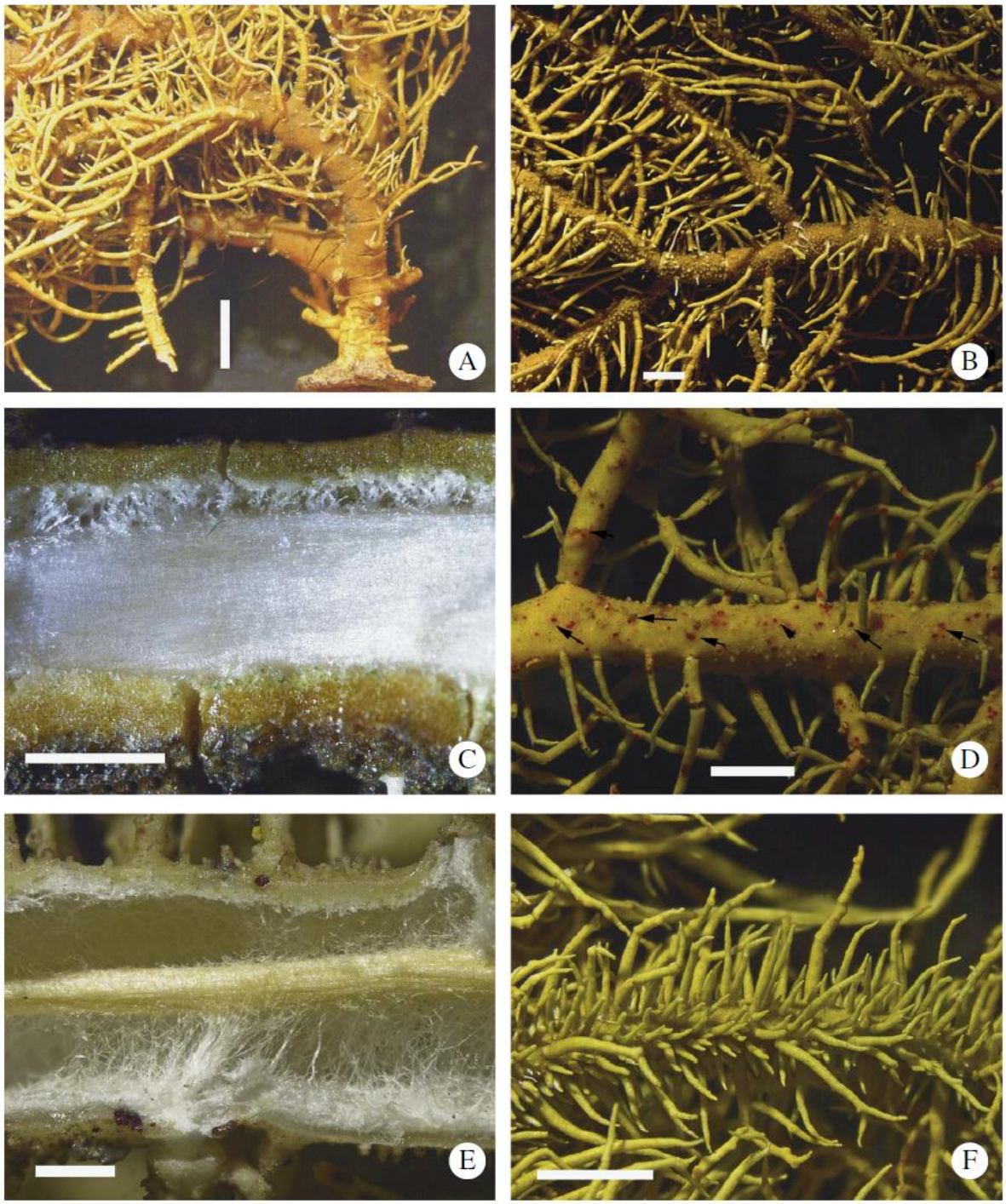


FIG. 6. A–C, *Usnea fleigiae*: A, trunk annulated, concolorous (holotype); B, branches annulated, slightly constricted and inflated at ramification (holotype); C, section through branch (isotype). D & E, *Usnea meridionalis*: D, fusiform branches with dark, red-pigmented dots on the cortex surface (arrows) (*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.

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

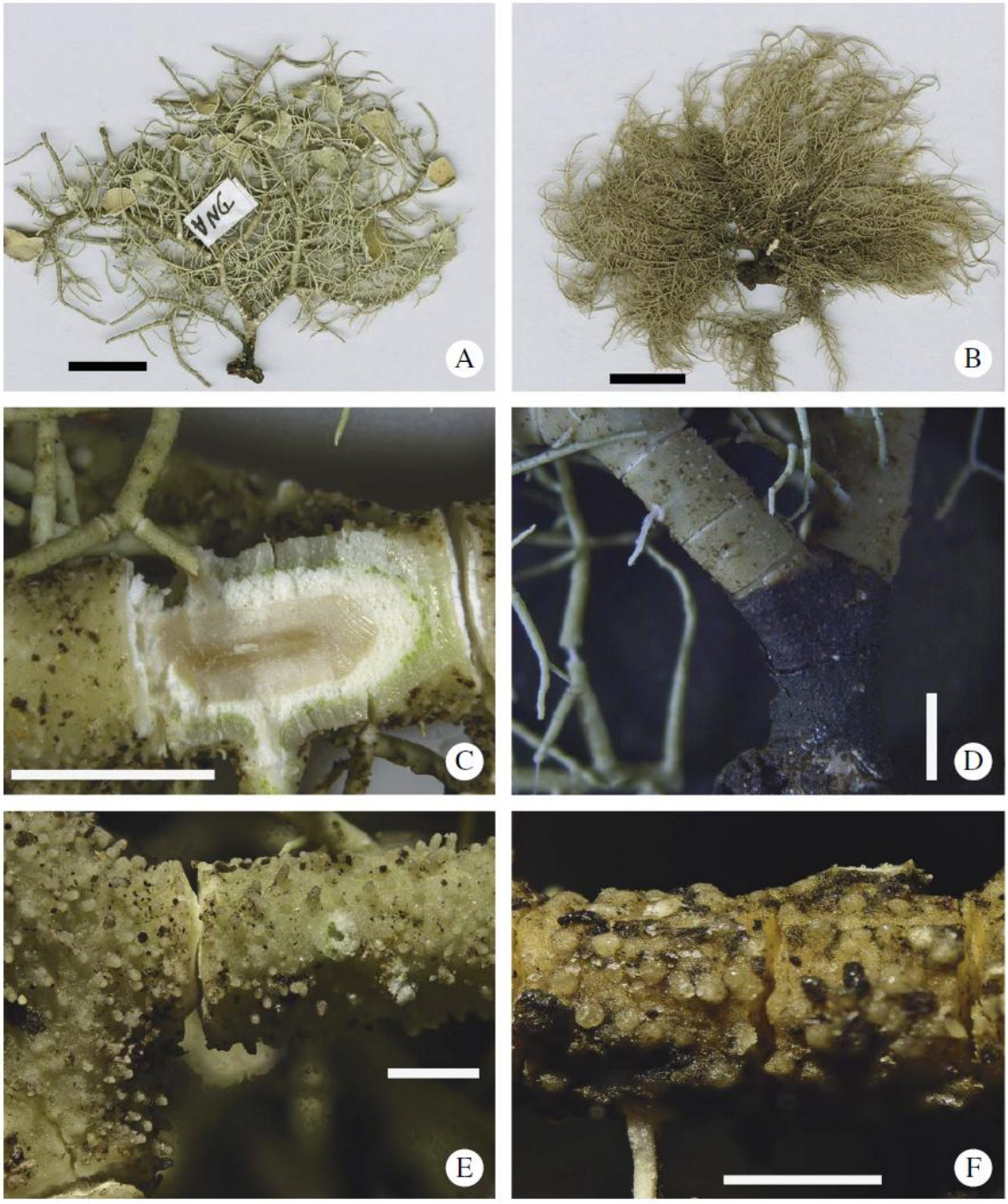


FIG. 7. *Usnea grandispora*. A, thallus (holotype); B, thallus with dense ramifications (*A. Gerlach* 1009); C, section through branch (holotype); D, trunk annulated, jet black (isotype); E, cylindrical papillae (holotype); F, verrucose tubercles, often not eroded at the apex. Scales: A & B = 1 cm; C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

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*

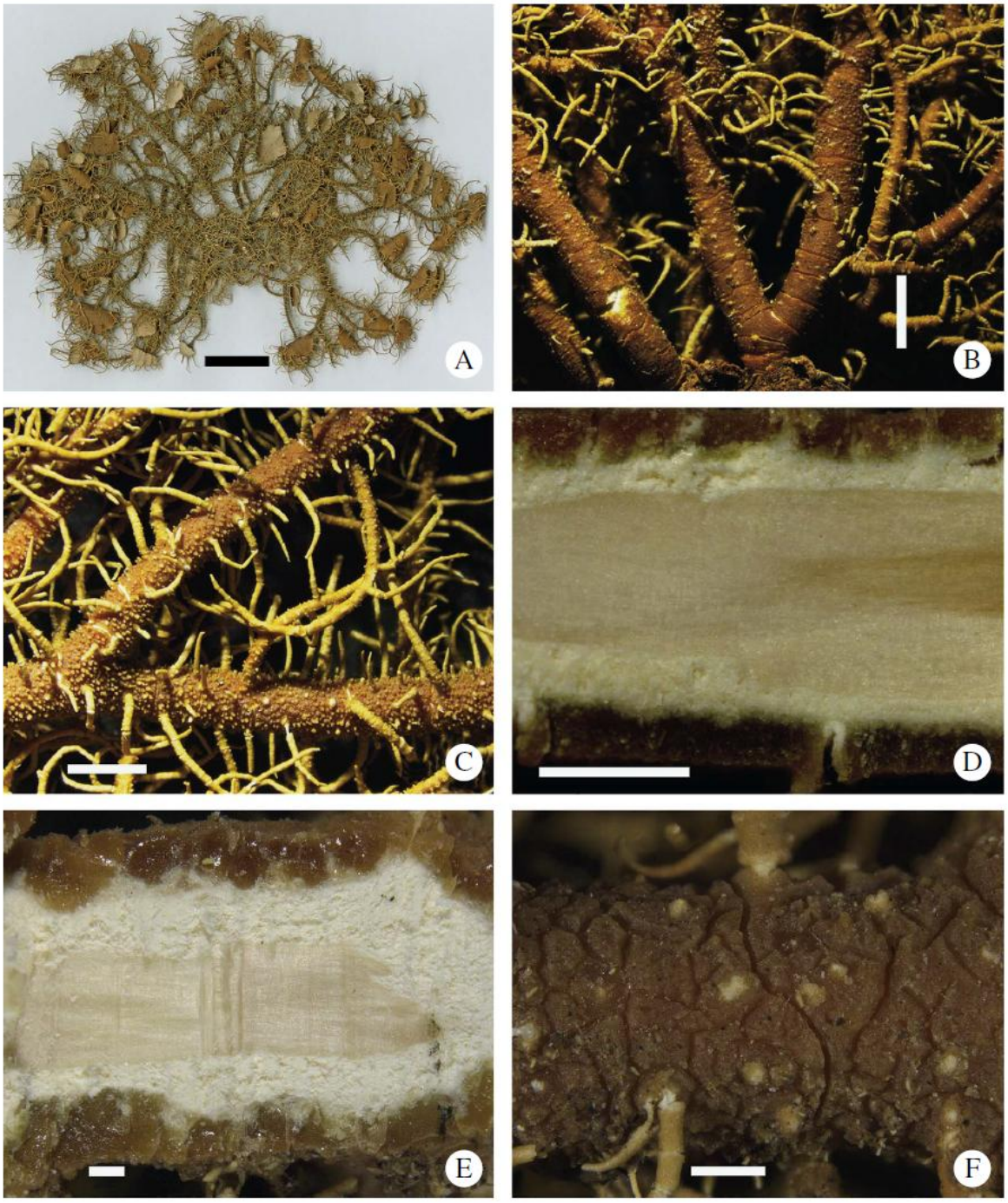


FIG. 8. A–D, *Usnea kalbiana* (holotype): A, thallus; B, trunk concolorous with branches with conspicuous annular cracks; C, branches annulated, terete and cylindrical, tubercles cone-shaped; D, section through branch with matt cortex. E & F, *Usnea lunaria* (holotype): E, section through branch with 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

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

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

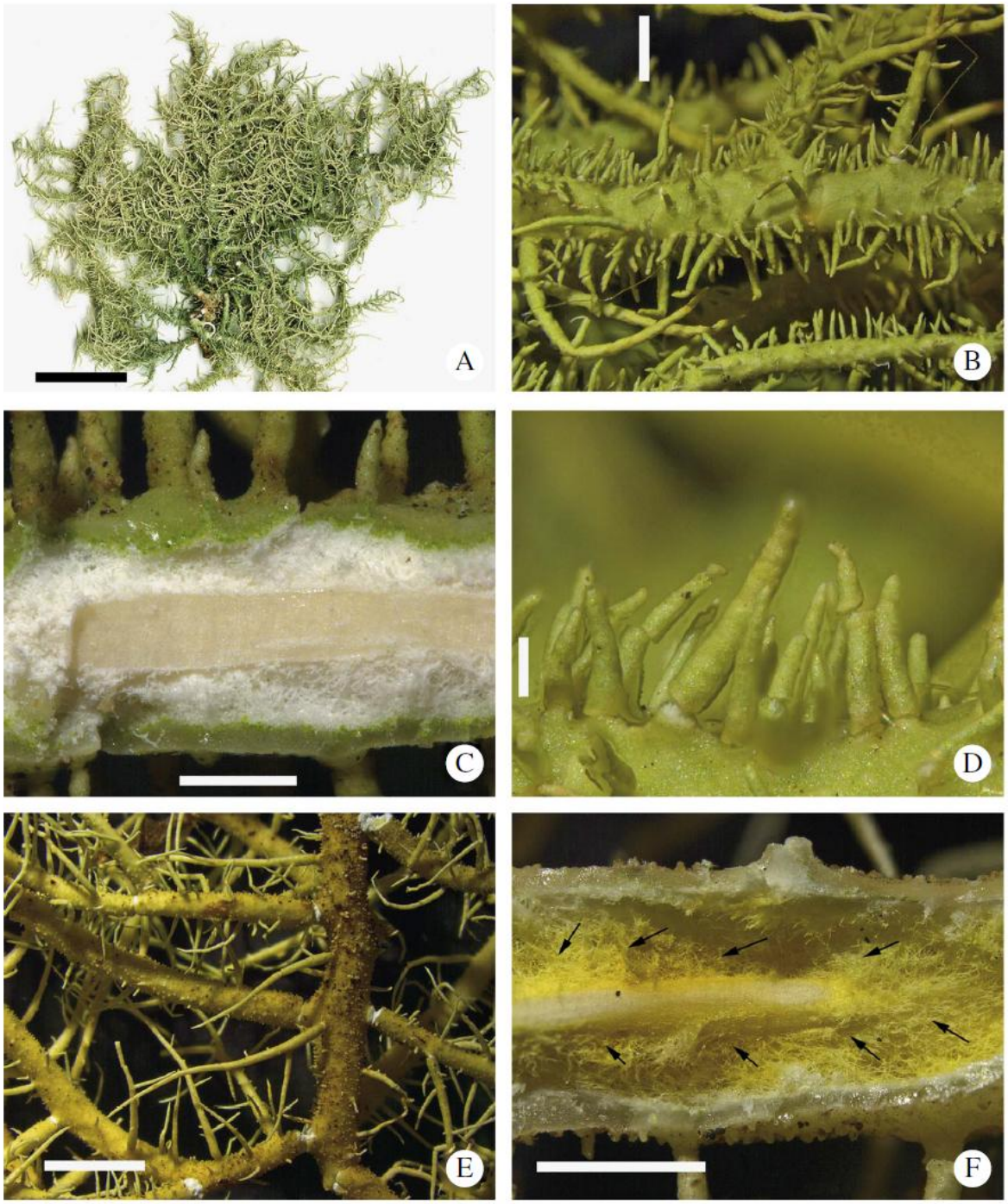


FIG. 9. A, *Usnea parvula*. A, branches densely covered with fibrils (*A. Gerlach* 870). B–D, *Usnea subparvula* (holotype): B, branches wider at ramification point, covered with spinulose fibrils; C, section through branch; D, details of conical spinulose fibrils. E & F, *Usnea* sp. 1 (*A. Gerlach* 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.

Baumpflanzung, 100 m, 1986, *Schäfer-Verwimp* L/7884 (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, *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 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. Canêz* 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 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-esorediate *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

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, pigment red 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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References

Akaike, H. (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control* **19:** 716–723.

Alvares, C. A., Stape, J. L., Sentelhas, P. C., de Moraes Gonçalves, J. L. & Sparovek, G. (2013) Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift* **22**(6)**:** 711–728.

Articus, K., Mattsson, J. E., Tibell, L., Grube, M. & Wedin, M. (2002) Ribosomal DNA and β-tubulin data do not support the separation of the lichens *Usnea florida* and *U. subfloridana* as distinct species. *Mycological Research* **106:** 412–418.

Awasthi, G. (1986) Lichen genus *Usnea* in India. *Journal of the Hattori Botanical Laboratory* **61:** 333–421.

Benjamini, Y. & Yekutieli, D. (2001) The control of the false discovery rate in multiple testing under dependency. *Annals of Statistics* **29:** 1165–1188.

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

Clerc, P. (1984*b*) *Usnea wirthii* – a new species of lichen from Europe and North Africa. *Saussurea* **15:** 33–36.

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

Clerc, P. (1998) Species concepts in the genus *Usnea* (lichenized Ascomycetes). *Lichenologist* **30:** 321–340.

Clerc, P. (2004) Notes on the genus *Usnea* Adanson. II. *Bibliotheca Lichenologica* **88:** 79–90.

Clerc, P. (2007) *Usnea*. In *Lichen Flora of the Greater Sonoran Desert Region* (T. H. Nash III, C. Gries & F. Bungartz, eds): 302–335. Tempe, Arizona: Lichens Unlimited, Arizona State University.

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

Clerc, P. (2011*b*) *Usnea*. In *Nordic Lichen Flora*, Vol. 4 (A. Thell & R. Moberg, eds): 107–127. Uppsala: Nordic Lichen Society.

Clerc, P. & Herrera-Campos, M. A. (1997) Saxicolous species of *Usnea* subgenus *Usnea* (lichenized Ascomycetes) in North America. *Bryologist* **100:** 281–301.

Crespo, A. & Lumbsch, H. T. (2010) Cryptic species in lichen-forming fungi. *IMA Fungus* **1:** 167–170.

Crespo, A., Blanco, O. & Hawksworth, D. L. (2001) The potential of mitochondrial DNA for establishing phylogeny and establishing generic concepts in the parmelioid lichens. *Taxon* **50:** 807–819.

Crespo, A., Lumbsch, H. T., Mattsson, J. E., Blanco, O., Divakar, P. K., Articus, K., Wiklund, E., Bawingan, P. A. & Wedin, M. (2007) Testing morphology-based hypotheses of phylogenetic relationships in *Parmeliaceae* (Ascomycota) using three ribosomal markers and the nuclear *RPB1* gene. *Molecular Phylogenetics and Evolution* **44:** 812–824.

Crespo, A., Divakar, P. K. & Hawksworth, D. L. (2011) Generic concepts in parmelioid lichens, and the phylogenetic value of characters used in their circumscription. *Lichenologist* **43:** 511–535.

Culberson, C. F. & Ammann, K. (1979) Standardmethode zur Dünnschichtchromatographie von Flechtensubstanzen. *Herzogia* **5:** 1–24.

Culberson, C. F. & Johnson, A. (1982) Substitution of methyl tert-butyl ether for diethyl ether in the standardized thin-layer chromatographic method for lichen products. *Journal of Chromatography* **238:** 483–487.

Darriba, D., Taboada, G. L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9:** 772.

Divakar, P. K., Crespo, A., Wedin, M., Leavitt, S. D., Hawksworth, D. L., Myllys, L., McCune, B., Randlane, T., Bjerke, J. W., Ohmura, Y. *et al.* (2015) Evolution of complex symbiotic relationships in a morphologically derived family of lichen-forming fungi. *New Phytologist* **208:** 1217–1226.

Fleig, M. & Grüninger, W. (2008) *Lichens of the Araucaria Forest of Rio Grande do Sul*. Pro-Mata: Field Guide No. 3: University of Tübingen, Germany.

Gutierrez, G., Blanco, O., Divakar, P. K., Lumbsch, H. T. & Crespo, A. (2007) Patterns of group I intron presence in nuclear SSU rDNA of the lichen family

Parmeliaceae. *Journal of Molecular Evolution* **64:** 181–195.
Hale, M. E. Jr. (1979) *How to Know the Lichens*, 2nd edition. Dubuque, Iowa: William C. Brown.
Hale, M. E. Jr. (1983) *The Biology of Lichens*, 3rd edition. London: Edward Arnold.
Halonen, P., Clerc, P., Goward, T., Brodo, I. & Wulff, K. (1998) Synopsis of the genus *Usnea* (lichenized Ascomycetes) in British Columbia, Canada. *Bryologist* **101:** 36–60.
Herrera-Campos, M. A., Clerc, P. & Nash, T. H. III, (1998) Pendulous species of *Usnea* from the temperate forests in Mexico. *Bryologist* **101:** 303–329.
Herrera-Campos, M. A., Nash, T. H., III & Garcia, A. Z. (2001) Preliminary study of the *Usnea fragilescens* aggregate in Mexico. *Bryologist* **104:** 235–259.
Iganci, J. R. V., Heiden, G., Miotto, S. T. S. & Pennington, R. T. (2011) Campos de Cima da Serra: the Brazilian Subtropical Highland Grasslands show an unexpected level of plant endemism. *Botanical Journal of the Linnean Society* **167:** 378–393.
IBGE (Instituto Brasileiro de Geografia e Estatística) (2004) *Mapa da vegetação do Brasil e Mapa de Biomas do Brasil*. Available from: http://www.ibge.gov.br
Katoh, K. & Standley, D. M (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30:** 772–780.
Kelly, L. J., Hollingsworth, P. M., Coppins, B. J., Ellis, C. J., Harrold, P., Tosh, J. & Yahr, R. (2011) DNA barcoding of lichenized fungi demonstrates high identification success in a floristic context. *New Phytologist* **191:** 288–300.
Kirika, P. M., Divakar, P. K., Crespo, A., Mugambi, G., Orock, E. A., Leavitt, S. D., Gatheri, G. W. & Lumbsch, H. T. (2016) Phylogenetic studies uncover a predominantly African lineage in a widely distributed lichen-forming fungal species. *Mycokeys* **14:** 1–16.
Knudsen, K. & Lendemer, J. C. (2006) Changes and additions to the North American lichen mycota–V. *Mycotaxon* **95:** 309–313.
Kroken, S. & Taylor, J. W. (2001) A gene genealogical approach to recognize phylogenetic species boundaries in the lichenized fungus *Letharia*. *Mycologia* **93:** 38–53.
Laundon, J. R. (1965) Lichens new to the British Flora: 3. *Lichenologist* **3:** 65–71.
Lücking, R., Del-Prado, R., Lumbsch, H. T., Will-Wolf, S., Aptroot, A., Sipman, H. J. M., Umaña, L. & Chaves, J. L. (2008) Phylogenetic patterns of morphological and chemical characters and reproductive mode in the *Heterodermia obscurata* group in Costa Rica (Ascomycota, *Physciaceae*). *Systematics and Biodiversity* **6:** 31–41.
Lumbsch, H. T. & Leavitt, S. D. (2011) Goodbye morphology? A paradigm shift in the delimitation of species in lichenized fungi. *Fungal Diversity* **50:** 59–72.
Marcano, V., Morales Méndez, A., Sipman, H. & Calderon, L. (1996) A first checklist of the lichen-forming fungi of the Venezuelan Andes. *Tropical Bryology* **12:** 193–235.
Miller, M. A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In *Proceedings of the Gateway Computing Environments Workshop (GCE), 14 November 2010, New Orleans, Louisiana*, pp. 1–8.
Motyka, J. (1936) *Lichenum Generis Usnea Studium Monographicum. Pars Systematica I*. Leopoldi: privately printed.
Motyka, J. (1938) *Lichenum Generis Usnea Studium Monographicum. Pars Systematica II*. Leopoldi: privately printed.
Nöske, N. M. & Sipman, H. J. M. (2004) Cryptogams of the Reserva Biológica San Francisco (Province Zamora-Chinchipe, southern Ecuador) II. Lichens. *Cryptogamie, Mycologie* **25:** 91–100.
Ohmura, Y. (2001) Taxonomic study of the genus *Usnea* (lichenized Ascomycetes) in Japan and Taiwan. *Journal of the Hattori Botanical Laboratory* **90:** 1–96.
Ohmura, Y. (2002) Phylogenetic evaluation of infrageneric groups of the genus *Usnea* based on ITS regions in rDNA. *Journal of the Hattori Botanical Laboratory* **92:** 231–243.
Ohmura, Y. (2012) A synopsis of the lichen genus *Usnea* (*Parmeliaceae*, Ascomycota) in Taiwan. *Memoirs of the National Museum of Nature and Science* **48:** 91–137.
Oliveira-Filho, A. T., Budke, J. C., Jarenkow, J. A., Eisenlohr, P. V. & Neves, D. R. M. (2015) Delving into the variations in tree species composition and richness across South American subtropical Atlantic and Pampean forests. *Journal of Plant Ecology* **8:** 242–260.
Osório, H. S. (1992) Contribution to the lichen flora of Brazil XXIX. Lichens from Ponta Porã, Mato Grosso do Sul. *Comunicaciones Botánicas del Museo de Historia Natural de Montevideo* **98:** 1–6.
Overbeck, G. E., Müller, S. C., Fidelis, A., Pfadenhauer, J., Pillar, V. D., Blanco, C., Boldrini, I. I., Both, R. & Forneck, E. D. (2007) Brazil's neglected biome: the South Brazilian Campos. *Perspectives in Plant Ecology, Evolution and Systematics* **9:** 101–116.
Poelt, J. (1970) Das Konzept der Artenpaare bei den Flechten. *Deutsche Botanische Gesellschaft, neue Folge* **4:** 187–198.
Poelt, J. (1972) Die taxonomische Behandlung von Artenpaare bei den Flechten. *Botanische Notiser* **125:** 77–81.
R Development Core Team (2016) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna. Available from: https://www.r-project.org/
Rambaut, A. (2009) *FigTree. v.1.4*. Available from: http://tree.bio.ed.ac.uk/software/figtree/
Rambaut, A. & Drummond, A. J. (2007) *Tracer version 1.5*. Available from: http://beast.bio.ed.ac.uk/Tracer
Rizzini, C. T. (1952) Species Organenses generis lichenum *Usneae*. (Omnes acidum usnicum

praebentes). *Revista Brasileira de Biologia* **12**(4): 337–348.

Rodriguez, J. M., Estrabou, C., Truong, C. & Clerc, P. (2011) The saxicolous species of the genus *Usnea* subgenus *Usnea* (*Parmeliaceae*) in Argentina and Uruguay. *Bryologist* **114:** 504–525.

Ronquist, F. & Huelsenbeck, J. P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19:** 1572–1574.

Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22:** 2688–2690.

Stevens, G. N. (2004) *Usneaceae.* In *Flora of Australia Vol. 56A, Lichens 4* (P. M. McCarthy & K. Mallett, eds): 78–98 & 107–115. Melbourne: ABRS/CSIRO.

Swinscow, T. D. V. & Krog, H. (1979) The fruticose species of *Usnea* subgenus *Usnea* in East Africa. *Lichenologist* **11:** 207–252.

Talavera, G. & Castresana, J. (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology* **56:** 564–577.

Tavares, I. I. & Sanders, W. B. (1998) Preliminary report on the short, apotheciate taxa of *Usnea* in the southwestern United States. In *Lichenographia Thomsoniana: North American Lichenology in Honor of John W. Thomson* (M. G. Glenn, R. C. Harris, R. Dirig & M. S. Cole, eds): 171–185. Ithaca, New York: Mycotaxon Ltd.

Truong, C. & Clerc, P. (2012) The lichen genus *Usnea* (*Parmeliaceae*) in tropical South America: species with a pigmented medulla, reacting C+ yellow. *Lichenologist* **44:** 625–637.

Truong, C. & Clerc, P. (2013) Eumitrioid *Usnea* species (*Parmeliaceae*, lichenized Ascomycota) in tropical South America and the Galapagos. *Lichenologist* **45:** 383–395.

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

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

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

Truong, C., Rodriguez, J. M. & Clerc, P. (2013*b*) Pendulous *Usnea* species (*Parmeliaceae*, lichenized Ascomycota) in tropical South America and the Galapagos. *Lichenologist* **45:** 505–543.

Vainio, E. A. (1890) Étude sur la classification naturelle et la morphologie des lichens du Brésil, pars prima. *Acta Societatis pro Fauna et Flora Fennica* **7:** i–xxix, 1–247.

Vareschi, V. (2001) El genero *Usnea* en Venezuela. *Boletin de la Academia de Ciencias Fisicas, Matematicas y Naturales de Venezuela* **61:** 9–63.

Wiens, J. J. (1998) Combining data sets with different phylogenetic histories. *Systematic Biology* **47:** 568–581.

Zahlbruckner, A. (1909) Lichenes (Flechten). In Ergebnisse der botanischen Expedition der Kaiserlichen Akademie der Wissenschaften nach Südbrasilien (V. Schiffner, ed.), 1901, Band 2. *Denkschriften der Mathematisch-Naturwissenschaftlichen Klasse der Kaiserlichen Akademie der Wissenschaften* **83:** 85–211.

CHAPTER 2

Notes on new and interesting pendulous species of the genus *Usnea* (Parmeliaceae: Lichenized Ascomycota) in Southern Brazil
To submit a *Phytotaxa*

Pendulous species of *Usnea* growing on *Araucaria angustifolia*. Brazil, Santa Catarina, São Joaquim National Park

Notes on new and interesting pendulous species of the genus *Usnea* (Parmeliaceae: Lichenized Ascomycota) in Southern Brazil

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Abstract: The diversity of pendulous *Usnea* species in Brazil is upgraded with reference to 23 species. *Usnea subsilesiaca* is newly described. *Usnea chilensis* and *U. transitoria* are new reported for Brazil; *Usnea dodgei*, *U. mexicana*, *U. papillata*, *U. subflammea* and *U. subscabrosa* for Southern Brazil. Modern descriptions are provided for *U. chilensis*, *U. disjuncta* and *U. venusta*. We propose the new combination *U. disjuncta*. Taxonomical notes for the new recorded species are provided, as well information about chemistry and distribution, together with an illustrative identification key.

Key words: corticolous, macrolichens, Neotropics, taxonomy

Introduction

Usnea is a hyperdiverse genus with more than 350 species in the Parmeliaceae the largest family of lichenized fungal species (Lücking *et al.* 2016). The genus is well-characterized by a fruticose thallus, branches with a cartilaginous central axis and the presence of usnic acid in the cortex. These characteristics are however shared with the genus *Protousnea*, that differs by the presence of dark brown apothecial discs without fibrils on the margin, the paucity of secondary morphological characters, the frequent presence of divaricatic acid in the medulla, its restricted geographical area in Southern South America (Krog 1976; Calvelo *et al.* 2005) and by molecular characters (Divakar *et al.* 2015).

The thallus of pendulous *Usnea* species exhibits branches running parallel almost from the basal part to the apices, and distinctly hanging downwards. Young thalli of pendulous species might look like shrubby to subpendulous species and it might be sometimes difficult to link them with the mature pendulous species. Exactly the same happens with shorter subpendulous thalli growing in bad or in suboptimal environmental conditions (Clerc 1998, Truong *et al.* 2013).

Modern taxonomical studies involving pendulous species of *Usnea* are available for Africa (Swinscow & Krog 1976, 1978), Australia (Stevens 2004), India (Awasthi 1986), Japan (Ohmura 2001), Mexico (Herrera-Campos *et al.* 1998, Herrera-Campos 2016), South America, especially for the Andes and the Galapagos islands (Truong *et al.* 2013), New England (Hinds & Hinds 2007), New Zealand (Galloway 2007) and Taiwan (Ohmura 2012).

In Southern Brazil, pendulous species of *Usnea* are remarkable and abundant, especially in Araucaria forest where they cover the trunks and the canopy of *Araucaria angustifolia* and other exotic trees as *Pinus* spp. and *Eucalyptus* spp.

The present study aims at continuing the ongoing investigation of the genus *Usnea* in tropical South America by formally describing one new species, reporting new records for Brazil (*U. chilensis, U. transitoria*), for Southern Brazil (*U. dodgei, U. mexicana, U. subflammea, U. subscabrosa*), resurrecting *Usnea venusta* and proposing a new combination for a badly known species (*U. disjuncta*). We provide full descriptions for the new species here proposed as well as for *U.chilensis*, and *U. disjuncta*. An illustrate key with diagnostic characters is provided.

Material and Methods

The following account is based on herbarium specimens deposited in the following herbaria: B, CESJ, CGMS, G, H, HAS, ICN, JPB, LBL, S, TUR, and W. Type material of all species discussed in this paper was studied. All voucher specimens collected during the field trips are deposited in the Federal University of Rio Grande do Sul (ICN) and some duplicates in G. For details of field trips that were carried out in Southern Brazil, see Gerlach *et al.* (2017).

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. Measures of the relative thickness of the cortex, medulla, and axis (CMA) were performed following Clerc (1984), at the widest part of the main branch under the stereoscope at 40×and are expressed as percentages of the total width of the branch.

The morphology of specimens was examined using a stereomicroscope Leica MS5, with measurements done using a Leica DM2000 microscope. The species concept and terminology used in this study follow Clerc (1987, 1998, 2006, 2011), Herrera-Campos *et al.* (1998), Truong *et al.* (2013). 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, were made according to Hale (1979), and directly applied to the medulla in longitudinal sections of the branches.

Results

A total of 23 species was found in Southern Brazil (415 specimens studied) (Table 1). *Usnea subsilesiaca* is new for science. *Usnea chilensis* Motyka and *U. transitoria* Motyka are new records for Brazil; *U. mexicana* Vain., *U. papillata* Motyka, *U. subflammea* P. Clerc s. l., and *U. subscabrosa* Motyka are new for Southern Brazil. The species *U. malmei*, *U. angulata* and *U. subscabrosa* are the most abundant species on Southern Brazil (Table 1).

Almost all species mentioned here have a thallus that is primarily pendulous to subpendulous with the exception of *U. dasaea* and *U. perhispidella* (rarely pendulous). The habitus is modulated by environmental conditions as discussed by Clerc (1998) and Truong *et al.* (2013). The cortex, medulla and axis (CMA) percentages are given for all the species found in Southern Brazil (Table 2).

Most species are characterized by one or two chemotypes, with the exception of *U. arthroclada* with three chemotypes (Table 3). With the exception of *U. chilensis* and *Usnea* sp. 1 that reproduce both sexually and asexually, all the species occurring in Southern Brazil reproduce either sexually or asexually.

Species	Descriptions	n	RS	SC	PR	ES
U. alata	Herrera–Campos *et al.*(1998)	7	■	■	■	
U. angulata	Herrera–Campos *et al.*(1998)	57	■	■	■	
U. arthroclada	Truong *et al.* (2013)	25	■			
*U. chilensis**	This study	35	■	■	■	
U. dasaea	Clerc & Herrera-Campos (1997)	8	■		■	
U. disjuncta	This study	2				■
U. aff. disjuncta	This study	4	■			
U. dodgei	Truong *et al.* (2013)	7	■	■	■	
U. geissleriana	Clerc (2006)	6	■		■	
U. malmei	Herrera–Campos *et al.* (1998)	62	■	■	■	
U. merrillii	Herrera–Campos *et al.* (1998)	26	■	■	■	
*U. mexicana***	Herrera–Campos *et al.* (1998)	6		■	■	
*U. papillata***	Herrera–Campos *et al.* (1998)	11	■	■	■	
U. perhispidella	Truong *et al.* (2013)	9	■	■	■	
U. regia	Truong *et al.* (2013)	25	■	■	■	
U. sanctaeritae	Herrera–Campos *et al.* (1998)	7	■			
*U. subflammea***	Clerc (2006)	42	■	■	■	
U. subgracilis	Herrera–Campos *et al.* (1998)	11	■	■	■	
*U. subscabrosa***	Herrera–Campos *et al.* (1998)	46	■	■	■	
U. subsilesiaca	This study	13	■	■		
*U. transitoria**	Herrera–Campos *et al.* (1998)	3		■	■	
U. venusta	This study	5	■	■		
U. sp. 1	This study	8	■		■	

TABLE 1. Specimens of *Usnea* analyzed in this study and their distribution in Southern Brazil. RS = Rio Grande do Sul, SC = Santa Catarina; PR: Paraná; ES = Espírito Santo. Bold = newly proposed species. *New records for Brazil. ** New records for Southern Brazil. n = number of specimens analyzed in this study.

***Usnea chilensis* Motyka** (Fig. 1)
Lich. Gen. Usnea Stud. Monogr. Pars Sys. 2: 578 (1938).
Type:–CHILE. MARGA, 700 m elev., 1933, *R. P. Anastase Pirion* (holotype, isotype LBL!; isotypes G!, S!). % C/M/A: 11/22/34 (holotype). Chemistry: usnic, salazinic, galbinic and norstictic acids (by Truong in 2011).

Description (n=35). *Thallus* pendulous, rarely entangled, up to 30 cm long, rough; *ramifications* mostly anisotomic, becoming isotomic–dichotomous towards the apices; *basal part* discrete to numerous when thallus entangled, often short (ca. 1mm), concolorous with the branches, with irregular and annular cracks; *main branches* up to 2 mm thick, irregular, sinuous, distinctly segmented; segments cylindrical, weakly to strongly ridged, rarely becoming slightly alate, often with cortical regeneration areas between segments; *lateral branches* not (main branches) to sometimes slightly constricted (secondary branches) at ramification point; *foveolae and depressions* scattered to numerous; *maculae* absent; *pseudocyphellae* absent (present only in young thalli and then linear); *papillae* cylindrical to

verrucose, often numerous, irregularly distributed; *tubercles* absent; *fibrils* few to numerous, slender, usually fish-bone like when numerous; *fibercles* rare; *soralia* (sorediate specimens, n=6) punctiform, ± circular, even with cortex to capitate, sometimes slightly stipitate, without distinct cortical margin, well delimited sometimes fusing together and looking like single linear soralia, numerous, ± regularly distributed, mainly on secondary and thinner branches, primarily originating *ad initio* on the cortex; *isidiomorphs* often present, bursting solitary on the cortex or in fasciculate clusters out of the soralia; *cortex* of the *ceratina*-type, thin to moderately thin [(2.5–)4.5–6%–7.5(–8.5)], slightly shiny, never vitreous; medulla ± thin to thick [(16.5–)22–26.5%–31(–38.5)], dense to somewhat looser towards the axis, orange around the axis (probably due to a high concentration of galbinic acid); axis thin to ± thick [(18–)26.5–34.5%–42.5(–57)], sinuous, brownish on older thallus, with an A/M ratio (0.5–)0,8–1.4–2(–3.5); *apothecia* up to 15 mm diameter, usually abundant, often present when soralia are absent (n = 29), lateral to rarely serial and terminal, disc whitish with marginal fibrils; *ascospores* (6–)6.8–7.5–8.2(–10.5) × (4–)4.5–5–5.5(–6) µm (n = 4); *pycnidia* rare, on terminal branches.

Chemistry. Medulla K+ yellow →red: salazinic, norstictic and galbinic acids (n = 37).

Habitat and distribution. Corticolous. Occurring in Argentina and Chile (Motyka 1938). Newly reported to Brazil, where it is quite frequent in the southern region.

Diagnostic characters. *Usnea chilensis* is characterized by irregular main branches that are distinctly segmented, not constricted at ramification point, weakly to strongly ridged and covered by cylindrical papillae, by punctiform soralia (when present), by a shiny cortex and an often sinuous central axis, and by the presence of galbinic acid in the medulla.

Variation. *Usnea chilensis* can be either sorediate (including the type) or non-sorediate and then caracterized by numerous apothecia growing laterally. Preliminary molecular studies (data not shown here) show that both morphotypes belong to the same taxon. Furthermore, the density of fibrils and papillae might strongly vary. Some specimens have a high density of fibrils in fish-bone-like arrangement whereas others have almost no fibrils. The shape of main branches might vary from weakly to strongly ridged and then ± alate. The ration A/M is most variable within this species.

Taxonomic notes. Specimens of *U. chilensis* with strongly ridged and almost alate main branches might resemble *U. angulata* or *U. alata*. However the central axis of the latter species is much larger and the medulla much thinner and compacter (Herrera-Campos *et al.* 1998). Moreover, galbinic is always absent in these species. *Usnea dasaea* Stirt., *Usnea dimorpha* (Müll. Arg.) Motyka, *U. feeana* Motyka, *U. filamentosa* Motyka, *U. furfurosula* Motyka and *U. subelegans* (Vain.) B. de Lesd. are other ± pendulous species with galbinic acid. *Usnea dasaea* and *U. furfurosula* are shrubby to subpendulous species with terete branches that are never ridged, with spinulose fibrils, without foveolae and papillae. They belong to the *Usnea cornuta* aggr. (Clerc & Herrera-Campos 1998). *Usnea dimorpha* has soralia that are mostly capitate at maturity and crowded on terminal/small lateral branches; fibrils and papillae are sparse or absent (Truong *et al.* 2013). *Usnea feeana* has large soralia with a distinct margin. The South African *Usnea filamentosa* is very similar to *U. chilensis*.

However we need to see more material from South Africa before synonymizing it with *U. chilensis*. *Usnea subelegans* is a non-sorediate species close to *U. dasaea* (Clerc 2007)

Specimens examined. BRAZIL. PARANÁ: Curitiba, en allant vers Vila Velha, 25°21′S, 49°34′W, 1989, *S. Vermont–Grundlehner s. n.* (G); Palmeira, margem do rio Cariú, Floresta Ombrófila Densa, mata ripária, 2013, *Engels & Lozano s. n.* (ICN); Ponta Grossa, beira de mata, 1975, *Krieger 13752* (CESJ). SÃO PAULO: Campos do Jordão, Parque Estadual do Horto Florestal, sobre *A. angustifolia*, 1600 m, 22°41′S, 45°31′W, 1989, *A. Chautems s. n.* (G). RIO GRANDE DO SUL: Bagé, BR 153, Casa de Pedra, 14 km estrada vicinal, capão no campo de pastagem, em ramos finos, 1988, *Fleig 3301* (ICN); Barracão, Espigão Alto, Parque Florestal Estadual, *Fleig* 3729b (ICN); Caçapava do Sul, arroio do Pessegueiro, próx. Cerro do Bugio, sobre ramos de arbusto, beira arroio, 1993, *Fleig 5698* (ICN); Cambará do Sul, Parque Nacional da Serra Geral, 2014, *Gerlach 1476* (ICN); Caxias do Sul, Distrito de Santa Lúcia do Piaí, localidade de Água Azul, 735 m, 29°11′48.6″S, 50°59′21.6″W, 2010, em galho caído, mata aberta que rodeia a fonte, *Spielmann & Giacornet 8666* (CGMS); *ibid*, 1990, *Mazzitelli s. n.* (HAS); Vacaria, Fazenda da Estrela, 900 m, 28°01′58″S, 50°58′17.5″W, corticícola na beira da estrada, 2003, *Canêz & Spielmann 441* (CGMS); Esmeralda, Estação Ecológica Aracuri, em arbusto, capão, 1983, *Fleig* (ICN); Porto Alegre, Guaíba, Estação Agronômica da UFRGS, 1985, *Balbueno s. n.* (ICN); São Francisco de Paula, Floresta Nacional de São Francisco de Paula, *Rosiak 06* (ICN); *ibid*, Hotel Veraneio Hampel, 2016, *Fazolino s. n.* (ICN); *ibid*, lago São Bernardo, derrière l'hôtel Cavalinho Branco, 1000 m, 29°27′34″S, 50°34′16″W, 1989, *Grundlehner s. n.* (G). SANTA CATARINA: Urubici, Parque Nacional de São Joaquim, Floresta de *Araucaria*, arredores do alojamento da Brigada Militar, 2014, *Gerlach & Alves 1375* (ICN); *ibid*, galho caído de *A. angustifolia*, 2015, *Alves s. n.* (ICN); Rio Negrinho, Fazenda Velha, 2007, *Gumboski 1025b* (ICN); *ibid*, Rio Preto, Fazenda Velha, 825 m, 26°17′56.8″S, 49°37′26″W, corticícola em borda de mata, 2012, *Spielmann 9966* (CGMS).

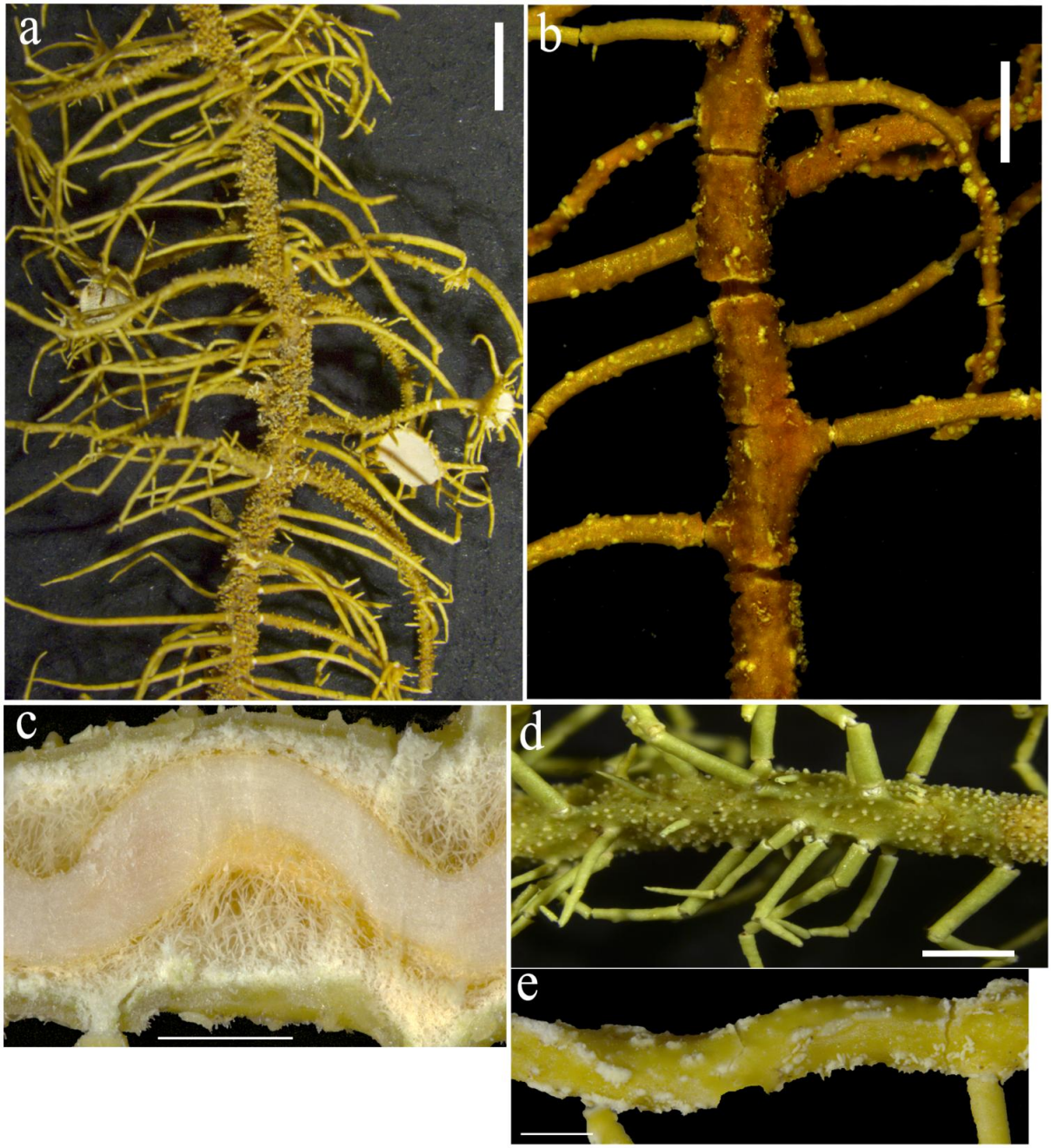


FIGURE 1. *Usnea chilensis*. a. Non-sorediated thallus with lateral apothecia (*Canêz & Spielmann 445*); b. Sorediated thallus, with irregular branches and punctiform soralia (*Gerlach 1476a*); c. Section through branch with sinuous central axis (*Gerlach 1476a*); d. Branches ridged with foveolae and papillae (*Gerlach 1375*); e. Soralia fusing together and looking like single linear soralia (*Gerlach 1476a*). Scales: a = 2 mm; b & d = 1 mm; c & e = 500 µm.

Usnea disjuncta **(Motyka) A. Gerlach & P. Clerc, comb. et stat. nov.** (Fig. 2a–b)
Usnea lethariiformis var. disjuncta Motyka. *Lich. Gen. Usnea Stud. Monogr. Pars Sys. 1* (1936: 12).

Type:–BRAZIL. ESPÍRITO SANTO: Prope Collatina, 1928, *Hartmann s.n.* (holotype, isotype W!). % C/M/A: 2/40.5/15 (holotype). Chemistry: usnic, barbatic, diffractaic, barbatolic and alectorialic acids (holotype and isotypes).

Thallus (description based on both the holotype and the isotypes specimens) pendulous, flaccid, up to 14 cm long; *ramification* anisotomic-dichotomous, terminal branches elongated; *basal part* indistinct, multiple (entangled thallus); *main branches* up to 1.8 mm diameter irregular; *branch segments* slightly swollen, ± sinuous and ± ridged; *annular cracks* rare, with irregular margin; *lateral branches* not constricted at attachment point; *foveolae and depressions* present on the whole thallus, numerous mainly on main branches; *maculae, pseudocyphellae, papillae and tubercles* absent; *fibrils* few, usually with black tips, mainly occurring on terminal branches; *fibercles* absent; *soralia and apothecia* not seen; *cortex* very thin [1.5–2%–2.5] (n=2), shiny; *medulla* thick [38.5–40%–41.5], lax; *axis* thin [14–16%–18] not sinuous; A/M < 0.5.

Chemistry. Medulla K+ brownish: barbatic, diffractaic, barbatolic and alectorialic acids (n=2).

Taxonomic notes. *Usnea disjuncta* was described as a variety of *Usnea lethariiformis* due to the absence of soralia and the slightly blackish apices (Motyka 1936:13). *Usnea lethariiformis* corresponds to a pendulous species with distinct elliptical soralia producing no isidiomorphs, with diffractaic acid as main medullary substance and with a strongly restricted distribution in the Andes–Patagonia region where it seems to be widespread (Rodriguez 2011). *Usnea lethariiformis* var. *disjuncta* Motyka is very similar to *U. lethariiformis* var. *lethariiformis*. However the var. *disjuncta* lacks soralia and seems to have a distinct distribution. For these reasons we propose here to elevate it at the species level. The occurence of this taxon in Brazil under a tropical climate in the Espirito Santo state is curious and deserves further investigations since we have no evidence that the botanist H. Hartman collected samples in Brazil. We might here well face a confusion of labels. *Usnea christinae* Bystr. (type, LBL!, sorediated thallus) and *U. kuehnemannii* Motyka (type LBL!, one sorediated thallus plus one non-sorediated thallus in the same packet) are two similar species with diffractaic acid described from Patagonia (Argentina). More material is needed to understand these two taxa that seem to be closely related to *U. disjuncta*.

FIGURE 2. *Usnea disjuncta* (holotype): a. Main branches with numerous foveolae; b. Section through branches. *Usnea* aff. *disjuncta* (*Jeeval s. n.*): c. Main branches with sparse foveolae; d. Section through branches. Scales: a = 2 mm; b = 500 µm; c = 1 mm; d = 200 µm.

***Usnea dodgei* Motyka** (Fig. 7D –H, in Truong *et al.* 2013)
Lich. Gen. Usnea Stud. Monogr. Pars Sys. 2: 610 (1938).
Type:–COSTA RICA. CARTAGO: pr. Naranjo, 1875, *Polakowsky* (holotype, LBL!; isotype, S!). %C/M/A: 7.5/28.5/28 (isotype). Chemistry: usnic, stictic, constictic, menegazziaic, traces of norstictic acids, unidentified triterpenoids (isotype; by Truong & Clerc in 2010)

***Description*.** Detailed description is available in Truong *et al.* (2013).
Chemistry: Medulla K+ yellow →red. TLC: salazinic, norstictic, galbinic acids and ± an unknown pinkish substance with Rf classes A/B/C: 2–3/3/? (n = 5).
Habitat and distribution. Corticolous on various substrates including *Araucaria angustifolia*, lignicolous on fences. Occurring in Costa Rica (Motyka 1936), in the Tropical Andes and in the Galapagos islands (Truong *et al.* 2013). In Brazil *U. dodgei* occurs in the Minas Gerais, São Paulo (Truong *et al.* 2013), Paraná and Santa Catarina states. *Usnea dodgei* is new to Southern Brazil.

Diagnostic characters. *Usnea dodgei* can be recognized by the capitate soralia that are large and crowded on terminal branches and fibrils, by the slightly inflated branch segments, by the lateral branches that are not or slightly constricted at ramification point, by the numerous papillae and tubercles on main branches, and by the shiny and thick [8–11%–14] cortex.
Variation. The constriction of lateral branches at ramification point can vary from not constricted to slightly but distinctly constricted. Branch segments vary from terete to slightly

irregular in transversal section. Although *Usnea dodgei* displays three chemotypes, the Brazilian specimens all belong to the galbinic acid chemotype.
Taxonomic notes. Three chemotypes were mentioned for *U. dodgei*: 1. stictic acid (holotype), 2. galbinic acid, 3. norstictic acid, all three chemotypes always with triterpenes (Truong *et al.* 2013). We found only the galbinic acid chemotype. *Usnea fallax* is a similar species with galbinic acid, described from Brazil. However, it has a distinct CMA, with a much thinner central axis (6/36.5/15, A/M<1; holotype W!) compared with the specimens of *U. dodgei* here analyzed (8–14/21–27/24.5–33.5, A/M = 0.8–1.2–1.6) or studied by Truong *et al.* (2013) (7–11/21.5–31.5/20.5–40, A/M = 0.5–2). Moreover, the type of *U. fallax* is colonized by a lichenicolous fungus that might be responsible for the special morphology of the soralia (large soralia looking like *U. dodgei*'s soralia). Morphological, chemical and molecular studies with a higher number of specimens collected in the whole distribution area of these species are needed.

Selected specimens studied. BRAZIL. PARANÁ: Curitiba, en allant vers Vila Velha, 25°21′S, 49°34′W, 1989, *Grundlehner s.n.* (G); *ibid*, Ópera de Arame, 1994, *Schatzmann* 33 (UPCB). RIO GRANDE DO SUL: Encruzilhada do Sul, Passo dos Coqueiros, Cerro dos mouros, fazenda Xafri sobre *A. angustifolia*, 1995, *Fleig 7011* (ICN). SANTA CATARINA: Campo Alegre, estrada das laranjeiras, mourão, 2013, *Gerlach & Beilke 1123* (ICN); Bergland bei Curitibanos, Waldwiede mit alten Araukarien, epiphytisch, 1030 m elev., 1987, *Schafer-Verwimp & Verwimp 9137* (G). SÃO PAULO, Bezirk Anhembí, Fazenda Barrero Rico. Cerrado-Insel in einem Primarregenwald. 450 m elev., 1979, *Kalb & Plöbst* (G).

Usnea mexicana **Vain.** (Fig. 11A–E, in Truong *et al.* 2013)
Dansk Botanisk Arkiv. 4: 3 (1926).
Type:–MEXICO. PASO DE DOÑA: 1841, *Liebman 7703* (lectotype designated by Herrera-Campos *et al.* 1998, TUR!, isotype S!). %C/M/A: 5.5/6.5/76. Chemistry: usnic, diffractaic and constictic acids (Herrera-Campos *et al.* 1998).
= *Usnea duriuscula* Motyka, *Lich. Gen. Usnea Stud. Monogr. Pars Sys*. 2: 401 (1938).
Type:–BRAZIL. Serra de Caldas, 1895, *Mosén* (holotpye UPS, isotype S!). %C/M/A: 4.5/11.5/68. Chemistry: usnic and protocetraric acids (Herrera-Campos *et al.* 1998)

Description. Detailed description is available in Herrera-Campos *et al.* (1998).
Chemistry. Medulla K–, P+ red: 1) protocetraric acid (n = 3). Medulla K+ pale yellow: 2) stictic, constictic, menegazziaic, crypstostictic, trace of norstictic and trace of eumitrin (n=2).
Habitat and distribution. Corticolous on various substrates including *Araucaria angustifolia*. This is a tropical and subtropical species. In the New World, it occurs from the North in Mexico (Herrera-Campos *et al.* 1998) to the South in Brazil (Truong *et al.* 2013). New to Southern Brazil (Santa Catarina State).

Diagnostic characters. *Usnea mexicana* is a highly variable taxon that can be recognized by the stiff thallus often with multiple attachment points, by the cylindrical to irregular branches

with terete to alate segments, by the punctiform and slightly stipitate soralia. It has a shiny, thin to ± thick cortex, [7.5–9.5], one of the largest ratio A/M [3.5–4] among the species studied here and a central axis that is typically brown to dark yellow pigmented.
Variation. The shape of branches varying from ± cylindrical with terete segments to slightly sinuous and ridged; lateral branches are often cylindrical at attachment point but specimens with lateral branches slightly enlarged at ramification point are also found; the presence of bead-like annular cracks with cortical regeneration areas as well as longitudinal or irregular cracks are also variable. Three chemotypes were reported (Truong *et al.* 2013; Herrera-Campos 2016) among which only the protocetraric acid chemotype was found in Brazil. However a new chemotype was found consisting of stictic with traces of eumitrin. It is possible that this group consists of a complex of several species.
Taxonomic notes. *Usnea transitoria* also with stiff thallus, cortical regeneration areas between segments, with slightly stipitate soralia and thick central axis, differs mainly by the unpigmented central axis, by the largest ratio axis/medulla (A/M > 3.5, Table 2) and by the presence of large tubercles scattered on the branches.

Selected specimens studied. BRAZIL. MATO GROSSO: Serra dos Coroados, ca. 6 km sudwestlich von Buriti, 600 m elev., 1980, *Kalb* (G). PARANÁ: Guaratuba, 1975, *Krieger 13875* (JPB, CESJ). SANTA CATARINA, Alfredo Wagner, Reserva Particular do Patrimonio Natural Rio das Furnas, 27°40′28.3″S, 49°10′37.9″W, ca. 870 m elev., em *A. angustifolia, Gerlach et al. 1254c*, *1255* (ICN); São Bento do Sul, Area Preservação Ambiental Rio Vermelho/Humboldt, em *A. angustifolia*, 2013, *Gumboski 4271a* (ICN).

***Usnea subflammea* P. Clerc** (Fig. 4 in Clerc 2006; Fig. 10C–G in Truong *et al.* 2013)
Lichenologist 38: 206 (2006).
Type:–PORTUGAL. AZORES: Pico [. . .], 700 m elev., 1993, *Purvis & James 5166* (holotype BM, isotype G!). % C/M/A: 13.5/13.5/47. Chemistry: usnic, stictic, constictic, menegazziaic and traces of norstictic acids (Clerc 2006).

Description. Detailed description is available in Clerc (2006).
Chemistry. Medulla: 1) K+ yellow, TLC=stictic, constictic, norstictic, ± crypstostictic, ± menegazziaic, ± barbatic acid (n=26); 2) K+ yellow→red, TLC= salazinic, norstictic, galbinic acids (n= 16).
Habitat and distribution. Corticolous on various substrates including *Araucaria angustifolia* and lignicolous on fences. It occurs in Macaronesia (Clerc 2006), Costa Rica, and in the tropical Andes (Truong *et al.* 2013). In Brazil, it was previously know to occur in the Minas Gerais, Rio de Janeiro and São Paulo states (Truong *et al.* 2013). New to Southern Brazil occurring in the Paraná, Rio Grande do Sul and Santa Catarina States.

Diagnostic characters. *Usnea subflammea* is characterized by the pendulous to subpendulous thallus with a pale basal part that is conspicuously annulated, by the cylindrical branches with terete segments and by the presence of numerous tubercles often eroded on top. The cortex is mat, moderately thick to thick and the medulla is thin to ± thin and compact (Table 2).

Variation. Three specimens (collected in Parque Estadual da Serra da Baitaca) have barbatic acid as accessory substance. Compared with typical *U. subflammea* specimens, their branches are more irregular, with slightly thinner cortex and wider medulla [%C/M/A: 9.5–11/26–28/25–27]. The stictic acid (holotype) and galbinic acid chemotypes (Truong *et al.* (2013) occur in Brazil. We were not able to find any correlation between these two chemotypes and any other morphological, ecological or geographical characters and we thus consider both chemotypes to belong to the same species. Molecular studies are needed to test the validity of this hypothesis.
Taxonomic notes. This species is very distinct among the species here studied. For differences with similar species see Clerc (2006) and Truong *et al.* (2013).

Specimens analyzed. BRAZIL. PARANÁ: Guaratuba, Morro dos Perdidos, ca. 1439 m elev., 2013, *Gerlach et al. 1018* (ICN); Quatro Barras, Parque Estadual da Serra da Baitaca, Morro do Anhangava, 25°23′13″S and 49°00′17″W, 1320 m elev., 2014, *Santos et al.* 98 (UPCB). RIO GRANDE DO SUL: Cambará do Sul, Parque Nacional da Serra Geral, cânion Itaimbezinho, vassoural nos ramos, 900 m elev.., 1989, *Fleig 3533* (ICN); São Francisco de Paula, Colinas de São Francisco, em Araucaria, 1000 m elev., 1989, *Grundlehner 4673* (ICN). SANTA CATARINA: Campo Alegre, estrada das Laranjeiras, em mourão, 2013, *Gerlach & Beilke 1128* (ICN); *ibid*, campos do Quiriri, 2012, *Gumboski 3609* (ICN).

Usnea subscabrosa **Nyl. ex Motyka** (Fig. 9D–E in Truong *et al.* 2013)
Lich. Gen. Usnea Stud. Monogr. Pars Sys. 2: 313 (1938).
Type:–PORTUGAL. 1877, *Newton* (holotype H!). %C/M/A: 14/13.5/45. Chemistry: usnic and protocetraric acids (Clerc 1992)
= *Usnea santae-annae* Motyka, *Lich. Gen. Usnea Stud. Monogr., Pars Syst.* 2: 315 (1938). Type:–BRAZIL. MATO GROSSO: Santa Anna da Chapada, in *cerrado et in margine silve*, 1894, *Malme* (holotype S); %C/M/A: 13/15.5/44. Chemistry: usnic, protocetraric and fumarprotocetraric acids (Clerc 1997).

Description. Detailed description is available in Herrera-Campos *et al.* (1998).
Chemistry. Medulla K-, P+ orange red: protocetraric acid (n=15).
Habitat and distribution. Corticolous in various substrates including *Araucaria angustifolia*, lignicolous on fences, occasionally saxicolous. It is the only species of *Usnea* forming large saxicolous populations on rocky shores in Southern Brazil. Widespread in Southwestern Europe (Clerc 1992), in Macaronesia (Clerc 2006), in North America (Clerc & Herrera-Campos 1997, Herrera-Campos *et al.* 1998, Clerc 2007), and in the tropical Andes (Truong *et al.* 2013). In Brazil, it was previously reported in the Mato Grosso (Motyka 1938), Minas Gerais, Rio de Janeiro and São Paulo states (Truong *et al.* 2013). New to the Southern region (Paraná, Rio Grande do Sul and Santa Catarina states).

Diagnostic characters. *Usnea subscabrosa* has cylindrical branches and can be easily identified by the basal part that is usually reddish tinged, by the typical thick and vitreous cortex and the thin and compact medulla reacting K–, P+ yellow-orange (Tables 1, 2).

Variation. Soralia are often punctiform and well-delimited at maturity, with the exception of the specimens collected on coastal rocks that have plane and more or less confluent soralia. This corroborate with Herrera-Campos *et al.* (1998) and Truong *et al.* (2013) describing soralia that vary from plane to punctiform, remaining well-delimited or strongly confluent when crowded. The thamnolic acid chemotype occurs in Macaronesia (Clerc 2006) and seems to be absent in South America. According to Clerc (1992) and Herrera-Campos *et al.* (1998) this species can has a shrubby to pendulous thallus. In Southern Brazil, this species is often subpendulous to pendulous.
Taxonomic notes. This species is very distinct from all other species studied here. See Clerc (1992) for differences with similar species.

Specimens analyzed. BRAZIL. PARANÁ: Curitiba, en allant vers Vila Velha, 25°21′S, 49°34′W, 1989, *Grundlehner* (G); Guaratuba, Morro dos Perdidos, 2013, *Gerlach et al. 1050* (ICN). RIO GRANDE DO SUL: Bagé, paredão rochoso, *Fleig 3934b* (ICN); Cambará do Sul, Parque Nacional dos Aparados da Serra, cânion Itaimbezinho, 2014, *Gerlach & Akkerman 1401* (ICN). Esmeralda, Estação Ecológica de Aracuri, em Araucaria, 900 m elev., 1984, *Fleig 2442* (ICN); São Francisco de Paula, Floresta Nacional, 2014, *Gerlach et al. 1518* (ICN). SANTA CATARINA: Alfredo Wagner, Reserva Particular do Patrimônio Natural Rio das Furnas, 2014, *Gerlach et al. 1226* (ICN); Caçador, margens da SC 455, 2013, *Gumboski 4709* (ICN); Campo Alegre, caminho para o Quiriri, sobre *A. angustifolia*, 2013, *Gerlach & Beilke 1168* (ICN); Fraiburgo, margens da rodovia SC 453, sobre *Syagrus romanzoffiana*, 2013, *Gumboski 4733* (ICN); Joinville, estrada das Laranjeiras, mourão, 2013, *Gerlach & Beilke 1124b* (ICN); Rio Negrinho, Rio dos Bugres, em *A. angustifolia*, 2012, *Gumboski 4102b* (ICN); São Bento do Sul, Area de Preservaçao Ambiental Rio Vermelho/Humboldt, em *A. angustifolia*, 2013, *Gumboski 4295* (ICN); São Francisco do Sul, Prainha, costão rochoso, growing with *Parmotrema tinctorum, Gerlach et al. 976* (ICN).

Usnea subsilesiaca* P. Clerc & A. Gerlach *sp. nov. (Fig. 3)
MycoBank No.: MB
Thallus stiff, shrubby to pendulous; basal part conspicuously jet-black; branches tapering never constricted at ramification point; segments cylindrical and terete; soralia punctiform, stipitate on top of tubercles, with isidiomorphs; cortex mat and thick (12.5%), medulla compact and thin (8.5%), axis thick (58%).
Type:–BRAZIL. RIO GRANDE DO SUL: Cambará do Sul, Parque Nacional dos Aparados da Serra, near the visitor center, ca. 29°09’31’’S, 50°04’47’’W, ca. 1000 m elev., growing on bark of *Araucaria angustifolia*, 13.iii.2014, *Gerlach & Akkerman* 1394 (holotype, G; isotypes, ICN, UPS). %C/M/A: 11/6/66 (holotype). Chemistry: salazinic acid (holotype and isotypes).

Syn.: *Usnea columbiana* sensu auct., non *U. columbiana* Motyka in Räsänen (1936) (= *U. silesiaca* Motyka).

Thallus shrubby, mostly subpendulous to sometimes pendulous, up to 20 cm long, stiff; *branching* isotomic or anisotomic dichotomous; *basal part* conspicuously blackened on 2–5 mm or more, black pigmentation often extending to the primary branches; *branches* 1–2 mm thick, tapering or cylindrical, not distinctly annulated, annular cracks thin without medullary extrusion, never constricted at ramification point; *terminal branches* long and thin, with few ramifications or fibrils; *segments* cylindrical and terete; papillae mostly absent; *tubercles* low, verrucous, usually numerous, irregularly distributed on every branches, often eroded and sorediated at their top; *fibercles* frequent when fibrils numerous, often sorediated; *fibrils* nearly absent to numerous, slender, 3 to 10 mm long; *pseudocyphellae* and *maculae*: absent; *soralia* punctiform, 70-120 µm, never enlarging, ± circular, distinctly stipitate, rarely fusing together, usually numerous on secondary and terminal branches, originating at the top of eroded tubercles or fibercles; *isidiomorphs* usually present, short, 0.1-0.2 mm; *cortex* mat, thick, (7.5–)10.5–12.5–14(–18)] (n=24); *medulla* compact and thin, (4–)4.5–8.5–12.5(–14) ; *axis* thick, (43–)48–58–68(–77) with an A/M ratio: (3.5–)2.7–8–13(–19.5); *apothecia* unfrequent, up to 11 mm diameter, flat, terminal, disc pale yellow covered with purine, with well-developed fibrils; *ascospores* 8 × 6 µm.

Chemistry. Medulla K+ yellow→red. TLC: salazinic acid (n = 21).
Habitat and distribution. This species is mainly corticolous, but can be sometimes found on rocks. It occurs in North, Central and South America, from Southern Mexico (Herrera-Campos 2016) to Northern Argentina (Rodriguez 2011). In Brazil it occurs in the Minas Gerais, Rio Grande do Sul, Santa Catarina and São Paulo States, in mountainous areas above 900 meters elevation, sometimes growing on bark of *Araucaria angustifolia.*

Diagnostic characters. The diagnostic characters of *U. subsilesiaca* are the stiff subpendulous thallus, the extended black pigmentation in the basal part, the stipitate and punctiform soralia growing on the top of eroded tubercles, the thick and mat cortex, the thin medulla and the thick central axis and the presence of salazinic acid in the medulla.

Variation. *Usnea subsilesiaca* displays some variation in the size and habitus of the thallus from small bushy to long pendulous. The extent of the black pigmentation in the basal part of the thallus might vary too, from 1−2 mm on the trunk to extending above first ramifications.

Taxonomical notes. *Usnea subsilesiaca* corresponds to what Rodriguez (2011), Rodriguez *et al.* (2011: 509), Truong *et al.* (2013: 507, 539) and Herrera-Campos (2016) called *Usnea columbiana* Motyka. *Usnea columbiana* was described in Räsänen (1936) from a manuscript Motyka sent to Räsänen. The correct citation of the name is thus *Usnea columbiana* Motyka in Räsänen (Räsänen 1936). The type mentioned in the protologue (H!) was collected by G. Looser in 1930, in the Province of Valdivia, Chile. However, this specimen corresponds morphologically, anatomically and chemically well to *Usnea silesiaca* Motyka. *Usnea columbiana* Motyka in Räsänen is thus a synonym of *U. silesiaca* Motyka (see Clerc 2011 and Herrera-Campos 2016 for modern descriptions of this taxon); Motyka (1938) gives a full description of *U. columbiana* mentioning correctly the protologue but pointing to a wrong citation of the type specimen. This description could fit the concept of *U. subsilesiaca*.

However we were unable to find one specimen of this species among the specimens kept under « *U. columbiana* » in LBL. The specimen named *U. columbiana* Motyka in Vezda's Lichenes Selecti Exsiccati nr. 573 [USA, California, Mendocino County] corresponds to a taxon of the *U. fragilescens* group (Clerc 1997).

Usnea subsilesiaca is very similar to *U. silesiaca*. However the latter species has larger mature soralia not growing on tubercles but *ad initio* on the cortex on the thinner and terminal branches (Fig. 3d–e). *Usnea tenuis* Motyka, a very common species in Costa Rica (Clerc unpublished) is very similar too but it is not sorediate. *Usnea subfloridana* Stirt. has another type of soralia and never produces salazinic acid. *Usnea praetervisa* (Asahina) P. Clerc has norstictic acid in the medulla and a different type of soralia as well.

Selected specimens studied *:* ARGENTINA : CORDOBA, Los Gigantes, sobre roca, 27 June 2004, *Rodriguez* 343 (G); TUCUMAN, El Infiernillo, on tree, 1500 m elev., 28 Mars 1989, *Grundlehner* 95.28.3, (G). BRAZIL: MINAS GERAIS: Serra da Mantiqueira, Itatiaia, zwischen, Registro do Picu und Agulhas Negras, 1978, *Kalb & Plobst* (G). RIO GRANDE DO SUL: Cambará do Sul, Fazenda Velha, Mata Atlântica, 1994, *Mazzitelli s. n.* (HAS); Canela, Hotel Continental, 29°16′S, 50°50′W, on bark of *A. angustifolia*, 420 m elev., 2013, *Dal-Forno 2072* (G); *ibid*, loteamento em Mata de *A. angustifolia*, 1988, *Osorio & Fleig 88/39* (ICN); São Francisco de Paula, Hotel Veraneio Hampel, 2016, *Fazolino et al. s.n.* (ICN); *ibid*, Flona de São Francisco de Paula, 29°23′S, 50°23′W, ca. 933 m elev., 2014, *Alves s. n.* (ICN). SANTA CATARINA: Urubici, Parque Nacional de São Joaquim, próximo ao alojamento, ca. 1300 m elev., 2014, *Gerlach & Alves 1308* (ICN). SÃO PAULO: Campos do Jordão, Parque Estadual de Campos de Jordão, Estrada para São José dos Alpes, após a Vila dos Funcionários, 1996, *Ribeiro 1002* (CESJ). RIO DE JANEIRO: Serra da Mantiqueira, Itatiaia, zwischen Registro do Picu and Agulhas Negras, 23 July 1978, *Kalb & Plöbst* (G), Serra da Mantiqueira, Vila Monte Verde, etwa 30 km östlich von Camanducaia, 7-11 September 1978, *Kalb & Plöbst* (G). COSTA RICA : San José, Pérez Zeledon Co, Parque Nacional Chirripo, 2502 m, forêt tropicale humide de montagne, sur *Quercus* sp., 13 September 2014, *Clerc & Loza* PC2015/576 (G). ECUADOR : PICHINCHA, Mital del Mundo, ruta hasta la reserva geobotanica Pululahua, rocas, 2695 m elev., *Truong & Shuguli* 508 (G). EL SALVADOR : SANTA ANA, Metapan, Parque Nacional Montecristo, stade de dégradation de la «Pine-Oak forest», sur *Trema micrantha*, 1587 m elev., 17 January 2013, *Clerc, Rojas & Morales* PC2015/1168 (G). PERU : CAJAMARCA, Parque nacional Cutervo, Cerca de La Capilla, bosque de neblina siempreverde (primario), 3031 m elev., 5 Mai 2007, *Truong & Ramirez-Ordaya* (G).VENEZUELA : MERIDA, Distr. Campo Elias, La Carboner, Finca San Isidro, 2200 m elev., Resten eines Wolkenwaldes, 4.viii.1989, *Kalb & Lopez-Figueras* 24203 (G); *ibid*, La Carbonera, restos de bosques primitivos, epifita, 2200 m elev., 17 December 1984, *Lopez-Figueiras & Brako* 31296 (G).

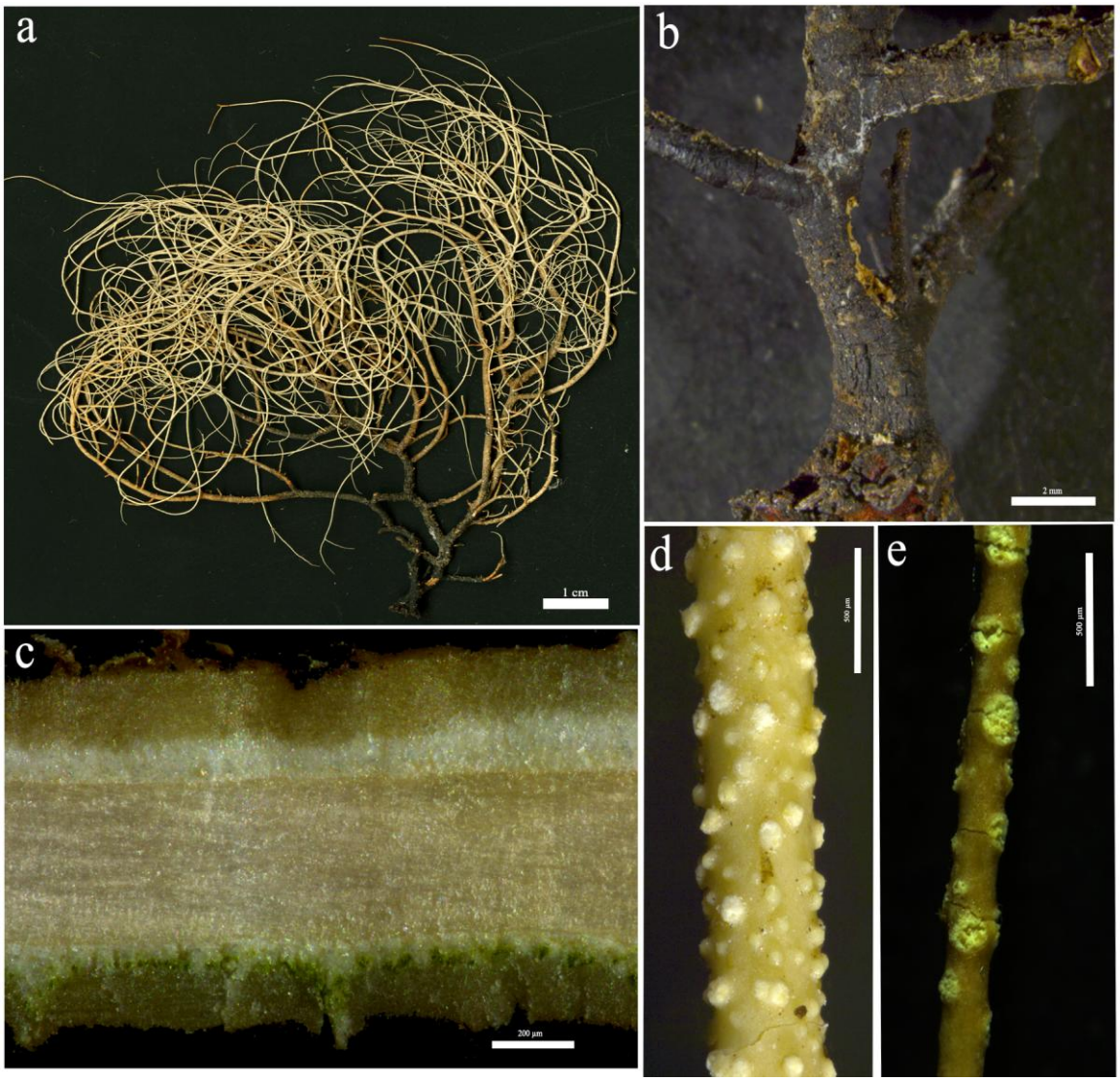


FIGURE 3. *Usnea subsilesiaca*. a. Thallus (holotype); b. Trunk with black pigmentation extending to the primary branches (holotype); c. Section through main branch (Clerc & Rojas 2015139); d. Soralia punctiform arising from tubercles (holotype). e. *Usnea columbiana* (holotype): soralia growing *ad initio* from the cortex and enlarging.

***Usnea transitoria* Motyka** (Fig. 9F–H in Truong *et al.* 2013)
Lich. Gen. Usnea Stud. Monogr. Pars Sys. 2: 375 (1938).
Type:–COSTA RICA. SANTIAGO DE CARTAGO: potrero above Rio Birris, 1220 m elev., 1929, *Dodge* (holotype, isotype LBL!).%C/M/A: 5.5/9/71.5. Chemistry: usnic, stictic, constictic, menegazziaic and traces of norstictic acids (Truong *et al.* 2013).

Description. Detailed description is available in Herrera-Campos *et al.* (1998).
Chemistry. Medulla K+ yellow: stictic, constictic, ± menegazziaic, ± cryptostictic and ± norstictic acids (n = 2).

***Habitat and distribution*.** Corticolous on various substrates including *Araucaria angustifolia*. Previously reported from Central America, the Caribbean islands and Venezuela (Motyka 1938, Herrera-Campos *et al.* 1998, Truong *et al.* 2013). Newly reported for Brazil where it seems to be rare.

Diagnostic characters. The cylindrical branches with areas of eroded cortex, the large and conical tubercles (not eroded at the summit), the convex to capitate soralia usually with many isidiomorphs, the slender fibrils in fish–bone pattern, the thin and compact medulla K+ yellow (stictic acid) and the large axis (> 50%) are characteristic for this seemingly rare species in Brazil.

Variation. The specimens analyzed from Brazil fit well with the description of Herrera-Campos *et al.* (1998), with the exception of the tubercles that are often not eroded at their summit and of the chemistry (presence of stictic acid in the Brazilian specimens as in the type). The branches of the Brazilian specimens are only slightly ridged but according to Herrera-Campos *et al.* (1998) the degree of development of the ridges are the most variable character in this species.

Taxonomic notes. Among the species studied here, *Usnea mexicana* is morphologically the most similar with *U. transitoria* which however differs by the typically brown to dark yellow pigmented axis and the absence of large tubercles on the cortex surface.

Specimens examined. BRAZIL. PARANÁ: São José dos Pinhais, 2013, collector unknown (ICN). SANTA CATARINA: Rio Negrinho, Rio dos Bugres, área rural, em *A. angustifolia*, 2012, *Gumboski 4088* (ICN); Urubici, Parque Nacional de São Joaquim, 2015, *Alves s. n.* (ICN).

Usnea venusta Motyka (Fig. 5A–C in Truong *et al.* 2013)

Lich. Gen. Usnea Stud. Monogr. Pars Sys. 2: 441 (1938).

Type:–BRAZIL. SANTA CATARINA: Serra do Oratorio, 1890, *Ule* (lectotype designated by Truong *et al.* 2013, S!, isolectotype LBL). %C/M/A: 2/43**.**5/9. Chemistry: usnic, salazinic and norstictic acids (Truong *et al.* 2013).

Description. (n=5) *Thallus* pendulous, entangled, soft, up to 20–30 cm long,; *ramification* isotomic-dichotomous often sharply tapering towards the apices; *apices* thin, with numerous ramifications; *basal part* often indistinct or discrete ca. 8 mm long, concolorous to the main branches to yellowish; *main branches* up to 1.8 mm thick, irregular; *branch* segments sausage-like, terete, with annular cracks, without bead-like cortical areas between segments; *lateral branches* distinctly constricted at the attachment point; *foveolae and depressions* present; *maculae* usually abundant, covering most of the surface (better seen on fresh material); *pseudocyphellae* absent; *papillae* rare, verrucose, usually red pigmented (or blackish); *tubercles* absent; *fibrils* scattered on the whole thallus, not abundant, slender; *fibercles* rare; *soralia and isidiomorphs* absent. *Apothecia* sometimes present (see on three thallus); *cortex* thin [1.5–2.5%–3.5(–4)] (n=5), shiny, glabrous, red-spotted; medulla moderately thick, [39–40.5%–42(–42.5)], very lax; axis thin [(11–)11.5–13.5%–15.5(–17)], often orange pigmented and sometimes slightly sinuous; A/M = 0.3–0.4.

Chemistry. Medulla K+ yellow→red: salazinic, norstictic and ± protocetraric acids (n=5).

Habitat and distribution. Corticolous. Occurs in Brazil (Rio Grande do Sul, Santa Catarina, and São Paulo states) and probably in Argentina (Rodriguez 2011, as *Usnea aff. articulata*).

Diagnostic characters. *Usnea venusta* is a pendulous non-sorediate species easily recognized by the distinctly constricted branches at attachment point, the sausage-like segments, the scarce papillae often reddish pigmented, the low ratio A/M (≤ 0.5), the spotted pigmented central axis (Fig 5C in Truong *et al.* 2013) and the medulla reacting K+ yellow→red (salazinic acid as main metabolite secondary).
Variation. The density of red minute dots on the cortex surface is variable on this species.
Taxonomic notes. *Usnea articulata* is a similar taxon but the absence of red dots and the distinct chemistry (protocetraric acid) in this species led us to the conclusion that it doesn't correspond to *U. venusta*, unlike what was stated by Truong *et al.* (2013). The presence of *U. articulata* in Brazil is doubtful (for more comments see below under *U. articulata*).

Specimens examined. BRAZIL. RIO GRANDE DO SUL: Cambará do Sul, Parque Nacional dos Aparados da Serra, cânion Itaimbezinho, 2014, *Gerlach & Akkerman 1426* (ICN); SANTA CATARINA: in silva Araucariarum in Serra Geral, 1891, *Ule 115* (G); Urubici, Parque Nacional de São Joaquim, 2014, *Gerlach & Alves 1393* (ICN, G). SÃO PAULO: Serra da Bocaina, 1952, *Mattick 16179* (B).

Uncertain species

Usnea articulata (L.) Hoffm.
Notes. This is an European species with protocetraric as main secondary metabolite, sausage-like branches with punctiform pseudocyphellae and a thin cortex with a large A/M ratio. The single specimen collected in Brazil (Frei Custodio, 1878, G!) that shows these features has diffractaic acid in the medulla and therefore remains unidentified. Other specimens collected in Brazil and identified as *U. articulata* correspond in fact to *U. venusta.* Usnea *articulata* is therefore excluded from the Brazilian mycota.

Usnea aff. disjuncta (Fig. 2c–d)
Chemistry. Medulla K+ yellow→red: salazinic, norstictic and galbinic acids (n = 4).
Taxonomic Notes. At first glance this taxon could be considered as a less developed thallus of *U. disjuncta* with thinner medulla (29.5–33.5%) and less pronounced foveolae. However, the distinct chemistry (galbinic acid instead of diffractaic and barbatic acids in *U. disjuncta*) and its distribution (it is so far known only in Southern Brazil and in Uruguay, in the Pampa grasslands biome) led us to the conclusion that it probably corresponds to a distinct still undescribed taxon (see under *U. disjuncta* for more details). *Usnea* aff. *disjuncta* is morphologically similar to *U. cavernosa* Motyka. It differs from this species mainly by the distribution, the chemistry and by subtle differences in the CMA measures [A/M < 1 in *Usnea* aff. disjuncta; A/M >1 in *U. cavernosa*). *Usnea cavernosa* shows a Holarctic distribution (USA–Europe) and produces salazinic acid in the medulla (Herrera-Campos *et al.* 1998).

Specimens examined. BRAZIL. RIO GRANDE DO SUL: Caçapava do Sul, Arroio Seival, mata ciliar, junto a campo de pastagem, 1993, *Fleig 5650* (ICN); Júlio de Castilhos, Passo do Felicio, nascente rio Saturno, 1989, *Osorio & Fleig 186* (ICN); Piratini, capão de mata, 2015, *Jeeval s. n.* (ICN). URUGUAY. Lavalleja, ruta 8 km, 209, Estancia Madeiros, sobre ramos de *Acacia farnesiana*, 1956, *Osorio 3689* (JPB).

Usnea sp. 1 (Fig. 4)

Chemistry. Medulla K+ yellow→red: 1) salazinic and ± trace of protocetraric acids (n = 5). Medulla K+ yellow: 2) stictic, crypstostictic, constictic, menegazziaic and trace of norstictic acids (n = 1).

Taxonomic notes. The branches are irregular with cylindrical segments, the lateral branches are not constricted (main branches) to slightly constricted (thin branches) at attachment point, the cortex is shiny, thin to ± thin [3.5–5%–6.5], the medulla is dense, ± thick to thick [28.5–33%–37.5], with an A/M ratio < 1.5. Soralia are circular to slightly irregular and delimited with a distinct cortical margin, becoming ± plane at the apices of branches and confluent like the soralia of *Usnea cornuta* Körb. Ascospores (when present) are (6–)7–8–9(–10) × (4.5–)5–5.5–6 µm (n=3). *Usnea sp. 1* can be either sorediate or not sorediate. We refrain here to describe this form as a new taxon before doing molecular analyses to test the validity of this taxon.

Specimens examined. BRAZIL: MINAS GERAIS: Lima Duarte, Parque Estadual do Ibitipoca, 1993, *Ribeiro 128* (CESJ); *ibid*, Serra da Mantiqueira, Itatiaia, zwischen Registro do Picú und Agulhas Negras, 1978, *Kalb & Plöbst* (G). PARANÁ: Curitiba, en allant vers Vila Velha, 25°21′S, 49°34′W, 1989, *Grundlehner s. n.* (G). RIO GRANDE DO SUL: Cambará do Sul, Itaimbezinho, em *Araucaria angustifolia*, 1989, *Fleig 3584* (ICN); São Francisco de Paula, Centro de Pesquisa e Conservação da Natureza Pro–Mata, em *Araucaria angustifolia*, 918 m elev., 1998, *Fleig & Grüninger 983151* (ICN); *ibid*, *Koch 33R* (ICN); *ibid*, Hotel Veraneio Hampel, 2014, *Rosiak 21* (ICN).

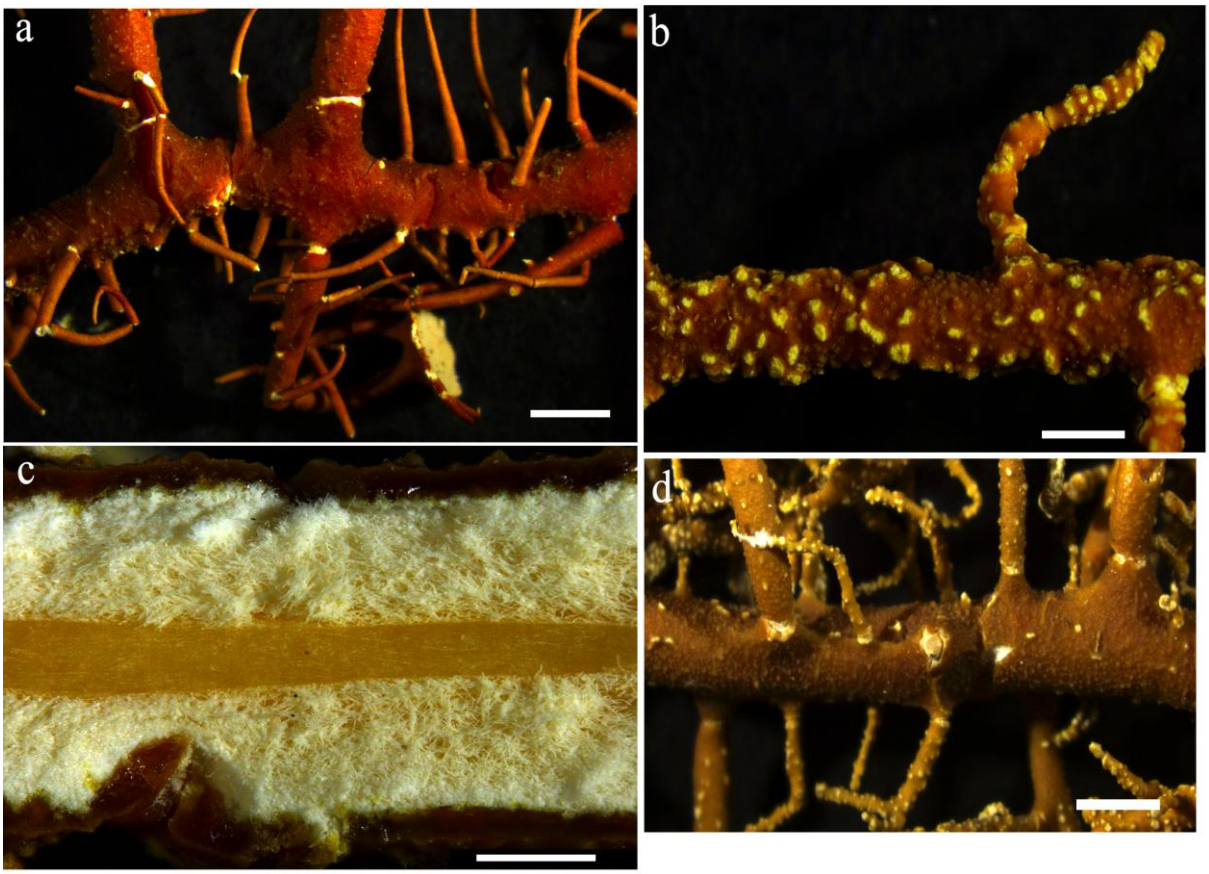
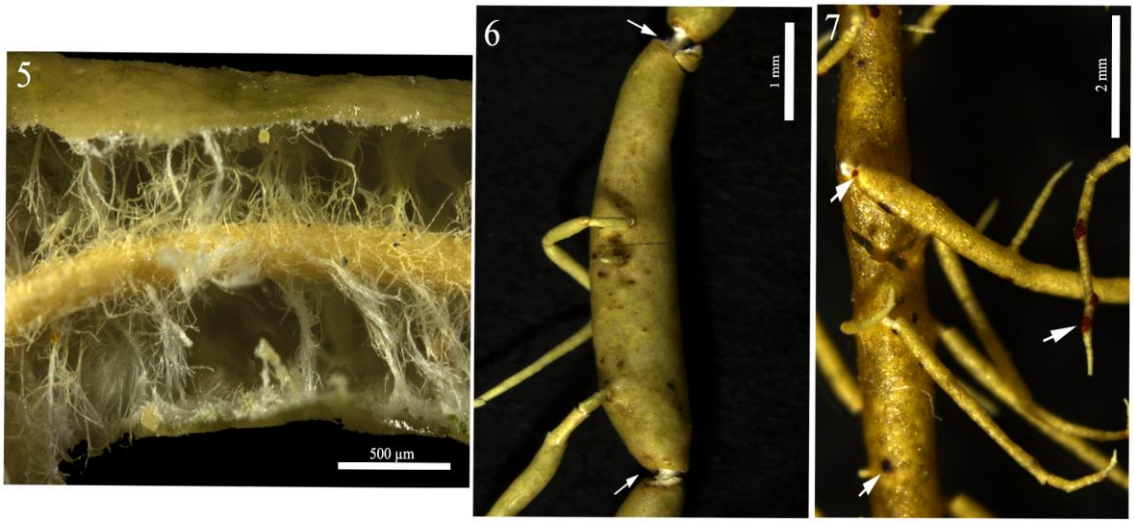


FIGURE 4. *Usnea* sp. 1: a. Non-sorediate thallus with lateral apothecia (*Ribeiro* 128). b. Sorediate thallus, soralia punctiform with distinct cortical margin (*Fleig & Grüninger* 983151); c. Section through branches (*Ribeiro* 128); d. Lateral branches constricted at attachment point (*Fleig & Grüninger* 983151). Scales: a & d = 2 mm; b & c = 500 µm.

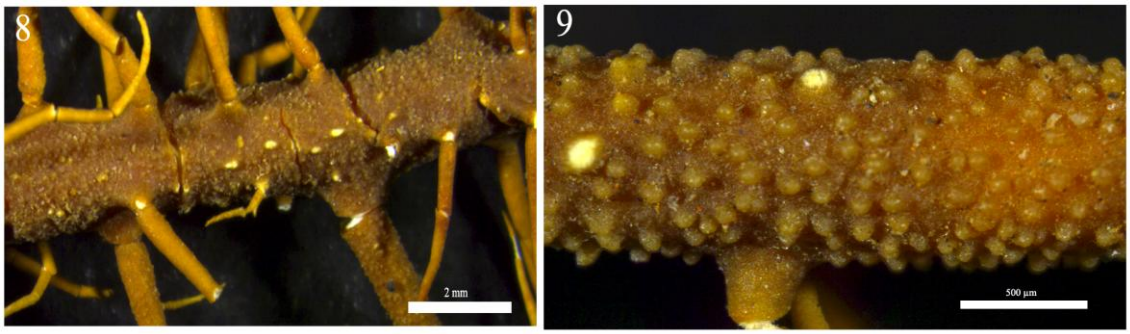
Key to pendulous *Usnea* species occurring in Southern Brazil

This key works only for mature specimens optimally developed with the typical characters of the species. If such characters are absent or the specimens are immature, TLC analyses are necessary and full descriptions should be checked. Finally a specialist should be consulted. Eumitrioid and pigmented species are not included.

1a. Thallus without soralia, usually with apothecia…….....2
1b. Thallus with soralia……..9
2a. Branches constricted at attachment point…….....3
2b. Branches not constricted at attachment point……..…….4
3a. Thallus flaccid; segments distinctly sausage–like [6]; cortex with minute red spots [7]; papillae rare; medulla lax [5], K+ red (salazinic and norstictic acids)……..……**U. venusta**



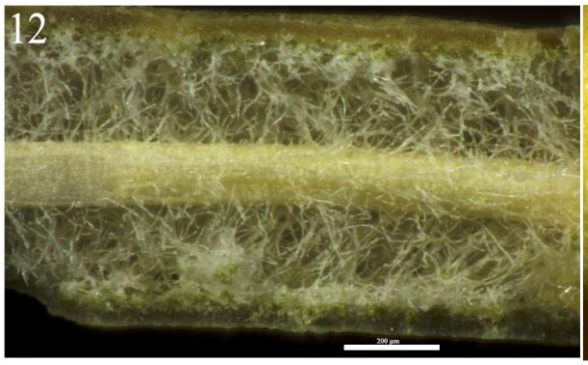
3b. Thallus stiff; segments not distinctly sausage–like **[8]**; surface of the cortex without red dots; papillae numerous **[9]**; medulla dense **[11]**, K+ yellow (stictic acid) or K+ red(salazinic acid)…….....**Usnea sp. 1**

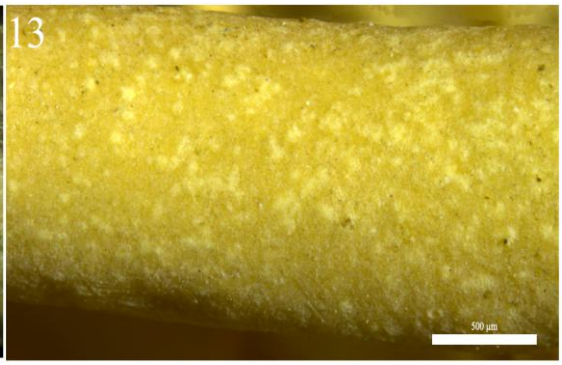


4a. Central axis large (≥50%) **[11]**; segments strongly ridged to alate, with large trapezoid segments **[10]**……...**U. alata**

4b. Central axis thinner (> 50%); branches not strongly alate……5

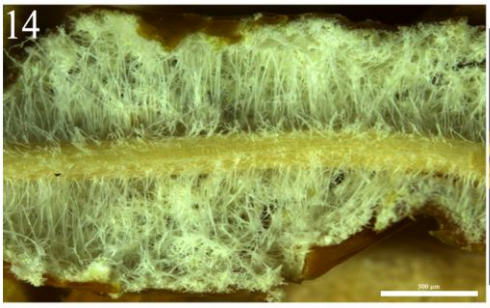
5a. Thallus pruinose with conspicuous maculae spotting the cortex **[13]**; cortex mat in section [**Fig. 12**] …….....**U. arthroclada**

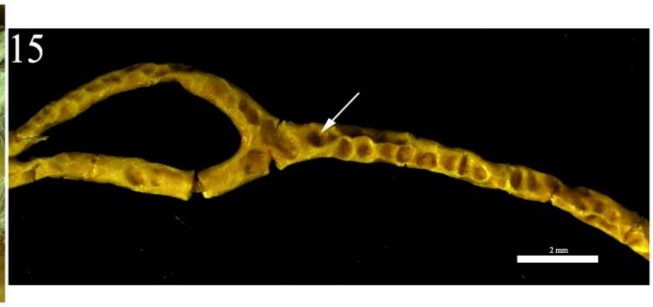




5b. Thallus not pruinose with or without maculae; cortex mat or vitreous in section……..6

6a. Main branches with numerous foveolae **[15]**; surface of the branches smooth; medulla lax (40% in average) **[14]** K+ brownish (diffractaic acid)**..……..……U. disjuncta**





6b. Foveolae absent to present; surface of the cortex rugose; medulla dense, thinner (20% in average) K+ or K– (diffractaic absent)……..7

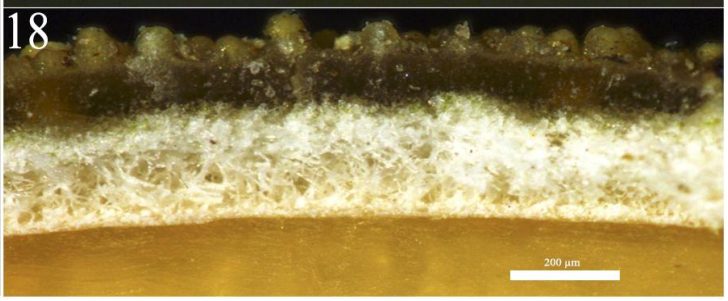
7a. Branches irregular; segments in main branches ridged to slightly alate; cortex thin (<8%); medulla K+ red (galbinic acid)…….....**U. chilensis**

7b. Branches regular; segments terete; cortex thicker (≥8%); medulla K+ or K– (galbinic acid absent)…… ……8

8a. Cortex vitreous **[23]**; pseudocyphellae conspicuous **[26]**, often in patches, in main branches with longitudinal cracks; medulla K– P– (Us2)…….....**U. papillata**

8b. Cortex mat and soft; conspicuous pseudocyphellae and longitudinal cracks absent; medulla K+ (salazinic acid)……..…….....**U. sancteritae**

9a. Base with a jet black pigmentation extending above first ramifications **[16]**; soralia punctiform, stipitate on top of tubercles **[17]**; cortex mat and thick (12–14%) **[18]** ……...……**U. subsilesiaca***



9b. Base unpigmented or brownish to reddish **[19]**; soralia punctiform to large; cortex mat or glossy……10

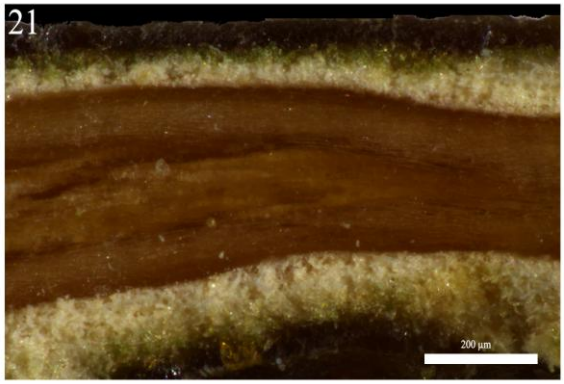
10a. Lateral branches distinctly constricted **[20]**……...**Usnea sp. 1**

10b. Lateral branches not to only slightly constricted……...……....11

11a. Medulla K– and P+ red (or K+ brownish), protocetraric acid as main substance……....12

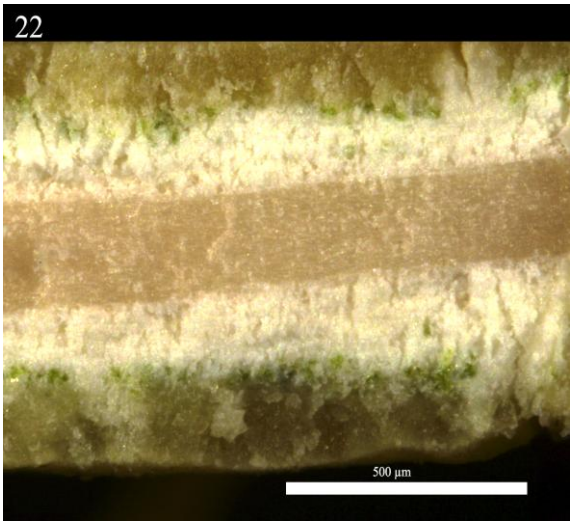
11b. Medulla K+ or K– and P–, protocetraric acid absent or as accessory substance……..15

12a. Central axis totally ochraceous (on thin branches as well) and thick (>50%) **[21]**……...**U. mexicana**



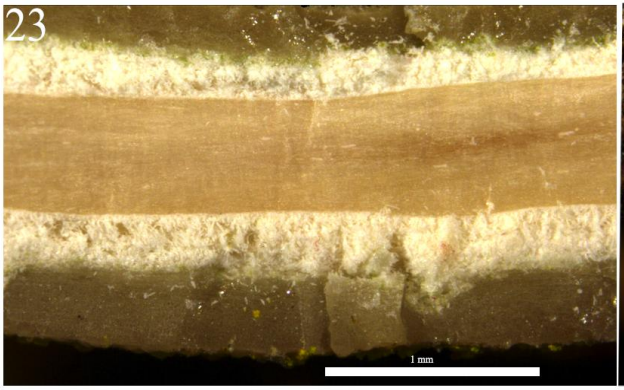
12b. Central axis not ochraceous (beware necrotic specimens!) thinner or thicker.....……...13

13a. Cortex mat in section **[22]**…….…..…..**U. subgracilis**



13b. Cortex glossy…….14

14a. Cortex vitreous and thick (10–18%) **[23]**; basal part often with red spots **[24]** ……..……...……**U. subscabrosa**



14b. Cortex glossy, not vitreous, thinner (3–10%); basal part without red spots ….....…**U. mexicana**

15a. Maculae very conspicuous on main branches, drawing a network on the cortex surface **[25]**…….....**U. regia**

15b. Maculae absent or faintly spotting the cortex surface…….....16

16a. Conspicuous and large pseudocyphellae covering main branches **[26]**; medulla K–, P– (Us2)…… ……...**U. malmei**

16b. Large pseudocyphellae absent or sparse on terminal branches; medulla K+, P+ (rarely K–, P–)…….....……17

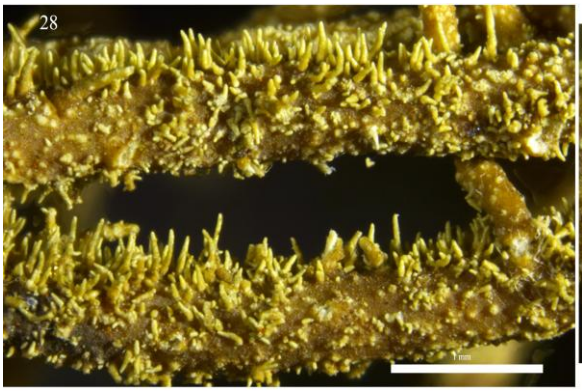
17a. Branch segments distinctly alate with large trapezoidal segment at ramification **[10]**; strongly ridged; central axis large (40–65%) **[21]**……**U. angulata**

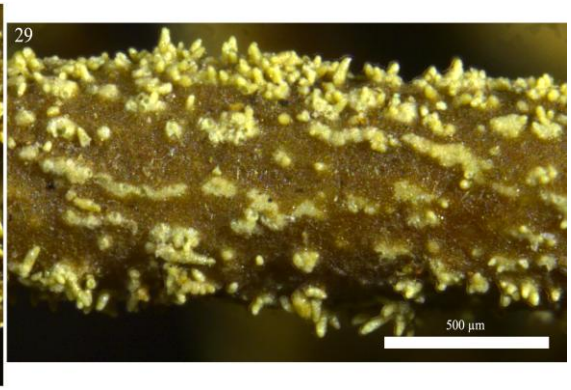
17b. Branch segments not or weakly alate; without large trapezoidal segments at ramification; ridged or not; central axis large or thinner……18

18a. Numerous curled or contorted branches and fibrils covered with numerous discrete to fusing soralia simulating skeletal fingers **[27]**……..**U. dodgei**

18b. Branch apices and fibrils not simulating skeletal fingers……...19

19a. Soralia minute, numerous, even with cortex, of irregular shape **[29]**, with numerous isidiofibrils (sometimes isidiofibrils are only present on some branches of the thallus) **[28]**…….**U. perhispidella**



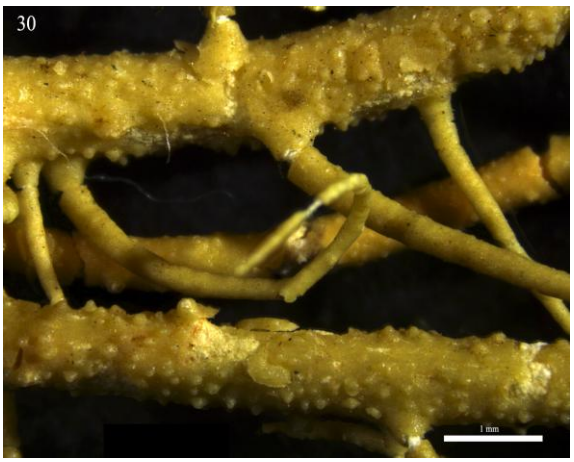


19b. Soralia minute or larger; of irregular or circular shape; without isidiofibrils…….....20

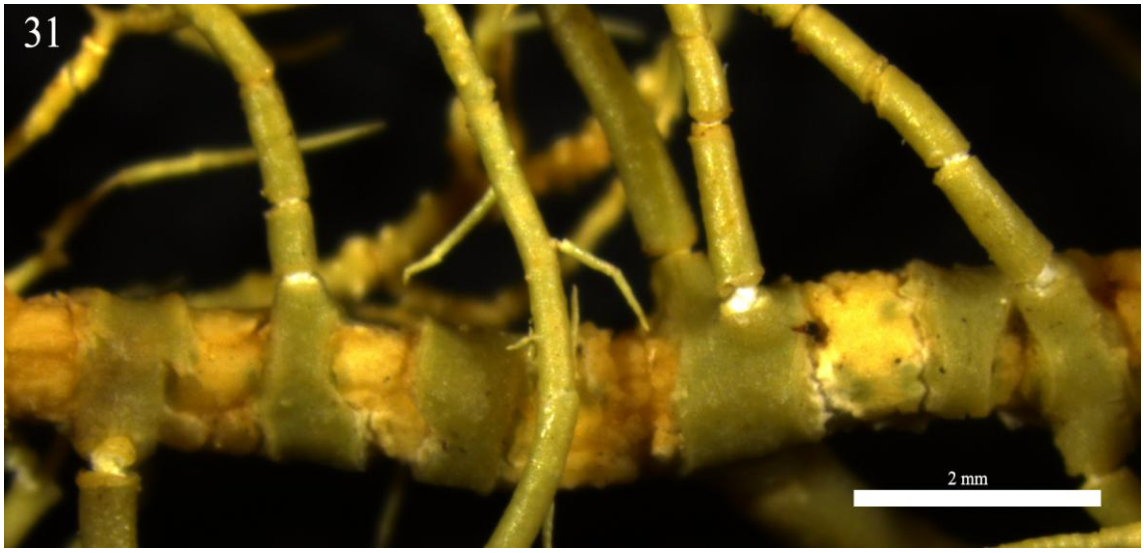
20a. Central axis > 50% of the total width of main branches……...21

20b. Central axis < 50% of the total width of main branches…….....22

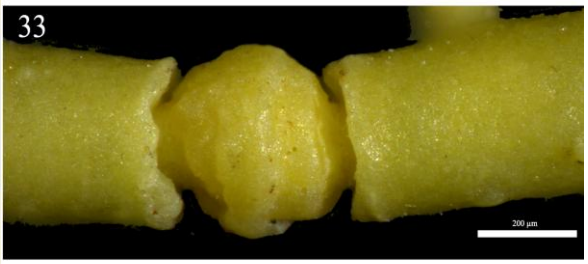
21a. Large and low tubercles slightly whitish at their top, rarely eroded on main branches **[30]** .....**U. transitoria**



21b. Large tubercles absent or rare……..**U. mexicana**



22a. Main branches distinctly segmented with ± large, paler and distinctly corticated beads–like regeneration areas in between the segments **[33]**; maculae distinctly present on thinner branches **[32]**; cortex mat in section **[22]**; medulla K+ red (salazinic acid)……..**U. merrillii**



22b. Regeneration areas absent or when present, even, not beads–like; maculae absent; cortex mat or glossy in section; medulla K+ yellow (stictic) or K+ red (galbinic acid)…….....23

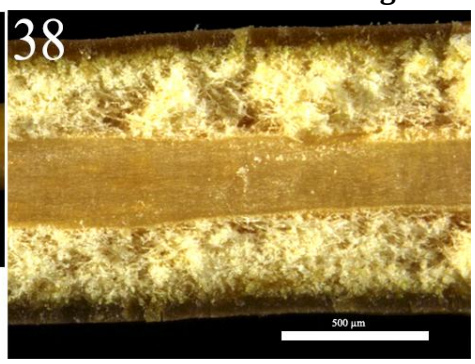
23a. Branch segments weakly to strongly ridged, often with numerous foveolae and papillae **[34]**; central axis often sinuous; cortex glossy; medulla K+ red (galbinic acid) …..**U. chilensis**

23b. Branch segment terete in cross section; never with numerous foveolae and papillae at the same time; central axis never sinuous; cortex mat or glossy; medulla K+ (galbinic or stictic acid)……. ……...……...……..24

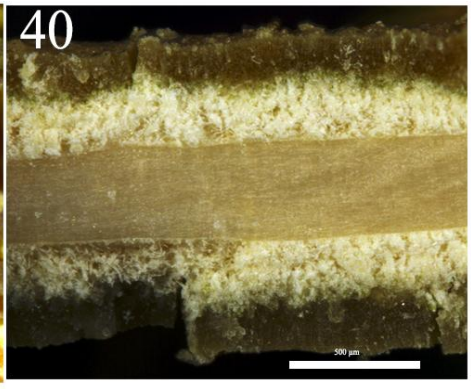
24a. Cortex glossy in section, thin (5–8%) **[36]**; branches with numerous and densely arranged spinulose fibrils **[35]**; medulla often faint orange periaxially **[36]**, K+ red (galbinic acid)…… **U. dasaea**

24b. Cortex mat in section; thin or thicker; without numerous spinulose fibrils; medulla K+ (galbinic or stictic acid)……..……...……...……25

25a. Soralia punctiform; numerous; even with the cortex; often aggregating together in packets **[37]**; cortex thin (5–7%) **[38]** ……..…...……...……**U. geissleriana**



25b. Soralia punctiform stipitate on top of eroded tubercles, not aggregating together **[39]**; cortex thick (12–18%) **[40]**……...……...……**U. subflammea**



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References

Awasthi, G. (1986) Lichen genus *Usnea* in India. *Journal of the Hattori Botanical Laboratory* 61: 333–421.

Calvelo, S., Stocker-Wörgötter, E., Liberatore, S., & Elix, J.A. (2005) *Protousnea* (Parmeliaceae, Ascomycota), a genus endemic to Southern South America. *The Bryologist* 108(1):1–15.

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

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

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

Clerc, P. (1997) Notes on the genus *Usnea* Dill. Ex Adanson. *Lichenologist* 29: 209–215.

Clerc, P. (1998) Species concepts in the genus *Usnea* (lichenized Ascomycetes). *Lichenologist* 30: 321–340.

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

Clerc, P. (2007) *Usnea*. In Lichen Flora of the Greater Sonoran Desert Region (T. H. Nash III, C. Gries & F. Bungartz, eds): 302–335. Tempe, Arizona: Lichens Unlimited, Arizona State University.

Clerc, P. (2011) *Usnea*. In Nordic Lichen Flora Vol. 4 (A. Thell & R. Moberg, eds.): 107–127. Uddevalla: Nordic Lichen Society.

Clerc, P. & Herrera-Campos, M.A. (1997) Saxicolous species of *Usnea* subgenus *Usnea* (lichenized Ascomycetes) in North America. *Bryologist* 100: 281–301.

Culberson, C.F. & Ammann, K. (1979) Standardmethode zur Dünnschichtchromatographie von Flechtensubstanzen. *Herzogia* 5: 1–24.

Culberson, C.F. & Johnson, A. (1982) Substitution of methyl tert-butyl ether for diethyl ether in the standardized thin-layer chromatographic method for lichen products. *Journal of Chromatography* 238: 483–487.

Divakar, P.K., Crespo, A., Wedin, M., Leavitt, S.D., Hawksworth, D.L., Myllys, L., McCune, B., Randlane, T., Bjerke, J.W., Ohmura, Y. *et al.*, 2015. Evolution of complex symbiotic relationships in a morphologically derived family of lichen forming fungi. *New Phytologist* 208: 1217–1226

Galloway, D. (2007) Flora of New Zealand Lichens. Revised Second Edition Including Lichen-Forming and Lichenicolous Fungi. Volumes 1 and 2. - Manaaki Whenua Press, Lincoln, New Zealand. i–cxxx + 2,261 pp

Gerlach, A., Clerc, P. & Borges da Silveira, R.M. (2017) Taxonomy of the corticolous, shrubby, esorediated, Neotropical species of *Usnea* Adans. (*Parmeliaceae*) with an emphasis on Southern Brazil. *The lichenologist* 49(3): 199–238.

Hale, M.E. Jr. (1979) How to Know the Lichens, 2nd edition. Dubuque, Iowa: William C. Brown.

Herrera-Campos, M.A. (2016) *Usnea* in Mexico. *Bibliotheca Lichenologica* 110: 505–620.

Herrera–Campos, M.A., Clerc, P. & Nash, T.H., III. (1998) Pendulous species of *Usnea* from the temperate forests in Mexico. *Bryologist* 101: 303–329.

Hinds, J.W. & Hinds, P.L. (2007) The Macrolichens of New England. - Memoirs of the New York Botanical Garden No. 96. New York Botanical Garden Press, Bronx, New York. 584 pp.

Krog, H. (1976) *Lethariella* and *Protousnea*, two new lichen genera in the Parmeliaceae. *Norwegian Journal of Botany* 23: 83–106.

Lücking, R., Hodkinson, B.P. & Leavitt, S.D. 2017(2016). The 2016 classification of lichenized fungi in the Ascomycota and Basidiomycota –Approaching one thousand genera. *The Bryologist* 119(4):361–416.

Motyka, J. (1936) Lichenum Generis *Usnea* Studium Monographicum. Pars Systematica I. Leopoldi: privately printed.

Motyka, J. (1938) Lichenum Generis *Usnea* Studium Monographicum. Pars Systematica II. Leopoldi: privately printed.

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

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

Räsänen, V. (1936) Collationes ad lichenologiam Chilensem pertinentes. *Rev. Univers. Santiago* 21(1): 137–148.

Rodriguez, J.M. (2011) El género *Usnea* (Ascomycetes liquenizados) en Argentina: estudio sistemático y biogeográfico. Tesis de doctorado en Ciencias Biologicas. Cordoba, Argentina.

Rodriguez, J.M., Estrabou, C., Truong, C., & Clerc, P. (2011) The saxicolous species of the genus *Usnea* subgenus *Usnea* (Parmeliaceae) in Argentina and Uruguay. *The Bryologist* 114(3): 504–525.

Stevens, G.N. (2004) *Usneaceae*. In Flora of Australia Vol. 56A, Lichens 4 (P. M. McCarthy & K. Mallett, eds.): 78–98 & 107–115. Melbourne: ABRS/CSIRO.

Swinscow, T.D.V. & Krog, H. (1976) The *Usnea articulata* aggregate in East Africa. *Norwegian Jour. Bot.* 23: 261–268.

Swinscow, T.D.V. & Krog, H. (1978) Pendulous species of *Usnea* in East Africa. Norwegian *Journal of Botany* 25: 221–241.

Truong, C., Rodriguez, J.M. & Clerc, P. (2013) Pendulous *Usnea* species (Parmeliaceae, lichenized Ascomycota) in tropical South America and the Galapagos. *Lichenologist* 45: 505–543.

Species	N	Cortex (%)	Medulla (%)	Axis (%)	Ratio A/M
U. angulata[c]	35	(4.5–) 6–8.5–11 (–15)	(2–)8–14–20(–28.5)	(31–) 41–53–65 (–83)	≥ 2
U. arthroclada[d]	19	5–7.5	28–34.5	19–30	≤ 1.25
U. chilensis*	27	(2.5–) 4.5–6–7.5(–8.5)	(16.5–) 22–26.5–31 (–38.5)	(18–) 26.5–34.5–42.5 (–57)	0,8–1.4–2
U. dasaea[b]	77	(2.5–) 5–6.5–8(–13)	(13–) 21–26.5–32 (–37.5)	(14–) 24–34–44 (–57)	≤ 1.5
U. disjuncta	2	2	40.5	15	0.4
U. aff. disjuncta	4	4.5–6–7.5 (–8)	29.5–31.5–33.5	(22–) 23.5–26–28.5	< 1
U. dodgei*	5	(7–) 8–11–14	(20–) 21–24–27 (–28)	(22–) 24.5–29–33.5 (–34)	0.8–1.2–1.6
U. geissleriana[a]	4?	(4–) 5–6–7 (–8.5)	22–26.5–31 (–31.5)	26–34.5–43 (–58)	≤ 1.5
U. malmei[c]	5	(6.5–) 8–13–18	9–14.5–20 (–24.5),	(32.5–) 35–45–55 (–60)	≥ 2
U. merrillii[c]	60	(6–) 10–13–16 (–21)	(7.5–) 14–18–22 (–28.5)	(25–) 32–38.5–45 (–53)	≥ 1
U. mexicana*	3	7.5–8	14–15.5	53.5–56.5	3.5–4
U. mexicana[c]	10	(2–) 3–6.5–10 (–15)	(12–) 16–20–24.5 (–26)	(30–) 36.5–48–59.5 (–71.5)	≥ 2.5
U. papillata[c]	1	12	20	36	1.8
U.perhispidella[d]	14	7–10	13–20	44.5–56	≥ 2
U. regia[d]	26	8.5–12.5	17–24	31.5–44.5	≥ 1.25
U. sanctaeritae[c]	20	(8–) 10–13–16 (–17)	(12–) 14–17–20 (–22.5)	(30)–33–39.5–46–(53)	> 2
U. subflammea[a]	38	(9.5–) 12–14–16 (–17)	(12.5–) 13–16–19 (–22.5)	(30–) 33–40–47 (–54)	≥ 1
U. subgracilis[c]	53	(3.5–) 8.5–11.5–14.5 (–20.5)	(6.5–) 10.5–14.5–18.5 (–23)	(27–) 37–47.5–58 (–80)	≥ 1
U. subscabrosa[c]	72	(9–) 11.5–15–18.5 (–24.5)	(4.5–) 7–9.5–12 (–15)	(21.5–) 33–40.5–48 (–55)	≥ 2
U.subsilesiaca*	24	(7.5–)10.5–12.5–14(–18)	(4–)4.5–8.5–12.5(–14)	(43–)48–58–68(–77)	3.5–8–13
U. transitoria*	2	4–4.5	10–17	58–71	≥ 3.5
U. transitoria[c]	32	(2.5–) 4–8–12 (–20)	(2.5–) 6–11–16 (–29.5)	(27.5–) 49–6–75 (–88)	≥ 5
U. venusta*	5	1.5–2.5–3.5 (–4)	39–40.5–42 (–42.5)	(11–) 11.5–13.5–15.5 (–17)	≤ 0.5
Usnea sp. 1*	8	(3–)3.5–5–6.5(–7)	(24.5–) 28.5–33–37.5 (–39.5)	(14.5–) 17–23.5–30.5 (–36.5)	≤ 1.5

TABLE 2. Cortex, medulla and axis (CMA) percentages of the pendulous species. Extreme values, values, and mean are given. N = total of individual thalli measured. [a]Clerc (2006); [b]Clerc & Herrera-Campos (1997); [c]Herrera-Campos *et al.* (1998); [d]Truong *et al.* (2013); *This study.

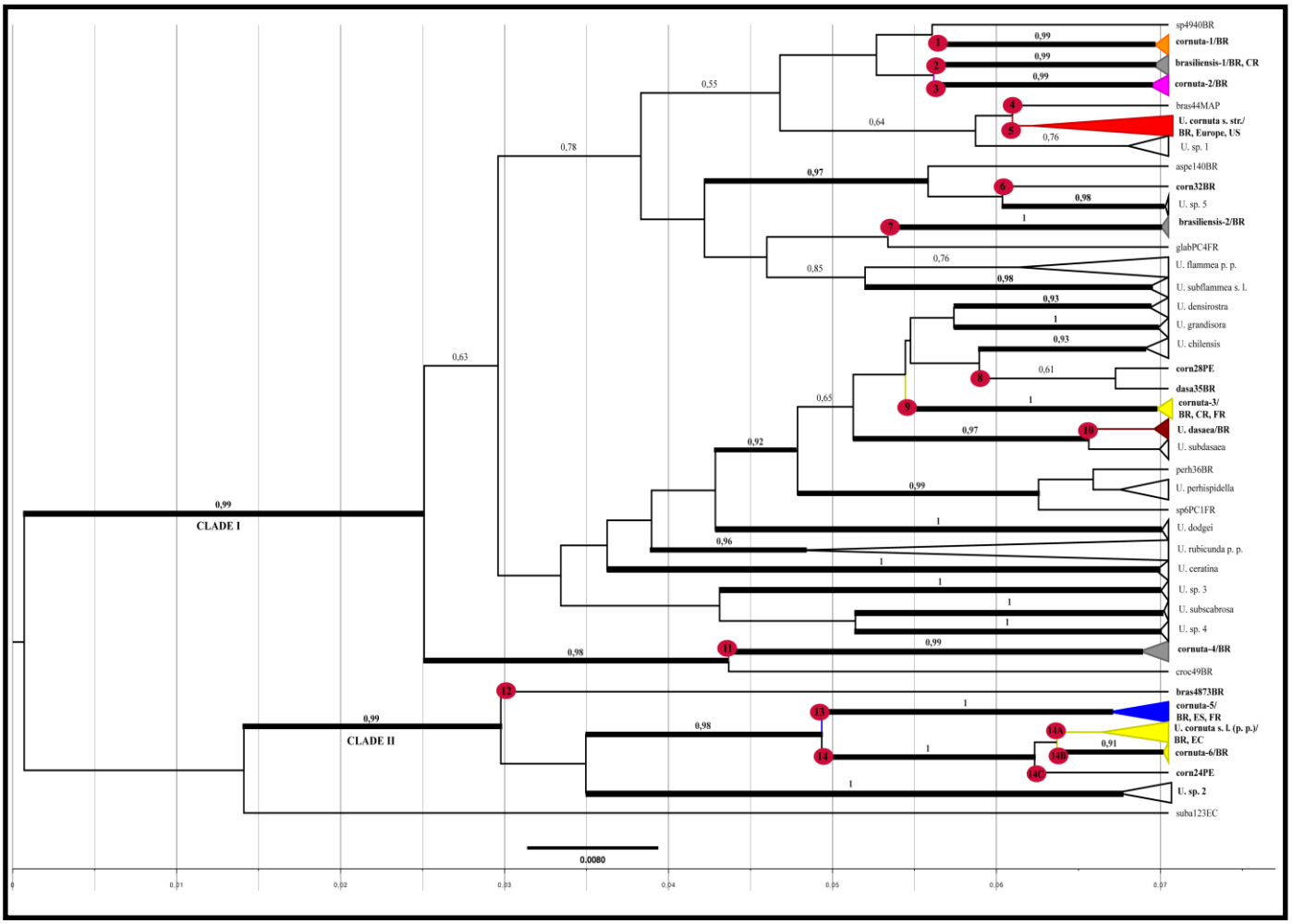
Species	n	SAL	STI	CST	CRY	ME	NOR	GAL	DIF	BAR	PRO	EUM	TER	FA	Us1	Us2	Ch0	Medulla color test
Usnea alata	16													±			+	K–, P–
U. angulata	10						+											K+ y→r
U. arthroclada	10		+	+	+	+	+											K+ y
	3	+																K+ y→r
	1						+											K+ y→r
U. chilensis	37	+					+	+										K+ y→r
U. dasaea	6	+					+	+										K+ y→r
U. disjuncta	2								+	+								K+ br
U. aff. disjuncta	4	+					+	+										K+ y→r
U. dodgei	5	+					+	+							±			K+ y→r
U. geissleriana	2		+	+	±	±	+											K+ y
	2	+					+											K+ y→r
U. malmei	15												±			+		K–, P–
	1																+	K–, P–
U. merrillii	4	+																K+ y→r
U. mexicana	3										+							K–, P+ y
	2		+	+	+	+	tr					tr						K+ y
U. papillata	5												±			+		K–, P–
U. perhispidella	3		+	+	+	+	+											K+ y
U. regia	5		+	+	+	+	tr											K+ y
U. sanctaeritae	3	+									± tr							K+ y→r
U. subflammea	25		+	+	±	±	+			rare								K+ y
	16	+					+	+										K+ y→r
U. subgracilis	7										+							K–, P+ or
U. subscabrosa	15										+							K–, P+ y
U. subsilesiaca	21	+																K+ y→r
U. transitoria	2		+	+	±	±	±											K+ y
	1																+	K–, P–
U. venusta	4	+					+				±							K+ y→r
Usnea sp. 1	5	+									± tr							K+ y→r
	3		+	±	+	±	± tr											K+ y

TABLE 3. Major secondary metabolites and chemotypes of Brazilian *Usnea* species. Abbreviations for secondary metabolites: SAL = salazinic, STI = stictic, CST = constictic, CRY = cryptostictic, ME = menegazziaic, NOR = norstictic, GAL = galbinic, DIF = diffractaic, BAR = barbatic, PRO = protocetraric, EUM = eumitrin, TER = unidentified terpenes, FA = unidentified fatty acid, Us1 = pigment rose Rf classes A/B/C = 6/5/6, Us2 = an unknown Rf classes A/B/C = 1–2/1–2/1–2, Ch0 = usnic acid alone, n = number of specimens studied; + = presence constant within species; ± = presence variable among specimens within species; tr = present in traces; rare = only in one/two specimens. Abbreviations for medulla color test: y→r = yellow turning red; br = brownish; or = orange; y = yellow

CHAPTER 3

New insights into the *Usnea cornuta* aggregate (Parmeliaceae, lichenized Ascomycota): molecular analysis reveals a high genetic diversity correlated with chemistry

Submitted to *Molecular Phylogenetics and Evolution*



Graphical abstract. *Usnea* collapsed species tree from Stacey analysis. Posterior probabilities higher than 0.5 are shown above branches. Branches and probabilities with strong support (PP ≥ 0.90) are marked in bold. Their equivalent OTU are indicated inside circles. Red circles refer to the *Usnea cornuta* aggr. Colors indicate the major secondary groups as follow: **orange**: constictic acid; **gray**: protocetraric acid; **rose**: thamnolic acid; **red**: salazinic acid (stictic and constictic acids can also be present in this OTU); **yellow**: stictic acid; **strong red**: galbinic acid; **blue**: lobaric acid. See Table 1 for complementary information.

New insights into the *Usnea cornuta* aggregate (Parmeliaceae, lichenized Ascomycota): molecular analysis reveals a high genetic diversity correlated with chemistry

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Abstract

Complexity in the evolutionary mechanisms can obscure the signals for the recognition of evolutionary distinct lineages, in particular in the recently diverged groups of organisms, therefore evaluation of the evidences from diverse sources may be critical for the accurate assessment of the species boundaries in such groups. The increasing availability of DNA sequences data provides much deeper insight on our understanding of the various biological processes and their consequences on the biodiversity.

In this study, we used DNA sequences, chemical, geographic, and morphological data, and applied an integrative approach to define species boundaries in the lichen-forming genus *Usnea* (Parmeliaceae), with a particular focus on the cosmopolitan species group *U. cornuta* aggr. We provide the first multi species coalescent model based species delimitation for the Neotropical *Usnea* species. Based on the ITS rDNA, and two protein-coding genes RPB1, and *Mcm7*, we estimated the species tree under the multi species coalescent model in a Bayesian framework using the STACEY method. Our results indicate presence of at least nine, strongly supported, distinct lineages in the *U. cornuta* aggr, which can be characterized by specific chemistry. We found strong evidence for the polyphyly of the three species, *Usnea brasiliensis*, *U. cornuta* and *U. dasaea*, all belonging to the *U. cornuta* aggr.

Keywords: Coalescent model, lichen-forming fungi, parallel evolution, protein-coding genes, STACEY, systematics, secondary metabolites, thin-layer chromatography

1. Introduction

Species delimitation is a central topic in systematics and refers to the process of delineation of species boundaries. Accurate identification of species boundaries is primordial in many areas of biology such as evolution, ecology, population genetic and conservation (Boykin et al., 2012; Brooks et al., 2006; Myers et al., 2000). Nevertheless, recognizing independent evolutionary lineages is challenging. Several evolutionary processes as for instance incomplete lineage sorting (ILS) (Mallo and Posada, 2016; Naciri and Linder, 2015) might blur species boundaries at the molecular level and lead to misinterpretation of species delimitation (Carstens et al., 2013). Incomplete lineage sorting is indeed a major source of discordance between the gene trees and species tree. This process mainly impacts the species with recent divergence histories and results in a lower probability of genealogical concordance across multiple loci, and reciprocal monophyly for a single locus (Leavitt et al., 2016b), the two prerequisites for segregating species according to the "general lineage concept" of de Queiroz (2007). The coalescence model takes ILS into account, and a derived framework known as the multi species coalescent (MSC; Rannala and Yang, 2003) is now implemented in several softwares used for species delimitation based on DNA sequences data (Bryant et al., 2012; Carstens et al., 2013; Heled and Drummond, 2010; Leavitt et al., 2015; Yang and Rannala, 2010, 2014) with or without the requirement of defining putative species *a priori*.

The improper assignment of individual sequences to the putative species prior to the analyses can seriously bias the outcomes (Leaché and Fujita, 2010; Olave et al., 2014). Therefore within groups for which taxonomy is poorly resolved, the choice of the method is critical for minimizing such bias. STACEY, implemented in BEAST2 (Bouckaert et al., 2014), is a recently developed program by Jones (2016), and it is one of the few approaches that do not require *a priori* assignment of individuals to putative species. This method has been used for species delimitation in plants (Naciri et al., 2017; Toprak et al., 2016; Wagner et al., 2017) as well as in lichens (Kanz et al., 2015; Mark et al., 2016). MSC model has over simplified assumptions, and so that all the methods based on MSC model are imperfect interpretations of the evolutionary process in nature (Carstens et al., 2013) and were reported to overestimate the number of potential species (Sukumaran and Knowles, 2017). For that reason, integrative morphological, anatomical, chemical, ecological and genetic data must be associated and carefully evaluated before any taxonomical conclusion can be drawn.

Usnea forms a strongly supported monophyletic lineage within the family Parmeliaceae (Crespo et al., 2007). The genus is characterized by a fruticose thallus with usnic acid in the cortex and an elastic central cord in the medulla (Clerc, 2011). *Usnea* is one of the most-species rich lichen genus with over 350 species distributed worldwide (Lücking et al., 2017). It displays a recent divergence history, with a diversification starting within the last 20-25 million years between the late Oligocene and the mid-Miocene (Divakar et al., 2017; Kraichak et al., 2015). Due to the its species high morphological variability, *Usnea* is known as one of the most difficult lichen genus (Clerc, 1998) and therefore species delimitation is highly controversial. Therefore, the use of genetic data is critical for the proper delimitation of the divergent lineages of *Usnea*.

Previous molecular studies in the genus (Kelly et al., 2011; Ohmura, 2008; Saag et al., 2011; Truong et al., 2013a) showed that species described based on morphological, and chemical characters are usually monophyletic, thus suggesting the traditional taxonomy as a suitable tool for species delimitation in *Usnea*. However, there still could be cryptic or overlooked species because of subtle phenotypical differences. Nevertheless, recent studies on some South American *Usnea* taxa (Truong et al., 2013a; Gerlach et al., 2017) revealed some species being polyphyletic. For instance, in Truong et al. (2013a: Fig. 2) *Usnea cornuta,* appears into two separate clades ("Usnea-3" and "Usnea-4") with good support.

Such data can ease the discovery of natural groups in many fungal lineages (Crespo and Pérez-Ortega, 2009), for instance by leading to detection of unseen, subtle morphological and anatomical differences beside the possible cryptic species. Furthermore, molecular studies are essential to test whether chemotypes are part of the intra-specific chemical variation on the genus but also other unrelated lichens (Gerlach et al., 2017; LaGreca, 1999; Stocker-Wörgötter et al., 2004; Truong et al., 2013a) and if the chemistry is a good predictor for delimiting species (Alors et al., 2016; Lendemer, 2012; Resl et al., 2016; Schmitt and Lumbsch, 2004).

The *Usnea cornuta* aggregate, as defined here includes *U. brasiliensis* (Zahlbr.) Motyka, *U. cornuta* Körb. and *U. dasaea* Stirt. These species are mainly corticolous, with an erect-shrubby to rarely subpendulous life form, composed of more or less inflated branches constricted at their point of attachment and relatively small, punctiform soralia usually covered with isidiomorphs. The CMA, defined as the relative thickness of the cortex (C), medulla (M) and axis (A) of the main branches (in a longitudinal branch section) is of the *cornuta*-type (Truong et al., 2011) or the *brasiliensis*-type (Gerlach et al., 2017). Truong et

al., (2013a) showed that this group is however artificial, based on homoplasious characters. Cases of parallel evolution are common in lichen-forming *Fungi* and have been documented by many studies (e.g., Lutzoni et al., 2004; Rivas-Plata and Lumbsch, 2011).

The taxa of this aggregate show a high degree of morphological variability (degree of branches inflation, form and ontogeny of soralia, frequency of isidiomorphs) as well as anatomical variability (transition forms between the *brasiliensis*- and the *cornuta*-type of CMA, density of the medullary hyphae). For this reason lichenologists often identified them as "*U. cornuta* s. l." Moreover the chemistry of this group was shown to be highly diverse with salazinic, stictic or norstictic acids (Clerc, 1987, 2006, 2011; Halonen et al., 1998; Herrera-Campos et al., 2001; Herrera-Campos, 2016), protocetraric, fumarprotocetraric or psoromic acids (Herrera-Campos et al., 2001; Herrera-Campos, 2016), galbinic acid (Clerc, 2004, Herrera-Campos, 2016) or lobaric acid (Caviró, 2015). Finally, this aggregate is known to occur worldwide on every continent, i.e. along the western coast of Europe (Caviró, 2015, as *Usnea* sp. 1; Clerc, 1987, 2011; Smith et al., 2009), in India (Awasthi, 1986), in Japan (Clerc, 2004; Ohmura, 2001, as *U. confusa* Asahina), in North eastern (Hinds and Hinds, 2007) and Western north America (Clerc, 2007), in Mexico (Herrera-Campos, 2016) in Australia (Stevens, 2004, as *U. confusa* Asahina), in eastern Africa (Swinscow and Krog, 1988, as *U. undulata* Stirt.) and in South America (Motyka, 1938, as *U. jelskii* Motyka; Rodriguez 2011).

As a consequence of this high variability and broad occurrence, numerous taxa were described in this aggregate, e.g. *U. confusa* Asahina, *U. inflata* (Duby) Motyka, *U. constrictula* Stirt., *U. intexta* Stirt., *U. subhirta* (Vain.) Motyka and *U. subpectinata* Stir. were synonymized with *U. cornuta* Körb. (Clerc, 1987, 2007, 2011); *U. confusa* subsp. *subconfusa* Asahina, *U. dolosa* Motyka, *U. galbinifera* Asahina, *U. kinkiensis* Asahina, *U. kyotoensis* Asahina, *U. pygmea* subsp. *kitamiensis* Asahina *U. spinigera* Asahina, *U. spinulifera* (Vain.) Motyka and *U. undulata* Stirt. were synonymized with *U. dasaea* Stirt. (Clerc & Herrera-Campos, 1997; Ohmura 2001).

In the last years, several molecular studies have shown that the diversity in many groups of lichens is underestimated, and numerous cryptic species were revealed (Alors et al., 2016; Crespo and Lumbsch, 2010; Leavitt et al., 2011b, 2016a; Singh et al., 2015; Wei et al., 2016). On the other hand, it was shown that molecular phylogenies generally support the current morphology-based species delimitations in lichens, suggesting that not all currently accepted species are necessarily repositories of hidden diversity (Geml et al., 2010). In this

context, the cosmopolitan *Usnea cornuta* aggregate requires a detailed molecular study to understand its species boundaries

Our goals, in this study of species delimitation in the *U. cornuta* aggregate based for the first time on the MSC model, were to access interspecific genetic diversity inside this aggregate and to see if there is a link between the genetic and the chemical diversities. Additional morphological studies and taxonomical conclusions will be presented in a further paper.

2. Material and Methods

2.1 Taxon sampling

We sampled from 156 *Usnea* specimens representing the three species of the *cornuta* aggr. known as *U. cornuta*, *U. brasiliensis* and *U. dasaea,* 21 species outside the *U. cornuta* group, plus 12 unidentified specimens (Supplementary data S1). In total, 451 sequences of which 410 were generated and used. The remaining sequences (41) were obtained from Genbank. Overall, our sampling focused on the *U. cornuta* aggr. (n = 82) specimens span over a broad geographical area including Brazil (55), Costa Rica (2), Ecuador (1), France (3), Peru (3), Portugal (3), Spain (9), United Kingdom (5) and USA (1). We furthermore included one representative of *U. subaranae* from Ecuador since it falls in the more basal group in Truong et al. (2013a). Only specimens that have information for at least two markers were included in the analyses.

2.2 Morphological and chemical studies

All specimens were checked for their morphological characters under a Leica MS5 stereomicroscope .The percentage of the thickness of the cortex, the medulla and the axis versus the total branch diameter (CMA), and the ratio axis/medulla (A/M) were calculated according to Clerc (1987). Secondary metabolites were determined by thin-layer chromatography (TLC) based on the standard procedures using solvent systems A, B, and C (Culberson and Ammann, 1979) with solvent B modified following Culberson and Johnson (1982).

2.3 DNA extraction and amplification

DNA was extracted from 230 freshly collected *Usnea* specimens (the majority of the samples were less than one year old) using the central axis as the source to avoid

contamination by lichenicolous *Fungi.* We used a modified protocol from Zolan and Pukkila (1986) i.e. an SDS-Phenol-Chloroform protocol with a 2% sodium dodecyl sulphate (SDS) to break the cell walls, and a phenol:chloroform:IAA mix (15:24:1) to remove proteins.

PCR amplifications were performed with the following primers: 1) USITS3-F and USITS4-R (Truong et al., 2013a) for nuITS; 2) UsRPB1-R and UsRPB1-F for the *RPB1* region and UsMCM7-R and UsMCM7-F for the *Mcm7* region (Gerlach et al., 2017). Amplified products were checked on a 1% agarose gel, stained with SYBER, then purified using NucleoFast© plates (Macherey-Nagel) before sent to Macrogene. Thermal cycling parameters were indicated in Gerlach et al. (2017).

We generated sequence data from three loci: the nuclear ribosomal marker nu*ITS* and fragments of the two low-copy nuclear genes encoding the DNA replication licensing factor mini-chromosome maintenance complex component 7 (*Mcm7*), and the largest subunit of the RNA polymerase II (*RPB1*). The internal transcribed spacer region (ITS1, 5.8S and ITS2) is widely used as barcode for *Fungi* (Schoch et al., 2012), and the two protein-coding markers are known to provide good phylogenetic signal (Divakar et al., 2015; Truong et al., 2013a).

2.4 Alignments

Multiple alignments were generated using MAFFT v.7.222 (Katoh et al., 2002) with default settings, implemented on Geneious version 9.1.5 (http://www.geneious.com, Kearse et al., 2012), and then corrected manually. For each locus, the substitution models were assessed using jModelTest2 (Darriba et al., 2012), and the models were selected based on the Akaike information criterion (AIC).

2.5 Species delimitation based on the Multispecies Coalescent Model

To infer the number of potential species in the *Usnea cornuta* aggr., we performed multispecies coalescent model based species tree analyses using the program STACEY, version 1.2.2 (Jones et al., 2015; Jones 2016) implemented in BEAST 2.4.5 (Bouckaert et al., 2014). STACEY eliminates the need of assigning individual samples to the putative species *a priori*. The input files were prepared using the STACEY template supplied by BEAUTI2 (Bouckaert et al., 2014).

The species tree analyses were run with the GTR substitution model and assuming a relaxed exponential molecular clock, for each of the locus. The species tree prior was set to a Yule model with a "collapse height" (ε) of 1.10^{-4}, and the relative death rate of 0.5, as

recommended by Jones (2016) (See the supplementary material from Jones et al. 2015; Jones, 2016). For the species tree growth rate (bdcGrowthRate.t:Species),a lognormal distribution was chosen with a mean of 1.0, and a standard deviation of 1.25. The initial value of the node "collapse weight" (ω), that is the parameter associated to the number of possible clusters in the data set (see Jones et al., 2015; Jones, 2016), was set to 0.5 and stated as a beta distribution range of [0.0, 1.0] with the parameters alpha, and beta set to 1.0. For the population sizes, we specified an initial value of [0.0, 2000] on the popPriorScale together with a Gamma distribution with the parameters alpha = 2, and beta = 259 (see Supplementary data, S2 for the settings of the input files). We used an exponential distribution on uncorrelated exponential clock mean for each of the locus. All other parameters were set as default.

The input file was run for 200 million generations by sampling for every 10000 log and 100000 tree, as four parallel chains. Convergence of each chain, and the ESS values for related parameters were checked using TRACER v1.6 (Rambaut et al., 2013). Estimated species trees were summarized via Tree Annotator v2.4.5 (supplied with the BEAST package) with the maximum clade credibility (MCC) tree option after discarding the 10% of the generations as burn-in. The tree was visualized in Figtree v1.4.3 (http:// tree.bio.ed.ac.uk/software/figtree/).

MCMC samples of the species trees from STACEY runs were further processed via Species Delimitation Analyzer tool (Jones et al., 2015; Jones, 2016) to check the probability of individual sequence pairs being belong to the same cluster, with the options of 10% as burn-in,1.10^{-4} collapse height, and 1.0 similarity cut off. The output from the species delimitation analyzer, was then upload to the R v2.15.1 (R Core Team 2014) using the script (available in the supplementary material of Jones et al., 2015) to construct the similarity matrix. Similarity matrix is a two dimensional pairwise matrix, where the cells are colored according to the probability of the pair of individuals being belong to the same cluster.

3. Results

3.1. Sequence data

We generated a total of 410 new sequences for the genus *Usnea* 137 of which are from the nuclear ITS rDNA, 136 from the mitochondrial *Mcm7*, and 137 from the RPB1 region (Supplementary data, S1). The data matrix consists in 503 nucleotide characters for ITS, 439 for *Mcm7*, and 534 for *RPB1* locus. Our dataset comprised 156 taxa with a small amount of

missing data (0.7% for ITS, 0.5% for *Mcm7* and 5.8% for RPB1). Alignment information is summarized in Table 1.

Table 1: Putative species, their posterior probabilities in the species tree, their equivalent operational taxonomical (OTU) and their associated chemistry.

Posterior Probabilities	Delimited species	OTU	Geography	Main chemistry
-	Usnea sp.	15	Brazil	unknown depsidone
0.99	cornuta-1	1	Brazil	constictic
0.99	brasiliensis-1	2	Brazil, Costa Rica	protocetraric, ± psoromic
0.99	cornuta-2	3	Brazil	thamnolic
-	U. brasiliensis s. l.	4	Madeira Island	protocetraric
0.29	U. cornuta s. str.	5	Brazil, Europe, Peru, USA	salazinic or constictic or stictic
0.76	Usnea sp. 1	16	Brazil	salazinic
-	U. aspera	17	Brazil	norstictic
-	U. cornuta s. l.	6	Brazil	constictic
0.98	Usnea sp. 5	18	Brazil	protocetraric, fumarprotocetraric
1.00	brasiliensis-2	7	Brazil	protocetraric, psoromic
-	U. glabrata	19	France	salazinic
0.76	U. flammea	20	Tenerife island	stictic, ± lobaric
0.98	U. subflammea s. l.	21	Brazil	stictic
0.93	U. densirostra	22	Brazil	norstictic, ± salazinic
1.00	U. grandisora	23	Brazil	galbinic
0.93	U. chilensis	24	Brazil	galbinic
0.61	U. cornuta aggr.	8	Brazil, Peru	galbinic
1.00	cornuta-3	9	Brazil, Costa Rica, Europe	stictic
0.97	U. dasaea	10	Brazil	galbinic
0.99	U. perhispidella	25	Brazil	stictic
1.00	U. dodgei	26	Brazil	norstictic, ± salazinic
0.96	U. rubicunda aggr.	27	Brazil, Tenerife island	stictic, salazinic
1.00	U. ceratina	28	Brazil	diffractaic
1.00	Usnea sp. 3	29	Brazil	squamatic
1.00	U. subscabrosa	30	Brazil	protocetraric
1.00	Usnea sp. 4	31	Brazil	galbinic
0.99	cornuta-4	11	Brazil	protocetraric, fumarprotocetraric
-	U. crocata	32	Brazil	protocetraric
-	U. brasiliensis s. str.	12	Brazil	protocetraric
1.00	cornuta-5	13	Brazil, Europe	± lobaric, ± fatty acid
1.00	cornuta-6	14	Brazil, Ecuador, Peru	stictic or norstictic or usnic only
1.00	Usnea sp. 2	33	Brazil	protocetraric or salazinic
-	U. subaranea	34	Ecuador	usnic only

3.2. Species tree and Operational Taxonomic Units (OTUs)

In the species tree estimated by STACEY the genus *Usnea* was subdivided into two main clades that are highly supported, (Fig. 1) showing no geographical structure. Relationships among the species within these clades, however, remain unresolved (Fig. 1). Gene trees were in general agreement with each other and the species tree, (Supplementary data S3, S4, S5, and S6) with the only exception of that support values on the OTU 9, which is moderately supported in the ITS, and *Mcm7* gene trees (both PP = 0.86), but strongly supported in the RPB1 gene tree (PP = 0.95).

All the specimens fall into 34 OTUs of which 22 are strongly supported (PP ≥ 0.90). four are not supported (PP < 0.90, OTU 5, 8, 16 and 20) and eight OTUs consist in singletons (Fig. 1, Table 1).

Out of the 22 strongly supported OTUs, nine contain specimens of the *U. cornuta* aggr., seven OTUs correspond to well known species not belonging to the *U. cornuta* aggr. (e.g., *U. ceratina* Ach. OTU 28, PP=1.00; *U. chilensis* Motyka OTU 24, PP = 0.93; *U. densirostra* Taylor OTU 22, PP = 0.93; *U. dodgei* Motyka OTU 26, PP = 1.00; *U. grandisora* Truong & P. Clerc OTU 23, PP= 1.00; *U. subaranea* Truong & P. Clerc OTU 34; *U. subscabrosa* Motyka OTU 30, PP= 1.00), four OTUs include unidentified specimens (*U. sp.* 2 to *U. sp.* 5), and two OTUs contain specimens belonging to different species (see Table 1).

The taxa traditionally identified as *Usnea cornuta* and *U. brasiliensis* are distributed into the two main clades (Clade I and II; Fig. 1). Specimens identified as *U. dasaea* only fall into Clade I, however within two different OTUs of which only one (OTU 10) is well supported. This OTU include specimens of both *U. dasaea* and *U. subdasaea*, its pigmented equivalent. The other OTU is poorly supported (PP = 0.65) (OTU8) and occurs as sister to a specimen of *U. cornuta* (corn 28PE).

The specimens of the *Usnea cornuta aggr*. are included in 14 OTUs of which nine are well supported (OTU 1, 2, 3, 7, 9, 10, 11, 13 and 14), two are poorly supported (OTU 5, 8), and three appear as single branches (OTU 4, 6 and 12) (Table 1).

3.3. Correlation with chemistry within the *Usnea cornuta* aggregate

One major results of our study is the high correlation between the occurrence of the nine strongly supported *U. cornuta* aggr. OTUs and a specific chemistry (Table 1; Fig. 1). This aggregate displays a highly diverse chemistry. The majority of the main secondary compounds belongs to β-orcinol depsidones, although orcinol depsidones (e.g. lobaric acid)

and β-orcinol depsides (e.g. thamnolic acid) occur as well. OTU 1 includes specimens with constictic acid in the medulla; OTU 2 with protocetraric acid; OTU 3 with thamnolic acid; OTU 7 with protocetraric and psoromic acids; OTU 9 with stictic acid usually accompanied by the related accessory depsidones (constictic, cryptostictic, menegazziaic, and norstictic acids); OTU 10 with galbinic acid; OTU 11 with protocetraric and fumarprotocetraric acids, OTU 13 mostly with lobaric acid and OTU 14 mostly with stictic acid (Table 1; Fig. 1). The complete chemistry for all specimens is provided in Supplementary Table (Table S1).

3.4. The 14 OTUs of the *U. cornuta* aggr.

Among the fourteen OTUs of *U. cornuta* aggr., OTU 9 appears as the largest strongly supported group (PP = 1), including specimens from Brazil (14 specimens), Costa Rica (1), France (1, Corsica). All these specimens share the stictic acid chemotype. The second largest group (OTU 13, Fig. 1), also strongly supported (PP = 1), contains specimens from Spain (5), France (1, Corsica) and Brazil (2). Most of them (63%) share the lobaric acid chemotype. The third largest group, (OTU 14, Fig. 1) with only Neotropical specimens from Brazil (9), Peru (1) and Ecuador (1) in their majority (86%) share the stictic acid chemotype. This group is further divided into three subgroups (14A-C; Fig. 2) of which only one (14B) is well supported (PP = 0.91, Fig. 1).

All the other strongly supported groups of the *U. cornuta aggr.* (OTUs 1-3, 7, 10, 11; Fig. 1, 2) comprise only three to five specimens without any clear geographical pattern. Each one of these OTUs displays a distinct and constant chemotype (Table 1).

Conversely, the core group (OTU 5, Fig. 1), meaning the group including the largest number of *U. cornuta* specimens, is not supported (PP = 0.29; Table 1). It includes three smaller subgroups whose relationships are poorly understood (OTU 5A-C; Figs. 1, 2). OTU 5 includes 24 specimens from distinct geographical regions: Europe (12 specimens), Brazil (10), USA (1) and Peru (1). Three different chemotypes were found in this group (the salazinic acid chemotype, the stictic acid chemotype and the constictic acid chemotype). It is worth mentioning that none of the Brazilian specimens of this OTU has stictic acid but constictic or salazinic acids as the main medullary secondary compounds (Fig. 1). OTU 8 is not supported and contains two different species of the *U. cornuta* aggr. traditionally identified as *U. cornuta* and *U. dasaea*.

The three remaining OTUs represented by a single specimen are identified as *U. cornuta* (OTU 6), and *U. brasiliensis* (OTUs 4, 12) (Fig. 1).

Regarding the geographical distribution of the specimens analyzed in the *U. cornuta aggr*., there are four specimens from the Andes, Ecuador, and Peru that are located in different OTUs (5A, 8, 14A and 14C). Specimens collected in Brazil (55 specimens) are dispersed within 13 OTUs. Specimens from Costa Rica (2) are recovered into two distinct OTUs (OTUs 2 and 9). Specimens from Europe (20) are displayed in three distinct OTUs (OTU 5, 9 and 13), whereas the single specimen from USA is placed into OTU 5A (Fig. 3).

3.4.1. The morphospecies *U. cornuta*

Specimens of the taxon morphologically identified as *U. cornuta* appear in 9 different OTUs (Table 1). The core group OTU 5, and OTU 8 are not supported while OTUs 1, 3, 9, 11, 13 and 14 are strongly supported. On specimen appears as a singleton (OTU 8).

3.4.2. The morphospecies *U. brasiliensis* and *U. dasaea*

Some specimens of the nominal *Usnea brasiliensis* are included in two strongly supported clades (OTUs 2 and 7, both in the main Clade I). Two further specimens collected in Portugal and in Brazil appear as single branches, respectively OTU 4 in Clade I, and OTU 12 in Clade II.

Three specimens of the nominal *U. dasaea* cluster with *U. subdasaea* as a strongly supported group (PP=0.97, OTU 10 in Clade I). All these samples share the same galbinic acid chemotype. The last specimen identified as *U. dasaea* with the galbinic chemotype as well appears as sister of *U. cornuta* from Peru (OTU 8), however with poor support (PP=0.61).

4. Discussion

We found two highly supported clades in the phylogeny of the genus *Usnea*: Clade I includes the majority of the samples and agrees well with "Usnea-4" one of the four clades presented in the most complete phylogeny published so far (Truong et al., 2013a). According to these authors this clade contains the core of the diversity of the genus *Usnea* and includes a part of the *U. cornuta* aggr. The species tree (Fig. 1) shows the relationships among the taxa within this clade as poorly resolved. According to Truong et al. (2013a) such lack of resolution is due to the low sampling, compared to the existing diversity in the genus. Clade II corresponds to the clade "Usnea-3" published by Truong et al. (2013a) in which part of the *U. cornuta* aggr. from the Neotropical Andes is nested. Furthermore our analyses confirm the

polyphyly of the *U. cornuta* aggr. already reported by Truong et al. (2013a). We further demonstrate that what is taxonomically considered as *U. brasiliensis* is also polyphyletic since this taxon appears both in Clade I and Clade II. *Usnea dasaea* appears in Clade I in two separate lineages (OTU 8 and OTU 10) but due to the lack of support of the internal nodes the polyphyly of this group needs to be confirmed with further investigations.

One of the most interesting results of our study is the correlation between the chemistry and the nine strongly supported lineages within the *Usnea cornuta* aggr. (Table 1, Fig. 1). In previous taxonomical studies of the (sub)tropical species of the genus *Usnea*, mainly based on the traditional delimitation of species (Awasthi, 1986; Clerc, 2007; Herrera-Campos, 2016; Stevens, 2004; Swinscow and Krog, 1979; Truong et al., 2011, 2013b), many taxa were shown to be chemically variable including several chemotypes. However our results show a different picture where strongly supported lineages and chemotype are more specifically connected. As a matter of fact, secondary chemistry was recently found to be a good predictor of phylogenetic relationships in some groups of lichenized Fungi, including Peltigeraceae (Miadlikowska and Lutzoni, 2000), Pertusariaceae (Schmitt and Lumbsch, 2004), Teloschistaceae (Arup et al., 2013), Graphidaceae (Lumbsch et al., 2014). In some cases, the chemistry was even a good marker to separate and highlight "cryptic" species (Alors et al., 2016; Elix et al., 2009; Ohmura et al., 2008; Spribille et al., 2011).

4.1 Species delimitation within the morphospecies *Usnea cornuta*

4.1.1. The core group of *U. cornuta* (OTU 5)

The core group of *Usnea cornuta* (OTU 5A-C) contains specimens collected in Brazil, Europe and the USA. They morphologically all correspond well with *U. cornuta* Körb. (G!—isolectotype) described from Germany [CMA of the *cornuta*-type, soralia typically minute, even with the cortex, numerous and confluent]. In addition to the characteristic chemotypes of this taxon (salazinic or stictic acids: Clerc, 1987; Herrera-Campos et al., 2001), this OTU contains a constictic acid chemotype. We were not able to sequence the type specimen, but nevertheless morphological, anatomical and chemical evidences show that this OTU would correspond to *U. cornuta s. str.*with the exception of specimen corn29PE (see below), no clear morphological, anatomical and chemical differences could be observed between the specimens of this OTU. Considering the lack of support of the relationships within this group, we consider so far all the specimens of this OTU (with the exception of corn29PE) to

belong to *U. cornuta s. str.*Disentangling the relationships within this group will need further studies at finer-scale with a larger sampling over the whole distribution range of this lineage. Moreover, our phylogenetic analyses indicate that *U. cornuta s. str.*is present in Western Europe along the coast from Norway to the Iberian Peninsula in the Mediterranean ecotone, in some Atlantic islands (Azores, Western Canary Islands, Madeira, and Porto Santo), in the eastern coast of the USA, as well as in the Atlantic Forest of Brazil (oceanic distribution)**,** and therefore displays an intercontinental distribution (Figure 3, Supplementary data S1). Although several lineages in the family Parmeliaceae are known to have restricted geographical distributions (Crespo et al., 2010; Divakar et al., 2010), there is evidence pointing out that trans-oceanic dispersal occurs and that it has played a major role in the species diversification within the family (Amo de Paz et al., 2011; Fernández-Mendoza et al., 2011). Long-distance dispersal (LDD) of vegetative diaspores (soredia, isidia, isidiomorphs, isidiofibrils or thallus fragments) can be carried by wind (Galloway and Aptroot, 1995; Geml et al., 2010; Muñoz et al., 2004). LDD could therefore explain the distribution of U. *cornuta s. str.*

Our data furthermore suggest that *Usnea cornuta s. str.*in South America mainly occurs in Brazil, since the only specimen collected in the Andes present within OTU 5 (corn29PE, Fig. 1) does not fit morphologically with *U. cornuta s. str.*(soralia not confluent, CMA not of the *cornuta*-type). In our analysis the assignment of this Peruvian specimen to OTU 5A is not supported, whereas other evidence suggests that it may belong to OTU16 (see the similarity matrix, Fig. 2). This could be explained by the stochastic effects of the method. This OTU 5 probably has a complex and ancient evolutionary history with gradual accumulation of diversity over time as indicated by the relatively long internal branch lengths within OTU 5 (Richardson et al., 2001). All demographic changes have an influence on the effective population size and therefore on the time of coalescence (Naciri and Linder, 2015). Large effective population sizes are expected to lead to more ILS which can have an obscuring effect on species boundaries (Naciri and Linder, 2015). Therefore the weak support of OTU 5 (PP = 0.29; Fig. 1, value not shown) might be due to past demographic regimes.

Still, in this large group (OTU 5) it is interesting to see that none of the specimens collected in Brazil have the depsidone stictic acid in the medulla whereas it is a well-known chemotype of *U. cornuta s. str.*. Instead of stictic acid, we found the depsidone constictic acid as a new chemotype for *U. cornuta s. str.* Further studies with a larger sampling are needed to confirm the absence of stictic acid in *U. cornuta s. str.* in Brazil.

4.1.2. The second largest group of the *U. cornuta* (OTU 9)

On the contrary to OTU5, the second largest group of the U. *cornuta* aggr. (OTU 9, Fig. 1) is strongly supported (PP = 1). However the identity of the specimens of this group still needs to be investigated and it is here provisionally named *cornuta*-3. It is genetically not structured (Fig. 2), and the presence of samples from Brazil, France (Corsica) and Costa Rica indicates the ancient or ongoing gene flow, probably *via* LDD, among these oceanic regions that are geographically far apart from each other. The ancestral branch lengths between the node where diversification began and branch tips indicate a rapid and recent burst of diversification (Richardson et al., 2001). This contrasts with the core group of *U. cornuta* (OTU 5) where diversity was gradually accumulated. The presence of European specimens in this group is the first evidence that what is called *U. cornuta s. str.*in Europe consists in more than one species.

4.1.3. The third largest group of the *U. cornuta* (OTU 13)

The third largest group of the *U. cornuta* (OTU 13, Fig. 1) is also strongly supported (PP = 1) containing specimens from Brazil, France (Corsica), continental Spain, Canary Islands (Tenerife). This lineage was previously named as "*Usnea* sp.1" by Caviró (2015) who studied specimens from the Iberian Peninsula and reported that 75% of the specimens contained lobaric acid in the medulla. In our study, most of the specimens (63%) of this OTU have lobaric acid which is a rare secondary compound in *Usnea*. This compound was found in *U. flammea* Stirt. (Clerc and May, 2007) in 80% of the specimens analyzed and in *U. dorogawensis* Asahina (Ohmura, 2001). The identity of the specimens of this group still needs to be investigated and it is here provisionally named *cornuta*-5.

4.1.4. The "Neotropical group" of the *U. cornuta* (OTU 14)

The "Neotropical group" of the *U. cornuta* (OTU 14, Fig. 1) is strongly supported, but the relationships within the group are poorly understood. Preliminary morphological analyses suggest that at least five different morphospecies are present in this OTU.

OTU 14A is not supported and includes some specimens that do not belong to the *U. cornuta aggr*. (lateral branches not constricted). In contrast, OTU 14B is well supported (Fig. 1) including only specimens collected in Brazil mostly with the stictic acid chemotype. This lineage, referred here as "cornuta-6", is morphologically identical with *Usnea cornuta s. str.*(OTU 5). In Brazil specimens of "cornuta-6" seems to differ from *U. cornuta s. str.*only by

the presence of stictic acid. We may well be here in the presence of a cryptic species (Crespo and Lumbsch, 2010). OTU 14C is represented by a single specimen collected from Peru, that is morphologically dissimilar from specimens in OTUs 14A and 14B (Fig. 2).

The difficulties of delineating putative species in OTU 14 might be due, as discussed for OTU 5, to large effective population sizes with consequent incomplete lineage sorting (Naciri and Linder, 2015).

4.1.5. The Usnea cornuta from Neotropical Andes

Specimens of *U. cornuta* from the Andes (corn29PE in OTU 5A, corn28PE in OTU 8, corn27EC in OTU 14A and corn24PE in OTU 14C) are scattered in Clade I and Clade II. However, within each of these clades, their position is not supported and their relationships with the Brazilian specimens remain unclear. However their occurrences in the two main Clades indicate the strong divergence existing among them. The fact each of these specimens are more closely related to Brazilian specimens than to each other would suggest a possible common origin outside of the Andes. Further studies are needed to access this hypothesis. In each of the OTUs in which they occur, these specimens are genetically similar (Fig. 2) but morphologically dissimilar with the corresponding Brazilian specimens. This could be explained by the recent orogeny of the Andes (uplift of Andes ca. 20 MYA) that was related with recent and rapid radiations within the Neotropical Andes (Antonelli, 2009; Linder, 2008). According to Antonelli (2009), the presence of dispersal barriers between the Andes and Amazonian lowlands can lead to local speciation and isolation. This could explain the high level of endemism found in the Neotropical Andes, as already evidenced by Truong et al. (2013a) who showed a highly supported clade including only specimens from the Andes. The former authors described several new species from this area (Truong et al., 2011, 2013b; Truong and Clerc, 2012, 2013, 2016) with for instance *U. subdasaea* from Peru which differs from *U. subdasaea* from the Galapagos (Truong et al., 2013a). Similarly, Tehler et al. (2009) separated *Roccella lirellina* (Darb.) M. Choisy a Peruvian endemic species from the *Roccella galapagoensis* aggregate. Several endemic lichens were recently reported from the Galapagos archipelago (Dal Forno et al., 2017), which displays the genetic divergence among the lichens from the Galapagos and the ones from the Neotropical Andes.

4.2 Species delimitation within the morphospecies *Usnea brasilensis*

We found a high diversity within *U. brasiliensis* with four putative species (OTUs 2, 4, 7, 14), falling into Clade I and II, all of them with the chemotype protocetraric acid sometimes accompanied by psoromic acid (possibly an accessory substance). Among the eight specimens analyzed, the single specimen (bras4873BR, OTU 12) in Clade II, fits morphologically well with *U. brasiliensis* (Zahlbr) Motyka (holotype—W!). We were unable to sequence the type specimen but, based on our morphological analysis, we hypothesize that OTU12 corresponds to *U. brasiliensis s. str.*

4.3 Species delimitation within the morphospecies *Usnea dasaea*

Usnea dasaea specimens were found within two OTUs (8 and 10). Although the polyphyly of *U. dasaea* is not supported ((Fig. 1), preliminary morphological analyses suggest that specimens from the two OTUs correspond to two different species. The specimen dasa35BR (OTU 8) is morphologically similar to the holotype of *U. spinulifera* (Vain.) Motyka (TUR!) collected in Brazil which was synonymized with *U. dasaea* (holotype—BM!) by Clerc and Herrera-Campos (1997). Sequences of the type material of both species are needed to verify this synonymy. The three other specimens traditionally identified as *Usnea dasaea* fall within a strongly supported clade (OTU 10, PP = 0.97, Fig. 1) which is sister group to a lineage that includes two specimens of *U. subdasaea* Truong & P. Clerc (Fig. 1, 2). As suggested by the names, both species are morphologically similar and *Usnea subdasaea* differs mainly by its red subcortical pigment (Truong et al., 2011). However, the subcortical pigment can be faint and discontinuous which sometimes makes it difficult to separate the two species. Truong et al. (2013a) reported *U. subdasaea* as being polyphyletic. Each clade was reported to have a specific chemistry: one with triterpenoids and the other one with galbinic acid (type specimen). Our data suggest an alternative hypothesis where *U. subdasaea* specimens with galbinic acid and the specimens of *U. dasaea* of the OTU 10 (considered thus as "pigment deficient") would belong to the same species. In this study, we analyzed only four specimens based on a three loci which is not enough to resolve the species boundaries for this group. Miralles and Vences (2013) suggest using at least five specimens for per putative lineages for an efficient species delimitation analyses.

4.4. Miscellaneous results

First, it is interesting to note that all the species with a red cortical pigment including a specimen of *U. steineri* (stei97BR) with a red subcortical pigmentation, are included within a strongly supported group (OTU 27, Fig. 1, 2).. It is noteworthy that, in *U. steineri*, the pigment often goes into the cortex as well. Species delimitation in this group will require a much larger sampling to be resolved.

Second, our data suggest that *U. subflammea* P. Clerc is polyphyletic since the specimens from Brazil and Tenerife do not cluster together (OTUs 20-21, Fig. 1), however with low support.

5. Conclusions

We conclude that the *Usnea cornuta* aggr. represents a complex of nine strongly supported lineages correlated with secondary chemistry, as well as two unsupported groups (possibly including *Usnea cornuta s. str.*) and three singletons making a total of at least 14 distinct lineages. This shows that the diversity in the *U. cornuta* aggr., especially in Brazil was so far underestimated. Future investigations will show whether this genetic diversity correlates or not with morphology and anatomy.

The use of species delimitation under the Multi Species coalescent model provide powerful insights for understanding the species limits in this difficult group, especially in the clades with rapid and recent diversification .In difficult groups like *Usnea*, such a species delimitation approach finds its entire usefulness. Accordingly, STACEY provides us with a strong basis for future morphological analyses. It will be furthermore necessary to add more samples and more genes in the future analyses to improve the resolution within the clades.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at xxxx

References

Amo de Paz, G., Cubas, P., Divakar, P.K., Lumbsch, H.T., Crespo, A., 2011. Origin and diversification of major clades in parmelioid lichens (Parmeliaceae, Ascomycota) during the Paleogene inferred by Bayesian analysis. Plos One 6(12) e28161. https://doi.org/10.1371/journal.pone.0028161

Alors, D., Lumbsch, H.T., Divakar, P.K., Leavitt, S.D., Crespo, A., 2016. An Integrative approach for understanding diversity in the *Punctelia rudecta* species complex (Parmeliaceae, Ascomycota). Plos One 11(2): e0146537.https://doi.org/10.1371/journal.pone.0146537

Antonelli, A., Nylander, J. A. A., Persson, C. Sanmartín, I., 2009. Tracing the impact of the Andean uplift on Neotropical plant evolution. PNAS 106(24): 9749–9754. doi: 10.1073/pnas.0811421106

Arup, U., Søchting, U., Frödén, P., 2013. A new taxonomy of the family Teloschistaceae. Nordic Journal of Botany 31(1): 016–083.

Awasthi, G., 1986. Lichen genus *Usnea* in India. Journal of the Hattori Botanical Laboratory 61, 333–421.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu C-H, et al., 2014. BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. Plos Comput Biol 10(4): e1003537. doi:10.1371/journal.pcbi.1003537.

Boykin, L. M., Armstrong, K. F., Kubatko L, De Barro P. 2012. Species delimitation and global biosecurity. Evolutionary bioinformatics : 8:1-37. doi: 10.4137/EBO.S8532

Brooks, T. M., Mittermeier, R. A., da Fonseca, G. A., Gerlach, J., Hoffmann, M., Lamoreux, J. F., Mittermeier, C. G, Pilgrim, J. D., Rodrigues, A. S. 2006. Global biodiversity consertvation priorities. Science 7: 313(5783):58-61DOI:10.1126/science.1127609

Bryant, D., Bouckaert, R., Felsenstein, J., Rosenberg, N., RoyChoudhury, A. 2012 Inferring species trees directly from biallelic genetic markers: bypassing gene trees in a full coalescent analysis. *Molecular Biology and Evolution* 19(8):1917-1932.

Carstens, B.C., Pelletier, T.A., Reid, N.M., Satler, J.D., 2013. How to fail at species delimitation. Mol. Ecol. 22, 4369–4383.

Caviró, E. A., 2015. Sistemática integrada del género *Usnea* Dill. Ex Adans. (Parmeliaceae) en la Península Ibérica. PhD dissertation. Madrid, Spain.

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

Clerc, P., 1998. Species concepts in the genus *Usnea* (lichenized Ascomycetes). Lichenologist 30, 321–340.

Clerc, P., 2004. Notes on the genus *Usnea* Adanson. II. Bibliotheca Lichenologica 88: 79–90.

Clerc, P., 2006. Synopsis of *Usnea* (lichenized Ascomycetes) from the Azores with additional information on the species in Macaronesia. The Lichenologist 38(3): 191–212 doi:10.1017/S002428290600569X

Clerc, P., 2007. *Usnea*. In: Nash, T.H., III, Gries, C., Bungartz, F. (Eds.), Lichen Flora of the Greater Sonoran Desert Region. Arizona State University, Tempe, Lichen Unlimited, pp. 302–335.

Clerc, P., 2011. *Usnea*. In: Thell, A., Moberg, R. (Eds.), Nordic Lichen Flora. Nordic Lichen Society, Uddevalla, pp. 107–127.

Clerc, P., Herrera-Campos, M.A., 1997. Saxicolous species of *Usnea* subgenus *Usnea* (lichenized Ascomycetes) in North America. Bryologist 100, 281–301. https://doi.org/10.1639/0007-2745-114.3.504.

Clerc, P., May, P.F., 2007. *Usnea flammea* (Lecanorales) new for North America. Bryologist 110(1):126-128. https://doi.org/10.1639/0007-2745(2007)110[126:UFLNFN]2.0.CO;2

Crespo, A., Lumbsch, H.T., Mattsson, J.E., Blanco, O., Divakar, P.K., Articus, K., Wiklund, E., Bawingan, P.A., Wedin, M., 2007. Testing morphology-based hypotheses of phylogenetic relationships in Parmeliaceae (Ascomycota) using three ribosomal markers and the nuclear RPB1 gene. Molecular Phylogenetics and Evolution 44, 812–824. https://doi.org/10.1016/j.ympev.2006.11.029

Crespo, A., Pérez-Ortega, S., 2009. Cryptic species and species pairs in lichens: a discussion on the relationship between molecular phylogenies and morphological characters. Anales del Jardín Botánico de Madrid 66, 71–81. doi: 10.3989/ajbm.2225

Crespo, A., Ferencova, Z., Pérez-Ortega, S., Argüello, A., Elix, J.A., Divakar, P.K., 2010. *Austroparmelina*, a new Australasian lineage in parmelioid lichens (Parmeliaceae, Ascomycota). Systematics and Biodiversity 8, 209–221. http://dx.doi.org/10.1080/14772001003738320

Crespo, A., Lumbsch, H.T., 2010. Cryptic species in lichen-forming fungi. IMA Fungus 1: 167–170. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3348775/

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

Culberson, C. F., Johnson, A., 1982. Substitution of methyl tert-butyl ether for diethyl ether in the standardized thin-layer chromatographic method for lichen products. Journal of Chromatography 238: 483–487.

Dal Forno, M., Bungartz, F., Yánez-Ayabaca, A., Lücking, R., Lawrey, J. D., 2017. High levels of endemism among Galapagos basidiolichens. Fungal Diversity doi:10.1007/s13225-017-0380-6

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9, 772. doi:10.1038/nmeth.2109.

de Queiroz, K., 2007. Species concepts and species delimitation. Systematic Biology 56: 879–886. doi: 10.1080/10635150701701083.

Divakar, P.K., Lumbsch, H.T., Ferencova, Z., Del Prado, R., Crespo, A., 2010. *Remototrachyna*, a newly recognized tropical lineage of lichens in the Hypotrachyna clade (Parmeliaceae, Ascomycota), originated in the Indian subcontinent. American Journal of Botany 97, 579–590. DOI: 10.3732/ajb.0900140

Divakar, P. K., Crespo, A., Wedin, M., Leavitt, S. D., Hawksworth, D. L., Myllys, L., McCune, B., Randlane, T., Bjerke, J. W., Ohmura, Y. et al., 2015. Evolution of complex symbiotic relationships in a morphologically derived family of lichen forming fungi. New Phytologist 208: 1217–1226. doi: 10.1111/nph.13553.

Divakar, P.K., Crespo, A., Kraichak, E., Leavitt, S.T., Singh, G., Schmitt, I., Lumbsch, H. T., 2017. Using a temporal phylogenetic method to harmonize family and genus-level classification in the largest clade of lichen-forming fungi. Fungal Diversity DOI 10.1007/s13225-017-0379-z

Elix, J.A., Corush, J., Lumbsch, H.T. 2009. Triterpene chemosyndromes and subtle morphological characters characterize lineages in the *Physcia aipolia* group in Australia

(Ascomycota). Systematics and Biodiversity 7(4): 479487. http://dx.doi.org/10.1017/S1477200009990223

Fernández-Mendoza, F., Domaschke, S., García, M.A., Jordan, P., Martín, M.P., Printzen, C., 2011. Population structure of mycobionts and photobionts of the widespread lichen *Cetraria aculeata*. Molecular Ecology 20, 1208–1232. DOI: 10.1111/j.1365-294X.2010.04993.x

Galloway, D.J., Aptroot, A., 1995. Bipolar lichens: a review. Cryptogamic Botany 5: 184–191.

Geml, J., Kauff, F., Brochmann, C., Taylor, D.L., 2010. Surviving climate changes: high genetic diversity and transoceanic gene flow in two arctic–alpine lichens, *Flavocetraria cucullata* and *F. nivalis* (Parmeliaceae, Ascomycota). J. Biogeogr., 37: 1529–1542. doi:10.1111/j.1365-2699.2010.02287.x

Gerlach, A. da C.L., Clerc, P., Borges da Silveira, R.M., 2017. Taxonomy of the corticolous, shrubby, esorediate, neotropical species of *Usnea* Adans. (Parmeliaceae) with an emphasis on southern Brazil. The Lichenologist 49(3): 199–238. https://doi.org/10.1017/S0024282917000196

Halonen, P., Clerc, P., Goward, T., Brodo, I., Wulff, K., 1998. Synopsis of the genus *Usnea* (lichenized Ascomycetes) in British Columbia, Canada. Bryologist 101: 36–60. DOI: 10.2307/3244073.

Heled, J., Drummond, A. J., 2010. Bayesian inference of species trees from multilocus data. Mol. Biol. Evol. 27: 570–580.

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

Herrera-Campos, M. A., 2016. *Usnea* in Mexico. Bibliotheca Lichenologica 110: 505-620.

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

Hinds, J. W., Hinds, P. L., 2007. The Macrolichens of New England. Memoirs of the New York Botanical Garden No. 96. New York Botanical Garden Press, Bronx, New York. 584 pp.

Jones, G., Aydin, Z., Oxelman, B., 2015. DISSECT: an assignment-free Bayesian discovery method for species delimitation under the multispecies coalescent. Bioinformatics. doi: 10.1093/bioinformatics/btu770.

Jones, G. 2016. STACEY: species delimitation and phylogeny estimation under the multispecies coalescent. doi:http://dx.doi.org/10.1101/010199.

Kanz, B., Wolfgang von Brackel, Cezanne, R., Eichler, M., Hohmann, M-L., Teuber, D., Printzen, C., 2015. DNA Barcodes for the distinction of reindeer lichens: A case study using *Cladonia rangiferina* and *C. stygia*. Herzogia, 28(2):445-464.

Katoh, K., Misawa, K., Kuma, K., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucl. Acids Res. 30, 3059–3066

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., Drummond, A., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28(12), 1647-1649.

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

Kraichak, E., Divakar, P.K., Crespo, A., Leavitt, S.D., Nelsen, M.P., Lücking, R., Lumbsch, H.T., 2015. A tale of two hyper-diversities: diversification dynamics of the two largest families of lichenized fungi. Scientific Reports | 5:10028 | DOI: 10.1038/srep10028

LaGreca, S., 1999. A phylogenetic evaluation of the *Ramalina americana* chemotype complex (lichenized Ascomycota, Ramalinaceae) based on rDNA ITS sequence data. Bryologist 102: 602–618.

Leaché A.D., Fujita M.K. 2010. Bayesian species delimitation in West African forest geckos (*Hemidactylus fasciatus*). Proc. Biol. Sci. 277:3071–3077.

Leavitt, S. D., Johnson, L. A., St. Clair, L. L., 2011a. Species delimitation and evolution in morphologically and chemically diverse communities of the lichen-forming genus *Xanthoparmelia* (Parmeliaceae, Ascomycota) in western North America. American Journal of Botany 98, 175–188.

Leavitt, S.D., Fankhauser, J.D., Leavitt, D.H., Porter, L.D., Johnson, L.A., Clair. L.L., 2011b. Complex patterns of speciation in cosmopolitan “rock posy” lichens – Discovering and delimiting cryptic fungal species in the lichen-forming *Rhizoplaca melanophthalma* species-complex (Lecanoraceae, Ascomycota). Molecular Phylogenetics and Evolution 59(3), 587–602. https://doi.org/10.1016/j.ympev.2011.03.020.

Leavitt, S.D., Moreau, C.S., Lumbsch, H.T., 2015. The dynamic discipline of species delimitation: progress toward effectively recognizing species boundaries in natural populations. In: Upreti DK, Divakar PK, Shukla V, Bajpai R, editors. Recent Advances in Lichenology: Springer India. v. 2, pp. 11–44.

Leavitt, S.D., Esslinger, T.L., Divakar, P.K., Crespo, A., Lumbsch, H.T. 2016a. Hidden diversity before our eyes: Delimiting and describing cryptic lichen-forming fungal species in camouflage lichens (Parmeliaceae, Ascomycota). Fungal Biology 120: 1374-1391. doi: 10.1016/j.funbio.2016.06.001.

Leavitt, S.D., Divakar, P.K., Crespo, A., Lumbsch, H.T., 2016b. A matter of time – understanding the limits of the power of molecular data for delimiting species boundaries. Herzogia 29 (2) Teil 1. https://doi.org/10.13158/heia.29.2.2016.479

Lendemer, J.C., 2012. A tale of two species: molecular data reveal the chemotypes of *Lepraria normandinoides* (Stereocaulaceae) to be two sympatric species. The Journal of the Torrey Botanical Society 139(2):118-130. https://doi.org/10.3159/TORREY-D-11-00059.1

Linder, H. P., 2008. Plant species radiations: where, when, why? Phil. Trans. R. Soc. B 363: 3097–3105. doi:10.1098/rstb.2008.0075.

Lücking, R., Hodkinson, B.P., Leavitt, S.T., 2017(2016). The 2016 classification of lichenized fungi in the Ascomycota and Basidiomycota –Approaching one thousand genera. The Bryologist, 119(4):361-416. https://doi.org/10.1639/0007-2745-119.4.361

Lumbsch, H.T., Parnmen, S., Kraichak, E., Papong, K., Lücking, R., 2014. High frequency of character transformations is phylogenetically structured within the lichenized fungal family Graphidaceae (Ascomycota: Ostropales). Systematics and Biodiversity 12: 271–291. http://dx.doi.org/10.1080/14772000.2014.905506

Lutzoni, F., Kauff, F., Cox, C.J., McLaughlin, D., Celio, G., Dentinger, B., Padamsee, M., Hibbett, D., James, T.Y., Baloch, E., Grube, M., Reeb, V., Hofstetter, V., Schoch, C., Arnold, A.E., Miadlikowska, J., Spatafora, J., Johnson, D., Hambleton, S., Crockett, M., Shoemaker, R., Hambleton, S., Crockett, M., Shoemaker, R., Sung, G.H., Lucking, R., Lumbsch, H.T., O'Donnell, K., Binder, M., Diederich, P., Ertz, D., Gueidan, C., Hansen, K., Harris, R.C., Hosaka, K., Lim, Y.W., Matheny, B., Nishida, H., Pfister, D., Rogers, J., Rossman, A., Schmitt, I., Sipman, H.J.M., Stone, J., Sugiyama, J., Yahr, R., Vilgalys, R., 2004. Assembling the fungal tree of life: progress, classification and evolution of subcellular traits. American Journal of Botany 91, 1446–1480. doi: 10.3732/ajb.91.10.1446

Mark, K., Saag, L., Leavitt, S.D., Will-Wolf, S., Nelsen, M.P., Tõrra, T., Saag, A., Randlane, T., Lumbsch, H.T. 2016. Evaluation of traditionally circumscribed species in the lichen-forming genus *Usnea*, section *Usnea* (Parmeliaceae, Ascomycota) using a six-locus dataset Org Divers Evol 16: 497. doi:10.1007/s13127-016-0273-7.

Mallo D, Posada D. 2016. Multilocus inference of species trees and DNA barcoding. Phil. Trans. R. Soc. B 371: 20150335. http://dx.doi.org/10.1098/rstb.2015.0335

Miadlikowska, J., Lutzoni, F. 2000. Phylogenetic revision of the genus *Peltigera* (Lichen-Forming Ascomycota) based on morphological, chemical, and large subunit nuclear ribosomal DNA Data. International Journal of Plant Sciences 161(6): 925-958.

Motyka, J., 1938. Lichenum generis *Usnea* studium monographicum. Pars systematica. Leopoldi (privately printed).

Muñoz, J., Felicísimo, A.M., Cabezas, F., Burgaz, A.R., Martínez, I., 2004. Wind as a long-distance dispersal vehicle in the Southern Hemisphere. Science 21; 304(5674):1144-7. https://doi.org/10.1126/science.1095210

Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B., Kent, K. 2000. Biodiversity hotspots for conservation priorities. Nature **403**, 853-858 doi:10.1038/35002501

Naciri, Y., Linder, H. P., 2015. Species delimitation and relationships: The dance of the seven veils. Taxon 64: 3–16. https://doi.org/10.12705/641.24

Naciri, Y., Du Pasquier, P-E, Lundberg, M., Jeanmonod, D., Oxelman, B. 2017. A phylogenetic circumscription of *Silene* sect. *Siphonomorpha* (Caryophyllaceae) in the Mediterranean basin. Taxon 66(1): 91-108(18). http://www.ingentaconnect.com/content/iapt/tax/2017/00000066/00000001/art00006;jsessionid=2n5d16ou4hbs4.x-ic-live-02.

Miralles, A., Vences, M., 2013. New Metrics for Comparison of Taxonomies Reveal Striking Discrepancies among Species Delimitation Methods in *Madascincus* Lizards. PLoS ONE 8(7): e68242. https://doi.org/10.1371/journal.pone.0068242

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

Ohmura, Y., 2008. Molecular phylogeny of *Usnea rubicunda* and *U. rubrotincta* (Parmeliaceae, lichenized Ascomycotina) based on ITS rDNA sequences. Journal of Japanese Botany 83, 347–355.

Ohmura, Y.; Moon, K-H., Kashiwadani, H., 2008. Morphology and molecular phylogeny of *Ramalina pollinaria*, *R. sekika* and *R. yasudae* (Ramalinaceae, lichenized Ascomycotina). Journal of Japanese Botany 83: 156–164.

Olave M., Solà. E., Knowles L.L. 2014. Upstream analyses create problems with DNA-based species delimitation. Syst. Biol. 63:263–271.

Rambaut, A., Suchard, M.A., Xie, W., Drummond, A.J. 2013. Tracer v1.6. http://beast.bio.ed.ac.uk/

Rannala, B., Yang, Z., 2003. Bayes estimation of species divergence times and ancestral population sizes using DNA sequences from multiple loci. Genetics, 164, 1645–1656.

Resl, P., Mayrhofer, H., Clayden, S.R., Spribille, T., Thor, G., Tønsberg, T., Sheard, J.W., 2016. Molecular, chemical and species delimitation analyses provide new taxonomic insights into two groups of *Rinodina*. Lichenologis 48: 469-488. doi:10.1017/S0024282916000359

Richardson, J. E., Pennington, R.T., Pennington, T.D., Hollingsworth, P.M., 2001. Rapid diversification of a species-rich genus of neotropical rain forest trees. Science 21;293(5538):2242-5. DOI: 10.1126/science.1061421.

Rivas-Plata, E., Lumbsch, H.T., 2011. Parallel evolution and phenotypic divergence in lichenized fungi: a case study in the lichen-forming fungal family Graphidaceae (Ascomycota: Lecanoromycetes: Ostropales). Molecular Phylogenetics and Evolution 61, 45–63. https://doi.org/10.1016/j.ympev.2011.04.025

Saag, L., Tõrra, T., Saag, A., Del Prado, R., Randlane, T., 2011. Phylogenetic relations of European shrubby taxa of the genus *Usnea*. Lichenologist 43, 427–444.

Schmitt, I., Lumbsch, H. T., 2004. Molecular phylogeny of the Pertusariaceae supports secondary chemistry as an important systematic character set in lichen-forming ascomycetes. Molecular Phylogenetics and Evolution 33(1): 43–55. https://doi.org/10.1016/j.ympev.2004.04.014.

Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W.T., Consortium, F.B., 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences 109, 6241–6246. doi: 10.1073/pnas.1117018109.

Singh, G., Dal Grande, F., Divakar, P.K., Otte, J., Leavitt, S. D., Szczepanska, K., et al. 2015. Coalescent-based species delimitation approach uncovers high cryptic diversity in the

cosmopolitan lichen-forming fungal genus *Protoparmelia* (Lecanorales, Ascomycota). Plos One, 10(5), e0124625. doi:10.1371/journal. pone.0124625.

Smith, C. W., Aptroot, A., Coppins, B. J., Fletcher, A., Gilbert, O. L., James, P. W., Wolseley, P. A., 2009. The Lichens of Great Britain and Ireland: 1046.

Spribille, T., Klug, B., Mayrhofer, H., 2011. A phylogenetic analysis of the boreal lichen *Mycoblastus sanguinarius* (Mycoblastaceae, lichenized Ascomycota) reveals cryptic clades correlated with fatty acid profiles. Molecular Phylogenetics and Evolution 59: 603–614. doi: 10.1016/j.ympev.2011.03.021

Stevens, G.N., 2004. Usneaceae. In: McCarthy, P.M., Mallett, K. (Eds.), Flora of Australia. ABRS/CSIRO, Melbourne, pp. 78–98 and 107–115.

Stocker-Wörgötter, E., Elix, J.A., Grube, M., 2004. Secondary chemistry of lichen-forming fungi: chemosyndromic variation and DNA-analyses of cultures and chemotypes in the *Ramalina farinacea* complex. Bryologist 107: 152–162.

Sukumaran, J., Knowles, L.L., 2017. Multispecies coalescent delimits structure, not species. PNAS, Proceedings of the National Academy of Sciences 114(7): 1607–1612, doi: 10.1073/pnas.1607921114.

Swinscow, T. D. V., Krog, H., 1979. The fruticose species of *Usnea* subgenus *Usnea* in East Africa. Lichenologist 11, 207–252.

Swinscow, T. D. V., Krog, H., 1988. Macrolichens of East Africa. British Museum (Natural History), London. 390 pp.

Tehler, A., Irestedt, M., Bungartz, F., Wedin, M., 2009, Evolution and reproduction modes in the *Roccella galapagoensis* aggregate (Roccellaceae, Arthoniales). Taxon 58:438–456.

Toprak, Z., Pfeil, B.E., Jones, G., Marcussen, T., Ertekin, A.S., Oxelman, B., 2016. Species delimitation without prior knowledge: DISSECT reveals extensive cryptic speciation in the *Silene aegyptiaca* complex (Caryophyllaceae). Molecular Phylogenetics and Evolution 102: 1–8. http://www.sciencedirect.com/science/article/pii/S1055790316301130

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

Truong, C. and Clerc, P., 2012. The lichen genus *Usnea* (Parmeliaceae) in tropical South America: species with a pigmented medulla, reacting C+ yellow. Lichenologist 44: 625–637.

Truong, C. and Clerc, P., 2013. Eumitrioid *Usnea* species (Parmeliaceae, lichenized Ascomycota) in tropical South America and the Galapagos. Lichenologist 45: 383–395.

Truong, C., Divakar, P.K, Yahr, R., Crespo, A., Clerc, P., 2013a. Testing the use of ITS rDNA and protein-coding genes in the generic and species delimitation of the lichen genus *Usnea* (Parmeliaceae, Ascomycota). Molecular Phylogenetics and Evolution 68(2): 357-72. doi: 10.1016/j.ympev.2013.04.005.

Truong, C., Rodriguez, J. M., Clerc, P., 2013b. Pendulous *Usnea* species (Parmeliaceae, lichenized Ascomycota) in tropical South America and the Galapagos. Lichenologist 45: 505–543.

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

Yang, Z., Rannala, B., 2010. Bayesian species delimitation using multilocus sequence data. Proc. Natl. Acad. Sci. USA 107, 9264–9269.

Yang, Z., Rannala, B., 2014. Unguided species delimitation using DNA sequence data from multiple loci. Mol. Biol. Evol. 31, 3125–3135.

Zolan, M.E., Pukkila, P.J., 1986. Inheritance of DNA methylation in *Coprinus cinereus*. Molecular and Cellular Biology 6: 195–200.

Wagner, F., Härtl, S., Vogt, R., Oberprieler, C., 2017. "Fix Me Another Marguerite!": Species delimitation in a group of intensively hybridizing lineages of ox-eye daisies (Leucanthemum Mill., Compositae-Anthemideae). Molecular Ecology, online in advance of print. http://dx.doi.org/10.1111/mec.14180

Wei, X., McCune, B., Lumbsch, H.T., Li, H., Leavitt, S., Yamamoto, Y., et al., 2016. Limitations of species delimitation based on phylogenetic analyses: a case study in the *Hypogymnia hypotrypa* Group (Parmeliaceae, Ascomycota). Plos one 11(11): e0163664. https://doi.org/10.1371/journal.pone.0163664.

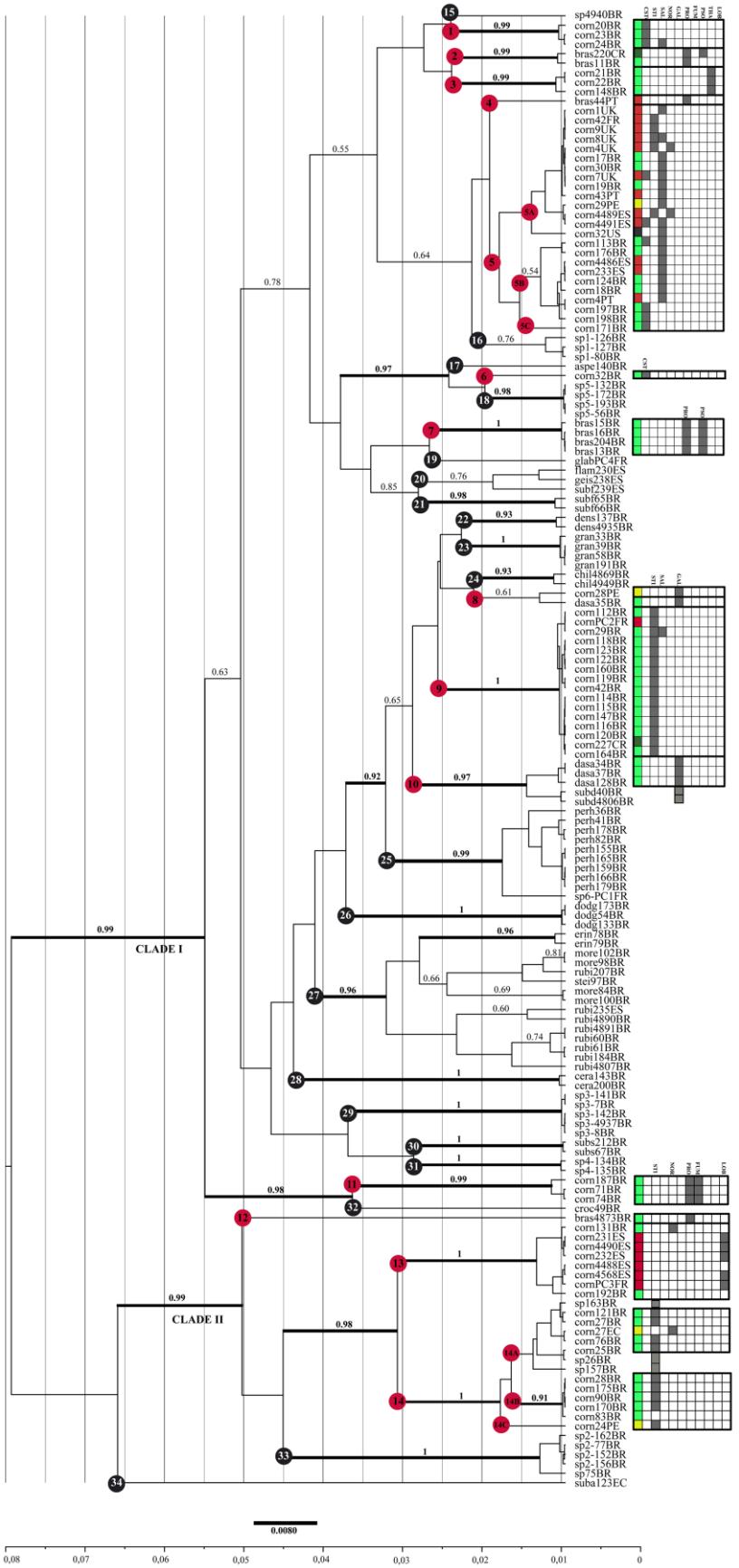


Fig. 1. Species tree from Stacey analysis.

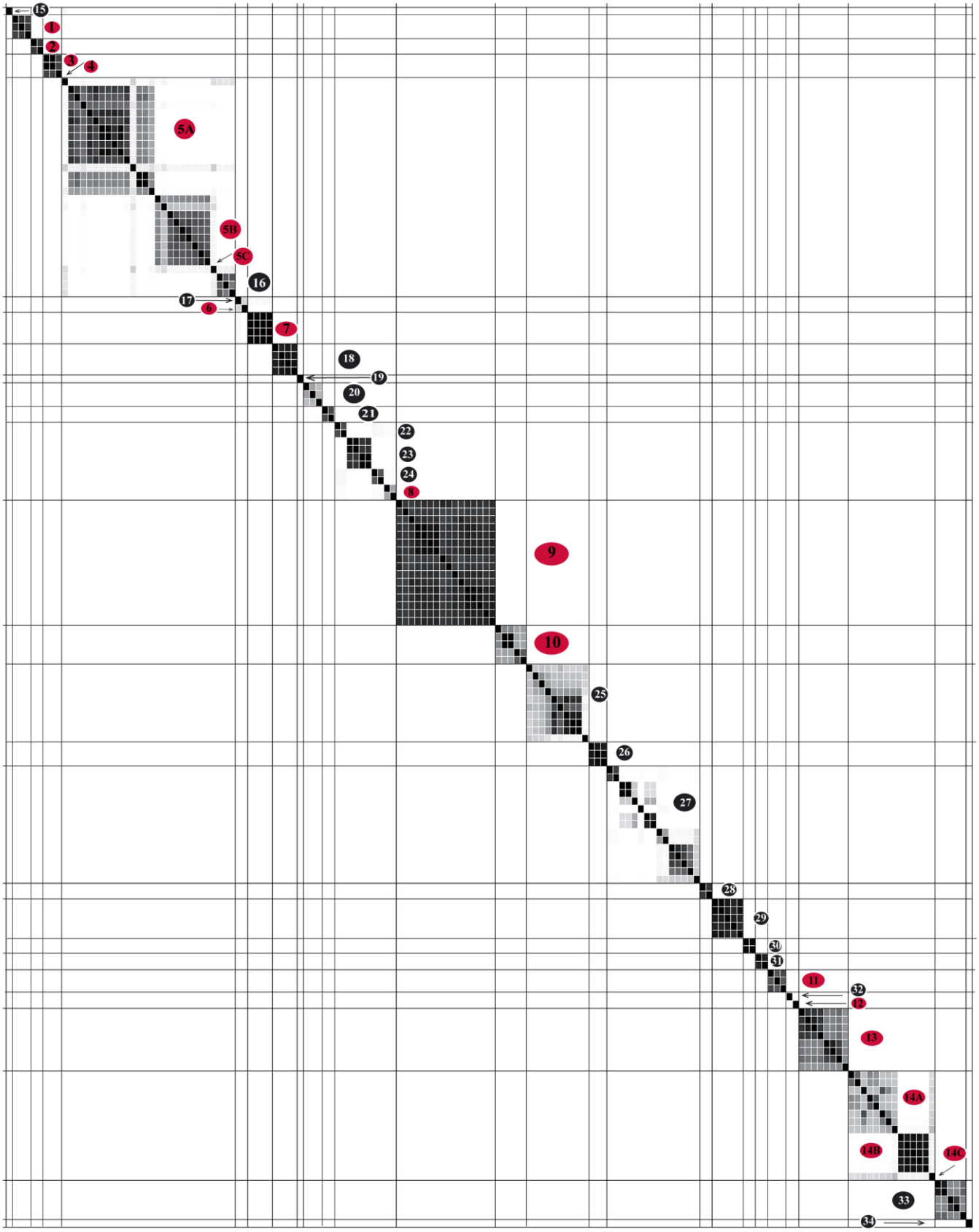


Fig. 2. Similarity matrix from Stacey analysis

Fig. 3. Map showing the geographical localities and respective OTUs of the samples of the *Usnea cornuta* group.

Fig. 1. Species tree from Stacey analysis. Posterior probabilities (PP > 0.5) are shown above branches. Branches and probabilities with strong support (PP≥0.90) are marked in bold. The genus *Usnea* is subdivided in two highly supported clades (I and II). The operational taxonomical units (OTU) for each clade are indicated inside circles. Red circles refer to the *U. cornuta* aggr. Countries of origin are indicated in color on the first column as follow: South and Central Americas in tones of green-yellow: Brazil (BR), Costa Rica (CR), Ecuador (EC), Peru (PE); Europe in red: France (FR), Portugal (PT), Spain (ES), United Kingdom (UK); United States (US) in black. The following major secondary chemistry are indicated in gray: constictic acid (CST); stictic acid (STI); salazinic acid (SAL); norstictic acid (NOR); galbinic (GAL); protocetraric acid (PRO), fumarprotocetraric acid (FUM); psoromic acid (PSO) acid; thamnolic acid (THA); lobaric acid (LOB). All individuals also contain usnic acid. When not indicated only usnic acid or an unidentified fatty acid were found. Species are abbreviated with the first four letters as follow: *U. cornuta* (corn), *U. brasiliensis* (brasi), *U. aspera* (aspe), *U. glabrata* (glab), *U. flammea* (flam), *U. geissleriana* (geis), *U. subflammea* (subf), *U. densirostra* (dens), *U. grandisora* (gran), *U. chilensis* (chil), *U. dasaea* (dasa), *U. subdasaea* (subd), *U. perhispidella* (perh), *U. dodgei* (dodg), *U. erinacea* (erin), *U. moreliana* (more), *U. rubicunda* (rubi), *U. steineri* (stei), *U. ceratina* (cera), *U. subscabrosa* (subs), *U. crocata* (croc), *U. subaranea* (suba). For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.

Fig. 2. Similarity matrix from Stacey analysis. The *squares* represent posterior probabilities (white=0, black=1) for pairs of individual to belong to the same cluster. The *lines* in the matrix separate major groups which numbers are indicated within circles. Red circles refer to the *U. cornuta aggr*.. Arrows indicated OTU with a single sample.

Fig. 3. Map showing the geographical localities and respective OTUs of the samples of the *Usnea cornuta* group. All samples and complete voucher information used on the Stacey analysis are show in Supplementary Table S1

Supplementary data S1. Samples used in the study, (bellow)

Supplementary data S2. Beauti file **(**xml file)

Supplementary data S3. Maximum Clade Credibility (MCC) species tree (fasta)

Supplementary data S4. Maximum Clade Credibility (MCC) gene tree-ITS, (fasta)

Supplementary data S5. Maximum Clade Credibility (MCC) gene tree-Mcm7, (fasta)

Supplementary data S6. Maximum Clade Credibility (MCC) gene tree-RPB1, (fasta)

NO	Species	Voucher	Chemotype	ITS	MCM7	RPB1
140	aspera	Brazil, MG, Gonçalves, A. Gerlach 1965 (P36)	SALNOR	**MF669805**	**yes**	**yes**
44	brasiliensis	Portugal, Madeira, Clerc (G)	PRO	JQ837294	JQ837338	n/a
4873	brasiliensis s. str.	Brazil, RS, São Francisco de Paula, A. Gerlach 1517 (ICN 179133)	PRO	**MF669810**	**yes**	**yes**
11	brasiliensis-1	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, A. Gerlach-P38 (G)	PRO	**MF669811**	**yes**	**yes**
220	brasiliensis-1	Costa Rica, P. Clerc 2015/644(G)	PROPSO	**MF669809**	**yes**	**yes**
13	brasiliensis-2	Brazil,SC, Alfredo Wagner, RPPN Rio das Furnas, trilha em torno da sede, galho caido, Maio 2016, A. Gerlach-P52 (G)	PROPSO	**MF669812**	**yes**	**yes**
15	brasiliensis-2	Brazil, MG, Gonçalves, em torno do Chalé Canario, Restaurante Tres irmas, sobre tronco, 22°43'56.75"S, 45°52'46.67"O, 1604 m. Junho 2016, A. Gerlach & R. Penati P1-3(G)	PROPSO	**MF669806**	**yes**	**yes**
16	brasiliensis-2	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, trilha em torno da sede, 27°40'28.3"S, 49°10'37.9"W, Maio 2016, A. Gerlach-P54 (G)	PROPSO	**MF669807**	**yes**	**yes**
204	brasiliensis-2	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, trilha em torno da sede, Maio 2016, A. Gerlach-P3 (G)	PROPSO	**MF669808**	**yes**	**yes**
143	ceratina	Brazil, MG, Gonçalves, caminho Pedra de Sao Domingos, 1480 m., tronco de Araucaria, Junho 2016, A. Gerlach & R. Penati-1974-P20 (G)	BARDIF	**MF669813**	**yes**	**yes**
200	ceratina	Brazil, MG, Gonçalves, em torno do Chalé Canario, Restaurante Tres irmas, sobre mourao, 22°43'56.75"S, 45°52'46.67"O, 1604 m. Junho 2016, A. Gerlach & R. Penati P1-8	DIF	**MF669814**	**yes**	**yes**
4869	chilensis	Brazil, RS, São Francisco de Paula, Flona de São Francisco de Paula, J. Rosiak 06 (G)	GAL	**MF669815**	**yes**	**yes**
4949	chilensis	Brazil, SC, Urubici, Parque Nacional de São Joaquim, galho de Araucaria angustifolia caido, Fevereiro 2015, C. Alves s. n. (G)	GAL	**MF669816**	**yes**	**yes**
24	cornuta s. lat.	Peru, Cajamarca, cordillera central, Parque Nacional Cutervo, Cerca de San Andres de Cutervo, 6°13'24.9"S, 78°45'11"W, 2665 m., tronco de Aliso, C. Truong 1628 (G)	STI	JQ837296	JQ837339	n/a
25	cornuta s. lat.	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P45 (G)	STI	**MF669823**	**yes**	**yes**
27	cornuta s. lat.	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P9 (G)	STI	**MF669825**	**yes**	**yes**
27	cornuta s. lat.	Ecuador, Imbabura, Reserva Ecologica Cotacachi-Cayapas Laguna Piñan, 0°31'3.4"N, 78°26'18"W, 2786 m., Bosque de nemblina montano, Set 2007, C. Truong 3253 (G)	NOR	JQ837340	JQ837340	n/a
28	cornuta s. lat.	Peru, Cusco, Cordillera Carabaya, Parque Nacional Manu Trocha Erikson, 13°11'45.4"S, 71°37'12.7"W, 3417 m., Páramo de Pajonal, 10/2007, Truong 2289 (G)	GAL	JQ837298	JQ837341	n/a
29	cornuta s. lat.	Peru, Pasco, Cordilera oriental, Parque nacional Yanachaga-Chemillén, 10°32'40.8"S, 75°21'24"W, 2419 m., Bosque de neblina monatno, 12/2007, Truong 2473 (G)	SAL	JQ837299	JQ837342	n/a
32	cornuta s. lat.	Brazil,SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P19 (G)	CSTTER	**MF669829**	**yes**	**yes**

76	cornuta s. lat.	Brazil, MG, Gonçalves, Apa Fernao Dias, mourao, Junho 2016, A. Gerlach & R. Penati-P43 (G)	STI	**MF669919**	**yes**	**yes**
121	cornuta s. lat.	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P34 (G)	STI	**MF669842**	**yes**	**yes**
192	cornuta s. lat.	Brazil, MG, Gonçalves, em torno do Chalé Canario, Restaurante Tres irmas, sobre mourao, 22°43'56.75"S, 45°52'46.67"O, 1604 m. Junho 2016, A. Gerlach & R. Penati P1 (G)	USN	**MF669929**	**yes**	**yes**
1	cornuta s. str.	Ireland	SAL	JN943562	n/a	JN992604
4	cornuta s. str.	United Kingdom, England	STI	JN943559	n/a	JN992601
4	cornuta s. str.	Portugal, Madeira island, Porto Santo, A. Gerlach & al 1577 (G)	SAL	n/a	**yes**	**yes**
7	cornuta s. str.	United Kingdom, Scotland	SALCST	JN943526	n/a	JN992572
8	cornuta s. str.	United Kingdom, Wales	SALSTI	JN943532	n/a	JN992578
9	cornuta s. str.	United Kingdom, Scotland	STI	JN943507	n/a	JN992557
17	cornuta s. str.	Brazil, MG, Gonçalves, Apa Fernao Dias, mourao, 06/2016, A. Gerlach & R. Penati P54 (G)	SALPRO	**MF669859**	**yes**	**yes**
18	cornuta s. str.	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P41 (G)	SAL	**MF669860**	**yes**	**yes**
19	cornuta s. str.	Brazil, MG, Gonçalves, Apa Fernao Dias, mourao, 06/2016, A. Gerlach & R. Penati P44 (G)	SAL	**MF669861**	**yes**	**yes**
30	cornuta s. str.	Brazil, MG, Gonçalves, Apa Fernao Dias, mourao, 09.06, A. Gerlach & R. Penati P52 (G)	SALPRO	**MF669828**	**yes**	**yes**
32	cornuta s. str.	USA, Maine, Washington Co., Humboldt Field Research Institute, Eagle Hill, Dyer Bay, alogn the Red Trail, 44°27'269"N, 67°55'487"W, 08/2006, P. Clerc (G)	SAL	JQ837300	JQ837343	n/a
42	cornuta s. str.	France, Bretagne, Finistere, Reserve Naturelle du Venec, sobre Salix sp., 12/2007, B. Lorella (G)	STI	JQ837301	JQ837344	n/a
43	cornuta s. str.	Portugal, Madeira, Ribeiro Frio, Miradores dos Balcoes, 32°44'24,7"N, 16°53'24,9"W, 828 m., Castanea abattu en travers du chemin, 10.2009, P. Clerc (G)	SAL	JQ837302	JQ837345	n/a
113	cornuta s. str.	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P7 (G)	SAL, CST	**MF669835**	**yes**	**yes**
124	cornuta s. str.	Brazil, MG, Gonçalves, Apa Fernao Dias, mourao, 09.06, A. Gerlach & R. Penati P47 (G)	SALFA	**MF669922**	**yes**	**yes**
171	cornuta s. str.	Brazil,SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P47(G)	CST	**MF669869**	**yes**	**yes**
176	cornuta s. str.	Brazil, MG, Gonçalves, Apa Fernao Dias, mourao, Junho 2016, A. Gerlach & R. Penati P46 (G)	SAL	**MF669847**	**yes**	**yes**
197	cornuta s. str.	Brazil,SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P10 (G)	CST	**MF669871**	**yes**	n/a
198	cornuta s. str.	Brazil,SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P18(G)	CST	**MF669872**	**yes**	**yes**

233	cornuta s. str.	Spain, Tenerife, Anaga, 1 km E of Casa Florestal, 28°32'25"N, 16°13'35"W, c. 850m., on Erica arborea, 01/2017, Aptroot 75462 (G)	SAL	**MF669862**	**yes**	**yes**
4489	cornuta s. str.	España, Orense	STI, NOR	**MF669854**	**yes**	**yes**
4491	cornuta s. str.	España, Orense	SALCST	**MF669856**	**yes**	**yes**
20	cornuta-1	Brazil,SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P36 (G)	CST	**MF669818**	**yes**	**yes**
23	cornuta-1	Brazil,SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P39 (G)	CST	**MF669821**	**yes**	**yes**
24	cornuta-1	Brazil,SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P12 (G)	SALCST	**MF669822**	**yes**	**yes**
21	cornuta-2	Brazil,SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P25 (G)	THAM	**MF669819**	**yes**	**yes**
22	cornuta-2	Brazil,SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P46 (G)	THAM	**MF669820**	**yes**	**yes**
148	cornuta-2	Brazil,SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P48 (G)	THAM	**MF669867**	**yes**	**yes**
29	cornuta-3	Brazil, MG, Gonçalves, Pedra chanfrada, tronco, area sombreada, Montana semidecidua, 22°43'31.09"S, 45°51'20.01"O, Junho 2016, A. Gerlach & R. Penati-P59 (G)	SALSTI	**MF669827**	**yes**	**yes**
42	cornuta-3	Brazil, MG, Gonçalves, Junho 2016, A. Gerlach & R. Penati (G)	STI	**MF669884**	**yes**	**yes**
112	cornuta-3	Brazil, MG, Gonçalves, em torno do Chalé Canario, Restaurante Tres irmas, sobre tronco, 22°43'56.75"S, 45°52'46.67"O, 1604 m. Junho 2016, A. Gerlach & R. Penati P1 (G)	STI	**MF669834**	**yes**	**yes**
114	cornuta-3	Brazil, MG, Gonçalves, Pedra Chanfrada, tronco, decidua montana, 22°43'31.09"S, 45°51'20.01"O, Junho 2016, A. Gerlach & R. Penati P69 (G)	STI	**MF669836**	**yes**	**yes**
115	cornuta-3	Brazil,SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P11 (G)	STI	**MF669837**	**yes**	**yes**
116	cornuta-3	Brazil,SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P20 (G)	STI	**MF669838**	**yes**	**yes**
118	cornuta-3	Brazil, MG, Gonçalves, sobre Araucaria, Junho 2016, A. Gerlach & R. Penati P56 (G)	STI	**MF669839**	**yes**	**yes**
119	cornuta-3	Brazil, MG, Gonçalves, em torno do Chalé Canario, Restaurante Tres irmas, sobre tronco, 22°43'56.75"S, 45°52'46.67"O, 1604 m. Junho 2016, A. Gerlach & R. Penati P1 (G)	STI	**MF669840**	**yes**	**yes**
120	cornuta-3	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, 27°40'28.3"S, 49°10'37.9"W, Maio 2016, A. Gerlach P1 (G)	STI	**MF669841**	**yes**	**yes**
122	cornuta-3	Brazil,SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P55 (G)	STI	**MF669843**	**yes**	**yes**
123	cornuta-3	Brazil,SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P16 (G)	STI	**MF669844**	**yes**	**yes**
147	cornuta-3	Brazil,SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach (G)	STI	**MF669863**	**yes**	**yes**

160	cornuta-3	Brazil, MG, Gonçalves, mourao, Junho 2016, A. Gerlach & R. Penati P24 (G)	STI	**MF669927**	**yes**	**yes**
164	cornuta-3	Brazil, MG, Gonçalves, em torno do Chalé Canario, Restaurante Tres irmas, sobre mourao, 22°43'56.75"S, 45°52'46.67"O, 1604 m. Junho 2016, A. Gerlach & R. Penati P1 (G)	STI	**MF669901**	**yes**	**yes**
227	cornuta-3	Costa Rica, Province of San Jose, Pérez Zeledon Co. Cordillera de Talamanca, San Gerardo de Rivas, 9°28'13.5"N, 83°35'7.0"W, 1486 m, sur Alnus acuminata, 09/2014, P. Clerc PC2015/638 (G)	STI	**MF669849**	**yes**	**yes**
PC2	cornuta-3	France, Corse du Sud, Alta Rocca, Bavella, San-Gavino-di-Carbini Pont de Fiumicelelli, 41°50'18"N, 9°18'31.4"E, 170 m., branches de Phillyrea angustifolia, July 2016, P. Clerc (G)	STI	**MF669906**	**yes**	**yes**
71	cornuta-4	Brazil,SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P46 (G)	PROFUM	**MF669832**	**yes**	**yes**
74	cornuta-4	Brazil,SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P10 (G)	PROFUM	**MF669833**	**yes**	**yes**
187	cornuta-4	Brazil,SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P50 (G)	PROFUM	**MF669848**	**yes**	**yes**
131	cornuta-5	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach P3 (G)	NORFA	**MF669817**	**yes**	**yes**
231	cornuta-5	Spain, Tenerife, Erjos, along track to Los Silos, 28°19'56"N, 16°18'23"W, c. 900m., on Erica arborea, 01/2017, A. Aptroot 75517 (G)	LOBFA	**MF669850**	**yes**	**yes**
232	cornuta-5	Spain, Tenerife, Erjos, along track to Los Silos, 28°19'56"N, 16°18'23"W, c. 900m., on Erica arborea, 01/2017, A. Aptroot 75514 (G)	LOBFA	**MF669851**	**yes**	**yes**
4488	cornuta-5	Spain, Ourense,, Parque Natural Baixa Limia-Serra do Xurés, Entrimo, Mirador de San Rosendo do Pedreirinho, sobre Pinus pinaster, UTM 29T57400 464385, 740 m., 11/2013, R. Arroyo (EA329(5) (MAF)	FA	**MF669853**	n/a	**yes**
4490	cornuta-5	Spain, Spain, Ourense,, Parque Natural Baixa Limia-Serra do Xurés, Entrimo, Mirador de San Rosendo do Pedreirinho, sobre Pinus pinaster, UTM 29T57400 464385, 740 m., 11/2013, R. Arroyo (EA338(3) (MAF);	LOB	**MF669855**	**yes**	**yes**
4568	cornuta-5	Spain, Orense (MAF 20041)	LOB, ATR	**MF669857**	n/a	**yes**
PC3	cornuta-5	France, Corse du Sud, Alta Rocca, Bavella, San-Gavino-di-Carbini Pont de Fiumicelelli, au bord de la riviere en amont du pont, 41°50'30.68"N, 9°18'28.46"E, 195 m., grands Quercus ilex, 2016, P. Clerc (G)	LOB	**MF669858**	**yes**	**yes**
28	cornuta-6	Brazil, MG, Gonçalves, Apa Fernao Dias, mourao, Junho 2016, A. Gerlach & R. Penati P45 (G)	STIFA	**MF669826**	**yes**	**yes**
83	cornuta-6	Brazil,SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P12 (G)	USN	**MF669865**	**yes**	**yes**
90	cornuta-6	Brazil,SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P47 (G)	STI	**MF669866**	**yes**	**yes**
170	cornuta-6	Brazil,SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P50 (G)	STIFA	**MF669868**	**yes**	**yes**
175	cornuta-6	Brazil, MG, Gonçalves, em torno do Chalé Canario, Restaurante Tres irmas, galho caido, 22°43'56.75"S, 45°52'46.67"O, 1604 m. Junho 2016, A. Gerlach & R. Penati P1 (G)	STI, US STI	**MF669870**	**yes**	**yes**

49	crocata	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P47 (G)	PRO	**MF669877**	**yes**	**yes**
35	dasaea	Brazil, MG, Gonçalves, em torno do Chalé Canario, Restaurante Tres irmas, sobre tronco, 22°43'56.75"S, 45°52'46.67"O, 1604 m. Junho 2016, A. Gerlach & R. Penati P1(G)	GAL	**MF669878**	**yes**	**yes**
34	dasaea s. lat.	Brazil,SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach (G)	GAL	**MF669831**	**yes**	**yes**
37	dasaea s. lat.	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P41 (G)	GAL	**MF669880**	**yes**	**yes**
128	dasaea s. lat.	Brazil, MG, Gonçalves, trilha para a Pedra do Forno, tronco de Araucaria, Junho 2016, A. Gerlach & R. Penati P27 (G)	GAL	**MF669885**	**yes**	**yes**
137	densirostra	Brazil, MG, Gonçalves Pedra de Sao Domingos, proximo a antena, 22°41'29.06"S, 45°57'35.15"W, 2050 m., 09.06 A. Gerlach & R. Penati P33 (G)	SALNOR	**MF669804**	**yes**	**yes**
4935	densirostra	Brazil, RS, Viamao, Itapuã, Agosto 2014, A. Gerlach et al. 1494 (ICN)	NOR	KY021906	KY204417	KY204438
54	dodgei	Brazil, MG, Gonçalves, Caminho pousada Lua da Pedra, 1540 m. Mourao, Junho 2016, A. Gerlach & R. Penati P18 (G)	SAL, NOR	**MF669886**	**yes**	**yes**
133	dodgei	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach P28 (G)	NOR	**MF669923**	**yes**	**yes**
173	dodgei	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach P50 (G)	SAL, NOR, TER	**MF669887**	**yes**	**yes**
78	erinacea s. lat.	Brazil, MG, Gonçalves, Junho 2016, A. Gerlach & R. Penati (G)	PRO	**MF669888**	**yes**	**yes**
79	erinacea s. lat.	Brazil, MG, Gonçalves, Junho 2016, A. Gerlach & R. Penati (G)	PRO	**MF669889**	**yes**	**yes**
230	flammea	Spain, Tenerife, Erjos, along treck to Los Silos, 28°19'56"N, 16°18'23"W, c. 900m., on Erica arborea, 01/2017, Aptroot 75512 (G)	STILOB	**MF669890**	**yes**	**yes**
238	geissleriana	Spain, Tenerife, Anaga, 1 km E of Casa Florestal, 28°32'25"N, 16°13'35"W, c. 850m., on Erica arborea, January 2017, Aptroot 75487 (G)	SALNOR	**MF669934**	**yes**	**yes**
PC4	glabrata s. lat.	France, Corse du Sud, Alta Rocca, Bavella, San-Gavino-di-Carbini Pont de Fiumicelelli, 41°50'18"N, 9°18'31.4"E, 170 m., branches de Phillyrea angustifolia, July 2016, P. Clerc (G)	SAL	**MF669864**	**yes**	**yes**
33	grandisora	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach P55 (G)	GAL	**MF669830**	**yes**	**yes**
39	grandisora	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach P34 (G)	GAL	**MF669881**	**yes**	**yes**
58	grandisora	Brazil, MG, Gonçalves, caminho pousada Lua da Pedra , araucaria, Junho 2016, A. Gerlach & R. Penati P21 (G)	GAL	**MF669891**	**yes**	**yes**
191	grandisora	Brazil, MG, Gonçalves, em torno do Chalé Canario, Restaurante Tres irmas, sobre mourao, 22°43'56.75"S, 45°52'46.67"O, 1604 m. Junho 2016, A. Gerlach & R. Penati P1 (G)	GAL	**MF669892**	**yes**	**yes**
84	moreliana	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach (G)	TER	**MF669893**	**yes**	**yes**
98	moreliana	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach P35 (G)	TER	**MF669894**	**yes**	**yes**

102	moreliana	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach P48 (G)	TER	**MF669895**	**yes**	**yes**
100	moreliana_NIS	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach (G)	TER	**MF669896**	**yes**	**yes**
36	perhispidella	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, 27°40'28.3"S, 49°10'37.9"W, Maio 2016, A. Gerlach (G)	GAL	**MF669879**	**yes**	**yes**
41	perhispidella	Brazil, MG, Gonçalves, Junho 2016, A. Gerlach & R. Penati (G)	STI	**MF669883**	**yes**	**yes**
82	perhispidella	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach P55 (G)	STI	**MF669898**	**yes**	**yes**
155	perhispidella	Brazil, MG, Gonçalves, Pefra Chanfrada, 22°43'31.09"S, 45°51'20.01"W, Junho 2016, A. Gerlach & R. Penati P58 (G)	STI	**MF669899**	**yes**	**yes**
159	perhispidella	Brazil, MG, Gonçalves, em torno do Chalé Canario, Restaurante Tres irmas, sobre mourao, 22°43'56.75"S, 45°52'46.67"O, 1604 m. Junho 2016, A. Gerlach & R. Penati P1-13 (G)	STI	**MF669900**	**yes**	**yes**
165	perhispidella	Brazil, MG, Gonçalves, em torno do Chalé Canario, Restaurante Tres irmas, sobre mourao, 22°43'56.75"S, 45°52'46.67"O, 1604 m. Junho 2016, A. Gerlach & R. Penati P1-17 (G)	STI	**MF669902**	**yes**	**yes**
166	perhispidella	Brazil, MG, Gonçalves, em torno do Chalé Canario, Restaurante Tres irmas, sobre mourao, 22°43'56.75"S, 45°52'46.67"O, 1604 m. Junho 2016, A. Gerlach & R. Penati P1-17 (G)	STI	**MF669903**	**yes**	**yes**
178	perhispidella	Brazil, MG, Gonçalves, em torno do Chalé Canario, Restaurante Tres irmas, sobre mourao, 22°43'56.75"S, 45°52'46.67"O, 1604 m. Junho 2016, A. Gerlach & R. Penati P1-4 (G)	STI	**MF669904**	**yes**	**yes**
179	perhispidella	Brazil, MG, Gonçalves, em torno do Chalé Canario, Restaurante Tres irmas, galho caido, 22°43'56.75"S, 45°52'46.67"O, 1604 m. Junho 2016, A. Gerlach & R. Penati P1-5(G)	STI	**MF669905**	**yes**	**yes**
60	rubicunda	Brazil, MG, Gonçalves, inicio da trilha pedra do Forno, galho de Araucaria, Junho 2016, A. Gerlach & R. Penati P19 (G)	STI	**MF669908**	**yes**	**yes**
61	rubicunda	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, tronco de arvore, Maio 2016, A. Gerlach P30 (G)	STI	**MF669909**	**yes**	**yes**
207	rubicunda	Brazil, MG, Gonçalves, Proximo ao chale tres irmas, 22°43'56.75"S, 45°52'46.67"W, ca. 1400 m, 08.06.2016, A. Gerlach & R. Penati P1-3 (G)	TER	**MF669910**	**yes**	**yes**
235	rubicunda	Spain, Tenerife, Anaga, 1 km E of Casa Florestal, 28°32'25"N, 16°13'35"W, 850 m elev., on Erica arborea, 7 january 2017, A. Aptroot 75478 (G)	STI	**MF669911**	**yes**	**yes**
4890	rubicunda	Brazil, SC, Urubici, PARNA Sao Joaquim, Floresta de Araucaria prox. Ao alojamento, ca. 1300 m., Fevereiro 2015, C. Alves s. n. (ICN)	SAL	KY021923	KY204428	KY204445
184	rubicunda s. lat.	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, tronco de arvore, Maio 2016, A. Gerlach P30 (G)	STI	**MF669912**	**yes**	**yes**
4807	rubicunda s. lat.	Brazil, RS, Caraá, APA do Caraá, Setembro 2014, A. Gerlach et al. 1499 (ICN)	STI	KY021925	KY204430	KY204447
4891	rubicunda s. lat.	Brazil, RS, Caraá, APA do Caraá, Setembro 2014, A. Gerlach et al. 1497 (ICN)	STI	KY021924	KY204429	KY204446
26	sp	Brazil, MG, Gonçalves, Apa Fernao Dias, mourao, Junho 2016, A. Gerlach & R. Penati-P44 (G)	STI	**MF669824**	**yes**	**yes**
157	sp	Brazil, MG, Gonçalves, Apa Fernao Dias, mourao, Junho 2016, A. Gerlach & R. Penati-P49 (G)	STI	**MF669926**	**yes**	**yes**

163	sp	Brazil, MG, Gonçalves, Apa Fernao Dias, mourao, Junho 2016, A. Gerlach & R. Penati-P47 (G)	STI	**MF669928**	**yes**	**yes**
4940	sp	Brazil, SC, Guaratuba, Morro dos Perdidos, campo de altitude, saxicola, 03.06.2013, A. Gerlach et al. 1005a (G)	unknow yellow	**MF669803**	**yes**	**yes**
80	sp1	Brazil,SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach-P23 (G)	SAL	**MF669920**	**yes**	**yes**
126	sp1	Brazil, MG, Gonçalves, mourao, 06/2016, A. Gerlach & R. Penati P26 (G)	SAL	**MF669845**	**yes**	**yes**
127	sp1	Brazil, MG, Gonçalves, trilha para a Pedra do Forno, tronco de Araucaria, 22°42'59.84"S, 45°52'7.78"W, , 06/2016, A. Gerlach & R. Penati P27 (G)	SAL	**MF669846**	**yes**	**yes**
75	sp2	Brazil, MG, Gonçalves, Apa Fernao Dias, lignicola em mourao, 09.06.2016, A. Gerlach & R. Penati P46 (G)	SAL	**MF669918**	**yes**	**yes**
77	sp2	Brazil, MG, Gonçalves, lignicola em mourao, 09.06.2016, A. Gerlach & R. Penati P2 (G)	PRO	**MF669921**	**yes**	**yes**
152	sp2	Brazil, MG, Gonçalves, Apa Fernao Dias, mourao, Junho 2016, A. Gerlach & R. Penati-P54 (G)	PROFUM	**MF669924**	**yes**	**yes**
156	sp2	Brazil, MG, Gonçalves, Apa Fernao Dias, mourao, Junho 2016, A. Gerlach & R. Penati-P44 (G)	SAL	**MF669925**	**yes**	**yes**
162	sp2	Brazil, MG, Gonçalves, Apa Fernao Dias, mourao, Junho 2016, A. Gerlach & R. Penati-P47 (G)	PRO, FUM, US PROTO	**MF669907**	**yes**	**yes**
7	sp3	Brazil, MG, Gonçalves Pedra de Sao Domingos, proximo a antena, 22°41'29.06"S, 45°57'35.15"W, 2050 m., 09.06 A. Gerlach & R. Penati P33 (G)	SQUA	**MF669913**	**yes**	**yes**
8	sp3	Brazil, MG, Gonçalves Pedra de Sao Domingos, proximo a antena, 22°41'29.06"S, 45°57'35.15"W, 2050 m., 09.06 A. Gerlach & R. Penati P33 (G)	SQUABARTHAM	**MF669914**	**yes**	**yes**
141	sp3	Brazil, MG, Gonçalves Pedra de Sao Domingos, proximo a antena, 22°41'29.06"S, 45°57'35.15"W, 2050 m., 09.06 A. Gerlach & R. Penati P33 (G)	SQUA	**MF669915**	**yes**	**yes**
142	sp3	Brazil, MG, Gonçalves Pedra de Sao Domingos, proximo a antena, 22°41'29.06"S, 45°57'35.15"W, 2050 m., 09.06 A. Gerlach & R. Penati P36 (G)	SQUA	**MF669916**	**yes**	**yes**
4937	sp3	Brazil, SC, Garuva, Alto Quiriri, ca. 1200 m., A. Gerlach 1188 (ICN)	SQUA	**MF669917**	**yes**	**yes**
134	sp4	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, tronco de arvore, Maio 2016, A. Gerlach P27 (G)	GAL	**MF669938**	**yes**	**yes**
135	sp4	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, tronco de arvore, Maio 2016, A. Gerlach P41 (G)	GAL	**MF669939**	**yes**	**yes**
56	sp5	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach P19 (G)	PROFUM-US36	**MF669873**	**yes**	**yes**
132	sp5	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach P37 (G)	PROFUM US36	**MF669874**	**yes**	**yes**
172	sp5	Brazil, SC, Alfredo Wagner, Floresta de Araucaria, 13.05.2016, A. Gerlach P36 (G)	PROFUM-US36	**MF669875**	**yes**	**yes**
193	sp5	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach P47 (G)	PROFUM	**MF669876**	**yes**	**yes**

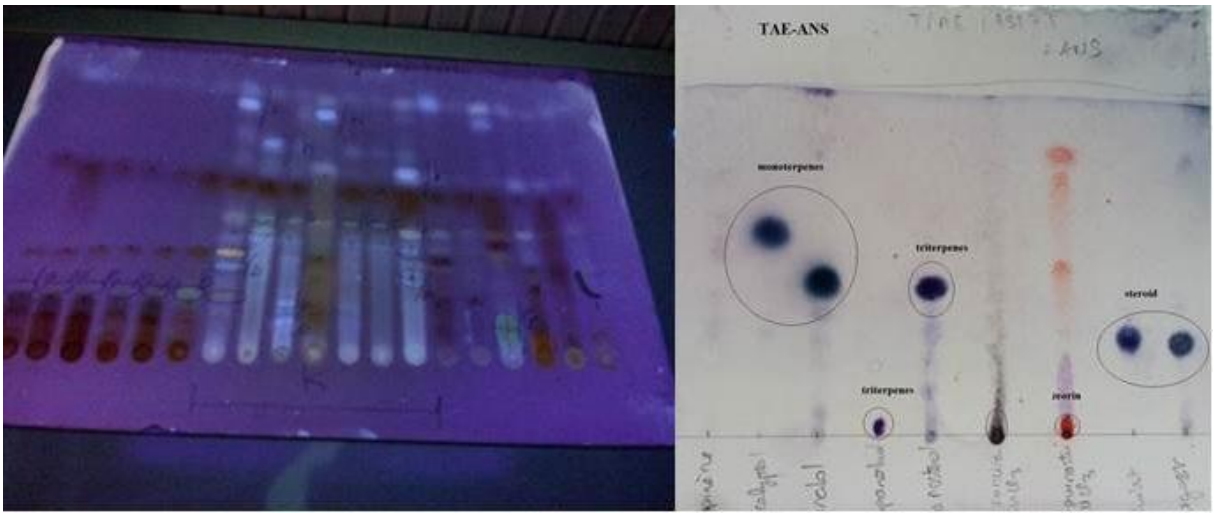
PC1	sp6	France, Corse du Sud, Alta Rocca, Bavella, San-Gavino-di-Carbini Pont de Fiumicelelli, au bord de la riviere en amont du pont, 41°50'30.68"N, 9°18'28.46"E, 195 m., bracnehs de Quercus Ilex, P. Clerc (G)	GAL	**MF669897**	**yes**	**yes**
97	steineri	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, Maio 2016, A. Gerlach (G)		**MF669930**	**yes**	**yes**
123	subaranea	Ecuador, Azuay, Cuenca, alredores del parque nacional Cajas Chuspipununa, sector de la virgen de Cajas cerca de la entrada del parque, 2°26'46.7S, 79°10'58.2"W, 3434 m, pasto con arboles, poste de madera, 15.09.2007, C. Truong 313 (G)	USN	JQ837292	JQ837337	JQ837416
40	subdasaea	Brazil, SC, Alfredo Wagner, RPPN Rio das Furnas, 27°40'28.3"S, 49°10'37.9"W, Maio 2016, A. Gerlach P1 (G)	GAL	**MF669882**	**yes**	**yes**
4806	subdasaea	Brazil, RS, Porto Alegre, Morro Santana, Campus do Vale da UFRGS, 02.10.2014, A.Magnago 1099 (ICN)	GAL	**MF669931**	**yes**	**yes**
65	subflammea s. lat.	Brazil, MG, Gonçalves, proximo ao Chale tres irmas, 22°43'56.75"S, 45°52'46.67"W, ca. 1400 m elev. , 06.2016, A. Gerlach & R. Penati P1-2 (G)	STI	**MF669932**	**yes**	**yes**
66	subflammea s. lat.	Brazil, SC, Alfredo Wagner , RPPN Rio das Furnas, 14.05.2016, A. Gerlach P39 (G)	STI	**MF669933**	**yes**	**yes**
239	subflammea s. str.	Spain, Tenerife, Anaga, 1 km E of Casa Florestal, 28°32'25"N, 16°13'35"W, c. 850m., on Erica arborea, January 2017, Aptroot 75474 (G)	STI	**MF669935**	**yes**	**yes**
67	subscabrosa	Brazil, MG, Gonçalves, inicio da trilha pedra do Forno, galho de Araucaria, Junho 2016, A. Gerlach & R. Penati P19 (G)	PRO	**MF669936**	**yes**	**yes**
212	subscabrosa	Brazil, RS, São Francisco de Paula, Hotel Veraneio Hampel, Maio 2016. E. Fazolino s. n. (G)	PRO	**MF669937**	**yes**	**yes**
				155	**149**	**147**
			Total	**156**		

Supplementary data S1. Samples used in the study

CHAPTER 4

The use of anisaldehyde sulfuric acid as an alternative spray reagent in TLC analysis reveals three classes of medullary compounds in the genus *Usnea* Adans. (Parmeliaceae, lichenized Ascomycota)

Short Note to be submitted to *Preprints* (www.preprints.org)



Left: TLC plate (solvent system C-H_2SO_4) showing the possible presence of terpenes as fluorescent spots under long wavelength (350 nm) (Photo: A. Gerlach). **Right:** TLC plate (solvent system TAE-ANS) showing monoterpenes, triterpenes and steroids (Photo: F. Lohézic-le Dévéhat).

The use of anisaldehyde sulfuric acid as an alternative spray reagent in TLC analysis reveals three classes of medullary compounds in the genus *Usnea* Adans. (Parmeliaceae, lichenized Ascomycota)

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Abstract

Presence and identity of secondary metabolites is one of the main components of lichen taxonomy, essential for many species, and the presence of substances is often mentioned and required in taxonomic keys. The presence of triterpenes in the medulla of *Usnea* specimens has been mentioned in several taxonomical papers, but their identities remain uncertain. We found difficulties to detect terpenes by the standardized thin layer chromatography (TLC) method with solvents A, B, or C, and sulfuric acid as spray solvent, probably due the instability of these compounds. In an attempt to solve this problem, we then tested another spray reagent, anisaldehyde sulfuric acid. We conclude that i) aside from the previously recognized terpenes; sugars and steroids are also detected in the medulla of some *Usnea* specimens; ii) the sulfuric anisaldehyde greatly improved the detection of terpenes, sugars and steroids compared with the sulfuric acid routinely used in chemotaxonomy; iii) among sugars, we detected arabitol and saccharose on the medulla, but the steroids and terpenes remain unidentified and deserve further investigations. This chemical study is framed within a bigger project of systematics on the genus *Usnea* from Brazil, and it is the first attempt to better understand chemistry on Neotropical species of *Usnea*, especially on samples without reaction on color tests.

Key words: Color tests, thin layer chromatography, chemotaxonomy, secondary metabolites, solvent system, spray solvents.

Introduction

The identification of secondary metabolites in lichens is usefull for the identifications of various groups of lichens (Lumbsh 2002) and to detect potential bioactive compounds, e.g., antioxidants and UV filters (Huneck & Yoshimura 1996; Lohézic-Le Dévéhat et al. 2007, 2013). There is an extensive literature on the chemistry of lichens (Elix 2014; Orange et al. 2001; Huneck & Yoshimura 1996) and ca. 1050 lichen (Huneck & Yoshimura 1996) compounds are known. The majority of them are aromatic compounds such as depsides and

depsidones. However, several other classes of substances were found as for example dibenzofurans, chromones and xanthones, pulvinic acid derivatives, fatty acids, steroids, and terpenes (Huneck & Yoshimura 1996).

Among the terpenes, the triterpenes (e.g., zeorin) constitute the largest number of compounds isolated from lichens (Honda & Vilegas 1998). The presence of terpenes in the genus *Usnea* was mentioned in several systematics studies (Halonen 2000, Halonen et al. 1998, Ohmura 2001, Herrera-Campos et al. 1998). More recently, Truong et al. (2013) detected distinct patterns of these terpenes in Neotropical species of *Usnea*, highlighting their importance for species identification in this genus. However, most of these terpenes remain unidentified and even their presence is sometimes dubious. Accurate detection of triterpenes is not an easy task using traditional thin-layer chromatography. Despite being frequently mentioned in the literature, terpenes in the lichen genus *Usnea* still remain poorly understood, and their presence is for instance not mentioned by Elix (2014).

Terpenes do not give any reaction with the classical color tests used in lichenology (K, C, KC, P). Moreover they are not visible until the TLC plates are sprayed with H_2SO_4 and heated. They appear as fluorescent spots under long wavelength (350 nm) with different colors. However the intensity of the spots is fading quite rapidely after being revealed by sulfuric acid (10%) and heat treatments. It can appear as dark-blue spots after charring only if present in sufficient concentrations. Terpenes might be however confused with steroids which look very similar (Elix 2014). Because accurate detection of lichen triterpenes with the sulfuric acid is doubtful we tested another spray reagent called anisaldehyde sulfuric acid-ANS) instead. In order to simplify the terminology we call all these K–, P– compounds "hidden compounds".

This study corresponds to one of a series of publications about the systematics of the genus *Usnea* in Brazil (PhD dissertation from the first author) and it is the first attempt to evaluate the presence of terpenes in Brazilian samples of this genus.

Material & Method

Lichen Material. A total of 20 specimens of *Usnea malmei* Motyka *(*H18, H19, H20, H21, H22, H52, H54, H55, H58, H59, H60, H61*), U. moreliana* Motyka (H13, H14, H62, H64) and *U. papillata* Motyka (H51, H53, H56, H57) (Parmeliaceae) collected in Brazil were analyzed. All these specimens have a K–, P–, C–, KC– reacting medulla (Hale 1979).

Thin-layer chromatography (TLC): A first round of TLC was done at the CJBG (Switzerland) as follows: Secondary compounds were extracted in acetone, boiled and spotted several times (ca. 10) onto Merck silica gel 60 F254 glass plates. Chemical analyses were performed in three routinely used solvent systems: A (toluene/ dioxane/ acetic acid: 180:45:5), B (*n*-hexane/MTBE/ formic acid–65:40:10) and C (toluene/ acetic acid–200:30) following Culberson & Ammann (1979) with solvent B modified according to Culberson & Johnson (1982). After brief drying, the plates are visualized on UV light (254 nm and 365 nm) and are sprayed with a stable solution of anisaldehyde sulfuric reagent (ANS: anisaldehyde/acetic acid/methanol/sulfuric acid–0.5:10:85:5) until wet, and then heated at

110º until development of spots, which were visualized on visible light (Le Pogam 2015). We identified when possible the family class according to the colors and retention value (Rf).

Because the presence of other family classes were suspected, fragments of samples were send to the University of Rennes (France), where a second round of TLC was done. First of all, several experimental tests were done within different solvent systems and spray reagents and at different concentrations. For specimens for which the presence of sugars was suspected the experimental tests were done as follows: Secondary compounds were extracted in chloroform/acetone/methanol (1/1/1), the extracts obtained were evaporated, weighted (data not showed), and solubilized in bidistilled tetrahydrofuran to obtain 10 mg/ml solutions (data not showed). The following four controls were used (10 mg/ml): arabitol, mannitol, ribitol, and saccharose. Automatic samples application was done for tested samples (6 µl) and controls (2 µl) on silica plates (Merck silica gel 60F254) thanks to the CAMAG automatic TLC sampler 3 (ATS3). The plates were then eluted using two solvent systems: i) D (ethyl acetate/formic acid /acetic acid /water -100/11/11/27); ii) E (butanol-1/acetone/water–5/4/1) (Culberson & Ammann 1979). Then, plates were sprayed with two different spray reagents: thymol sulfuric acid (Le Pogam 2015) and anisaldehyde sulfuric acid (anisaldehyde/acetic acid/methanol/sulfurique acid–0.5:10:85:5).

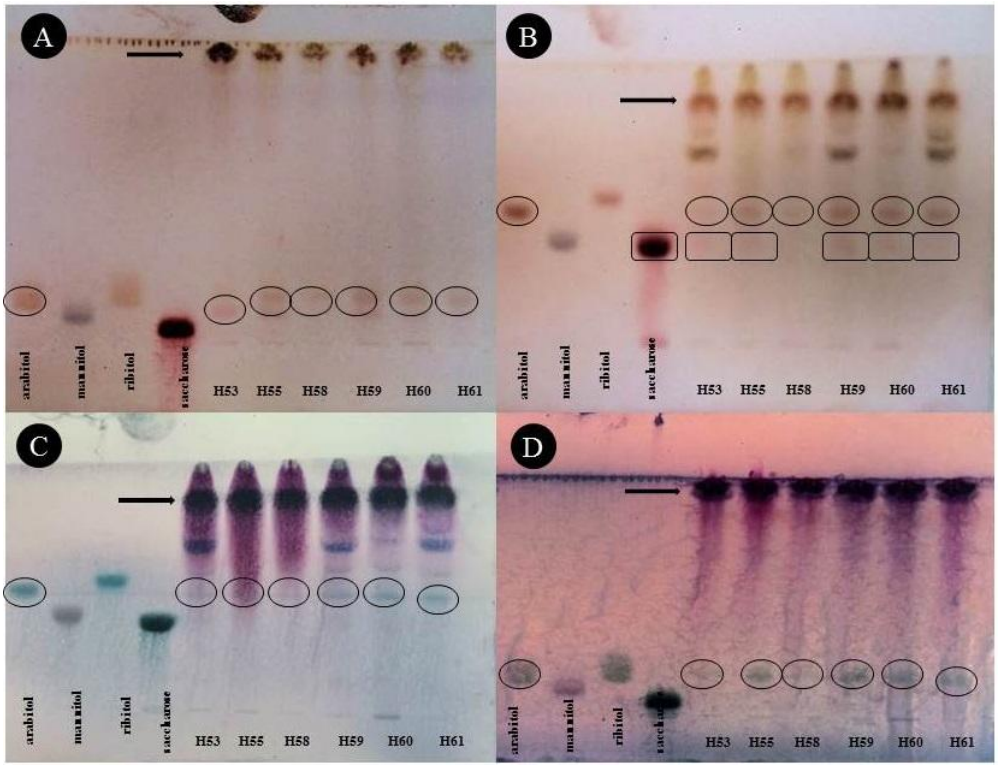
Specimens suspected to contain terpenes or steroids were tested in the following way: secondary compounds were extracted in chloroform/acetone/methanol (1/1/1); the extracts obtained were evaporated, weighted (data not showed), and solubilized in the bidistilled tetrahydrofuran to obtain 5 mg/ml solutions (data not showed). The following controls were used (3 µL to 5 mg/ml): eucalyptol, linalool and α-pinene (monoterpenes); hopane-triol, lanosterol and zeorin (triterpenes) as well as cholesterol and ergosterol (steroids). Automatic samples application on silica plates was used for the tested samples (15 µl) and the controls (3 µl). The plates were then eluted using two solvent systems: i) TAE (toluene/ethyl acetate–97/3) and ii) G (Toluene/ethyl acetate/formic acid–139/83/8) (Orange 2001). Finally, the plates were sprayed with two different spray reagents: anisaldehyde sulfuric acid vanillin/ phosphoric acid.

Results

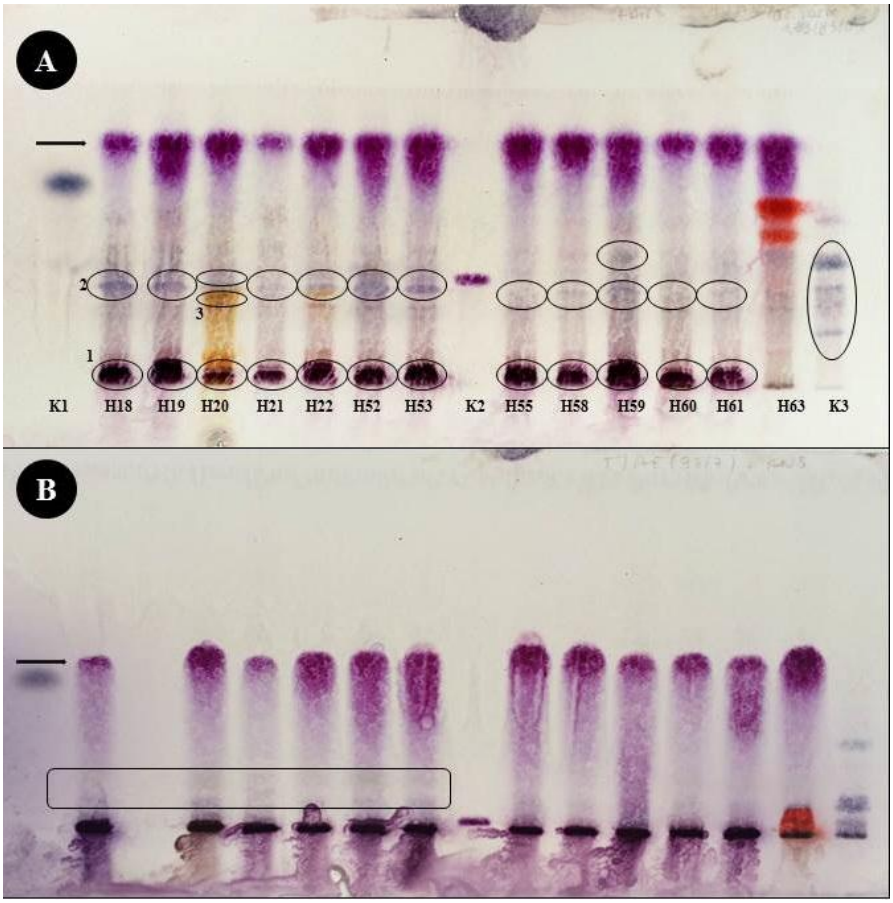
We detected the presence of sugars in all samples of the three species analyzed. Arabitol and saccharose are often present in *U. malmei*, *U. moreliana* and *U. papillata*; while mannitol seems to be rare since it was only found in one specimen of *U. malmei*). Unidentified steroids and monoterpenes were found in *U. malmei* and *U. papillata*. Unidentified triterpenes were found in *U. moreliana*. (Table 1).

Table 1: Three classes of substances found in *U. malmei, U. moreliana* and *U. papillata*. N = indicate the number of specimens analyzed.

CLASSES		**SUGARS**			**STEROIDS**	**TERPENES**	
species		**arabitol**	**mannitol**	**saccharose**		**monoterpenes**	**triterpenes**
U. malmei	n=12	+	±	+	+	+	
U. papillata	n=4	+		+	+	+	
U. moreliana	n=4	+					+



Figures 2A–**D:** Detection of sugars: TLC analysis of *U. papillata* (H53) and *U. malmei* (H55, H58, H59, H60, H61) showing the presence of sugars (arabitol) in four combinations of solvents (D, E)-spray (thymol, ANS) systems: **A)** D-thymol. **B)** E-thymol. **C)** E-ANS. **D)** D-ANS. Arrows on the top indicates usnic acid; circles indicate presence of arabitol; squares indicate presence of saccharose.



Figures 2A–**B:** Detection of terpenes and steroids: TLC analysis in two solvents-spray reagent systems of *U. malmei* (H18, H19, H20, H21, H22, H52, H55, H58, H59) and *U. papillata* (H53) with three controls: K1=linalool (monoterpene), K2=hopane-triol (triterpene); K3=ergosterol and related compounds (steroids). Arrows on the top indicates usnic acid. **A)** Plate run in solvent G and sprayed with ANS - Spots surrounded by circles of line 1 indicate the presence of sugars; spots surrounded by circles of line 2 indicate the presence of unidentified steroids (gray spots) and spots surrounded by circles of line 3 indicate the presence of the depsidone derivate stictic acid. **B)** Plate run in solvent TAE and sprayed with ANS - Spots surrounded by a rectangle indicate the presence of unidentified monoterpenes in faint concentration.

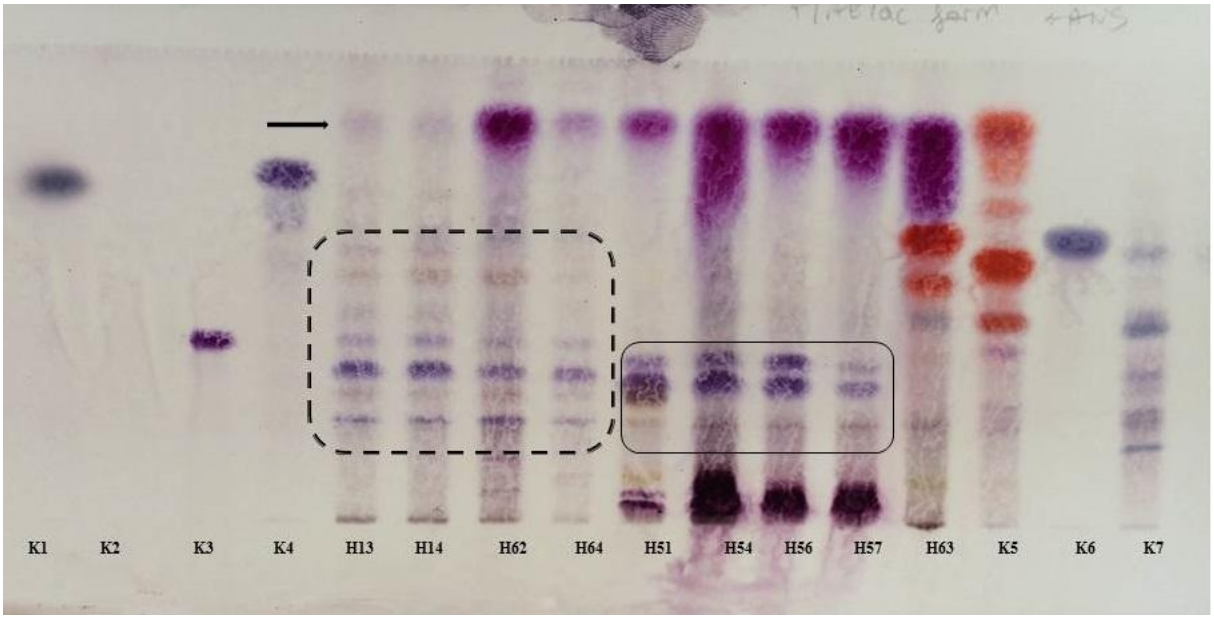


Figure 3: Detection of terpenes and steroids: TLC analysis in the solvent-spray reagent system G-ANS of *U. moreliana* (dashed square) and *U. malmei* (H54)*/U. papillata* (H51, H56, H57) (solid square) with two distinct chemical patterns: a) unidentified triterpenes in *U. moreliana* and b) steroids in *U. malmei/U. papillata* (as already showed in Figure 2). The controls were the following: K1=linalool, K2=eucalyptol (monoterpenes); K3=hopane-triol, K4=lanosterol, K5=zeorin (triterpenes, extracted from *Evernia prunastri*); K6=cholesterol; K7=ergosterol and related compounds (steroids). H63 = *U. longissima* with zeorin. Arrows on the top indicates the presence of usnic acid.

Discussion and conclusions

Prior to this study, several patterns of substances considered as being triterpenes were detected in Neotropical *Usnea* species (Truong et al. 2013) especially specimens showing no reactions with the traditional reagents (K, C, and P) used in lichenology. Our study, however, proved that among these suspected triterpenes, sugars and steroids are also present and that they are even relatively frequent among the tested species. Our study also shows that steroids and terpenes display the same kind of spots in forms and colors in the three solvent TLC system traditionally used by lichenologists. Therefore terpenes and steroids might have been confused in the taxonomical literature so far.

Among the **sugars**, arabitol and saccharose are relatively frequent in the species studied (Figures 1A–D). These primary metabolites are relatively common in lichens (Honda & Vilegas 1998). However, detection of sugars by the traditional TLC analysis (Culberson & Ammann 1978) is difficult because they are often present in faint concentrations. Another difficulty is due to their polar nature which prevents their migration on the silica plates with the solvent systems (A, B, C) traditionally used. For example, the detection of saccharose in the analyzed sample was only possible with the butanol solvent system (Figure 1B, very weak spots), while solvent D allowed a better separation of arabitol and saccharose.

In the traditional TLC solvent systems, the use of ANS as spray reagent allowed us to detect a strong gray spot at the base of the plates already heated in several species of lichens. On the plates sprayed with sulfuric acid (10%), routinely used as spray solvent, the same

substance (sugar) appears only under ultraviolet light (365 nm) as a fluorescent spot after charring. It was probably due to its fluorescence that this substance was misinterpreted as a terpene by Truong et al. (2013, Fig. 2 as *U. malmei/U. papillata*). The same substance was not detected by the same authors in *U. moreliana* (Fig. 2 as *U. rubricornuta*). Using the alternative spray solvent ANS, we were however able to show the presence of this sugar in this species (data not showed, very weak).

Besides sugars, two other classes were found: **steroids** (Fig. 2A; gray spots) and **terpenes**: monoterpenes (Fig. 2B, very faint) and triterpenes (Fig. 3; violet spots). We found a high diversity of medullar compounds related to steroids/terpenes, none of which corresponding to the controls used in this study. Both terpenes and steroids displayed very similar pattern visualized as several gray-violet spots on the middle of the plates. Despite our efforts, these compounds remained unidentified and deserve further investigations. For instance, *Usnea moreliana* exhibited a typical pattern of unidentified triterpenes in the medulla (Figure 3), also found by Truong et al. (2013, Figure 2, as *U. rubricornuta*) diagnostic for this species.

Terpenes are common in various groups of lichens and were used for instance in the taxonomy of *Nephroma* (James & White 1987) and *Peltigera* where 35 distinct terpenes were recognized (Miadlikowska & Lutzoni 2000). Terpenes were also identified and used in the taxonomy of *Physcia* (Elix et al. 2009).

In *Usnea*, however, although widely mentioned in the literature, the identity of terpenes remains poorly known. We found only reference to zeorin (Rogers & Stevens 1988; Ohmura 2001; Kaa et al. 2013; Truong & Clerc 2013; Prateeksha et al. 2016) and four other terpenes in *Usnea longissima* Ach. (Prateeksha et al. 2016). The presence and the nature of the terpenes in this genus, therefore, deserve further investigations using two-dimensional chromatography, doing experimental tests with several solvent systems, comparative literature, or even using more powerful methods (HPLC with a differential refractive index detector as suggested by Sato et al. 2001 or gas chromatography).

Finally, this study reveals the presence of two other classes of medullary compounds besides the terpenes widely mentioned in the literature. A few sugars were identified but terpenes and steroids remain unidentified and deserve further investigations. Spraying the plates with ANS before charring improves the visualization of these classes of substances not easyly seen with traditional methods. We therefore strongly recommend the use of the ANS spraying system as routine analysis aiming at detecting for taxonomy useful patterns especially in specimens with K–, C– and P– medullary reactions.

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Reference

Elix, J.A. 1996. Biochemistry and secondary metabolites. In: Lichen Biology; Nash III, T. H. Ed.; Cambridge University Press, Cambridge, p 154.

Elix, J.A.; Corush, J.; Lumbsch, H.T. **2009.** Triterpene chemosyndromes and subtle morphological characters characterize lineages in the *Physcia aipolia* group in Australia (Ascomycota). *Systematics and Biodiversity* 7(4):479–487. http://dx.doi.org/10.1017/S1477200009990223

Elix, J.A. 2014. A catalogue of standardized chromatographic data and biosynthetic relationships for lichen substances, 3rd edn. Published by the author, Canberra. http://www.anbg.gov.au/abrs/lichenlist/Chem%20Cat%203.pdf

Hale, M.E, Jr. 1979. How to Know the Lichens. 2nd. Edition. Wm. C. Brown Co., Dubuque, Iowa. 246 pp.

Halonen, P. 2000. Studies on the lichen genus *Usnea* in East Fennoscandia and Pacific North America. PhD thesis. Oulu: University of Oulu.

Herrera-Campos, M.A.; Clerc, P.; Nash, T.H. III. 1998. Pendulous species of *Usnea* from the temperate forests in Mexico. *Bryologist 101*: 303–329.

Honda, N.K.; Vilegas, W. 1998. A química dos liquens. *Química Nova 21(6):* 110–125.

Huneck, S.; Yoshimura, I. 1996. Identification of Lichen Substances. Springer-Verlag, Berlin, Heidelberg. 493 pp.

James, P.W.; White F.J. 1987. Studies on the genus *Nephroma* I. The European and Macaronesian species. *Lichenologist 19(3):* 215–268.

Kaa, S.; Sangvichien, E.; Boonpragob, K.; Tee, D.C. 2013. Secondary metabolic profiling and antibacterial activities of different species of *Usnea* collected in Northern Philippines. *Mycosphere* Doi 10.5943/mycosphere/4/2/10

Le Pogam, P.; Herbette, G.; Boustie, J. 2015. Analysis of Lichen Metabolites, a Variety of Approaches. In: Recent Advances in Lichenology. Eds. Upreti, D.K.; Divakar, P.K.; Shukla, V.; Bajpai, R. Springer India. pp. 229–261.

Lohézic-Le Dévéhat, F. ; Tomasi, S. ; Elix, J.A.; Bernard, A.; Rouaud, I.; Uriac, P.; Boustie, J. 2007. Stictic acid derivatives from the lichen *Usnea articulata* and their antioxidant activities. *J. Nat. Prod.70*: 1218–1220.

Lohézic-Le Dévéhat, F. ; Legouin, B. ; Couteau, C. ; Boustie, J. ; Coiffard, L. 2013. Lichenic extracts and metabolites as UV filters. *Journal of Photochemistry and Photobiology B: Biology 120*: 17–28.

Lumbsch, H.T. 2002. Analysis of phenolic products in lichens for identification and taxonomyc. In: Protocols in Lichenology. Culturing, Biochemistry, Ecophysiology and Use in Biomonitoring. Eds. Kranner, I., Beckett, R.P., Varma, A.K. Springer-Verlag, Berlin, Heidelberg, pp. 281–295.

Miadlikowska, J.; Lutzoni, F. 2000. Phylogenetic revision of the genus *Peltigera* (Lichen-Forming Ascomycota) based on morphological, chemical, and large subunit nuclear ribosomal DNA Data. *International Journal of Plant Sciences 161(6):* 925–958.

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

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

Prateeksha, B.S.P.; Bajpai, R.; Jadaun, V.; Kumar, J.; Kumar, S.; Upreti, D.K.; Singh, B.R.; Nayaka, S.; Joshid, Y.; Singh, B.N. 2016. The genus *Usnea*: a potent phytomedicine with multifarious ethnobotany, phytochemistry and pharmacology. *RSC Adv.6:* 21672–21696.

Rogers, R.W.; Stevens, G.N. 1988. The *Usnea baileyi* complex (Parmeliaceae, Lichenised Ascomycetes) in Australia. *Australian Systematic Botany 1:* 355–361.

Sato, H.; Hara, K.; Komine, M.; Yamamoto, Y. 2001. Analysis of lichen substances including triterpenoids by high performance liquid chromatography with a differential refractive index detector and a photodiode array detector. *Mycosystema 30(6):* 944–949.

Truong, C.; Clerc, P. 2013. Eumitrioid *Usnea* species (Parmeliaceae, lichenized Ascomycota) in tropical South America and the Galapagos. *Lichenologist 45*: 383–395.

Truong, C.; Rodriguez, J.M.; Clerc, P. 2013. Pendulous *Usnea* species (Parmeliaceae, lichenized Ascomycota) in tropical South America and the Galapagos. *Lichenologist 45*: 505–543.

CHAPTER 5

Integrative taxonomy reveals six new corticolous species in the *Usnea cornuta* aggregate (Parmeliaceae, lichenized Ascomycota)

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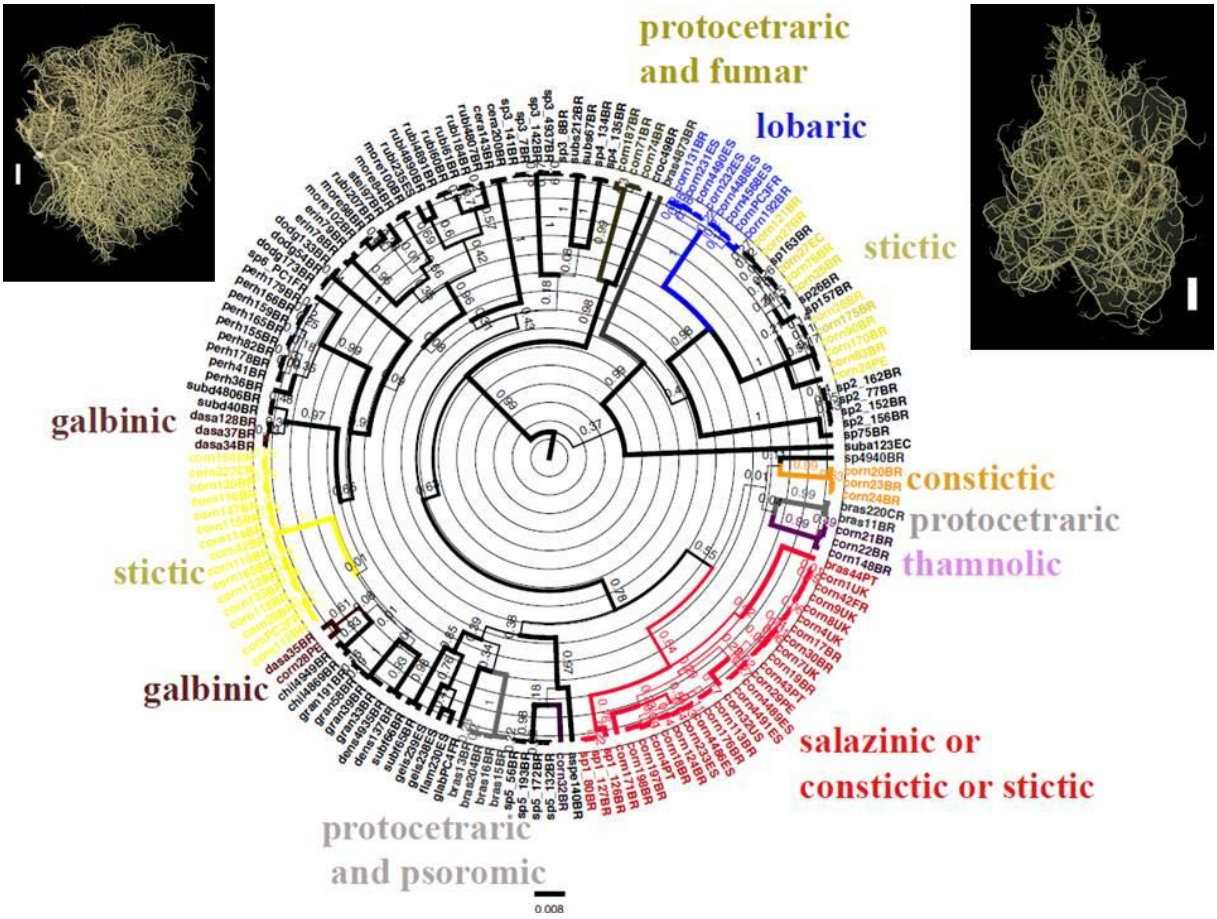

The *Usnea cornuta aggregate*: *Usnea brasiliensis* s.str. (left thallus with protocetraric acid); *Usnea cornuta* s. str. (right thallus with salazinic acid); *cornuta*-1 with constictic acid; *cornuta*-2 with thamnolic acid; *cornuta*-3 with stictic acid; *cornuta*-4 with protocetraric and fumarprotocetraric; *cornuta*-5 with lobaric acid and brasiliensis-2 with protocetraric and psoromic acid.

Integrative taxonomy reveals six new corticolous species in *Usnea cornuta aggr.* (Parmeliaceae, lichenized Ascomycota)

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

ABSTRACT. Based on previous multi-locus species delimitation analyses we describe six new corticolous species from the *Usnea cornuta aggr*. The majority of species occurs so far only on Brazil (with exception of *U. arianae sp. nov.* occurring also on Canarian Islands and *U. isidiofibrillosa sp. nov.* occurring also on Costa Rica and France (Corsica). The newly recognized species are not cryptic species but well characterized with a combination of a morphological and anatomical characters and also chemically. *Usnea arianae sp. nov.* is characterized by the efflorescent soralia, the *cornuta*-type CMA and by the lobaric acid often present in the medulla; *U. catarinensis sp. nov.* is characterized by the convex and well delimited soralia, the *cornuta*-type CMA and the presence of thamnolic acid in the medulla; *U. furnensis sp. nov.* is characterized by the punctiform and ± stipitate soralia, the *cornuta*-type CMA and by the presence of protocetraric and fumarprotocetraric acids in their medulla; *U. isidiofibrillosa sp. nov.* is characterized by the branches covered with isidiofibrils, the *cornuta*-type CMA and the presence of stictic acid in their medulla; *U. pseudobrasiliensis sp. nov.* is characterized by the large soralia, the *cornuta*-type CMA and by the presence of protocetraric and psoromic acids in the medulla; *U. stipitata sp. nov.* is characterized by the stipitate soralia, the *brasiliensis*-type CMA and the presence of constictic acid in the medulla. Full description with morphological, anatomical (CMA) and chemical features (TLC), as well as geographical distribution, is provided for each species along with an identification key to all species reported. *Usnea kriegeriana sp. nov.*, not belong to the *Usnea cornuta aggr.*, was also described and is characterized by the annulate basal part, the eroded tubercles, the large soralia and the presence of fumarprotocetraric in the medulla. Additional descriptions of the *U. cornuta* Körb and *U. brasiliensis* Zahlbr. as well illustrations for all the species were provided.

KEYWORDS. Brazil, erect-shrubby, taxonomy, thin-layer chromatography (TLC), species delimitation analyses

INTRODUCTION

The species investigated herein belong to the *U. cornuta aggregate* sharing a shrubby-erect thallus (i.e. the branches remain erect and divergent up to the apices) to subpendulous

(i.e. branches are first diverging and erect, then run rapidly parallel hanging down) with lateral branches constricted and inflated at their point of attachment, usually with numerous punctiform soralia variable in shapes, and a CMA of the *cornuta*- or *brasiliensis*-type as defined by Truong *et al.* (2011) and Gerlach *et al.* (2017). The medullar chemistry is variable, often with combinations of β-orcinol depsidones (e. g. stictic, salazinic) but orcinol depsidones (e. g. lobaric acid) and β-orcinol depsides (e. g. thamnolic acid) can also be found. The species of this group mostly grow on a variety of corticolous substrata (bark and twigs of trees or bushes, as well as fences).

Usnea cornuta Körb. was considered in a broad sense, morphologically speaking (*sensu latu*) in Europe and North America by several authors (Clerc & Herrera-Campos 1997; Halonen *et al.* 1998; Brodo *et al.* 2001; Herrera-Campos *et al.* 2001). *Usnea brasiliensis* Zahbr. was considered as being a subspecies of *U. cornuta* by Clerc (2004, 2007) due to the presence of seemingly intermediate forms between the two taxa . These two species were considered to belong to the *Usnea fragilescens* aggr. as defined by Clerc (1987), Halonen *et al.* (1998) and Herrera-Campos *et al.* (2001). However *U. fragilescens* s.str. differs in having large, not punctiform soralia and belongs to the *U. florida* clade (Usnea-2 in Truong et al. 2013) whereas members of the *U. cornuta* aggr. belong to the Usnea-3&4 clades (Truong et al. 2013). Studying the genus *Usnea*, it is convenient to use such morphologically defined aggregates even though they are shown to be polyphyletic (Truong et al. 2013).

In South America *U. cornuta* was mentioned growing on rocks in Argentina by Rodriguez *et al.* (2011) and according to Truong (2012) it corresponds to a very common species in the neotropics occuring at a wide altitudinal range. Nevertheless the only supposedly member of this group ever mentioned for Brazil, was *Usnea jelskii* Motyka (described from Chile and synonymized under *U. cornuta* by Clerc 2004), which was cited for Rio de Janeiro by Rizzini (1952, 1956). Among the species described from Brazil *U. brasiliensis* Zalhbr., *U. bornmüelleri var. brasiliensis f. inactiva* Zahlbr. and *U. spinulifera* (Vain.) Motyka belong to the *Usnea cornuta* aggregate as defined here.

This group shows a strong phenotypical plasticity and detailed molecular and species delimitation studies with such methods using the theory of coalescence as background were used in this group. We were then able to segregate nine lineages that are potentially good candidate at the species level (Gerlach et al., chapter 3). An *a posteriori* research of subtle and before the molecular study unseen characters, with a larger number of specimens was successful and allowed us to propose the description of six of new species that are

molecularly, chemically, morphologically and anatomically well defined. Two lineages deserve further investigation ("brasiliensis-1" and "*cornuta*-6", referred on Chapter 3) and will be treated elsewhere.

Below, we formally describe these six new species building strong supported clades that seem to be unrelated with the *U. cornuta s. str.* clade. Furthermore, we described a lineage not belonging to the *U. cornuta* aggr. based on molecular and phenotypically features. Description of *U. cornuta* s.str., *U. brasiliensis* s.str. and of all the new proposed species as well illustrations and a species key are provided.

MATERIALS AND METHODS

This study take account specimens mostly collected in Brazil, Southern region as indicated in Gerlach *et al.* (2017). Field trips were made also during Mai-June 2016 in two localities: Santa Catarina State (Alfredo Wagner municipality, Reserva Particular do Patrimonio Natural Rio das Furnas) and in Minas Gerais States (Gonçalves municipality on the Serra da Mantiqueira). A few specimens collected in Costa Rica, France (Corsica) and Spain (Canarian Islands, Tenerife) were also included in this study.

Specimens deposited in the following herbaria were also studied: G, HAS, ICN, JPB, LBL, MACB, and W. The morphology of specimens was examined using a stereomicroscope Leica MS5, with measurements done using a Leica DM2000 microscope.

The species concept and terms for morphological characters used in this study follows Clerc (1998, 2006), Clerc & Herrera-Campos (1997), Herrera-Campos *et al.* (1998), Ohmura (2001), Truong *et al.* (2011, 2013). 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.

Molecular analyses were carried with multi-species delimitation analyses template Stacey with three markers, ITS, *Mcm7* and RPB1 as indicated in Gerlach *et al.* (2018, chapter 3 of this thesis). Clades with posterior probabilities (PP) > 0.90 were considered to be highly supported. At least one specimen per locality is included in the list of selected specimens.

RESULTS AND DISCUSSION

We described six new species belonging to the *Usnea cornuta aggregate*: *Usnea arianae, U. catarinensis, U. isidiofibrillosa, U. furnensis, U. pseudobrasiliensis* and *U.*

stipitata. Additionally we describe *U. kriegeriana*, which not belong to the *U. cornuta aggr.* All of these newly proposed species are highly supported by our previous molecular studies.

All these new species are described based on phenotypical and molecular features. Molecular analyses were done on the previous work on this group of corticolous, sorediate shrubby *Usnea* species (Gerlach et al., chapter 3).

Main morphological and anatomical characters analyzed

Shape of soralia. Most of the species described here have punctiform soralia arising from tubercles or from fibrils scars (fibercles): *U. catarinensis, U. furnensis, U. isidiofibrillosa,* and *U. stipitata*. *Usnea brasiliensis* also has punctiform and irregularly shaped soralia that become confluent (agglomerating but remaining clearly separated). Convex soralia bursting into numerous isidiomorphs and/or granular soredia (efflorescent sensu Clerc 1987) occur in *U. arianae*. Fusing soralia (agglomerating, losing their individuality, and looking like a large soralia) that are even with the cortex are typical of *U. cornuta s. str.* Large individual soralia were found in *U. pseudobrasiliensis* and *U. kriegeriana*. It is very important to analyze mature soralia usually found on the terminal branches of the thallus. Isidiomorphs are often present in all species treated here and thus do not seem to have any taxonomical value.

Cortex and CMA values. Almost all the species described in this study have a CMA of the *cornuta*- or the brasiliensis type or a variation of one of these two main types. Only *U. kriegeriana* has another type of CMA, a mat cortex, that is the thickest cortex among the species presented here. A typical CMA *cornuta-type* was defined with 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) by Truong *et al.* (2011). This CMA type was found in *U. catarinensis, U. cornuta* and *U. isidiofibrillosa.* While a typical CMA of the *brasiliensis-type* 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) (Gerlach *et al.* 2017) was found only in *U. brasiliensis*. Two species display a variation of the *cornuta-type*: *U. arianae* with a slightly thinner cortex than usual in the *cornuta*-type CMA and *U. furnensis* with slightly thinner central axis than usual in the *cornuta*-type CMA). *Usnea stipitata* displays a variety of *brasiliensis*-type, with a slightly thicker central axis than usual in the *brasiliensis*-type (Table 1).

Species	N	%Cortex	% Medulla	%Axis	A/M	Type
U. arianae	13	4–5.5–7	33–35–37	17–20–23	< 1	cornuta- with thinner cortex
U. brasiliensis	13	3.5–4.5–5.5	38.5–40–43	9.5–11.5	< 0.5	brasiliensis-
U. catarinensis	9	5.5–8.5–11.5	26.5–31–35.5	17–21–25	< 1.3	cornuta-
U. cornuta	13	6–7– 8	28.5–31–33.5	20–24–28	< 1.3	cornuta-
U. furnensis	6	5–6–7	34.5–36.5–38.5	11–14–17	< 0.5	cornuta- with thinner axis
U. isidiofibrillosa	12	7–6.5–8	29–31.5–34	21.5–24.5–27.5	< 1.3	cornuta-
U. kriegeriana	16	7.5–9–10.5	19–25.5–32	24–31.5–38.5	< 2	not cornuta- neither brasiliensis-
U. pseudobrasiliensis	7	6–7–8	32–34–36	16–18–20	< 0.7	cornuta-
U. stipitata	28	4.5–5.5–6.5	33.5–37.5–41.5	9.5–14.5–19.5	< 0.8	brasiliensis- with thicker axis

Table 1. Cortex, medulla and axis (%CMA) percentages of the *Usnea* species. Values, and mean values (underlined) are given. For extreme values see description under each species. N = total of individual thalli measured.

Chemistry

Most of the species treated here are characterized by only one chemotype with the exception of *U. arianae* with four chemotypes (often K─, P─) and *U. cornuta* (K+) with three chemotypes (Table 2). Stictic acid can be found in two species (*U. cornuta and U. isidiofibrillosa*); protocetraric acid in four species (*U. brasiliensis*, *U. furnensis*, *U. kriegeriana*, and *U. pseudobrasiliensis*). In these species with protocetraric it is therefore important to check the presence of psoromic, fumarprotocetraric acid or of an unknown substance (refered here as US2).

Species	N	SAL	NOR	STI	CST	PRO	FUM	PSO	LOB	THA	FA	TER	US1	US2	Medulla color test
U. arianae	8**								+		±				K─, P─
	4**		+						±		+				K+ y→r
	2										+				K─, P─
	2												+		K─, P─
U. brasiliensis	20					+		±							K− or K+ dirty y, P+ r
U. catarinensis	8									+					K+ br. y
U. cornuta	35	+		±	±	± tr					+				K+ y→r
	10	± tr			+										K+ y or K+ y→r
	5**			+	±										K+ y
U. furnensis	14					+	±								K− or K+ dirty y, P+ r
U. isidiofibrillosa	25	±		+	±							±			K+ y or K+ y→r
U. kriegeriana	20					+	+ *							+ *	K+ y→r
U. pseudobrasiliensis	4					+		+							K− or K+ dirty y, P+ r
U. stipitata	34	±			+	± tr					±	±			K+ y or K+ y→r

Table 2. Major secondary metabolites (columns) and chemotypes (lines) detected by TLC in the medulla were showed. Abbreviations for secondary metabolites: **SAL** = salazinic, **NOR** = norstictic, **STI** = stictic, **CST** = constictic, **PRO** = protocetraric, **FUM** = fumarprotocetraric, **PSO** = psoromic, **LOB** = lobaric, **THA** = thamnolic, **FA** = unidentified fatty acid, **TER** = unidentified terpenoids, **US1** = an unknown with grey bluish spot [Rf classes A/B/C: 5/5/5], **US2** = an unknown with orange spot [Rf classes A/B/C: 1–2/2–3/1–2]. Abbreviations for medulla color test: y→r = yellow turning red; br. y = bright yellow. N = number of specimens studied; + = presence constant within species; ± = presence variable among specimens within species; tr = present in traces; * absent only on one specimen. ** This chemotype was found only on specimens from Europe

TAXONOMY

Usnea arianae P. Clerc, E. Caviró & A. Gerlach, *sp. nov. ad int.*

MycoBank No.: MB

Differs from U. cornuta Körb. by its individual and efflorescent convex soralia, the lax medulla without reaction on color tests (K−, P−) often with lobaric acid and by its molecular phylogenetic position, in 18 parsimony-informative alignment positions of the internal transcribed spacer (ITS) and four indels (Supplementary figure)

Type: Spain, Canarian Islands, Tenerife, Erjos, along track to Los Silos, 28°19’56’’N 16°18’23’’W, 900 m., on *Erica arborea*, Jan. 2017, *A. Aptroot* 75514 (G—holotype). %C/M/A: 5.5/34/20. TLC: usnic, lobaric acid (trace) and an unknown fatty acid (Rf class A: 3–4, 4–5). DNA code: corn5-232TEN.

(Fig. 1A−C)

Description. *Thallus* erect-shrubby, up to 5 cm long; *ramifications* anisotomic-dichotomous; basal part fusiform, often blackish only in the first 0.5-1 mm, more rarely concolorous with branches, without distinct annular cracks; main *branches* up to 1.9 mm diameter (n=13), strongly irregular in shape, often ±flattened, with depressions , with thin and sparse annular cracks; *branch segments* distinctly swollen, terete to flattened, *lateral branches* distinctly constricted at attachment points; *foveolae and depressions* often present on main branches; *maculae* and *pseudocyphellae* absent; *papillae* indistinct to small verrucous, numerous, irregularly, regularly distributed, mainly on main branches; *tubercles* absent; *fibrils* slender, few, irregularly distributed on apices; *fibercles* present; *soralia* remaining punctiform or enlarging to more than 1/2 branch diameter when well developed, slightly stipitate, ±circular, usually remaining discrete, but becoming confluent and efflorescent-convex when numerous and well developed, with granular soredia, covering the apices, developing from the cortex *ad initio* or from fibercles, *isidiomorphs* often numerous on well developed soralia; *isidiofibrils* absent; *cortex* glossy, thin to moderately thin, (3.5–)4–5.5%–7(–7.5) (n=13); *medulla* lax, thick (31–)33–35%–37(–39); *axis* thin, (14–)17–20%–23(–27), A/M = (0.4–)0.5–0.6–0.7(–0.8).

Apothecia rare (one apothecia on a Brazilian specimen), subterminal, 7 mm of diameter, with few fibrils. *Spores* not seen. ***Pycnidia*** not seen.

Chemistry. Medulla: K−, P−. TLC: 1) lobaric acid, ± fatty acid (Rf classes A: 3–4, 4–5) (n=8); 2) fatty acid (Rf classes B: 5, 6) (n=2); 3) an unknown spot grey bluish (Rf classes A/B/C: 5/5/5 (n=2); 4) usnic only (n=2). Medulla: K+ yellow→red. TLC: 5) norstictic acid, ±lobaric acid, fatty acid (Rf classes A/B: 4–5/5–6) (n=4).

Etymology. This taxon is named in honour to Alice's dear sister, Ariane, who passed away during this study.

Habitat and distribution. Corticolous on a variety of trees (e. g. *Erica arborea*, *Quercus robur*, *Pinus pinaster*). *Usnea arianae* show an oceanic-type of distribution. It occurs in France (Bretagne, Corsica), Portugal (Parque Natural Peneda-Gerês), Spain (Ourense and Canarian Islands: Tenerife, La Gomera) and also in Brazil (Santa Catarina and Minas Gerais).

Diagnostic characters. When well developed, *Usnea arianae* can be recognized by the main branches that are ± irregularly swollen or flattened, with depressions, (Fig. 1A), the convex and efflorescent discrete soralia and the numerous isidiomorphs (Fig. 1B), the *cornuta-type* CMA with a slightly thinner cortex (3.5–)4.0–**5.5%**–7(–7.5) and a lax medulla (Fig. 1B). Lobaric acid is present in 74% of the specimens analyzed.

Variation. Two specimens from Brazil clustered together with the samples from Europe (see Chapter 3, referred as *cornuta*-5). They fit morphologically well with the type specimen but without lobaric acid (not detected by TLC). By the same way one specimen from Spain presents only fatty acid.

Taxonomic notes. *Usnea flammea* Stirt. also can have lobaric acid in the medulla (Clerc 2006). It differs from *U. arianae* mainly by the branches not constricted at attachment point, the pale basal part with numerous annulations, the large soralia, and the mat cortex (Clerc 2006). *Usnea peruviana* Motyka belongs to the *U. cornuta* aggr. and also has a lax medulla with lobaric acid and punctiform soralia. However, it differs from *U. arianae* by the thinner cortex and wider medulla [2.5/40/15, W!—holotype; 3.5/40/13, W!—isotype], the soralia with a distinct cortical margin, not convex and without the typical granular soredia and isidiomorphs of *U. arianae* and by the segment of branches which is not delimited by annular cracks and not swollen as in *U. arianae*.

Usnea cornuta s. str. displays a very typical habitus (± swollen branches and lateral branches forming 90° at ramification point as defined by Clerc 1987, Fig. 24) but differs from *U. arianae* mainly by the presence of minute, numerous and confluent soralia giving the impression of larger soralia, on the apices of the branches and by the chemistry (Table 2). Multi-locus species delimitation showed that U. arianae forms a strong supported clade unrelated to the *U. flammea* and *U. cornuta* (chapter 3, referred as *cornuta*-5).

Specimens examined. **Brazil**: *Santa Catarina*: Alfredo Wagner, RPPN Rio das Furnas, 27°40'28.3"S, 49°10'37.9"W, 2016, *Gerlach et al*. P3 (G). *Minas Gerais*: Gonçalves, proximo ao Chalé Três Irmãs, 22°43'56.75"S, 45°52'46.67"W, 2016, *Gerlach & Penati* (G). —**France**: *Corse du Sud*, Alta Rocca, Bavella, San-Gavino-di-Carbini Pont de Fiumicelelli, au bord de la riviere en amont du pont, 41°50'30.68"N, 9°18'28.46"E, 195 m, grands *Quercus ilex*, 2016, *Clerc* (G). —**Spain**: *Ourense*, Parque Natural Baixa Limia-Serra do Xurées, Entrimo, Mirador de San Rosendo do Pedreirinho, sobre *Pinus pinaster*, 740 m, 2013, *Arroyo* EA328(4) (MACB). *Canarian Islands*, La Gomera, versant N tu Mt. Garajonay, Barranco del Cedro, 1250 m, 2014, M-Mad (G) ; *ibid*, Hermigua El Cedro, chemin au nord du hameau, restes de Laurisilva, *Clerc* 11700 (G) ; *ibid*, *Clerc* 11755 (G); Garajonay, 17°14,49'W, 28°06,74'N, 1435 m., on *Erica sp*., Sept/2009, *Boom* 46196 (G); *ibid*, 17°15,18'W, 28°07,23'N, 1260 m,

Sept/2009, *Boom* 46426 (G); Tenerife, Erjos, along track to Los Silos, 28°19'56"N, 16°18'23"W, ca. 900 m, on *Erica arborea*, 01/2017, *Aptroot* 75517 (G).

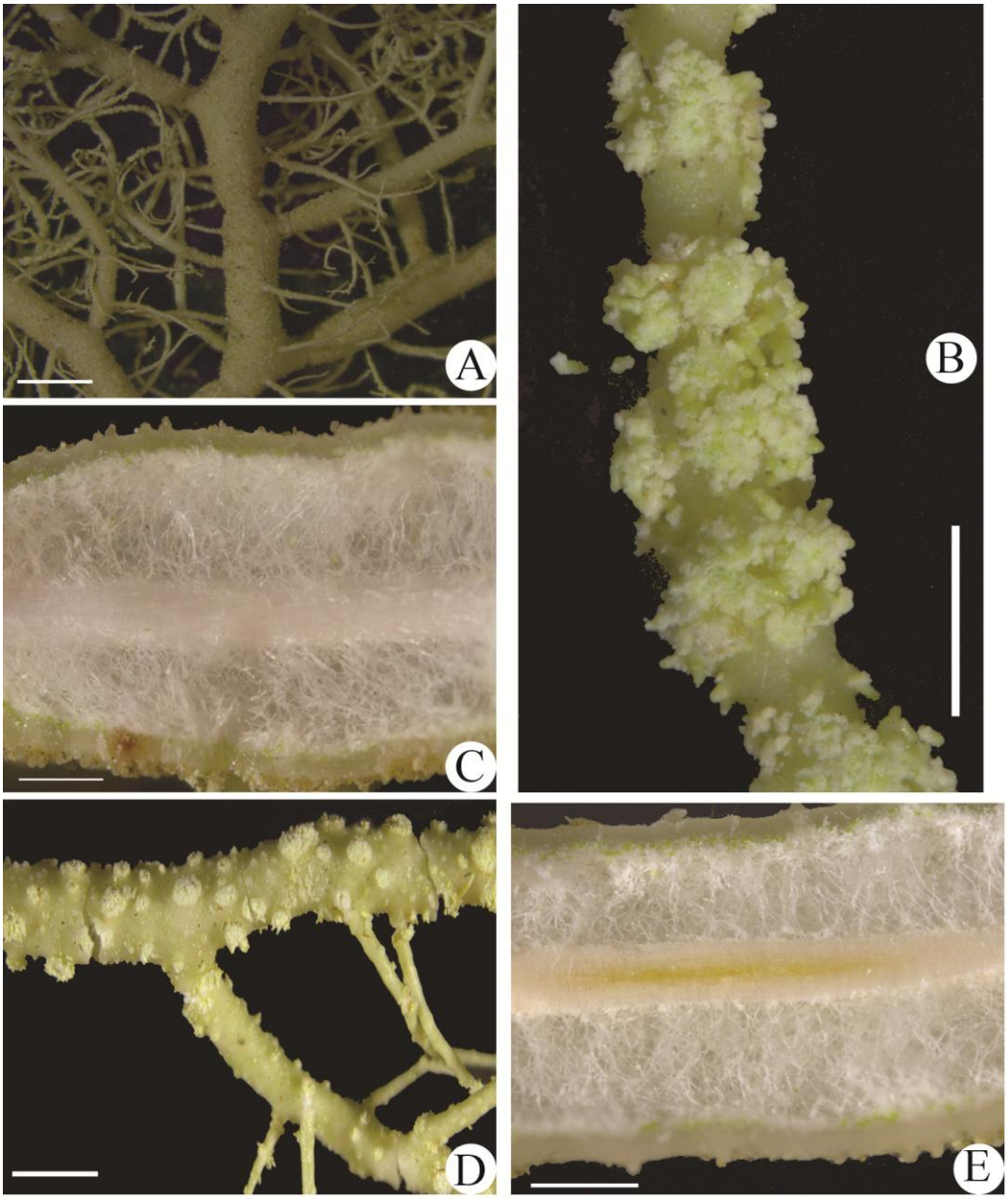

FIG. 1. **A–C, *Usnea arianae***: A, branch segments swollen with papillae (holotype); B, convex minute soralia with small isidiomorphs (*A. Aptroot* 75517); C, section through thallus with *cornuta*-type CMA with thin axis and lax medulla (holotype). **D–E, *Usnea catarinensis*** (holotype): D, soralia convex to stipitate; E, section through thallus, with *cornuta*-type CMA with lax medulla. Scales: A=2 mm; B & C=500 µm; D=1mm; E=500 µm. In colour online.

***Usnea* brasiliensis** (Zahlbr.) Motyka.

Lich. Gen. Usnea Stud. Monogr. Pars Syst. 2(1): 504 (1937); type: Brazil, São Paulo, near Lagoas, Exp. Acad. Vindobon, 1901, *Schiffner* (W!—holotype). %C/M/A: 4/42/7. Chemistry: usnic, protocetraric and psoromic acids (chemistry by Clerc in 1996).

=? *Usnea bornmuelleri var. brasiliensis f. inactiva* Zahlbr. *Denkschr. Kaiserl. Akad. Wiss., Wien. Math.-Naturwiss. Kl.*, 83: 187 (1909); type: Brazil, ad confines Rio de Janeiro et Minas Gerais, Monte Itatiaia, 2500 m, 1901, *Schiffner* (W!—holotype). %C/M/A: 6/37/14. Chemistry: usnic acid only (two thallus tested).

(Fig. 2A−C)

Description. *Thallus* shrubby, up to 8 cm long; *ramification* anisotomic- to isotomic-dichotomous; *basal part* usually short (ca. 2 mm long), concolorous to the main branches or blackish only on the first mm, sometimes with irregular cracks; *main branches* thick, up to 3.6 mm diameter (n=13), irregular longitudinally, somewhat fusiform to deeply deformed and trapezoidal in longitudinal section; *branches segments* irregular on longitudinal and ridged in transversal sections; *lateral branches* constricted, often with annular cracks at the attachment point often with medullar extrusion at margin; *foveolae and depressions* often present, sometimes numerous; *maculae and pseudocyphellae* absent; *papillae* (?) or *"bumps"* numerous, especially on terminal branches; *tubercles* absent; *fibrils* few, with base slightly constricted and then easily breakable; *fibercles* few; *soralia* punctiform, slightly stipitate, circular to ± irregular, often with well delimited cortical margin, discrete to often confluent towards the apices of branches, developing *ad initio* on the cortex or at the top of irregular "bumps"; *isidiomorphs* usually present on mature soralia; *isidiofibrils* absent; *apothecia* rare, terminal, ca. 2 mm diameter; *ascospores and pycnidia* not seen; *cortex* thin [(3–)3.5–4.5%–5.5(–6)] (n=13), glossy, surface irregular due to depressions of the cortex and eventually with some transversal ridges; *medulla* thick [(37.5–)38.5–40%–43], lax to dense; *axis* thin [7–9.5%–11.5(–13)], with a ratio A/M < 0.5.

Chemistry. Medulla: K−, P+ red. TLC: 1) protocetraric and ± psoromic acids (n=22).

Habitat and distribution. Corticolous on the native coniferous *Araucaria angustifolia* and on a variety of exotic trees (*Castanea sativa*, *Pinus* spp.). Widely mentioned in the American continent: Argentina, Bolivia, Brazil, Colombia, Costa Rica, Peru, Porto Rico, Venezuela (Motyka 1938), Mexico (Herrera-Campos *et al.* 2001), and rare in the Macarronesian area (Pérez-Vargas *et al.* 2010). In Brazil, it was mentioned for Minas Gerais (Motyka 1938), Rio de Janeiro (Rizzini 1952) and São Paulo (Motyka 1938). Newly reported here for Southern Brazil in the Rio Grande do Sul and Santa Catarina States.

Diagnostic characters. *Usnea brasiliensis* is characterized by the strongly irregular main branches (Fig. 2A), the lateral branches constricted often with annular cracks exposing the medulla at attachment point (Fig. 2A), the CMA of the *brasiliensis-type* (Fig. 2B), the slightly stipitate soralia producing typical irregularities on the surface of the cortex (Fig. 2C) and by the presence of protocetraric acid.

Variation. The density of annular cracks, papillae, tubercles and soralia are variable in this species as well the degree of irregularity of the main branches.

Taxonomic notes. *Usnea brasiliensis* differs from *U. cornuta* mainly by the branches that are

more distinctly irregular, by the confluent and slightly stipitate soralia giving the typical irregularity of the cortex surface, by the CMA of the *brasiliensis*-type with larger medulla [38.5−43%] and by the presence of protocetraric acid. In *U. cornuta* soralia are fusing and even with the cortex surface, the CMA is of the *cornuta*-type with thinner medulla [28.5−33.5%] and the chemistry is different. Molecular studies (chapter 3) show clearly that to two distinct lineages are here present.

Due the presence of intermediate forms (*U. brasiliensis* morphotypes without protocetraric acid or typical *U. cornuta* with protocetraric acid), Clerc (2004, 2007), proposed the combination *U. cornuta subsp. brasiliensis* (Zahlbr.) P. Clerc. Interestingly, intermediate forms were also found in Brazil and a critical molecular study reveals that they belong to two strongly supported lineages corresponding to two distinct species described as new in this study: *Usnea pseudobrasiliensis* sp, nov. ad int. with the same morphology as *U. cornuta* but with protocetraric acid in the medulla and *U. stipitata* with the same morphology as *U. brasiliensis* but with constictic acid in the medulla (see under these taxa for more comments).

The infra-specific taxon *U. bornmüelleri var. brasiliensis f. inactiva* corresponds well with *U. brasiliensis* except by the medulla without secondary compounds as suggested by the name *inactiva.* This form was synonymized with *Usnea jelskii* Motyka (Motyka 1938: 595) but it differs mainly by the chemistry (salazinic and norstictic acids) (LBL!—holotype, see under *U. isidiofibrillosa* for more comments). It possibly belongs to the same species. Therefore, morphotypes of *U. brasiliensis* without medullar compounds should be tested molecularly before further conclusions.

Selected specimens analysed. **Brazil**. *Paraná*: Guaratuba, Morro dos Perdidos, ca. 25°53'17"S, 48°57'43"W, ca. 1400 m, 2013, *Gerlach et al.* 1010 (ICN); Rio Branco do Sul, beira da rodovia, 2012, *Gerlach et al.* 804 (ICN). *Rio Grande do Sul*: Cambará do Sul, Parque Nacional dos Aparados da Serra, 1986, *M. Fleig* 2836 (ICN); *ibid*, mata nebular, proximo ao centro de visitantes, sobre *A. angustifolia*, 2014, *Gerlach & Akkerman* 1405 (G); *ibid*, Parque Nacional da Serra Geral, 2014, *Gerlach & Akkerman* 1446 (ICN); São Francisco de Paula, Flona São Francisco de Paula, ca. 900 m., mata de castanheira portuguesa *Castanea sativa*, 2014, *Gerlach* 1517 (ICN); *ibid*, lago São Bernardo, derrière l'hôtel Cavalinho Branco, 29°27'34"S, 50°34'16"W, 1000 m, 1989, *Grundlehner* (G). *Santa Catarina*: Alfredo Wagner, RPPN Rio das Furnas, 27°40'28.3"S, 49°10'37.9"W, ca. 870 m, 2014, *Gerlach & Alves* 1241 (ICN); *Campo Alegre*, Estrada de chão para o Quiriri, sobre *A. angustifolia*, 2013, *Gerlach & Beilke* 1174 (ICN); Garuva, Estrada das laranjeiras, mourão, 2013, *Gerlach & Beilke*1131 (ICN); Joinville, galhos caídos de *A. angustifolia*, *Gerlach & Beilke* 1149 (ICN); São Bento do Sul, APA Rio Vermelho, sobre *A. angustifolia*, 2013, *Gumboski* 4297b (ICN); Urubici, Parque Nacional de São Joaquim, arredores do alojamento, ca. 1300 m, *Gerlach & Alves* 1386 (ICN). *São Paulo*: Campos do Jordão, 150 km nordöstlich von São Paulo in einem hellen, feuchten Urwald, 1700 m, 1978, *Kalb & Plöbst* (G). Zwishen Guapira und Apiaí, in einem kleinen *Pinus* Forest. An *Pinus* spec., 800 m, 1980, *Kalb* (G). Serra do Mar, Serra de Paranapiacaba, 60 km südwestlich von São Paulo, oberhalb von Juquitiba, in einem hellen, feuchten Urwald am Rio Juquiá, ca. 800 m, 1978, *Kalb & Plöbst* (G).

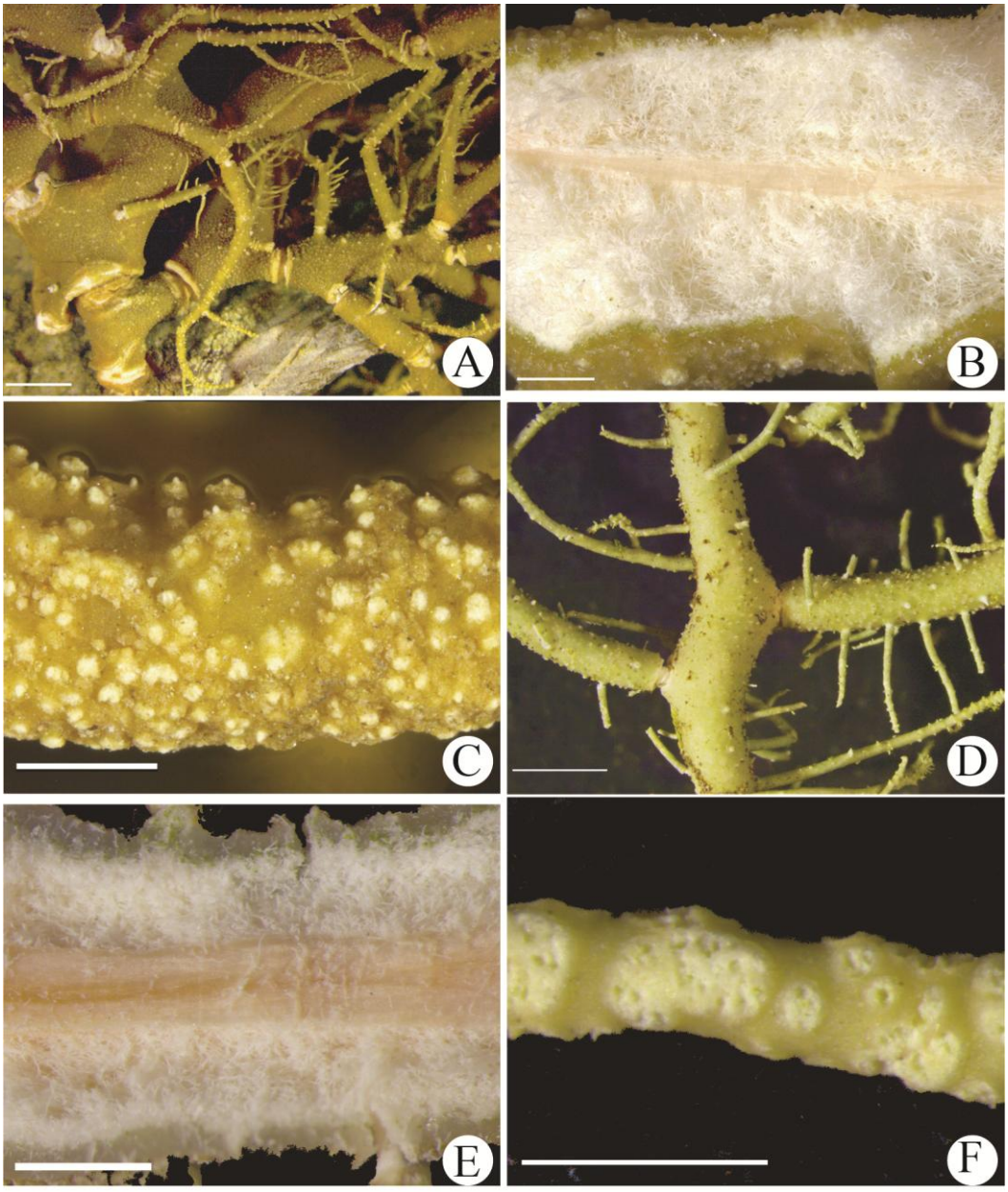

FIG. 2 **A–C, *Usnea brasiliensis*** (*Gerlach* 1172a): A, main strongly irregular main branches; B, CMA of the *brasiliensis*-type; C, soralia punctiform, slightly stipitate at the top of irregular "bumps" and confluent, but not fusing together. **D–F, *Usnea cornuta***: D, lateral branches constricted at attachment point (*Gerlach et al.* P18, DNA code: corn-198; E, CMA of the *cornuta*-type (*Gerlach & Penati* P47, DNA code: corn-124); F, soralia punctiform and fusing together (*Gerlach et al.* 1577, DNA code: corn-4). Scales: A=2 mm; B & C=500 µm; D=2 mm; E & F=500 µm. In colour online.

Usnea catarinensis A. Gerlach & P. Clerc, *sp. nov*. ad. int.

MycoBank No.: MB xxxxxxxx

Differing from Usnea cornuta by the convex soralia, the presence of thamnolic acid as main secondary metabolite and by its molecular phylogenetic position, in 10 parsimony-informative alignment positions of the internal transcribed spacer (ITS) (Supplementary figure)

Type: Brazil, Santa Catarina, Reserva Particular do Patrimônio Natural Rio das Furnas, ca. 27°40'28.3"S, 49°10'37.9"W, ca. 900 m, corticolous, 14.05.2016, *Gerlach et al.* P46 (ICN—holotype). %C/M/A: 5/35/21. Chemistry: usnic and thamnolic acids. DNA code: corn2-22BR.

(Fig. 1D–E)

Description. *Thallus* erect-shrubby, up to 6 cm long; *ramifications* mainly isotomic-dichotomous; *basal part* short, up to 5 mm long, concolorous to the branches to slightly yellowish, sometimes annulated exposing the medulla; main *branches* up to 2 mm diameter (n=9), slightly irregular, segmented; *branch segments* terete in cross-section; *lateral braches* constricted at attachment point; *annular cracks* few, sometimes with medullar extrusion; *foveolae and depressions* absent; *maculae* inconspicuous, visible only on fresh collections; *papillae and tubercles* few to numerous, cylindrical to verrucose; *tubercles* present, without eroded apices; *fibrils* usually numerous, slender; *fibercles* absent or rare ; *soralia* punctiform, slightly stipitate, convex at maturity towards the apices of the branches, circular, sometimes with well delimited cortical margin (when located on main branches), not fusing together, often numerous on the whole thallus, arising from tubercles and fibercles; *isidiomorphs* present, few to numerous; *isidiofibrils* absent; *apothecia* rare (one immature found); pycnidia not seen; cortex moderately thick [(4.5–)5.5–8.5%–11.5(–13)] (n=9), glossy; *medulla* ± thick to thick [(22–)26–30%–34(–35)], lax, often heterogeneous; *axis* thin [(19–)20–24%–28(–30)], A/M = 0.6–1.3.

Chemistry. Medulla: K+ bright yellow. TLC: 1) thamnolic acid, ± squamatic acid, ± unknown grey Rf Class A/B/C: 1/1/1 (n=13).

Etymology. The epithet *catarinensis* refers to the locality where this species was discovered, Santa Catarina State, southern Brazil.

Habitat and distribution. Corticolous on twigs or lignicolous on fences. So far found only in Brazil: Santa Catarina and São Paulo states.

Diagnostic characters. *Usnea catarinensis* is characterized by the convex and well delimited soralia (Fig. 1D), the *cornuta-type* CMA (sometimes with slightly thicker cortex) (Fig. 1E) and by the presence of thamnolic acid in the medulla.

Variation. The density of isidiomorphs and annular cracks can vary in this species. Immature soralia looks slightly stipitate, but on mature thallus soralia are clearly convex.

Taxonomic notes. *Usnea cornuta* differs by the shape of soralia, which are minute, numerous, even and confluent towards the apices of the branches, whereas soralia of *U. catarinensis* are well-delimited and convex. Both species have the same type of CMA of the *cornuta-type*, although in *U. catarinensis* the cortex is in average slightly thicker [(5–)5.5–8.5%–11.5(–12)]

compared with *U. cornuta* [(5–)6–7%– 8(–9)]. The chemistry is also distinct: *U. cornuta* produces salazinic acid or related depsidones in the medulla whereas *U. catarinensis* has the depside thamnolic acid as main medullar secondary compound.

Usnea trachyclada (Müll. Arg.) Zahlbr., described from Brazil and also with thamnolic acid, differs from *U. catarinensis* by the CMA of the *brasiliensis-type* (4.5/37.5/16), the irregular branches with foveolae and the soralia that are not convex, but rounded with well delimited cortical margin Moreover the soralia of this species develop on conspicuous fibercles (G!—holotype). Molecular analysis showed that *U. catarinensis* corresponds to a strongly supported lineage (chapter 3, referred as *cornuta-2*).

Selected specimens examined. **Brazil**: *Santa Catarina*: Alfredo Wagner, RPPN Rio das Furnas, em galhos caidos, 2016, *Gerlach et al.* P3 (ICN); Garuva, estrada de chão para a serra do Quiriri, 2013, *Gerlach & Beilke* 1158a (ICN); Joinville, estrada das Laranjeiras, on fences, 2013, *Gerlach & Beilke* 1108 (ICN); *ibid*, alto da Serra Dona Francisca, em palanque próximo a torre, 2013, *Gumboski* 4662 (ICN). *São Paulo*: Serra de Paranapiacaba, 60 km südwestlich von São Paulo, oberhalb von Juqitiba, in einem hellen, feuchten Urwald am Rio Juquiá, ca. 800 m, 1978, *Kalb & Plobst* (G).

Usnea cornuta Körb.

Parerga lichenologica, p. 2, Breslau (1865); type: Germany. ad saxa arenaria "des Regensteins" prope Blankenburg Hercyniae, Hampe s. n.; Koerber, Lich. Sel. Germ. n°181 (G!—isolectotype). %C/M/A (lectotype): 4/35.5/21. Chemistry (lectotype): usnic, salazinic, constictic, protocetraric acids (%C/M/A and chemistry by Clerc 1987).

(Fig. 2D–F)

Description. *Thallus* erect-shrubby, up to 8 cm long; *ramifications* anisotomic- becoming isotomic-dichotomous towards the apices; *basal part* concolorous to sometimes black pigmented only on the first mm below the first ramifications; *branches* up to 1.6 mm diameter (n=13), slightly irregular, irregularly segmented; *branches segments* often swollen; *lateral branches* constricted at attachment point; *annular cracks* sparse; *foveolae and depressions* sometimes present, not abundant; *maculae and pseudocyphellae* absent; *papillae* few, verrucous, sparsely distributed; *tubercles* few to rarely numerous, not eroded at apices,; *fibrils* scattered on whole thallus, slender; *fibercles* occasionally present; *soralia* minute, even with the cortex, circular, often without well delimited cortical margin, becoming confluent and fusing together looking like large soralia, numerous towards the apices of the branches, arising from tubercles or fibercles on main branches or on *ad initio* the cortex towards the apices of branches; *isidiomorphs* few to numerous; *isidiofibrils* rarely present; *apothecia* sometimes present, terminal, mostly on fibrils, ca. 2 mm diameter; *ascospores* 7–8.5(–10) × 5–6 µm (n=10); *pycnidia* not seen; *cortex* thin to moderately thick [(5–)6–7%–8(–9)], shine (n=13); *medulla* moderately thick to thick [(27.5–)28.5–31–33.5(–34.5)], lax to dense; *axis* thin to moderately thin [(18–)20–24–28(–33)]; A/M = 0.5–1.3.

Chemistry. Medulla: K+ yellow→red. TLC: 1) salazinic acid, ± stictic acid, ± constictic acid, ± menegazziaic acid, ± crypstostictic acid, ± norstictic acid, ± (trace) protocetraric acid, ± unidentified fatty acids Rf Classes A/B/C: 4/5/5 and 5/5/5 (n=35); 2) constictic acid, ± salazinic (trace) (n=10). Medulla: K+ yellow. TLC: 3) stictic acid, ± constictic acid, ± menegazziaic acid, ± crypstostictic acid, ± norstictic acid (n=5, this chemotype is so far absent on Brazil).

Habitat and distribution. Mainly corticolous, rarely saxicolous. In Brazil, it has a wide ecological range in the Atlantic forest, from the sea level to about 1800 m elevation in mountainous areas, growing on barks or fences. Previous molecular results (chapter 3) showed that *Usnea cornuta* s. str. occurs in Brazil, Europe (including the Macaronesian area) and in the USA. It corresponds an amphi–Atlantic disjunct lichen species. This species is also presents in Argentina growing secondarily on rocks (Rodriguez *et al*. 2011). In Europe it displays an oceanic distribution (Clerc 1987, 2011; Caviró 2016). This species is recorded here for the first time in Brazil in the Minas Gerais, Rio Grande do Sul, Santa Catarina and São Paulo States.

Diagnostic characters. *Usnea cornuta* is characterized by the erect-shrubby thallus with terminal branches crowded with minute fusioning soralia (Fig. 2F), the *cornuta-type* CMA (Fig. 2E) with a dense medulla containing the depsidones stictic, salazinic or constictic acids as main secondary metabolites. For more details see Clerc (1987).

Variation. Branches segments can vary from slightly swollen to distinctly swollen, as it is the case for the constriction of lateral branches. Exposed thallus can develop numerous isidiomorphs as well as isidiofibrils. Soralia are often minute and confluent and often look like a single large soralium. This species is quite variable chemically with three chemotypes clustered together in our previous molecular analyses (chapter 3). It is interesting to note that among the Brazilian samples studied there are no specimens with stictic acid in this group. Our Multi-locus species analyses (MSC) with three markers did not allow us to resolve the delimitation inside the group of *U. cornuta s. str*. (PP= 0.29; chapter 3). Although the presence of cryptic species in this clade cannot be ruled out, we so far consider that the specimens collected for instance in Brazil, Europe and in the USA correspond to the same taxa.

Taxonomic notes. Among the species described here as new for science *Usnea arianae*, *U. catarinensis*, *U. furnensis* and *U. isidiofibrillosa* have a CMA of the *cornuta*-type. They are therefore very similar to *U. cornuta*. The morphology of mature soralia and the chemistry are the most important characters to separate them from *U. cornuta s.str*. *Usnea cornuta* can be misidentified with *U. dasaea* Stirt. or with *U. perhispidella* J. Steiner. *Usnea dasaea* differs mainly by the presence of densely arranged spinulose fibrils on the branches and the galbinic acid in the medulla (Clerc & Herrera-Campos 1997; Clerc 2004a). *Usnea perhispidella* differs from *U. cornuta* by the non-inflated branches, covered by isidiofibrils and the thinner and compact medulla (A/M ratio > 2) (Truong et al. 2013) (see under *U. isidiofibrillosa* for more details).

Additional specimens examined. **Argentina**: Tucumán, El Infernillho, Km 519, 1750 m elev., on rocks, 1989, *Grundlehner* 60.28.3 (G). —**Brazil**: *Minas Gerais*: Serra da Mantiqueira, Fazenda São Mateus, östlich von Camanducaia, 1800 m, 1980, *Kalb* (G); Gonçalves, Apa Fernão Dias, lignicola em mourão, 06/2016, *Gerlach & Penati* P54 (G). *Rio Grande do Sul*: Barra do Ribeiro, Mariana Pimentel, 30°21'S, 51°35"W, 1989, *Grundlehner* (G); Esmeralda, Estação Ecológica Aracuri, sobre *A. angustifolia*, 1984, *Fleig* 2440 (ICN); Rio Grande, Estação Ecológica Taim, em ramos, 1985, *Fleig* 26886 (ICN); São Francisco de Paula, Pro-Mata, 2014, *Grüninger & Hamp* 20140323 (ICN); Viamão, Parque Estadual de Itapuã, 2013, *Martins* 2790 (HAS). *Santa Catarina*: Joinville, Alto da Serra Dona Francisca, as margens da rodovia, em palanque próx. a torre, 2013, *Gumboski 4653c* (ICN); *ibid*, Castelo dos Bugres, campo de altitude, ca. 900 m, 2013, *Gerlach & Beilke* 1192 (ICN); São Francisco do Sul, Acaraí, restinga arbórea, 2013, *Gerlach et al.* 983b (ICN); Urubici, Parque Nacional de São Joaquim, arredores do alojamento, ca. 1300 m, 2014, *Gerlach & Alves* 1352 (ICN); *ibid*, Campos de Santa Bárbara, ca. 1600 m, 2013, *Magnago* (ICN). *Paraná*: Guaratuba, Morro dos Perdidos, 25°53'11"S, 48°57'33"W, ca. 1300 m, campo de altitude, 2013, *Gerlach et al.* 1012b (ICN); Paranaguá, Parque Estadual da Ilha do Mel, Brasilia, em cemitério, 2012, *Gerlach & Feuerstein* 789 (ICN); Piraí do Sul, Fazenda Nova Era, em cerca de madeira, 2012, *Canestraro et al. 487* (ICN). *São Paulo*: Parque Nacional do Itatiaia, 1970, *Xavier Filho* (JPB); Campos do Jordão, 150 km nordöstlich von São Paulo in einem hellen, feuchten Urwald, 1700 m, 1978, *Kalb & Plöbst* (G); Zwishen Guapira und Apiaí, in einem kleinen *Pinus* Forest. An *Pinus* spec., 800 m, 1980, *Kalb* (G).

Usnea furnensis A. Gerlach & P. Clerc, *sp. nov*. ad int.

MycoBank No.: MB xxxxxxxx

Differs from U. cornuta by the faint reddish tinge due to an orange subcortical pigment, the irregular main branches with lateral branches constricted at ramification points, by its wide medulla [34.5–36.5%–38.5] reacting K−, P+ red (protocetraric acids) and by its position in the molecular phylogeny, in 7 parsimony-informative alignment positions of the internal transcribed spacer (ITS) (Supplementary figure).

Type: Brazil, Santa Catarina, Reserva Particular do Patrimônio Natural Rio das Furnas, 27°40'28.3"S, 49°10'37.9"W, ca. 900 m, corticolous, 14.05.2016, *Gerlach, G. Giovanka & R. Rizzaro* P46 (ICN—holotype; G—isotype). %C/M/A: 6/35/18 (holotype). Chemistry: usnic and protocetraric (holotype and isotype). DNA code: corn4-187BR.

(Fig. 3 A–C)

Desciption. *Thallus* erect-shrubby to subpendulous, up to 10 cm long, slighlty reddish especially close to the basal part; *ramifications* often anisotomic to isotomic-dichotomous; *basal part* somewhat fusiform, with a reddish tinge, sometimes black only in the first few mm above the holdfast, up to 0.5 cm long, sometimes with annulations; *branches* up to 2 mm

diameter (n=6), irregular, often ±terete to slightly flattened in transversal section; annulated; *branches segments* slightly inflated; *lateral branches* constricted at ramification points, sometimes first broadened and then constricted; *annular cracks* few to numerous, often exposing the medulla; *foveolae and depressions* usually present; *maculae and pseudocyphellae* absent; *papillae and tubercles* sparse, cylindrical to verrucose; *tubercles* present, not eroded at apices; *fibrils* few to numerous, slender; *fibercles* often present; *soralia* punctiform, slightly stipitate, rounded, usually with clusters of isidiomorphs, arising from tubercles or fibercles*; isidiomorphs* usually numerous especially on fibrils; *isidiofibrils* often present, mainly on the fibrils, sometimes also on the main branches, arising in minute soralia; *apothecia* rare, terminal to subterminal, up to 4 mm diameter; *ascospores* 7.5–8.5 × 5–6.5 µm (n=9); *pycnidia* not seen; *cortex* thin to ± thin [(4.5–)5–6%–7] (n=6), shiny and with an irregularly distributed and faint reddish tinge due to a thin subcortical orange pigment, mainly near the basal part and located only in the cortex surface; *medulla* thick [34.5–36.5%–38.5(–39)], lax, sometimes heterogeneous; *axis* thin [11–14%–17(–18)]; A/M < 0.5.

Chemistry. Medulla: K−, P+ red. TLC: protocetraric acid, ± fumarprotocetraric acid, ± an unknown spot gray Rf classes A/B/C: 4/4–5/5–6 (US24), ± an unknown spot gray Rf classes A/B/C: 2/5–6/? (US25) (n=14).

Etymology. The specific epithet refers to the *Private Reserve of Natural Heritage* (RPPN) *Rio das Furnas* with exuberant Atlantic Forest in the Santa Catarina State.

Habitat and distribution. Corticolous, mainly on twigs and bark of *Araucaria angustifolia*. It seems to be widespread in the Reserva Particular do Patrimônio Natural Rio das Furnas above 900 meters elevation. So far found only in Brazil in the Santa Catarina State.

Diagnostic characters. *Usnea furnensis* is characterized by the CMA of the *cornuta*-type with a slightly thinner axis central [11–14%–17] (Fig. 3B), the fusiform basal part often with reddish tinges (orange subcortical pigment) (Fig. 3A), the punctiform and ± stipitate soralia occurring mainly on fibrils (Fig. 3C) and by the medulla reacting K−, P+ red (protocetraric acid and often fumarprotocetraric acid).

Variation. Not a very variable species. This is probably, due to the few specimens collected and the so far restricted distribution area. The most remarkable variation in this species is the shape of the branches that can vary from ± terete to slightly flattened in cross-section.

Taxonomic notes. At the first glance *U. furnensis* makes us think to *U. moreliana* Motyka due the presence of the faint reddish tinge on the cortex, the constricted branches and the medulla reacting K− (Truong & Clerc 2016). However, in *U. furnensis* this is only the cortex surface that is mottled reddish, whereas in *U. moreliana* the reddish pigment is located also inside cortex (Truong et al. 2011). Moreover it has a thicker central axis [18–28%, Truong *et al.* 2011 as *U. rubricornuta*) and a medulla producing an unidentified triterpenes (K−, P−) (Truong *et al.* 2011; Chapter 4) which clearly separate both species. *Usnea* furnensis builds a strongly supported lineage (chapter 3, referred as *cornuta*-4) not related neither to *U. cornuta* nor to *U. moreliana*.

Selected specimens examined. **Brazil**: *Santa Catarina*: Alfredo Wagner, RPPN Rio das Furnas, Bosque das Araucarias, sobre *A. angustifolia*, 2016, *Gerlach et al.* P37 (G); *ibid*, near the house, 27°40'28.3"S, 49°10'37.9"W, em galhos caídos, 2016, *Gerlach et al.* P1 (ICN).

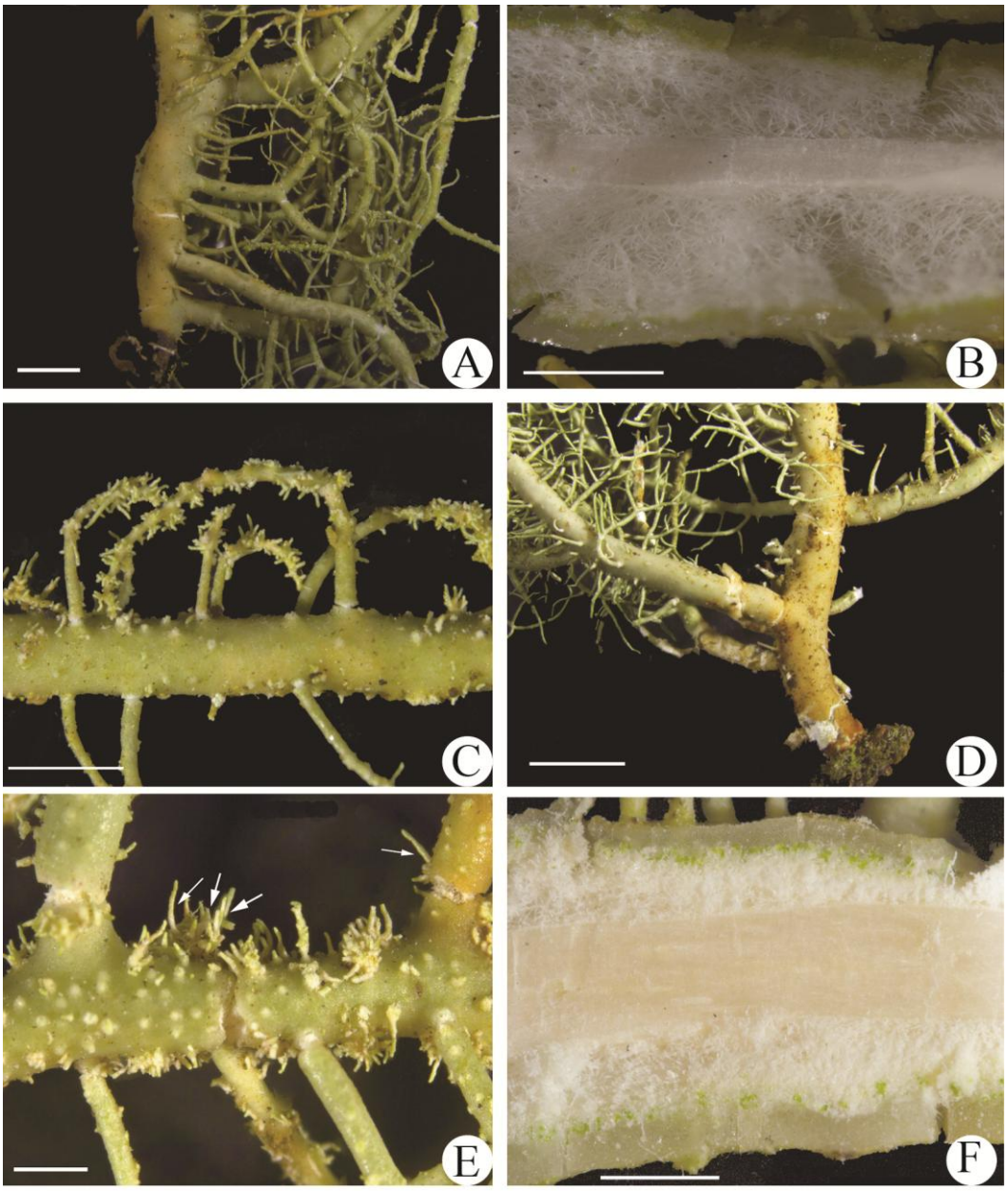

FIG. 3 A–C, ***Usnea furnensis*** (holotype): A, main branches fusiform with a faint mottled reddish tinge and anisotomic-dichotomous ramifications; B, CMA of the *cornuta*-type with thinner axis; C, punctiform and ± stipitate soralia. **D–F, *Usnea isidiofibrillosa*:** D, irregular main branches with faint mottled reddish tinges (isotype); E, punctiform soralia with isidiofibrils (white arrows) and isidiomorphs (holotype); F, CMA of the *cornuta*-type (*A. Gerlach* P11, DNA code: corn3-115). Scales: A=2 mm; B=500 µm; C=1 mm; D=5 mm; E & F=500 µm. In colour online.

Usnea isidiofibrillosa A. Gerlach & P. Clerc, *sp. nov*. ad int.

MycoBank No.: MB

Differs from U. cornuta by the often subpendulous thallus, the punctiform soralia that are not confluent, by the presence of isidiofibrils and isidiomorphs on whole thallus and by the K+ yellow medulla (stictic acid).

Type: Brazil, Minas Gerais, Gonçalves, Pedra chanfrada, tronco, area sombreada, Montana semidecidua, 22°43'31.09"S, 45°51'20.01"O, Junho 2016, *Gerlach & Penati* P59 (ICN—holotype; G—isotype). %C/M/A: 7.5/29/27. Chemistry: usnic, stictic, menegazziaic, norstictic and salazinic acids (holotype). DNA code: corn3-29BR.

(Fig. 3D–F)

Description. *Thallus* subpendulous to rarely shrubby, up to 14 cm long; *ramifications* isotomic-dichotomous; *basal part* up to 7 mm long, often pale reddish in the first mm, becoming concolorous with main branches, sometimes with thin annular cracks; *branches* up to 1.6 mm diameter (n=12), slightly irregular to tapering, irregularly segmented; *branch segments* ± terete in cross-section; *lateral branches* not to slightly constricted at ramification (main branches) to distinctly constricted; *annular cracks* sparse; *foveolae, depressions, maculae and pseudocyphellae* absent; *papillae and tubercles* rare; *fibrils* irregularly distributed, slender; *fibercles* sparse; *soralia* punctiform to seldom enlarging at the apices of the branches, but not more than half of the branch diameter, stipitate and circular, sparsely distributed on the whole thallus, arising from fibercles or tubercles; *isidiomorphs* usually numerous but rarely covering the apices of the branches, often growing in fasciculate clusters; *isidiofibrils* present on whole thallus, often numerous, growing in minute soralia, fragile and easily breakable; *apothecia* sometimes present; *ascospores* 7.5–10 × 5–6.5 μm (n=10); *pycnidia* not seen; *cortex* thin [(3.5–)7–6.5%–8(–8.5)] (n=12), shiny; *medulla* thick [(27–)29–31.5%–34(–35.5)], dense to lax; *axis* thin [(19.5–)21.5–24.5%–27.5(–29.5)]; A/M < 1.3.

Chemistry. Medulla: K+ yellow. TLC: stictic, ± constictic, ± crypstostictic, ± menegazziaic, ± norstictic and ± salazinic acids, ± possible an unidentified terpenes Rf Class A/B/C: 5/2-3/? (n=25).

Etymology. The epithet *isidiofibrillosa* refers to the constant presence of a special type of isidiomorphs growing and developing a central axis and named isidiofibrils (Truong *et al.* 2011).

Habitat and distribution. Corticolous. Brazil, Costa Rica and France. *Usnea isidiofibrillosa* is widespread in Brazil where it was found in the Minas Gerais, Rio Grande do Sul and Santa Catarina states.

Diagnostic characters. *Usnea isidiofibrillosa* can be recognized by the subpendulous thallus, the CMA of the *cornuta*-type (Fig. 3F), the branches covered with numerous isidiofibrils growing from minute soralia (Fig. 3E) and by the medulla reacting K+ yellow (stictic acid).

Variation. The density of isidiofibrils, isidiomorphs and fibrils shows some variation in this species.

Taxonomic notes. *Usnea perhispidella* is a similar species but it differs from *U. isidiofibrillosa* mainly by the thinner medulla and thicker central axis [C/M/A%: 7–10/13–20/44.5–56 with a ratio A/M > 2, Truong *et al.* 2013) and by the branches that are not constricted at the attachment point (Truong *et al.* 2013; G!—isotype). Molecular analysis showed clearly that these two similar species are unrelated phylogenetically. *Usnea isidiofibrillosa* builds a strongly supported lineage including specimens mainly from Brazil, one specimen from Costa Rica and one specimen from France (chapter 3, referred as *cornuta*-3).

Usnea jelskii Motyka described from Chile, belongs to the *U. cornuta* aggr., and also has a subpendulous thallus (S—isotype, Jstor Global plants). It differs from *U. isidiofibrillosa* mainly by the chemistry (salazinic and norstictic acids; LBL!—holotype) and by the absence of isidiofibrils (W!—holotype).

Selected specimens examined. **Brazil**: *Rio Grande do Sul*: São Francisco de Paula, Flona de São Francisco de Paula, 2014, *Gerlach et al.* 1523 (ICN); *ibid*, Paulinas de São Francisco, ca. 900 m, 29°27'S, 50°34"W, 1989, *Grundlehner* (G). *Santa Catarina*: Alfredo Wagner, RPPN Rio das Furnas, ca. 900 m, 2016, *Gerlach et al.* P47 (ICN). —**Costa Rica**: *Province of San Jose*, Pérez Zeledon Co. Cordillera de Talamanca, San Gerardo de Rivas, 9°28'13.5"N, 83°35'7.0"W, 1486 m, sur *Alnus acuminata*, 09/2014, *P. Clerc* 2015/638 (G). —**France**: *Corse du Sud*, Alta Rocca, Bavella, San-Gavino-di-Carbini Pont de Fiumicelelli, 41°50'18"N, 9°18'31.4"E, 170 m., branches de *Phillyrea angustifolia*, July 2016, *P. Clerc* (G).

Usnea pseudobrasiliensis A. Gerlach & P. Clerc, *sp. nov*. ad int.

MycoBank No.: MB xxxxxxxx

Differs from Usnea brasiliensis by the CMA of the cornuta-type, by the often confluent and large soralia on terminal branches and in 18 parsimony-informative alignment positions of the internal transcribed spacer (ITS) and one indel (Supplementary figure).

Type: Brazil, Minas Gerais, Gonçalves, near Chalé Canário, 22°43'57"S, 45°52'47"W, corticolous on bark, 07.VI.2016, *Gerlach & Penati* P1-3 (ICN—holotype, G—isotype). %C/M/A: 8.5/32/18. Chemistry: usnic, protocetraric, psoromic, conpsoromic acids and an unknown substance grey with Rf Classes A/B/C: 4/4-5/?. DNA code: bras2-15BR.

(Fig. 5A–C)

Description. *Thallus* shrubby, up to 5 cm long; *ramifications* anisotomic-dichotomous; *basal part* concolorous to the main branches, annulated; *branches* up to 2.2 mm diameter (n=7), irregular in longitudinal section, terete to more or less flattened in transversal section, segmented; *lateral branches* slightly constricted and annulated at attachment point; *foveolae and depressions* rare; *maculae and pseudocyphellae* absent; *papillae and tubercles* few; *fibrils*

slender; *soralia* large, ± concave, circular to irregular, without distinct cortical margin, confluent towards apices of branches, few to crowded, arising from the top of tubercles or fibercles on main branches and ad intio on the cortex and almost even on terminal branches; *isidiomorphs* few; *isidiofibrils* absent; *apothecia and pycnidia* not seen; *cortex* moderately thin [(5–)6–7%–8(–9)] (n=7), shiny; *medulla* thick [(29–)32–34%–36(–37)], dense; *axis* thin [(10–)16–18%–20], with an A/M ratio < 0.7.

Chemistry. Medulla: K−, P+ red. TLC: 1) protocetraric and psoromic acids (n=7).

Etymology. Refers to the chemical similarity with *U. brasiliensis*.

Habitat and distribution. Corticolous or lignicolous. So far only known from Brazil: Minas Gerais, Paraná, Santa Catarina, and São Paulo states.

Diagnostic characters. *Usnea pseudobrasiliensis* can be recognized by its irregular main branches and its lateral branches that are constricted at their attachment points (Fig. 5A), the confluent and large soralia towards the apices of the branches (Fig. 5B), the CMA of the *cornuta*-type with slightly thinner axis [(10–)16–18%–20] (Fig. 5C) and by the presence of protocetraric and psoromic acids in the medulla.

Variation. The degree of constriction of the lateral branches as well the intensity of deformations of the main branches can vary on this species. The density of annular cracks is also variable. Soralia, when mature, are fusing together in irregular patches, that might cover almost completely the branch apices.

Taxonomic notes. *Usnea brasiliensis* shares the production of protocetraric acid in the medulla but differs from *U. pseudobrasiliensis* mainly by the CMA of the *brasiliensis*-type and the large soralia. *Usnea pseudobrasiliensis* builds a strongl supported clade unrelated to *U. brasiliensis* (chapter 3, referred as brasiliensis-2).

Selected specimens analyzed. **Brazil**: *Paraná*: Prudentópolis, rural area, 2012, *A. Charnei* 550 (ICN). *Santa Catarina*: Alfredo Wagner, RPPN Rio das Furnas, trilha em torno da sede, 27°40'28.3"S, 49°10'37.9"W, Maio 2016, *Gerlach* P54 (G); Campo Alegre, on fences, *Gerlach & Beilke*1127 (ICN). *São Paulo*: Serra do Mar, Paranapiacaba, 60 Km sudwestlich von São Paulo, oberhalb von Juquitiba, in einem hellen, feuchten Urwald am Rio Juquiá, ca. 800 m, 1978, *Kalb & G. Plöbst* (G).

Usnea stipitata A. Gerlach & P. Clerc, *sp. nov*. ad int.

MycoBank No.: MB xxxxxxxx

Differs from U. cornuta by its stipitate soralia, a CMA of the brasiliensis-type and the presence of constictic acid as a main medullary secondary metabolite. *Differs from all other taxa of the U. cornuta aggr. in seven parsimony informative alignment positions of the internal transcribed spacer* (ITS) (Supplementary figure).

Type: Brazil, Santa Catarina State, Alfredo Wagner, Reserva Particular do Patrimônio Natural Rio das Furnas, ca. 27°40'28.3"S, 49°10'37.9"W, ca. 900 m, corticolous on *Araucaria angustifolia*, 14.05.2016, *Gerlach et al.* P39 (ICN—holotype). %C/M/A: 5.5/37/14. Chemistry: usnic and constictic acids. DNA code: corn1-23BR.

(Fig. 5D–F)

Description. *Thallus* shrubby to subpendulous, up to 13 cm long; *ramifications* usually anisotomic to isotomic-dichotomous; *basal part* often short, up to 2 mm long, fusiform, concolorous to main branches and often with a faint reddish tinge near the holdfast, rarely partially black, sometimes with annular cracks; *branches* up to 2.8 mm diameter (n=28), irregular in longitudinal section to slightly terete in transversal section, segmented with annular cracks; main *branches* segments slightly swollen; *lateral branches* constricted at attachment point (not in main branches), sometimes at the beginning slightly broadened and then constricted; *annular cracks* rarely with medullary extrusions; *depressions* often present on main branches; *maculae and pseudocyphellae* absent; *papillae* verrucose, sometimes densely covering the branches; *tubercles* absent; *fibrils* irregularly distributed, slender; *fibercles* numerous, giving a rough consistence to the thallus; *Soralia* punctiform, stipitate, circular, without cortical margin, remaining individual, denser on thin and terminal branches; developing at the top of fibercles; *isidiomorphs* few and present mainly in mature soralia; *isidiofibrils* rare; *apothecia and pycnidia* not seen; *cortex* thin to moderately thin [(3.5–)4.5–5.5%–6.5(–8.5)] (n=28), shiny; *medulla* thick [(32–)33.5–37.5%–41.5(–52)], lax, often heterogeneous; *axis* thin [(6.5–)9.5–14.5%–19.5(–26)]; A/M = 0.2–0.4–0.8.

Chemistry. Medulla K+ yellow or K+ yellow→red. TLC: constictic acid, ± salazinic acid, ± unidentified fatty acid, ± unidentified terpenoid, ± protocetraric acid (trace), ± unknown spot brownish Rf classes A/B/C: 1–2/2/1–2, ± unknown spot yellow Rf class A/B/C: 2/1–2/? (n=34).

Etymology. The epithet refers to the shape of the soralia which arise from cylindrical *papillae*-like tubercles or fibercles and are thus stipitate in shape.

Habitat and distribution. Corticolous on bark of *A. angustifolia* or exotical *Pinus* spp., and lignicolous on fences. Occurs so far only in Brazil (Southern and Southeast parts. It seems to be relatively frequent in the following states: Minas Gerais, Paraná, Rio Grande do Sul, Santa Catarina and São Paulo.

Diagnostic characters. The distinguishing features of *Usnea stipitata* are the CMA of the *brasiliensis*-type with slightly thicker central axis [9.5–14.5%–19.5] (Fig. 5E), the soralia that are distinctly stipitate with isidiomorphs (Fig. 5F), the segments of branches that are slightly swollen (Fig. 5D) and the presence of constictic acid as the major substance in the medulla.

Variation. This species has a quite constant morphology and chemistry.

Taxonomic notes. *Usnea stipitata* has the same type of CMA than *U. brasiliensis*. The main difference is the chemistry, with constictic acid in *U. stipitata* instead of protocetraric acid in

U. brasiliensis. The shape of soralia is also different: in *U. stipitata* soralia are often distinctly stipitate (developing at the top of fibercles) and not confluent whereas in *U. brasiliensis* they are only slightly stipitate and confluent. The CMA on *U. brasiliensis* is typically of the *brasiliensis-type* [3.5–4.5–5.5 / 38.5–40–43 / 7.5–9.5–11.5] whereas in *U. stipitata* the cortex and the central axis can be slightly thicker [4.5–5.5–6.5(–8.5) / 33.5–37.5–41.5 / 9.5–14.5%–19.5(–26)]. *Usnea cornuta* can also have constictic acid as secondary metabolite; it differs however from *U. stipitata* by the typical minute and fusioning soralia, and by the CMA of the *cornuta-type*. Molecular analysis shows that *U. stipitata* (chapter 3, referred as *cornuta*-1) corresponds to a strongly supported lineage unrelated to the *U. brasiliensis* and *U. cornuta* lineages (Chapter 3).

Selected specimens analyzed. **Brazil**: *Minas Gerais*: Gonçalves, caminho para a pousada Pedra da Lua, 1580 m, 2016, *Gerlach & Penati* P21 (ICN). *Paraná*: São José dos Pinhais, 2014, *Imig* s. n. (ICN). *Rio Grande do Sul*: Cambará do Sul, Parque Nacional dos Aparados da Serra, *Cânion* Itaimbezinho, mata de Araucária, 2014, *Gerlach & Akkerman 1418* (ICN); São Francisco de Paula, Floresta Nacional de São Francisco de Paula, 2014, *Alves et al.* s. n. (ICN). *Santa Catarina*: Alfredo Wagner, RPPN Rio das Furnas, 2016, *Gerlach* P12 (G); Campo Alegre, estrada de chão para a Serra do Quiriri, sobre *Pinus* sp., 2013, *Gerlach & Beilke*1166 (ICN, G); Joinville, Estrada das Laranjeiras, on fences, *Gerlach & Beilke*1104 (ICN); Rio Negrinho, Rio dos Bugres, rural area, on *A. angustifolia*, 2012, *Gumboski 4091* (ICN). *São Paulo*: Serra do Mar, Serra de Paranapiacaba, 60 km südwestlich von São Paulo, oberhalb von Juquitiba, in einem hellen, feuchten Urwald am Rio Juquiá, ca. 800 m, 1978, *Kalb & Plöbst* (G).

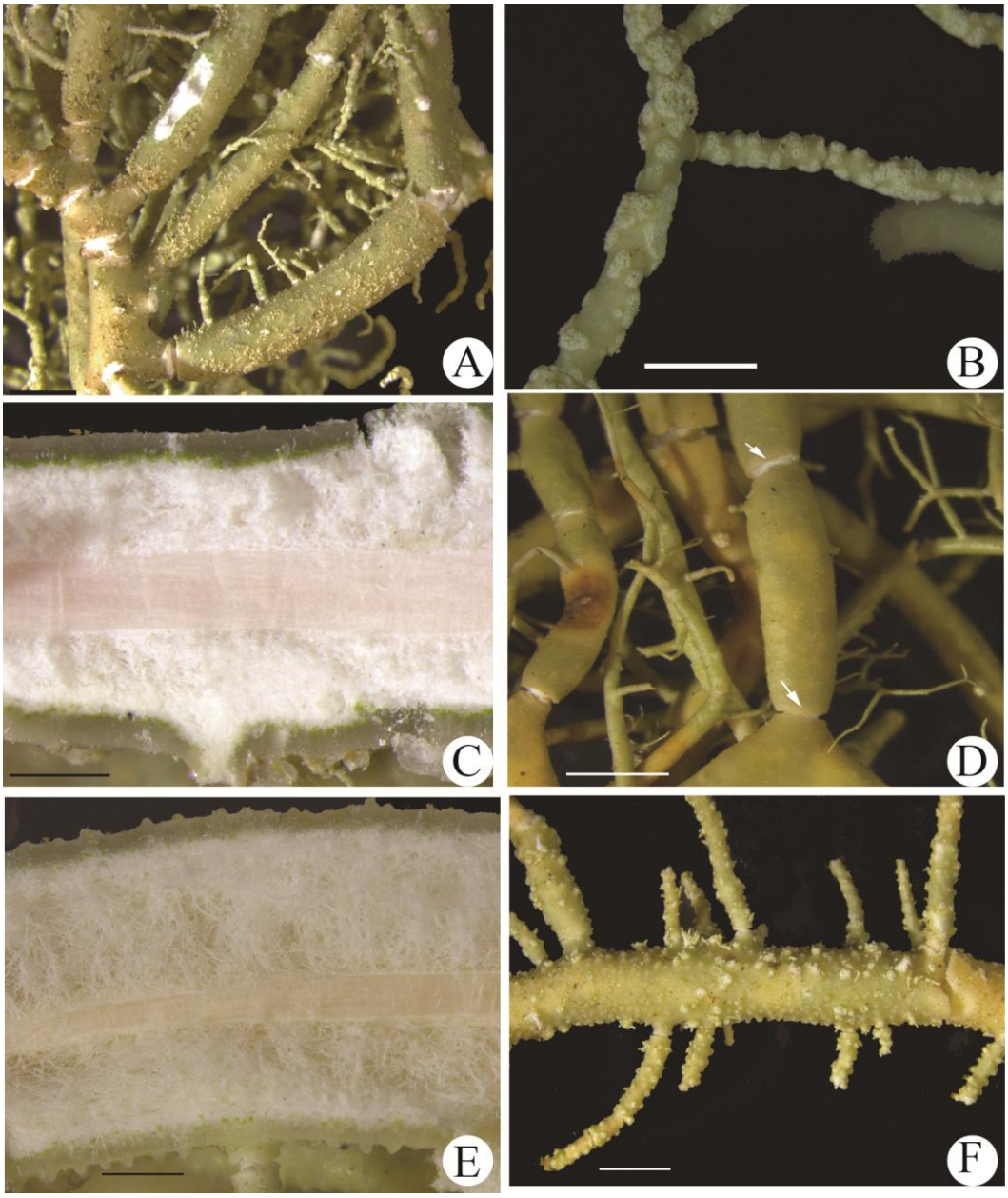

FIG. 5 **A–C,** ***Usnea pseudobrasiliensis*** (holotype): **A**, irregular main branches with constricted lateral branches at ramification point; **B**, large and confluent soralia on terminal branches; **C**, CMA of the *cornuta*-type with thin axis. **D–F,** ***Usnea stipitata*** (holotype): **D**, annular cracks (arrows) and swollen segments; **E**, CMA of the *brasiliensis*-type with thicker axis; **F**, punctiform and stipitate soralia with isidiomorphs. Scales: A=2 mm; B=1 mm; C=500 μm; D=2 mm; E=500 μm; F=1 mm. In colour online.

Key to corticolous mainly shrubby, sorediate *Usnea* species of the U. *cornuta* aggregate in 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.

1a. CMA of the *brasiliensis*-type on average; medulla K─, P+ (protocetraric acid) or K+ yellow (constictic acid) …….....……2

1b. CMA of the *cornuta*-type on average; medulla K─, P+ (protocetraric acid) or otherwise…….3

2a. CMA of the *brasiliensis* type; soralia slightly stipitate (developing at the top of irregular "bumps") and confluent; medulla K−, P+ red (protocetraric, ± psoromic acids) …….....**U. brasiliensis**

2b. CMA of the *brasiliensis* type but with thicker axis [9.5–14.5%–19.5(–26)]; soralia distinctly stipitate (developing at the top of fibercles), and not confluent; medulla K+ yellow (constictic acid)……**U. stipitata**

3a. Isidiofibrils and isidiomorphs present on whole thallus; thallus usually subpendulous, medulla K+ yellow (stictic acid) ……**U. isidiofibrillosa**

3b. Isidiofibrils absent to rare; thallus mainly shrubby; medulla K+ or K─……...4

4a. Soralia large and confluent on the apices of branches; medulla K─ P+ red (protocetraric and psoromic acids) ……**U. pseudobrasiliensis**

4b. Soralia punctiform, not enlarging (they can however fuse together, giving the false appearance of one single large soralia), confluent or not; medulla lax to dense, K+ or K- …….....……5

5a. Basal part and lower parts of main branches orangish-reddish due to the presence of faint subcortical-cortical (lowest part of the cortex) orange pigment, medulla K−, P+ orange red (protocetraric acid)…….....**U. furnensis**

5b. Basal part and lower parts of main branches without orange pigment, medulla K-, P− or K+ (protocetraric acid absent)……..…….....6

6a. Soralia even with the cortex, minute, confluent and fusing together (looking like a single large soralium), crowded on terminal branches; medulla K+ (salazinic acid or constictic or

stictic acid*).....**U. *cornuta* s. str.** [*The presence of the chemotype stictic acid in Brazil is uncertain].

6b. Soralia slightly to distinctly stipitate, if confluent not fusing together; medulla K-, P− or K+……7

7a. Medulla lax, K−, P− (lobaric acid or fatty acids or only usnic acid) or K+ yellow turning reddish orange (norstictic acid)……...**U. arianae**

7b. Medulla lax to dense, K+ bright yellow (thamnolic acid)……**U. catarinensis**

APPENDIX

Usnea kriegeriana A. Gerlach & P. Clerc, *sp. nov.* ad. int.

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Thallus shrubby, basal part with annular cracks, lateral branches not constricted at ramification point, main branches covered with eroded tubercles and convex soralia, cortex mat, thin to ± thick (6.5–)7.5–9%–10.5(–11.5) and medulla with fumarprotocetraric acids as main medullar secondary metabolite.

Type: Brazil, Santa Catarina State, Alfredo Wagner, Reserva Particular do Patrimônio Natural Rio das Furnas, ca. 27°40'28.3"S, 49°10'37.9"W, ca. 900 m, on *twigs*, 13.05.2016, *Gerlach et al.* P19 (ICN—holotype). %C/M/A: 8/22.5/39. Chemistry: usnic, fumarprotocetraric acid, ± protocetraric acid ± unknown orange Rf class A/B/C: 1-2/2-3/1-2 (US2). DNA code: sp5-56BR.

(Fig. 4A–D)

Description. *Thallus* shrubby, up to 10 cm long; *ramifications* mainly isotomic-dichotomous; *basal part* up to 0.5 cm long, concolorous to the main branches, sometimes paler or with a faint orange tinge, regularly with annular cracks;, main *branches* up to 1.8 mm diameter (n=16), cylindrical to slightly irregular; *branches segments* cylindrical, sometimes slightly swollen; *lateral branches* not (main branches) to sometimes slightly constricted (secondary branches) at ramification point; *annular cracks* present, thin, often with medullar extrusions, sparse to frequent on the whole thallus, 1–2 annular cracks/0.5 cm; *foveolae, depressions, maculae and pseudocyphellae* absent; *papillae* absent or sparse; *tubercles* present, often numerous, verrucous, eroded at top; *fibrils* slender, unevenly distributed; *fibercles* sparse; *soralia* punctiform to large, convex, often circular or building elliptical to irregular masses of soralia, usually numerous on main branches, well delimited to confluent towards the apices of the branches, arising from the top of tubercles, with granular soredia; *isidiomorphs* usually numerous; *isidiofibrils* rare; *apothecia and pycnidia* not seen; *cortex* moderately thin to ± thick [(6.5–)7.5–9%–10.5(–11.5)], mat (n=16); *medulla* ± thin to thick [19–25.5%–32(–48)], dense; *axis* sometimes with an orange tinge, thin to ± thin [(19–)24–31.5%–38.5(–40)]; A/M = 0.6–1–2.

Chemistry. Medulla K+ yellow→red. TLC: fumarprotocetraric acid, ± protocetraric acid, ± an unknown orange Rf classes A/B/C: 1–2/2–3/1–2 (US2) (n=20).

Etymology. This species is named in honour of the Brazilian Priest Leopoldo Krieger, retired botany teacher of the Federal University of Juiz de Fora (Brazil), who collected many *Usnea* specimens chiefly in the south of Minas Gerais and also in the Paraná State. The oldest specimen of this new species was collected by him in 1975.

Habitat and distribution. Corticolous on bark of *Araucaria angustifolia*, on exotical *Pinus* spp, or lignicolous on fences. It seems to be relatively frequent in the mountainous areas above 900 meters in *Araucaria* Forest. So far known only in Brazil (Southern and Southeast Region): Espírito Santo, Minas Gerais, Paraná, Rio Grande do Sul, Santa Catarina and São Paulo states.

Diagnostic characters. *Usnea kriegeriana* is characterized by the annulate and cracked basal part (Fig. 4A), the lateral branches that are not constricted at attachment point (Fig. 4B), the numerous tubercles eroded at the top (Fig. 4B), the large soralia (Fig. 4D), the mat cortex, the thin to thick medulla [22.5–25%–35] (Fig. 4C) and the presence of fumarprotocetraric acid as main secondary substance in the medulla.

Variation. The density of the annular cracks with medullar extrusion, as well as the isidiomorphs is variable in this species. The lateral branches are most of the time not constricted, only rarely slightly constricted at attachment point. The shape of the segments varies from slightly swollen to cylindrical. Soralia are convex and often well delimited on main branches. Well-developed specimens have confluent soralia that becom larger up to the half of the branches diameter towards the apices. *Usnea kriegeriana* produces fumarprotocetraric often accompanied by protocetraric acid and an unknown substance (refereed as US2; only one specimen without protocetraric and another one without US2 were found) in the medulla.

Taxonomic notes. *Usnea kriegeriana* is the only species described here that does not belong to the *Usnea cornuta* aggr. It is morphologically similar with *U. flammea* Stirt. which however differs mainly by the shape of the soralia, that are even with the cortes surface, and by the absence of tubercles (Clerc 2006). *Usnea subflammea* P. Clerc is similar by the presence of numerous tubercles, but it differs by the thicker cortex [12–16%; instead 7.5–9%–10.5(–11.5) in *U. kriegeriana*] and the soralia that do not enlarge (Clerc 2006). Moreover, *U. flammea* and *U. subflammea* have stictic acid in the medulla whereas *U. kriegeriana* has fumarprotocetraric acid. Moreover, *Usnea kriegeriana* builds a strongly supported clade (Chapter 3, referred as *Usnea* sp. 5), unrelated to *Usnea flammea* and *U. subflammea*.

Selected specimens examined. **Brazil**: *Espírito Santo*: Domingos Martins, in Weide am Morro do Cruzeiro, 1200 m, 20°26'S, 41°00'W, 11.10.1988, *Schäfer-Verwimp & Verwimp* (G). *Minas Gerais*: Serra de Ibitipoca, 19.05.1975, *Krieger* 13474 (JPB). *Paraná*: Curitiba, en allant vers Vila Velha, 25°21'S, 49°34'W, 04.03.1989, *Grundlehner* (G). *Rio Grande do Sul*: Cambará do Sul, Parque Nacional dos Aparados da Serra, Cânion do Itaimbezinho,

30.04.1989, *Fleig* 3577 (ICN); *ibid*, mata nebular próximo ao centro de visitantes, 14.03.2014, *Gerlach & Akkerman* 1403 (ICN). *Santa Catarina*: Alfredo Wagner, RPPN Rio das Furnas, Bosque das Araucarias, sobre *A. angustifolia*, ca. 900 m, 2016, *Gerlach et al.* P37 (G); Joinville, estrada das Laranjeiras, sobre mourão, 05.10.2013, *Gerlach & Beilke*1133a (ICN); Urubici, Parque Nacional de São Joaquim, arredores do alojamento, ca. 1300 m, 03.02.2014, *Gerlach & Alves* 1327 (ICN); São Bento do Sul, APA Rio Vermelho, on *A. angustifolia*, 12.03.2013, *Gumboski 4269* (ICN). *São Paulo*, Zwischen Guapira und Apiaí, in einem Kleinen *Pinus*-Forest, an *Pinus* spec., 800 m, 23.08.1980, *Kalb* (G); Serra da Mantiqueira, Campos do Jordão, etwa 150 km nordöstlich von São Paulo, an freistehenden *Pinus* spec. 1700 m, 26.05.1978, *Kalb & Plöbst* (G).

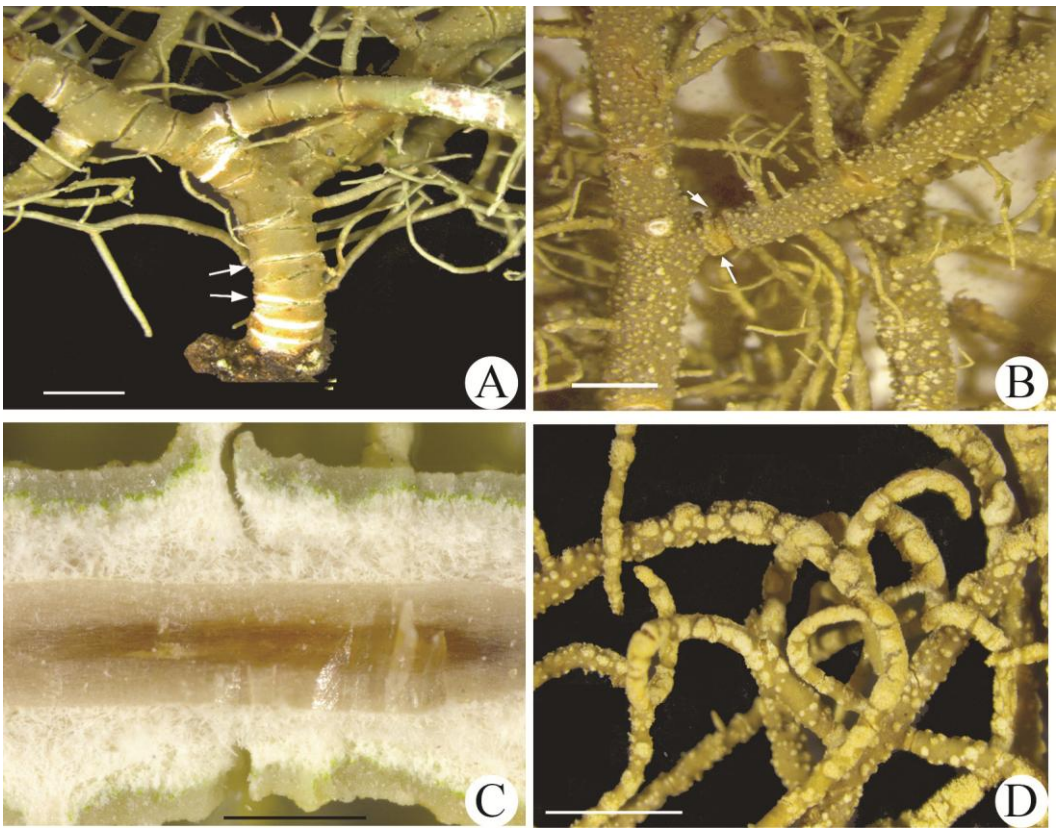

FIG. 4 A–D, *Usnea kriegeriana*: A, basal part with annular cracks (white arrows) (holotype); B, lateral branches slightly constricted at ramification point, with annular cracks (arrows) (*Krieger* 13474); C, section through thallus with matt cortex (holotype); D, large and confluent soralia (*Krieger* 13474). Scales: A & B=2 mm; C=500 μm; D=2 mm. In colour online.

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The first author acknowledges the financial support received from CNPq (Brazil) and Swiss Government Excellence Scholarship that supported her in her yearlong stay in Switzerland. The first author would also like to thank all her colleagues who helped during fieldwork specially Rodrigo Penati, Renato Rizzaro and Gabriela Giovanka as well as those from the *Conservatoire et Jardin Botaniques de la Ville de Genève*. We also acknowledge the Directors of the collection sites for granting collecting permits and the curators of the cited herbaria who kindly sent specimens on loan.

References

Brodo, I.M., Sharnoff, D., & Sharnoff, S. (2001) Lichens of North America. Yale University Press, New Haven & London. 795 pp.

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

Clerc, P. & Herrera-Campos, M. A. (1997) Saxicolous species of *Usnea* subgenus *Usnea* (lichenized Ascomycetes) in North America. *Bryologist* **100:** 281–301Clerc (1998

Clerc, P. (1998) Species concepts in the genus *Usnea* (lichenized Ascomycetes). *Lichenologist* **30:** 321–340.

Clerc, P. (2004) Notes on the genus *Usnea* Adanson. II. *Bibliotheca Lichenologica* **88:** 79–90.

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

Culberson, C. F. & Ammann, K. (1979) Standardmethode zur Dünnschichtchromatographie von Flechtensubstanzen. *Herzogia* **5:** 1–24.

Culberson, C. F. & Johnson, A. (1982) Substitution of methyl tert-butyl ether for diethyl ether in the standardized thin-layer chromatographic method for lichen products. *Journal of Chromatography* **238:** 483–487

Gerlach, A., Clerc, P. & Borges da Silveira, R.M. (2017) Taxonomy of the corticolous, shrubby, esorediated, Neotropical species of *Usnea* Adans. (Parmeliaceae) with an emphasis on Southern Brazil. *The lichenologist* **49(3):** 199–238.

Hale, M. E. Jr. (1979) How to Know the Lichens, 2nd edition. Dubuque, Iowa: William C. Brown

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

Herrera-Campos, M. A., Clerc, P. & Nash, T. H. III, (1998) Pendulous species of *Usnea* from the temperate forests in Mexico. *Bryologist* **101:** 303–329.

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

Motyka, J. (1938) Lichenum Generis *Usnea* Studium Monographicum. Pars Systematica II. Leopoldi: privately printed.

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

Pérez-Vargas, I, Hernández-Padrón, C, Arroyo, R. & Seriña, E. (2010) *Usnea brasiliensis* (Zahlbr.) Motyka (Parmeliaceae), a new amphi-Atlantic disjunct lichen species. *Bryologist* **113(2):** 308-312.

Rizzini, C.T. (1952) Species Organenses generis lichenum *Usneae.* (Omnes acidum usnicum praebentes). *Rev. Brasileira de Biol.* **12(4):** 337-348.

Rizzini, C.T. (1956) Flora Organensis. Lichenes. *Rev. Brasileira de Biol.* **16(4):** 387-402.

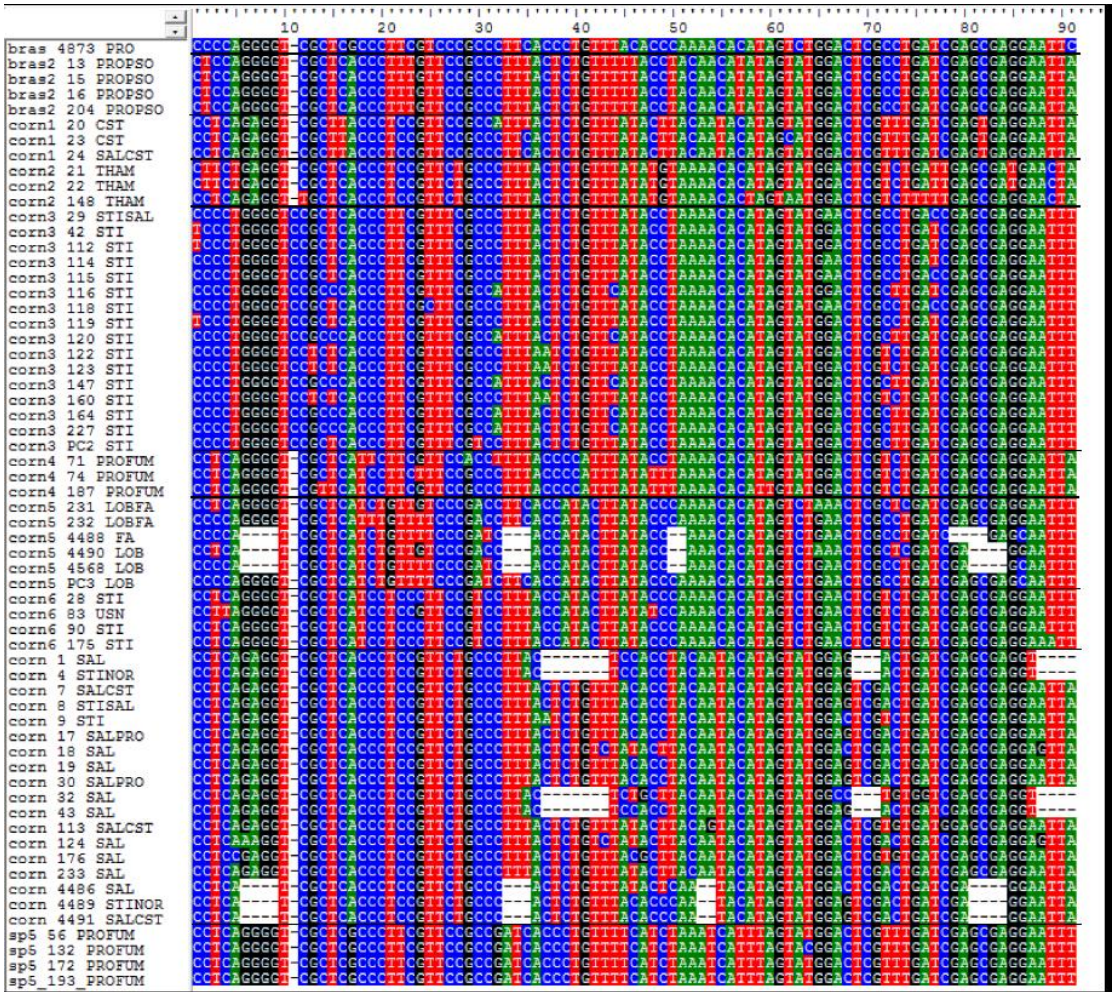
Rodriguez, J.M., Estrabou, C., Truong, C. & Clerc, P. (2011) The saxicolous species of the genus *Usnea* subgenus *Usnea* (Parmeliaceae) in Argentina. *Bryologist* **114:** 504–525.

Truong, C. (2012) Systematics of the lichen genus *Usnea* in tropical South America. PhD thesis. Geneva: University of Geneva. 285 pp.

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

Truong, C., Rodriguez, J. M. & Clerc, P. (2013). Pendulous *Usnea* species (Parmeliaceae, lichenized Ascomycota) in tropical South America and the Galapagos. *Lichenologist* **45:** 505–543.

Supplementary Data



Supplementary Data: Illustrations of the diagnostic ITS sequence features for the seven new species, six segregate from the *Usnea cornuta* aggr, showing the parsimony-informative columns only. The corresponding alignment file was obtained from the original alignment by deleting all constants and parsimony-uninformative columns. Species are abbreviated as follow: bras= *U. brasiliensis*; bras2= *U. pseudobrasiliensis*; corn1= *U. stipitata*; corn2= *U. catarinensis*; corn3= *U. isidiofibrillosa*; corn4= *U. furnensis*; corn5= *U. arianae*; corn= *U. cornuta*; sp5 = *U. kriegeriana*.

CHAPTER 6

A new saxicolous species of *Usnea* (Parmeliaceae: Lichenized Ascomycota) from the mountains of Brazil

To submit a *Phyotaxa*

A large population of *Usnea oreophila sp. nov. ad int.* growing on open rock outcrops (2050 meters elevation) in Serra da Mantiqueira (Minas Gerais State, Brazil)

A new saxicolous species of *Usnea* (Parmeliaceae: Lichenized Ascomycota) from the mountains of Brazil

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Abstract

Usnea oreophila A. Gerlach & P. Clerc is described as new to science. This saxicolous species is characterized by a patchy, faded reddish pigment above the jet–black basal pigmentation, branches with numerous cracks with downturned edges, numerous and sorediated fibercles, thick and fistulose central axis, as well as by the production of a complex chemistry with squamatic acid as main medullar compounds. This is a mountainous species living in high altitude grasslands above 1200 meters, so far found in the Minas Gerais, Paraná and Santa Catarina States of Brazil.

Key words: high–altitude grasslands, taxonomy, thin layer chromatography.

Introduction

Usnea is a hyperdiverse genus with more than 350 species in Parmeliaceae the largest family of lichenized fungal species (Lücking *et al.* 2016). The genus is well–characterized by a fruticose thallus, branches with a cartilaginous central axis and the presence of usnic acid in the cortex. The subgenus *Neuropogon* Nees & Flotow is exclusively saxicolous, differing from *Usnea* by the black pigmentation in the cortex, the dark pigmented apothecial discs, a *sphacelata*–type cortex (Ohmura & Kanda 2004), and a more restrict distribution occurring in polar regions and in the Southern Neotropical Andes (Truong *et al.* 2013)

Most *Usnea* species are primarily corticolous, occurring secondarily on rocks when environmental conditions are optimal as for instance *U. cornuta* Körb. (Clerc 1987). Strictly saxicolous species are rare in Europe (e.g. *U. fragilescens* var. fragilescens Lynge; Clerc

1987). In North America, four primarily saxicolous species were reported by Clerc & Herrera-Campos (1998). In Africa, three mainly saxicolous species were reported by Swinscow & Krog (1976). In South America, saxicolous species seems to be more frequent and eight species, among them three new for science, were reported for Argentina and Uruguay (Rodriguez & Estrabou 2008, Rodriguez *et al.* 2011).

In Brazil saxicolous *Usnea* species were never the subject of a detailed taxonomical study. Before this study only four strictly saxicolous species were mentioned on the literature (**Table 1**) but remaining yet badly known. The objective of this paper is describe a new saxicolous *Usnea* species found in the mountains of Minas Gerais, Paraná and Santa Catarina, on high altitude grassland and based on morphological, chemical and molecular evidence (showed in the Chapter 3 as *Usnea sp3*).

TABLE 1. Five primarily saxicolous species of *Usnea* recorded in Brazil.

Species	**Reference**
U. amblyoclada (Müll. Arg.) Motyka	Osorio *et al.* (1981); Fleig (1995)
U. aspera (Eschw.) Vain.	Vainio (1890) ; Zahlbruckner (1904, 1909) ; Rizzini (1952, 1956) ; Aptroot (2002)
U. complecta Motyka	Müller Argoviensis (1891b)
U. densirostra Taylor	Müller Argoviensis (1881,1891a–b); Motyka (1938) ; Rizzini (1952, 1956); Osorio (1981, 1997); Osorio & Homrich (1978); Osorio *et al.* (1981); Osorio & Fleig (1986, 1988, 1991); Fleig (1990, 1995)
U. laevis (Eschw.) Nyl	Vainio (1890); Müller Argoviensis (1891b)

Material and Methods

Specimens of *Usnea oreophila* were collected during the years 2013–2014 and June 2016 in three States in the Serra do Mar region: Minas Gerais (Municipality of Gonçalves), Paraná (Municipality of Guaratuba) and Santa Catarina (Municipality of Garuva). The Serra do Mar region includes the largest remnants of the Atlantic Forest in the slopes and at the tops of Serra do Mar and Serra da Mantiqueira with median altitudes varying between 1200 meters to 2800 meters elevation above sea level (Aguiar *et al.* 2003).

The morphology of specimens was examined using a stereomicroscope Leica MS5. Ascospores measurements were done using a Leica DM2000 microscope. The species concept and terminology used in this study follows Clerc (1998), Clerc & Herrera-Campos (1998) and Rodriguez *et al.* (2011). 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. We propose a new species based on morphological, chemical and molecular evidence.

Taxonomic treatment

Usnea oreophila A. Gerlach & P. Clerc. *sp. nov.* (Fig. 1A–D, 2)

MycoBank No.: MB

Thallus shrubby and rigid with jet black pigmented basal part, slightly irregular main branches with a patchy, faded reddish pigment above the jet–black pigmentation and numerous cracks with downturned edges, numerous and sorediated fibercles, thick and fistulose central axis, squamatic acid as main medullary substance.

Type. BRAZIL. MINAS GERAIS: Gonçalves, Pedra de São Domingos, proximo a antena, 22°41'06"S, 45°57'35.15"W, 2050 m elev., campo de altitude, 09.06.2016, *A. Gerlach & R. Penati* 1960–P33 (holotype ICN; isotypes G, UPS). %C/M/A: 5.5/10/68 (holotype). Chemistry: usnic, squamatic, thamnolic, barbatic acids, an unidentified terpenoid Rf Classes A/B: 5–6/5 (holotype). Ascospores: (7.5–)9–10–11 × 5–5.5–6 µm (holotype, n=12). DNA–code: sp3–8BR.

Description. *Thallus* erect–shrubby, up to 8 cm, rigid; *ramifications* mainly anisotomic dichotomous; *basal part* single or rarely with proliferating holdfast, up to 0.5 cm long, jet–black pigmented with pigmentation extending on main branches, often with numerous thin and irregular annular cracks; main *branches* 1–2.3 mm diameter (n=6), often with a patchy, faded reddish pigment above the jet–black pigmentation; distinctly irregular to cylindrical and slowly tapering, with thick, irregular, longitudinal to annular cracks whose sides are distinctly downturned; *lateral branches* not narrowed at point of attachment; *segments* cylindrical, terete to slightly obtuse–angled in cross section (numerous longitudinal cracks!), with conspicuous irregular, annular and longitudinal cracks; *foveolae and maculae* absent;

pseudocyphellae inconspicuous, only on thinner branches and fibrils; *papillae and tubercles* absent; *fibrils* numerous, both spinulose and slender, irregularly distributed, rapidly shed; *fibercles* numerous on the whole thallus; *soralia* punctiform, circular, ± capitate, stipitate to more rarely even with the cortex (on apices), without distinct cortical margin, well delimited, usually numerous, sometimes crowded on terminal branches, arising at the top of fibercles or seldom even with cortex; *isidiomorphs* often numerous, conspicuous, thick, spinulose–like, sometimes black at the tips; *isidiofibrils* absent; *cortex* shiny, with conspicuous irregular, annular and longitudinal cracks on the surface, thin to moderately thick, 5.5–7.5%–9.5(–11.5) (n=6); medulla compact, white, thin, (4–)6–9%–12; axis often fistulous in main branches, thick, (62–)63–68%–73(–76), with an A/M ratio: 6–12–18 (–22).

Apothecia sometimes present, up to 10 mm diameter, terminal, disc light brownish beige with fibrils and sorediate fibercles on the margins; *ascospores* (8–)9–10–11 × (5–)5.5–6–6.5 µm (n=4 thallus; 55 ascospores measured). *Pycnidia* not seen.

***Chemistry*.** Medulla K–, P–. **1)** TLC: squamatic acid, ± eumitrin Rf classes A/B/C: 6/2/5, ± an unknown orange after charring Rf class A: 4 (n=12). Medulla K+ bright yellow. **2)** TLC: squamatic acid, ± thamnolic, ± barbatic, ± eumitrin Rf classes A/B/C: 6/2/5, ± unidentified terpenoid (?) Rf classes A/B/C: 5–6/5/?, ± an unknown yellow after charring Rf classes A/B/C: 4/3/6?, ± an unknown orange after charring Rf class A: 4 (n=7).

Etymology. The epithet "*oreophila*" means "mountain lover" and refers to the occurrence of the species in the mountains of Brazil, on high altitude grasslands above 1200 meters.

Habitat and distribution. *Usnea oreophila* is locally frequent, on open acidic rock outcrops in high altitude grassland (also known as *Campo de altitude*) (Figure 2). Known so far only in Brazil: Minas Gerais (Serra da Mantiqueira), Paraná and Santa Catarina States (Serra do Mar).

Diagnostic notes. *Usnea oreophila* is characterized by the very rigid shrubby thallus, the jet black pigmented basal part, the slightly irregular main branches with a patchy, faded reddish pigment above the jet–black pigmentation;, the numerous cracks with downturned edges, the numerous and often sorediated fibercles, the thick and fistulose axis and by the presence of squamatic acid as main medullary substance.

Variation. The basal part is single or with proliferating holdfast. Beside squamatic acid that seems to be always present (sometimes as traces) there are many accessory substances appearing on the TLC plates.

Taxonomic notes. *Usnea durietzii* Motyka, also has a spot-like red pigment close to the basal part., It differs however from *U. oreophila* by the inflated branches, the type of soralia, the lax medulla, the moderately thin axis (27–33%–37) and the presence of norstictic acid in the medulla (Rodriguez *et al.* 2011). *Usnea bogotensis* Vain. and *U. laevigata* Vain. are chemically similar, also with squamatic acid in medulla. *Usnea bogotensis* has a different type of soralia and *U. laevigata* has a pruinose thallus.

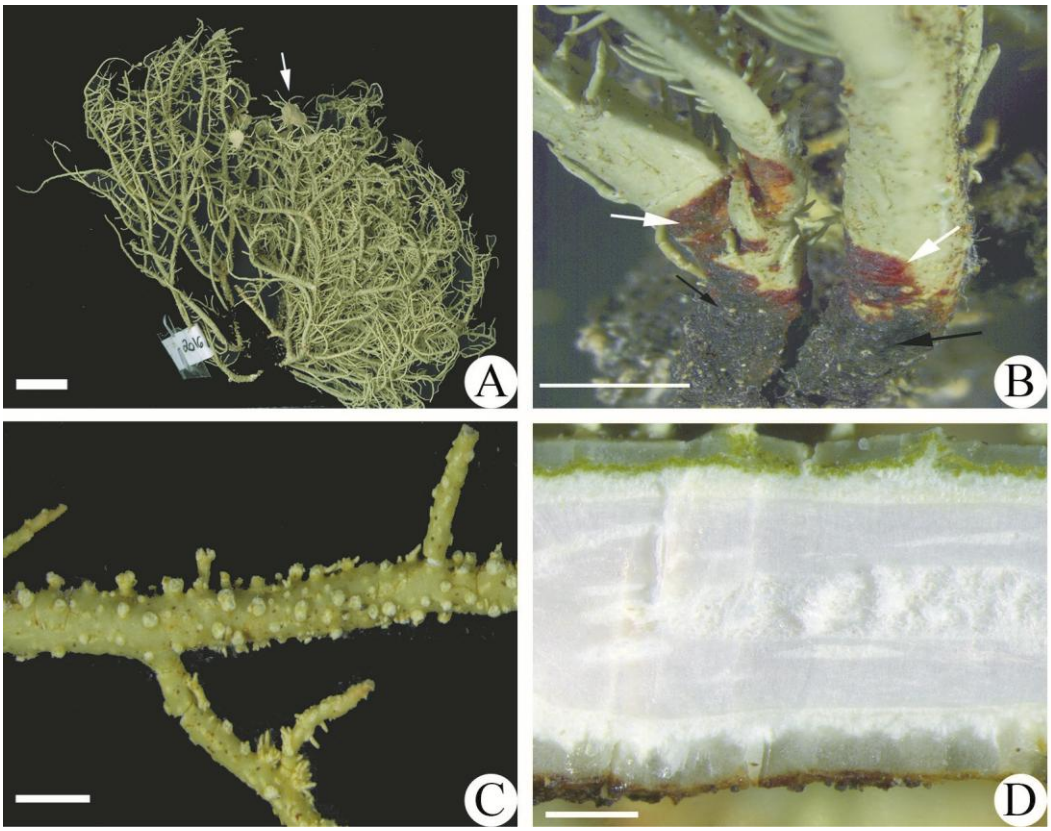

Figure 1. *Usnea oreophila*: A (holotype), thallus. B–D (*A. Gerlach* & *R. Penati 1959*). B, trunk jet–black (indicated by black arrows) becoming red fainted (white arrows). C, fibercles giving raise to the soralia. D, section through thallus with thick and fistulous axis central. Scales: A=1 cm; B=2mm; C=1 mm; D=500 µm. In colour online.

Figure 2. Type locality of *Usnea oreophila* growing on acidic rock outcrops in open grasslands at high altitude.

Specimens examined. BRAZIL. MINAS GERAIS: Gonçalves, Pedra de São Domingos, proximo a antena, 22°41'06"S, 45°57'35.15"W, 2050 m elev., campo de altitude, 09 June 2016, *A. Gerlach & R. Penati* 1959–P33 (G, ICN). PARANÁ: Guaratuba, Morro dos Perdidos, ca. 25º53'S, 48º57'W, ca. 1.260 m elev., 03 June 2013, *A. Gerlach et al.* 1005a (ICN). SANTA CATARINA: Garuva, Serra do Quiriri, ca. 26º03'S, 48º56'W, ca.1200 m elev., 05 october 2013, *A. Gerlach & F. Beilke* 1188 (ICN, G).

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References

Aguiar, A. P., Chiarello, A. G., Mendes, S.L. & Neri de Matos, E. 2003. The Central and Serra do Mar corridors in the Brazilian Atlantic Forest. In *The Atlantic Forest of South America: Biodiversity Status, Threats, and Outlook* (Leal, C.G., Câmara, I. de G., eds.): 488 pp. Center for Applied Biodiversity Science at Conservation International.

Aptroot, A. (2002) New and interesting lichens and lichenicolous fungi in Brazil. *Fungal Diversity* 9: 15–45.

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

Clerc, P. (1998) Species concepts in the genus *Usnea* (lichenized Ascomycetes). *Lichenologist* 30: 321–340.

Clerc, P. & Herrera-Campos, M.A. (1997) Saxicolous species of *Usnea* subgenus *Usnea* (lichenized Ascomycetes) in North America. *Bryologist* 100: 281–301.

Culberson, C.F. & Ammann, K. (1979) Standardmethode zur Dünnschichtchromatographie von Flechtensubstanzen. *Herzogia* 5: 1–24.

Culberson, C.F. & Johnson, A. (1982) Substitution of methyl tert–butyl ether for diethyl ether in the standardized thin–layer chromatographic method for lichen products. *Journal of Chromatography* 238: 483–487.

Fleig, M. (1990) Líquens saxícolas, corticícolas e terrícolas do Morro Santana, Rio Grande do Sul. II. Espécies e novas ocorrências. *Pesquisas, Botânica* 41: 33–50.

Fleig, M. (1995) Lichens from "Casa de Pedra" and surroundings, Bagé, Rio Grande do Sul, Brazil. – In: Daniëls, FJA,Schulz, M,Peine, J (eds.): Flechten Follmann. Contributions to lichenology in Honour of Gerhard Follmann. Geobotanical and Phytotaxonomical Study Group, Botanical Institute, University of Cologne, Cologne, pp. 415–426.

Hale, M.E. Jr. (1979) How to Know the Lichens, 2nd edition. Dubuque, Iowa: William C. Brown.

Lücking, R., Hodkinson, B.P. & Leavitt, S.D. 2017(2016). The 2016 classification of lichenized fungi in the Ascomycota and Basidiomycota – Approaching one thousand genera. *The Bryologist* 119(4):361–416.

Motyka, J. (1938) Lichenum Generis Usnea Studium Monographicum. Pars Systematica. Leopoldi: privately printed.

Müller Argoviensis, J. (1881) Lichenologische Beiträge 12 (N.º 228–247). Flora 64(6): 81–88.

Müller Argoviensis, J. (1891a). Lichenes Catharinenses a cl. E. Ule in Brasilia prov. Santa Catharina lecti. *Hedwigia* 30: 235–243.

Müller Argoviensis, J. (1891b). Lichenes Schenckiane a cl. Dr. H. Schenck, Bonnensi, in Brasiliae orientalis prov. Santa Catharina, Paraná, Rio de Janeiro, Minas Geraes et Pernambuco lecti. *Hedwigia* 30: 219–234.

Ohmura, Y. & Kanda, H. (2004) Taxonomic status of section *Neuropogon* in the genus *Usnea* elucidated by morphological comparisons and ITS rDNA sequences. *Lichenologist* 36(3–4): 217–225.

Osorio, H.S. (1981) Contribution to the lichen flora of Brazil VIII. Lichens from Morro do Coco, Viamao, Rio Grande do Sul. *Phytologia* 48: 72–76.

Osorio, H. S. (1997) Contribution to the lichen flora of Brazil. XXXIV. Lichens from Laguna, Santa Catarina State. *Comunicaciones Botánicas del Museo de Historia Natural de Montevideo* 6: 1–4.

Osorio, H.S. & Homrich, M. (1978) Contribution to the lichen flora of Brazil IV. Lichens from southern Rio Grande do Sul. *The Bryologist* 81: 452–454.

Osorio, H.S., Aguiar, L.W. & Homrich, M.H. (1981) Contribution to the lichen flora of Brazil VI. New or additional records from Rio Grande do Sul State. *The Bryologist 84*: 79–81.

Osorio, H.S. & Fleig, M. (1986) Contribution to the lichen flora of Brazil XVIII. Lichens from Itaimbezinho, Rio Grande do Sul State. *Comunicaciones Botanicas del Museo de Historia Natural de Montevideo 4(75)*: 1–8.

Osorio, H.S. & Fleig, M. (1988) Contribution to the lichen flora of Brazil. XXI. Lichens from Morro Santana, Rio Grande do Sul State. *Comunicaciones Botanicas del Museo de Historia Natural de Montevideo* 5(86): 103.

Osorio, H. S. & Fleig. M. (1991) Contribution to the lichen flora of Brazil. XXVIII. Lichens from northern Santa Maria, Rio Grande do Sul State. *Comunicaciones Botánicas del Museo de Historia Natural de Montevideo* 5(96): 1–7.

Rodriguez, J.M. & Estrabou, C. 2008. *Usnea amblyoclada* «Barba de piedra» (Ascomycetes liquenizados) en Argentina. *Bol. Soc. Argent. Bot. 43*: 221–225.

Rodriguez, J.M., Estrabou, C., Truong, C. & Clerc, P. (2011) The saxicolous species of the genus *Usnea* subgenus *Usnea* (Parmeliaceae) in Argentina. *Bryologist* 114: 504–525.

Rizzini, C.T. (1952) Species Organenses generis lichenum Usneae. (Omnes acidum usnicum praebentes). *Rev. Brasileira de Biol.* 12(4): 337–348.

Rizzini, C.T. (1956) Flora Organensis. Lichenes. *Rev. Brasil. Biol.* 16(4): 387–402.

Swinscow, T.D.V. & Krog, H. (1976) The *Usnea bornmuelleri* aggregate in East Africa. *Norwegian Jour. Bot.* 23: 23–31.

Truong, C., Divakar, P. K., Yahr, R., Crespo, A. & Clerc, P. (2013) Testing the use of ITS rDNA and protein–coding genes in the generic and species delimitation of the lichen genus *Usnea* (Parmeliaceae, Ascomycota). *Molecular Phylogenetics and Evolution* 68(2): 357–372.

Vainio, E.A. (1890) Étude sur la classification naturelle et la morphologie des Lichens du Brésil, pars prima. *Acta Societatis pro Fauna et Flora Fennica* 7(1): 1–247.

Zahlbruckner, A. (1904) Lichenes a cl. Damazio in montibus Serra do Ouro Preto Brasiliae lecti in herb. Barbey–Boissier asservati. *Bull. Herb. Boissier Ser.* 2, 4: 134–136.

Zahlbruckner, A. (1909) Lichenes (Flechten). in: V. Schiffner (ed.), Ergebnisse der botanischen Expedition der kaiserlichen Akademie der Wissenschaften nach Südbrasilien, 1901, 2. Band. –Denkschr. Kaiserl. Akad. der Wissensch. 83: 85–211.

GENERAL DISCUSSSION

1. Diversity of *Usnea* species in Brazil

1.1 Novelties

Overall, we identified during this study a total of 70 *Usnea* species for Brazil (**Appendix 2**). Among these 70 taxa, we provided detailed descriptions for 48 of them **(Table 1)**. Fourteen species were or will be newly described: *U. arianae ad. int.* (Chapters 3 and 5), *U. aurantiaca-parvula* (Chapter 1), *U. catarinensis ad int.* (Chapters 3 and 5), *U. fleigiae* (Chapter 1), *U. furnensis ad. int.* (Chapters 3 and 5), *U. grandispora* (Chapter 1), *U. isidiofibrillosa ad. int.* (Chapters 3 and 5), *U. kalbiana* (Chapter 1), *U. kriegeriana ad. int.* (Chapters 3 and 5), *U. oreophila ad. int.* (Chapter 3 and 6), *U. pseudobrasiliensis ad. int.* (Chapters 3 and 5), *U. stipitata ad. int.* (Chapters 3 and 5), *U. subparvula* (Chapter 1), and *U. subsilesiaca ad int.* (Chapter 2).

Five species were newly reported for Brazil: *U. chilensis* (Chapter 2), *U. cirrosa* (Chapter 1), *U. cornuta* (Chapter 5), *U. erinacea* (Chapter 1) and *U. transitoria* (Chapter 2). Four species were new for the Southern region: *U. mexicana*, *U. papillata*, *U. subflammea* and *U. subscabrosa* (Chapter 2).

Modern descriptions, based on modern tools, were provided for some "resurrected" species, originally described from Brazil but not mentioned again (after 1970) in the recent scientific literature: *Usnea cladocarpa* (= *U. ramillosa syn. nov.*), *U. concinna* (= *U. radiata syn. nov.*), *U. lunaria* (Chapter 1), *U. meridionalis* (= *U. michauxii syn. nov.*). Another resurrected species, *U. venusta*, was originally described from Brazil. It is here considered as a good species, distinct from *U. articulata* (Chapter 2). Modern descriptions were provided also for the "rare" *U. disjuncta* (with a new combination) and the widespread *U. chilensis*.

However, in the current state of knowledge, it is difficult to give the exact number of the *Usnea* species occurring in Brazil. Beside the 70 species treated in this study, there are four species that were recently mentioned for the country, but not found in the Southern states: *U. dimorpha* (Müll. Arg.) Motyka; *U. firma* Motyka, U. *humboldtii* Motyka and *U.*

subcornuta Stirt. (Truong *et al.* 2013b, Truong & Clerc 2016). There are furthermore five species originally described from Brazil but as well not found in the Southern states: *U. complecta* Motyka, *U. feeana* Motyka, *U. leioclada* (Zalhbr.) Motyka, *U. regnellii* Motyka and *U. trachyclada* (Müll. Arg.) Zahlbr.). The identity of two further species remains uncertain because their types could not be located (material probably lost): *U. ludicra* Rizzini and *U. elongate f. sorediifera* Rizzini (see **Appendix 1**). On the other hand, two taxa were excluded from the species list of Brazil: *U. articulata* (see Chapter 2) and *U. florida* (see Chapter 1). Finally, the status of some names recorded for Brazil is still in need of revision (e.g. *U. barbata* (L.) F.H. Wigg and their infra-specifical entities) and molecular studies will be necessary to access the status of some other species (e.g. Is *U. spinilufera* (Vain.) Motyka a synonym or not of *U. dasaea*?). Therefore, it is certain that 80 *Usnea* species occurs in Brazil. This is close to the number of species occurring in the whole tropical South America (ca. 100 species, Truong 2012). It is well probable that the diversity of the genus *Usnea* in South America is so far underestimated and Brazil is certainly a hot spot for the genus in South America. Around 200 species are added every year to the lichen biota of Brazil, and it will soon become the country with the highest lichen species number (over 4750 species known to date; Aptroot *et al.* 2017).

1.2 Distribution ranges of the species studied

The distribution per Brazilian states of the species studied and their occurrence outside of tropical South America is presented in **Table 1**.

Table 1. Distribution per States of the species found in Southern Brazil, in Brazil as well as their occurrence in other parts of the world

	Southern			BRAZIL																
species	**RS**	**SC**	**PR**	**BA**	**CE**	**DF**	**ES**	**MG**	**MS**	**MT**	**PE**	**RJ**	**SP**	SA	NCA	AF	MA	EU	AS	OC
U. alata	+	+	+					+					+	+						
U. angulata	+	+	+					+				+	+	+	+				+	+
U. arianae		+						+									+	+		
U. arthroclada	+							+				+	+		+					
U. aurantiaca-parvula				+	+			+	+		+									
U. brasiliensis s str.	+	+	+										+				+			
U. catarinensis		+											+							
U. cf. moreliana	+	+	+									+	+							
U. chilensis*	+	+	+											+						
U. cirrosa*	+	+	+					+				+	+	+	+					
U. cladocarpa			+					+				+	+	+	+					
U. concinna	+							+				+	+							
U. cornuta s.str. *	+	+						+					+	+	+			+		
U. cristatula*	+	+	+	+		+		+						+	+			+		
U. dasaea	+		+													+	+	+	+	+
U. disjuncta							+													
U. aff. disjuncta	+																			
U. dodgei	+	+	+					+					+	+	+					
U. erinacea s. lat.*	+	+	+					+				+	+	+	+	+		+		
U. fleigiae	+	+	+																	
U. furnensis		+																		
U. geissleriana	+		+					+				+	+	+			+	+		
U. grandispora	+	+	+																	
U. isidiofibrillosa	+	+						+							+			+		
U. kalbiana		+	+					+				+	+							
U. kriegeriana	+	+	+				+	+					+							
U. lunaria								+				+								
U. malmei	+	+	+					+				+	+	+	+					
U. meridionalis	+	+	+					+				+	+	+	+					
U. merrillii	+	+	+									+	+		+				+	
U. mexicana**		+	+							+		+		+	+					
U. oreophila		+	+					+												
U. papillata**	+	+	+	+				+						+	+					
U. parvula	+	+	+										+	+	+					
U. perhispidella	+	+	+											+		+				
U. pseudobrasiliensis		+	+					+					+							
U. regia	+	+	+					+				+	+	+						
U. sanctaeritae	+											+	+		+					
U. steineri	+	+	+									+	+	+		+				
U. stipitata	+	+	+					+					+							
U. subelegans	+	+	+					+	+			+	+	+	+					
U. subflammea**	+	+	+					+				+	+	+	+		+			
U. subgracilis	+	+	+									+	+	+	+				+	+
U. subparvula	+		+						+				+	+						
U. subscabrosa**	+	+	+					+				+	+		+	+	+	+	+	
U. subsilesiaca	+	+						+				+	+	+	+					
U. transitoria*		+	+											+	+					
U. venusta	+	+											+	+						
	RS	**SC**	**PR**	**BA**	**CE**	**DF**	**ES**	**MG**	**MS**	**MT**	**PE**	**RJ**	**SP**	SA	NCA	AF	MA	EU	AS	OC

Squares (+) in gray indicated specimens analyzed in this study; squares in white (+) indicates presence mentioned on the modern literature (Ohmura 2001; Clerc 2004, 2006, 2007, 2011b;

Truong 2012; Herrera-Campos 2016; Pérez-Vargas *et al.* 2010); red squares (+) indicates origin where the type was described. The types of *U. arthroclada*, *U. cladocarpa* and *U. concinna* are from unknown localities in Brazil and therefore were not indicated.

RS = Rio Grande do Sul; **SC** = Santa Catarina; **PR** = Paraná; **BA** = Bahia, **CE** = Ceará; **DF** = Distrito Federal; **ES** = Espírito Santo; **MG** = Minas Gerais; **MS** = Mato Grosso do Sul; **MT** = Mato Grosso; **PE** = Pernambuco; **RJ** = Rio de Janeiro; **SP** = São Paulo; **SA** = South America; **NCA** = North & Central America; **AF** = Africa; **MA** = Macaronesia; **EU** = Europe species in **bold** = newly described species; *newly reported for Brazil; **newly reported for Southern region. Species within black thick squares = mentioned by Rodriguez (2011) **and** Truong (2012); species highlighted in green = mentioned by Rodriguez (2011); species highlighted in purple = mentioned by Truong (2012).

Most of the species found in this study (Table 1, 15 spp.) are endemic to the American continent (*U. arthroclada, U. cirrosa s. lat., U. cladocarpa, U. dodgei, U. malmei, U. meridionalis, U. cf. moreliana, U. papillata, U. parvula, U. sanctaeritae, , U. subelegans, U. subsilesiaca* and *U. transitoria*); four species are endemic to South America (*Usnea alata, U. chilensis, U. regia, U. venusta*). Finally 15 taxa are so far known to be endemic to Brazil (*Usnea aurantiaca-parvula, U. catarinensis, U. concinna, U. disjuncta, Usnea aff. disjuncta, U. fleigiae, U. furnensis, U. grandispora, U. kalbiana, U. kriegeriana, U. lunaria, U. oreophila, U. pseudobrasiliensis, U. stipitata* and *U. subparvula*).

From the species studied, **nine** were originally described from **North and Central America** (*U. cirrosa, U. cristatula, U. dodgei, U. erinacea, U. merrillii, U. mexicana, U. sanctaeritae, U. subgracilis* and *U. transitoria*); three species from South America (*U. angulata, U. chilensis, U. parvula*), three species from the Macaronesian islands (*U. arianae, U. dasaea, U. geissleriana, U. subflammea*) (Clerc 2006); two species from Europe (*U. cornuta s. str., U. subscabrosa*) and one species from **Africa** (*U. perhispidella*).
Additionally (**Appendices II**) we found a few specimens that we were able to identify as *U. macaronesica* P. Clerc a macaronesian species (Clerc 2006) new for South America.

The combination of elements from various origins was showed by Truong (2012) and was corroborated with their phylogenetic analyses (Truong *et al.* 2013a) where species were generally distributed across the tree without clear geographical structure. For instance our molecular data confirm the distribution of *U. arianae, U. cornuta s. str.*, and *U. isidiofibrillosa* occurring in Brazil as well as in Europe **(Chapter 3)**.

Our results show 41% of similarity (21 spp., **Table 1,** highlighted in purple **and** within black thick squares) between the species mentioned in this study (48 spp.) and the species found in the Neotropical Andes and the Galapagos islands (51 spp; Truong 2012). Among the twelve new species recently described from these regions, only two occurs in Brazil so far: *U. subglabrata* Truong & P. Clerc (only one specimen) and *U. subdasaea* Truong & P. Clerc (Truong & Clerc 2016). Therefore the similarity found is mainly due to the presence of more cosmopolitan elements, occurring for instance in Europe, Central, North America and even in Brazil). The number of species in common may increase as new studies are done; for instance we recently documented for the first time the presence of *U. grandisora* Truong & P. Clerc in Brazil (**Chapter 3, Appendix II**).

Future phylogenetic studies in the genus *Usnea* will probably change this picture. For instance, a spectacular number of endemic lichens were recently described, from the Neotropical Andes and the Galapagos islands (Moncada & Lücking 2012; Lücking *et al.* 2014; Yasmín *et al.* 2014; Dal-Forno *et al.* 2017). Finally, in the *Usnea cornuta* aggr. the lineages found in the Neotropical Andes (n=4 specimens) seem to diverge from those found in Brazil (**Chapter 3**).

There is 36% of similarity (16 spp., **Table 1,** highlighted in green **and** within black thick squares) between the species mentioned in this study (48 spp.) and the species found in Argentina (44 spp; Rodriguez 2011). Among them *U. parvula* is the only species originally described from this country (Cordoba). The newly described species *U. subparvula* was discovered also occurring in Argentina (Cordoba) and probably was overlooked before this study **(Chapter 1)**. *Usnea venusta* seems to occur also in Argentina (as *U. aff. articulata*; Rodriguez 2011). Unfortunately we could not see the cited specimens but the morphology and chemistry described by Rodriguez (2011) fits well with *U. venusta* **(Chapter 2).** *Usnea subsilesiaca ad. int.* occurs also in Argentina where was found occurring frequently on rocks (as *U. columbiana*; Rodriguez *et al.* 2011). We agree with Rodriguez (2011) that *U. cornuta* could be present also in Argentina. However, only one herbarium specimen from Tucuman was analyzed in this study **(Chapter 5)**.

Regarding the general distribution, there is increasing evidence that some species have a narrower distribution than thought before, since they have been shown to be polyphyletic. This is especially the case for *U. cornuta, U. erinacea and U. rubicunda* (Truong *et al.* 2013b; Caviró 2015). For instance the Brazilian specimens of *U. erinacea* are polymorphic with distinct patterns of cortical pigmentation and several chemotypes. *Usnea subdasaea*, recently

described from the Galapagos and the Neotropical Andes (Truong *et al.* 2011), was shown to be polyphyletic as well (Truong *et al.* 2013a). Furthermore we found the same kind of evidence for *U. cirrosa* (Gerlach et al. 2017, **Chapter 1)** and for *U. dasaea* and *U. subflammea* **(Chapter 3).** It is possible that future integrative taxonomical studies will show that more than one species is involved in each of these species so far defined with traditional characters.

1.3 Ecology

The majority of the species mentioned in this study are corticolous growing on trees (bark, twigs) and lignicolous growing on fences posts, with no specific preferences for one or the other type of substrate. Two species were originally described as growing on rocks (*U. cornuta* and *U. lunaria*) but they were found only growing as corticolous or lignicolous. In Europe, *U. cornuta* was found mainly on tree bark (Clerc 1987b).

Usnea is frequent on coniferous trees (e.g. *Araucaria angustifolia* (Bertol.) Kuntze, *Pinus* spp.) in mountainous and rural areas. In the Araucaria forest the presence of pendulous species is usually very notable. It is also frequent also on bark of palm trees (e.g. *Syagrus romanzoffiana* (Cham.) Glassman) in rural areas or in the vicinity of urban areas) and it can be frequently found on old fences posts.

It grows also frequently on bushes or trees near the sea and lagoons. However, it grows rarely on rocks shores and despite an intense sampling along the rock shores from Paraná to Southern Santa Catarina, only one large population of *U. subscabrosa* could be found in such habitat. It was growing on coastal cliffs a few meters away from the beach, and only in one place. Close to the seashore, *Usnea* may be found growing on shrubs on sandbanks or at the edge of lagoons (e.g. *U. parvula*, *U. subelegans*). A large population of the saxicolous species *U. densirostra* and *U. ambyloclada* were found also growing on rocks at the edge of lagoons.

It is worth to mention that the distribution of some of the species found in Brazil occur in specific biomes. So, for instance, *U. aurantiaca-parvula* occurs mainly in the Caatinga and Cerrado biomes in the north-eastern and south-eastern parts of Brazil. This species could not be found in Southern Brazil despite our intense sampling in this area covered mainly by the Atlantic forest biome and the study of numerous herbarium specimens. This could be explained by the very distinct climatic conditions characterizing these biomes: Cerrado and

Caatinga correspond to a tropical savanna with a hot and dry climate, while the Atlantic forest is warm and wet (see Wittmann *et al.* 2017, for more details about the Brazilian biomes).

Another case is found in the *U. subparvula*/*U.parvula* species complex. In Brazil, *Usnea subparvula* is mainly found in the Cerrado biome with a few exceptions (one specimen collected in the continental subtropical seasonal Atlantic forest at border of the Mato Grosso do Sul and Paraná States and one specimen collected within the Pampa biome near the border of Argentina and Uruguay. On the other hand *U. parvula* is frequent in the Southern Region, occurring mainly in coastal habitats close to the seashore (Atlantic forest s. str., wet forest). These two species are morphologically very similar differing mainly by the chemistry. It is possible that *U. subparvula* was overlooked by Rodriguez (2011) who mentioned *U. parvula* with two distinct chemotypes. Biogeographical studies involving these two similar species are still necessary to better understand their ecological specificities (**Figure 1**).

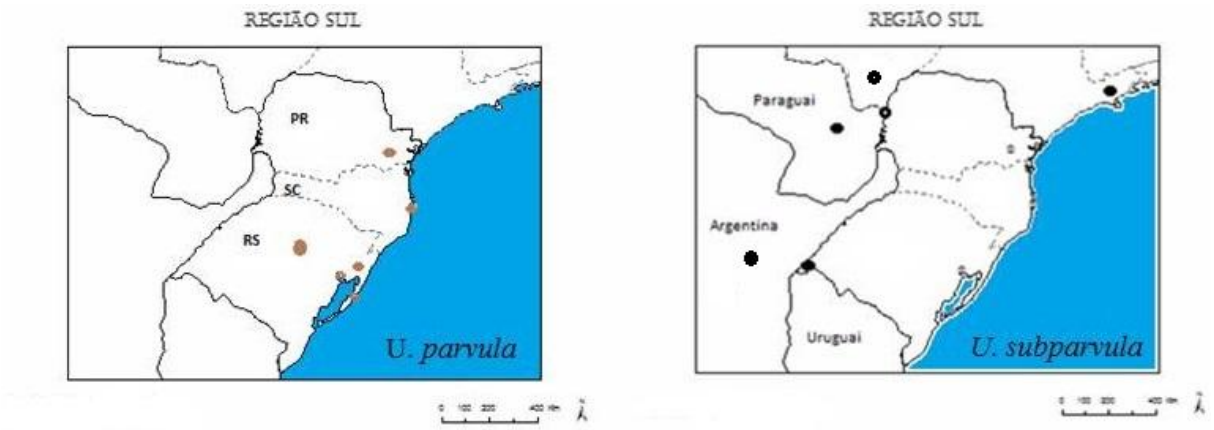

Figure 1: Distribution of *Usnea parvula* (right, brown circles) and *U. subparvula* (left, black circles): **RS**: Rio Grande do Sul, **SC**: Santa Catarina, **PR**: Paraná. The circles indicate the number of localities sampled.

2. Chemistry

2.1 Thin Layer Chromatography analyses (TLC)

The use of more than one solvent system is especially important to recognize compounds with similar Rf distances. For instance, solvent B is the best one to separate stictic from galbinic acid and protocetraric from fumarprotocetraric acid. The same solvent is useful to separate thamnolic acid from another very similar orange substance that proved to be important taxonomically (Us2-krie; Chapter 5). Solvent A and C are useful to check the presence of eumitrins since in solvent B they can hide behind several substances with low Rf commonly

found in the genus. Therefore, in cases where it is not possible to perform TLC in the three solvent systems, solvents A and B should be used first.

Several specimens are P–, meaning that they do not show any reactions on the classical color tests used in lichenology (K, C and P). We recommend here to spray the plates with anisaldehyde sulfuric acid (see **Chapter 4**). This allows revealing such substances as terpenes, steroids and sugars that are not depsides or depsidones. Sugars seem to be diagnostic for *U. malmei* and *U. papillata* (n=20 specimens); terpenes are diagnostic for *U. moreliana* (n=22); the presence of terpenes in *U. arianae*, *U. aurantiaca-parvula*, *U. erinacea*, *U. meridionalis*, *U. steineri* and *U. parvula* are in need of further investigations.

2.2 The chemotypes

Chemistry, i.e. the study lichen secondary compounds was shown to be very useful in the taxonomy of the genus *Usnea* (Clerc 1998, Ohmura 2001). In this study we describe several examples where distinct chemotypes were correlated molecular data as well as with (sometimes subtle) morphological or anatomical differences as for instance: *Usnea parvula* versus *U. subparvula*; *U. cirrosa* versus *U. cladocarpa*; *U. cornuta* versus *U. brasiliensis* and other examples in the *U. cornuta aggr.* (**Chapters 1 and 3**). These results show that chemistry is helpful indeed to delimit and recognize species. The same situation occurred for example in the fruticose genus, *Ramalina*, where three chemotypes were recognized as distinct species based on molecular data correlated with subtle morphological differences in their propagules and habitats (Ohmura *et al.* 2008). This is however not always the case: distinct species supported by molecular data might contain several different chemotypes as it is the case for instance for *U. fleigiae*, *U. grandispora*, and *U. cornuta s. str.* Furthermore, our preliminary phylogenetical analyses show also that the thamnolic acid chemotype found in *U. subscabrosa* collected in Porto Santo (Portugal) clusters together in a well supported clade (data not showed) with the protocetraric acid chemotype of specimens collected in Brazil.

All these results showed that distinct chemotypes should be carefully analyzed (with a large sampling) before taxonomical decisions are taken.

In our study we found 27 species with only one chemotype, 13 species with two chemotypes, five species with three chemotypes, two species with four chemotypes et one species with five chemotypes. The details of these species and chemotypes are presented below:

a) Species with one chemotype (Table 2) – Almost all species identified in this study were characterized by the presence of only one chemotype. Most of the time the chemotype of the type specimen corresponds to the chemotype of the species, except for Brazilian specimens of *U. dodgei* that have galbinic acid whereas the type of *U. dodgei* has stictic acid (**see Chapter 2)**. *Usnea kriegeriana* is characterized by a constant chemistry with protocetraric often accompanied by fumarprotocetraric and an unknown substance (Us2-krie). *Usnea alata* and *U. papillata* have a K–, P–reacting medulla with an unidentified fatty acid in *U. alata* and a sugar-like substance accompanied or not by steroids and terpenes in *U. papillata.* Unlike what was found by Truong *et al.* (2013b) in Andean specimens, we were unable to detect terpenes in the Brazilian specimens of *U. alata* (16 specimens tested). On the other hand the same chemical pattern mentioned by these authors was found in *U. papillata*. The nature of these compounds was clarified and they correspond to three distinct classes of chemical products (Chapter 4).

Table 2. *Usnea* species with one chemotype

Species	chemotype	species	chemotype	species	chemotype
U. aff. disjuncta	GAL	U. brasiliensis s str.	PRO	U. alata	±FA
U. chilensis	GAL	U. cladocarpa	PRO	U. papillata	SUG,±TER,±STE
U. dasaea	GAL	U. furnensis	PRO	U. catarinensis	THA
U. angulata	NOR	U. lunaria	PRO	U.isidiofibrillosa	STI
U. cirrosa	SAL	U. pseudobrasiliensis	PRO,PSO	U. perhispidella	STI
U. merrillii	SAL	U. subgracilis	PRO	U. regia	STI
U. sanctaeritae	SAL	U. subscabrosa	PRO	U. stipitata	CST
U. subsilesiaca	SAL	U. kriegeriana	PRO,FUM,Us2krie	U. venusta	SAL,NOR

CST = constictic; FA = fatty acid; FUM = fumarprotocetraric; GAL= galbinic; NOR = norstictic; PRO = protocetraric; PSO = psoromic; SAL= salazinic; STI = stictic, SUG = sugar (named Us2 in Chapter 2); STE = steroid; TER = terpenoids; THA = thamnolic; Us2Krie = unknown substance.

b) Species with two chemotypes (Table 3) – *Usnea malmei* and *U. moreliana* have a K−, P− reacting medulla and each of them present two distinct chemical pattern that are the same than those mentioned by Truong *et al.* (2013b, Fig. 2): *U. malmei* with a sugar-like substance accompanied or not by steroids and monoterpenes and *U. moreliana* with triterpenes accompanied or not by a sugar-like substance **(Chapter 4).** The second chemotype (II) of both species consists in the absence of medullary compounds (Ch0); the "Ch0" should be carefully investigated to found possible overlooked substances (fatty acid, terpenes etc).

Usnea subelegans has an almost constant chemistry with galbinic acid and only one specimen could be found with stictic acid. *Usnea subflammea* is known to have stictic acid in the medulla but a chemotype with galbinic acid was recently reported in South America by Truong *et al.* (2013b). We have reported it in Brazil as well. *Usnea oreophila* presents a complex chemistry with several accessory substances (e.g. eumitrin, thamnolic, barbatic).

Table 3. *Usnea* species with two chemotypes.

	chemotypes			chemotypes	
Species	**I)**	**II)**	**Species**	**I)**	**II)**
U. malmei	SUG,±TER,±STE	Ch0	U. cristatula	DIF	BAR
U. moreliana	TER, ±SUG	Ch0	U. disjuncta	DIF	BAR
U. concinna	STI	NOR	U. mexicana	PRO	STI
U. geissleriana	STI	SAL	U. subparvula	PRO	PSO
U. subflammea	STI	GAL	U. subelegans	GAL	STI
U. transitoria	STI	Ch0	U. grandispora	SAL	PRO
U. fleigiae	NOR	SAL	U. oreophila	SQU	SQU, ±THA

BAR = barbatic, DIF = diffractaic, GAL = galbinic, NOR = norstictic, PRO = protocetraric, PSO = psoromic, SAL = salazinic, STI = stictic, STE = steroid, SQU = squamatic, SUG = sugar, THA = thamnolic, TER = terpenes.

c) Species with three chemotypes (Table 4) – Three species (highlighted in gray) have a K−, P− reacting medulla with substances whose exact nature is still unclear: *U. arianae, U. aurantiaca-parvula* and *U. steineri*. Two further species (*U. arthroclada* and *U. cornuta*), produce a combination of compounds issued from the same biosynthetical pathway and present in different concentration. This is known as "chemosyndrome variation" (e.g. salazinic, constictic, stictic, norstictic), a special type of chemotype: *Usnea cornuta s. str.* is already well known to present a combination of salazinic or stictic acids in their medulla (Clerc 1987b, 2006, 2011a); moreover a new chemosyndrome was found for this species, constictic acid corroborated with molecular data but still in need of further analysis. In *U. arthroclada*, we found two new chemotypes, salazinic or norstictic acids and we interpret this as a chemosyndromic variation as well.

Table 4. *Usnea* species with three chemotypes.

	chemotypes		
species	**I)**	**II)**	**III)**
U. aurantiaca-parvula	Us2-aura	Ch0	TER
U. arianae	LOB	FA	TER, Us1-aria
U. steineri	TER	FA	Ch0
U. arthroclada	STI	SAL	NOR
U. cornuta s.str.	SAL	CST	STI

SAL = salazinic, STI = stictic, LOB = lobaric; NOR = norstictic; FA = fatty acid, TER = terpenes, Ch0 = no medullary substances detected, Us1-aria, Us2-aura = unknown substances found respectively in *U. arianae* and *U. aurantiaca-parvula*.

d) Species with four and five chemotypes (Table 5) – Both *Usnea meridionalis* and *U. parvula* have a K–, P– reacting medulla without any compounds detected by TLC (Ch0). *Usnea meridionalis* has three further chemotypes, one with a K–, P– reacting medulla (terpenes). *Usnea parvula* has a chemotype characterized by the presence of an unidentified compound (Us1-parv; found also on the type specimen), a fatty acid chemotype (caperatic acid) and a fourth chemotype characterized by the presence of an unidentified fatty acid and/or terpenes. *Usnea erinacea* is the species with the highest number of chemotypes found in this study. All were mentioned by Truong *et al.* (2011) except the protocetraric acid, a new chemotype discovered in this study. However, this species is most probably polyphyletic.

Table 5. *Usnea* species with four and five chemotypes.

	Chemotypes				
species	**I)**	**II)**	**III)**	**IV)**	**V**
U. meridionalis	TER	SAL	NOR	Ch0	
U. parvula	Us1-parv	CAP	TER,FA	Ch0	
U. erinacea s. lat.	PRO	TER	NOR	SAL	STI

CAP = caperatic; SAL = salazinic, STI = stictic, NOR = norstictic; PRO = protocetraric; FA = atty acid, SUG = TER = terpenes, Ch0 = no medullary substances detected, Us1-parv = unknown substance found in *U. parvula*.

2.3 The taxonomical significance of "hidden" compounds

Fatty acids (or aliphatic acids), terpenes, steroids and sugars are referred here as "hidden" compounds because they does not show any positive reaction when the traditional chemical reagents (K, C and P) used in lichenology are applied to the medulla. Moreover their presence is not always easily revealed on the TLC plates **(Chapter 4)**. For this reason they may be easily overlooked or misinterpreted.

Fatty acid (FA). The presence of fatty acid is already known to be important in the taxonomy of *Usnea* (Clerc 1987b, 1992; Halonen *et al.* 1999; van den Boom *et al.* 2015; Caviró 2015). For example, caperatic acid is often found in *U. lapponica* (Halonen *et al.* 1999) and it is diagnostic for *U. boomiana* P. Clerc (van den Boom *et al.* 2015). The murolic acid gr. is diagnostic for *U. hirta* (Clerc 1987b) and *U. mutabilis* (Clerc 2007, Caviró 2015). Bourgeanic acid is diagnostic for *U. esperantiana* (Clerc 1992; Caviró 2015). Finally the same

unidentified fatty acid was found in *U. flavocardia* by Clerc (1984) and Caviró (2015). In South America, however, their importance is still unclear. Fatty acids were mentioned for *U. meridionalis*, *U. steineri* and *U. subdasaea* by Rodriguez (2011). Among these three species we were only able to find fatty acids in *U. steineri*. Truong (2012) does not report any fatty acid from South American specimens, despite an intensive sampling. We found several unidentified fatty acids in Brazilian specimens but we were not able to clarify their taxonomical value. Among them only caperatic acid was identified. It corresponds to one of the four chemotypes present in *U. parvula* (Chapter 1). In the crustose lichen *Mycoblastus sanguinarius* for instance, distinct fatty acid profiles were correlated with molecularly well supported clades, and were consequently considered as being a good criteria to recognize cryptic species in this group (Spribille *et al.* 2011). So the presence and characterization of fatty acids in South American specimen of the genus *Usnea* is still in need of further studies.

Terpenes. The presence of terpenes in the genus *Usnea* is known to have some taxonomic value as for instance zeorin that was reported by Halonen *et al.* (1999) in some shrubby species in Eastern Fennoscandia. More recently Truong *et al.* (2013b) highlighted their importance in the identification of some pendulous species (*U. alata, U. angulata*, *U. crenulata, U. deformis, U. dodgei, U. flaveola, U. malmei, U. moreliana, U. papillata* and *U. rubicunda*). Each of these species was tested by spraying anisaldehyde sulfuric on the wet plates which brought to light a much more complex chemistry. HPLC studies were then conducted but more specific studies are still needed to interpret correctly the results. Monoterpenes were found in faint concentration in *U. malmei/U. papillata* while triterpenes were found in strong concentration in *U. moreliana* (Chapter 4).

Steroids - As far as we know it, and it the genus, sterols were mentioned only for *U. longissima* (Prateeksha *et al.* 2016). We were not able to identify the steroids found in the specimens analyzed with the two controls used (cholesterol and ergosterol). Moreover, both terpenes and steroids presented very similar TLC pattern, appearing as several gray- (steroids) to blue-violet (terpenes) spots in the middle of the plates. Their presence, identification and taxonomic importance deserve further investigations. So far, steroids were detected only in *U. malmei* and *U. papillata*.

Sugar. We identified sugars (mainly arabitol and saccharose) in *U. malmei/U. papillata*. One of these substances identified as sugar was refered as "Us2" in the chapter 2 of this study. It

seems to occur in others species and even in specimens from Costa Rica (Clerc, com. pers.). Their taxonomic importance remains not well understood. For instance it seems to be diagnostic for *U. malmei* /*U. papillata* but can also be found in *U. moreliana* as an accessory substance.

Chemistry correlated with geography. Our molecular data indicated that *U. cornuta s.str.* collected in Brazil has no stictic acid in the medulla while this chemotype occurs frequently in specimens collected in Europe. All Brazilian specimens with stictic acid and identified as *U. cornuta s. l.* (n=21) cluster in two other unrelated clades (Chapter 3).

A strong geographical correlation was found by Truong *et al.* (2013) with the chemotypes of *U. mexicana.* The protocetraric acid chemotype (n=13) was found only in continental South America, whereas the diffractaic acid chemotype was found to occur mainly on the Galapagos Islands (n=10), rarely on the continent (n=2). These authors also mentioned *U. erinacea* with two chemotypes occurring only in the Andes (Truong *et al.* 2011). The same chemotypes were found in Brazil, as well as a new one, the protocetraric acid chemotype.

3. Molecular studies

DNA sequences data were generated for 320 *Usnea* specimens assigned to ca. 48 species. The majority of the sequences were obtained from Brazilian specimens. A small number of sequences (n=39) were obtained from specimens collected in other countries (Corsica, Costa Rica, India, Porto Santo island, continental Spain, Switzerland, Tenerife).

In the genus *Usnea,* phylogeny was mostly inferred via a concatenation approach (Ohmura 2002, Ohmura & Kanda 2004; Kelly *et al.* 2001, Velásquez 2012, Caviró 2015, Truong *et al.* 2013a, Truong & Clerc 2016; Gerlach *et al.* 2017) with few markers (not more than 4) and with only one to very few specimens per species. These approaches have contributed to understand the relationships among some taxa and may provide information to identify the problematic taxa. However, when it comes to the resolution of the species boundaries in the recently diverged groups, this approach may not be accurate because of its assumption that all genes have the same history which is a wrong assumption.

3.1. Species delimitation in the Neotropical *Usnea*: Starting from scratch

The multi-species delimitation analyses using the coalescence approach (MSC) with numerous specimens per species provides a better resolution compared with the concatenation

approach. This approach was first used in the genus *Usnea* by Mark *et al.* (2016) who European and North American specimens. Our study is the first one to study Neotropical Usnea species with the help of the coalescence approach. We found clear differences in the results of these two different approaches [concatenation (not shown here) *versus* coalescence **Chapter 3**] especially regarding the lineages with rapid and recent diversification (e.g. OTU 9, **Chapter 3**).

As one of the main outcomes of this study, we show that the *Usnea cornuta* aggregate is genetically highly diverse and that this diversity is strongly correlated with chemistry as well as with subtle morphological and anatomical characters. This aggregate could be splitted in nine highly supported clades. Out of theses nine clades, six species were newly described (Chapter 5). The remaining three clades are in need of further investigations. We also show that what is known in Europe as *U. cornuta* may be correspond to more than one species.

3.2 Cryptic species

They are well defined genetically but not supported by morphological characters and display no obvious features to identify them (Crespo & Pérez-Ortega 2009). The interpretation of cryptic species is therefore subjective, requiring some degree of qualitative judgment and individual interpretation. In order to better define these cryptic species, the term '**semi-cryptic**' was introduced for genetically distinct species that are morphologically and chemically identical but that can be identified on the base of their distinct ecology and/or their specific distribution areas (Vondrák *et al.* 2009, Hodkinson & Lendemer 2011). Another criteria to define cryptic species more accurately is the phylogenetical position: the term '**pseudo-cryptic**' was used for morphologically similar species but phylogenetically not closely related. **Allopatric cryptic species** was defined for phylogenetically closely related species (sister species) occurring however in different geographic areas (Lücking *et al.* 2016; Dal-Forno *et al.* 2017).

In this study, two unrelated and well supported clades, differing only by the chemistry and co-occurring in two distinct localities were found (OTU 5 and OTU 14, Chapter 3). More studies with larger sampling are needed to decide if they correspond to cryptic species or not. Therefore, cryptic species were not found in this study, but further detailed molecular studies might change this in the future. So far and in most cases, "a posteriori" searches for phenotypic characters, with larger sampling including specimens from herbarium, always

revealed subtle morphological differences (*U. parvula versus U. subparvula*; and the six new species proposed segregate from the *U. cornuta* aggr.).

3.3 Morphological studies

The taxonomy of the genus *Usnea* requires a wide experience and specialists should be consulted to correctly identify samples used in molecular studies. Only this will allow evaluating the value of the phenotypical features on the light of the evolution towards an integrative taxonomy. The molecular approach provide very useful working hypothesis and *a posteriori* searching for phenotypic characters revealed well-defined phenotypic features in the *U. cornuta aggr*., often based on the combination of morphological (e.g. shape of soralia) and anatomical characters (%CMA).

To understand the extent of the intraspecific variation is the great challenge of taxonomists. It requires analyses of numerous specimens under distinct aspects (ecology, morphology, anatomy, chemistry and geography). Before the advent of molecular phylogeny this intraspecific variation was a more subjective concept difficult to test. Nowadays, the molecular approach is used by the majority of taxonomists, and the species diversity may be studied more accurately. A bulk of newly recognized cryptic species was recently proposed among different types of organisms (e.g. Blanquer & Uriz 2008; Crespo & Pérez-Ortega 2009). Sometimes the newly recognized taxa, revealed by molecular tools, are phenotypically well characterized (Moncada *et al.* 2014; Dal-Forno *et al*. 2016, 2017; Chapters 1 and 5) sometimes not (Leavitt *et al.* 2016a). The genetical diversity seems to be often higher than the morphological/anatomical diversity found by traditional taxonomists. One should be however careful because of the danger of overestimating the number of taxa using molecular analysis; (Sukumaran & Knowles 2016). A high number of analytical methods are available to test species boundaries, but their results can be contradictory (Leavitt et al. 2016b). This point out the importance of an accurate analyze of the phenotypical characters in order to interpret correctly the molecular data to pave the way towards an integrative taxonomy and the understanding of biological diversity.

4. Conclusions and Perspectives

Systematics

► All the newly recognized species in this study are not cryptic species. They have rather well-defined phenotypic features, often a combination of morphological, anatomical and chemical characters.

► Some subtle morphological and anatomical features proved to be useful to recognize some species: i. e. density, size and shape of fibrils; size of ascospores;

► The chemistry is a good marker to recognize morphologically similar species;

► Most of species found are endemic to South America; some species show distinct pattern of distribution within Brazil. These patterns are correlated with the biomes.

► Despite their morphological similarity, *Usnea cirrosa* (a paraphyletic species) and *U. cladocarpa* are not closely related. In the same way *U. brasiliensis* and *U. cornuta* are not closely related and both are polyphyletic.

► The *U. cornuta* aggregate is splitted into 9 supported lineages that are strongly correlated with a specific chemistry, and subtle phenotypical characters.

► Diversity in the *U. cornuta* aggr., especially in Brazil (but also in Europe) was so far strongly underestimated;

► The taxa belonging to the *U. cornuta aggr.* in the Neotropical Andes are morphologically similar but genetically divergent. They seem to differ from the Brazilian specimens of the *U. cornuta aggregate*;

► The use of species delimitation under the MSC model provide powerful insights to understand the species limits in this difficult genus; this especially in the clades with rapid and recent diversification;

► Studies using the MSC model are still necessary to accurately delimit some taxa, especially *Usnea cornuta s. str.*, *U. dasaea*, *U. erinacea/U. rubicunda*, *U. cirrosa*, *U. dasaea*, *U. subflammea.* A larger sampling including all geographic and chemical ranges is needed.

Chemistry

► The nature of the “terpenes” mentioned in the literature for *U. malmei*, *U. moreliana* and *U. papillata* was clarified. These “terpenes” include three classes of compounds: sugars

(mainly arabitol and saccharose), steroids, terpenes (monoterpenes and triterpenes). These results demonstrate that the chemistry in *Usnea* is more complex than thought before;

► The presence of sugar seems to be diagnostic for *U. malmei/U. papillata*; the presence of triterpenes is diagnostic for *U. moreliana*;

► Some unidentified compounds were shown to be important taxonomically: Us1aria in *U. arianae*; Us1parv in *U. parvula*; Us2aura in *U. aurantiaca-parvula* and Us2krie in *U. kriegeriana*;

► This study shows that *Usnea* species have a narrower chemical variability that thought before and that many chemotypes might in reality be good species.

The next steps are to refine species delimitation using the MSC models with more markers. This might help to resolve relationships and strengthen support throughout the tree, increasing our knowledge of species diversification in the genus, and then conduct a classical Bayesian phylogenetic analysis with dating as recently suggested by Divakar *et al.* (2017).

In the genomics era, systematics studies are prone to produce a huge quantity of data and whole genomes are now sequenced. For instance, the recent whole genome and transcriptomes sequencing in *Usnea florida* in the framework of the project 1000 Fungal Genomes (GOLD-Genomes online database) will lead to promising results for the *Usnea* systematics in future studies using Next Generation Sequencing.

REFERENCES (cited in the introduction and discussion parts)

Alors, D., Lumbsch, H.T., Divakar, P.K., Leavitt, S.D. & Crespo, A. 2016. An integrative approach for understanding diversity in the *Punctelia rudecta* species complex (Parmeliaceae, Ascomycota). **PLoS ONE 11(2)**: e0146537.

Aptroot, A. 2002. New and interesting lichens and lichenicolous fungi in Brazil. **Fungal Diversity 9:** 15–45

Aptroot, A., Gumboski, E. & Cáceres, M.E. da S. 2017. Ocean view: a first assessment of the littoral, crustose lichen biota of south Brazil. **Lichenologist** (in press).

Articus, K., Mattsson, J.E., Tibell, L., Grube, M. & Wedin, M. 2002. Ribosomal DNA and β–tubulin data do not support the separation of the lichens *Usnea florida* and *U. subfloridana* as distinct species. **Mycological Research 106(4)**: 412–418

Articus, K. 2004. *Neuropogon* and the phylogeny of *Usnea s.l.* (*Parmeliaceae,* Lichenized Ascomycetes). **Taxon 53**: 925–934.

Awasthi, G. 1986. Lichen genus *Usnea* in India. **Journal of the Hattori Botanical Laboratory 61**: 333–421.

Blanco, O., Crespo, A., Ree, R.H. & Lumbsch, H.T. 2006. Major clades of parmelioid lichens (Parmeliaceae, Ascomycota) and the evolution of their morphological and chemical diversity. **Molecular Phylogenetics and Evolution 39:** 52-69.

Blanquer, A. & Uriz, M.J. 2008. 'A posteriori' searching for phenotypic characters to describe new cryptic species of sponges revealed by molecular markers (Scopalina: Dictyonellidae). **Invertebrate Systematics 22**: 1–14.

Boluda, C.G., Rico, V.J., Crespo, A., Divakar, P.K. & Hawksworth, D.L. 2015. Molecular sequence data from populations of *Bryoria fuscescens* s. lat. in the mountains of central Spain indicates a mismatch between haplotypes and chemotypes. **Lichenologist 47**: 279–286.

Boluda, C.G. 2017. **Species, phylogeography and extrolite production in *Bryoria* and *Pseudephebe* (Parmeliaceae)**. PhD dissertation. Spain, Madrid, 431 pp.

Brodo, I. M. 1986. Interpreting chemical variation in lichens for systematic purposes. **The Bryologist 89**: 132–138.

Büdel, B. & Scheidegger, C. 1996. Thallus morphology and anatomy. In: **Lichen Biology**, 1st Ed. (T.H. Nash, III, ed.): 155–180. Cambridge: Cambridge University Press.

Calvelo, S., Stocker-Wörgötter, E., Liberatore, S. & Elix, J.A. 2005. *Protousnea* (Parmeliaceae, Ascomycota), a genus endemic to southern South America. **Bryologist 108**: 1–15.

Carstens, B.C., Pelletier, T.A., Reid, N.M. & Satler, J.D., 2013. How to fail at species delimitation. **Molecular Ecology 22:** 4369–4383.

Caviró, E.A. 2015. **Sistemática integrada del género *Usnea* Dill. Ex Adans. (Parmeliaceae) en la Península Ibérica**. PhD dissertation. Madrid, Spain, 328 pp.

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

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

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

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

Clerc, P. 1994. Comment *Usnea mutabilis* Stirton, une espèce nord-américaine, se cache en Europe sous le nom d'*Usnea marocana* Motyka. Une contribution à la systématique du genre *Usnea* (Ascomycètes lichénisés). **Bulletin de la Société Linnéenne de Provence 45:** 309–316.

Clerc, P. 1998. Species concepts in the genus *Usnea* (lichenized Ascomycetes). **Lichenologist 30**: 321–340.

Clerc, P. 2004. Notes on the genus *Usnea* Adanson. II. **Bibliotheca Lichenologica 88**: 79–90.

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

Clerc, P. 2007: *Usnea*. In: **Lichen Flora of the Greater Sonoran Desert Region** Vol. 3 (T. H. Nash, III, C. Gries & F. Bungartz, eds.): 302–335. Tempe: Lichen Unlimited, Arizona State University.

Clerc, P. 2011a. *Usnea*. In: **Nordic Lichen Flora** Vol. 4 (A. Thell & R. Moberg, eds.): 107–127. Uddevalla: Nordic Lichen Society.

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

Clerc, P. 2016. Notes on the genus *Usnea* (lichenized Ascomycota, Parmeliaceae). IV. **Herzogia 29:** 403–411.

Clerc, P. & Herrera-Campos, M.A. 1997. Saxicolous species of *Usnea* subgenus *Usnea* (lichenized Ascomycetes) in North America. **Bryologist 100**: 281–301.

Crespo, C., Lumbsch, H.T., Mattsson, J.E., Blanco, O., Divakar, P.K., Articus, K., Wiklund, E., Bawingan, P.A. & Wedin, M. 2007. Testing morphology–based hypotheses of phylogenetic relationships in Parmeliaceae (Ascomycota) using three ribosomal markers and the nuclear RPB1 gene. **Molecular Phylogenetics and Evolution 44**: 812–824.

Crespo, A. & Pérez-Ortega, S. 2009. Cryptic species and species pairs in lichens: a discussion on the relationship between molecular phylogenies and morphological characters. **Anales del Jardin Botanico de Madrid 66**: 71–81.

Crespo, A., Kauff, F., Divakar, P.K., del Prado, R., Pérez-Ortega, S., de Paz G.A., Ferencova, Z., Blanco, O., Roca-Valiente, B., Núñez-Zapata, J., Cubas, P., Argüello, A., Elix, J.A., Esslinger, T.L., Hawksworth, D.L., Millanes, A., Molina, M.C., Wedin, M., Ahti, T., Aptroot, A., Barreno, E., Bungartz, F., Calvelo, S., Candan, M., Cole, M., Ertz, D., Goffinet, B., Lindblom, L., Lücking, R., Lutzoni, F., Mattsson, J.E., Messuti, M.I., Miadlikowska, J., Piercey-Normore, M., Rico, V.J., Sipman, H.J.M., Schmitt, I., Spribille, T., Thell, A., Thor, G., Upreti, D.K. & Lumbsch, H.T. 2010. Phylogenetic generic classification of parmelioid lichens (Parmeliaceae, Ascomycota) based on molecular, morphological and chemical evidence. **Taxon 59***:* 1735–1753.

Culberson, W.L. 1969. The use of chemistry in the systematics of the lichens. **Taxon 18**: 152–166.

Culberson, C.F. & Culberson, W.L. 1976. Chemosyndromic variation in lichens. **Systematic Botany 1**: 325–339.

Culberson, C.F. & Ammann, K. 1979. Standard-methode zur Dünnschichtchromatographie von Flechtensubstanzen. **Herzogia 5**: 1–24.

Culberson, C.F. & Johnson, A. 1982. Substitution of methyl tert-butyl ether for diethyl ether in the standardized thin-layer chromatographic method for lichen products. **Journal of Chromatography 238:** 483–487.

Dal Forno, M., Lücking, R., Bungartz, F. *et al*. 2016. From one to six: unrecognized species diversity in the genus *Acantholichen* P.M. Jørg. (lichenized Basidiomycota: Hygrophoraceae). **Mycologia 108**: 38–55.

Dal Forno, M., Bungartz, F., Yánez–Ayabaca, A., Lücking, R., Lawrey, J.D. 2017. High levels of endemism among Galapagos basidiolichens. **Fungal Diversity 85**: 45–73.

Dal Grande, F., Widmer, I., Wagner, H.H. & Scheidegger,, C. 2012. Vertical and horizontal photobiont transmission within populations of a lichen symbiosis. **Molecular Ecology 21**: 3159–3172.

Dal Grande, F., Alors, D., Divakar, P., Bálint, M., Crespo, A. & Schmitt, I. 2014. Insights into intrathalline genetic diversity of the cosmopolitan lichen symbiotic green alga *Trebouxia decolorans* Ahmadjian using microsatellite markers. **Molecular Phylogenetics and Evolution 72**: 54–60.

Dayrat, B. 2005. Towards integrative taxonomy. **Biological Journal of the Linnean Society 85:** 407–415.

de Queiroz, K., 2007. Species concepts and species delimitation. **Systematic Biology 56:** 879–886.

Del Prado, R., Cubas, P., Lumbsch, H.T., Divakar, P.K., Blanco, O., Amo, de Paz G., Molina, M.C. & Crespo, A. 2010. Genetic distances within and among species in monophyletic lineages of Parmeliaceae (Ascomycota) as a tool for taxon delimitation. **Molecular Phylogenetics and Evolution 56**: 125–133.

Divakar, P.K., Crespo, A., Wedin, M., Leavitt, S.D, Hawksworth, D.L., Myllys, L., McCune, B., Randlane, T., Bjerke, J.W., Ohmura, Y., Schmitt, I., Boluda, C.G., Alors, D., Roca–Valiente, B., Del–Prado, R., Ruibal, C., Buaruang, K., Núñez–Zapata, J., Amo de Paz, G., Rico, V.J., Molina, M.C., Elix, J.A., Esslinger, T.L., Tronstad, I.K.K., Lindgren, H., Ertz, D., Gueidan, C., Saag, L., Mark, K., Singh, K., Dal Grande, F., Parnmen, S., Beck, A., Benatti, M.N., Blanchon, D.J., Candan, M., Clerc, P., Goward, T., Grube, M., Hodkinson, B.P., Hur, J.S., Kantvilas, G., Kirika, P.M., Lendemer, J., Mattsson, J.E., Messuti, M.I., Miadlikowska. J., Nelsen, M., Ohlson, J.I., Pérez–Ortega, S., Saag, A., Sipman, H.J.M., Sohrabi, M., Thell, A., Thor, G., Truong, C., Yahr, R., Upreti, D.K., Cubas, P. & Lumbsch, H.T. 2015. Evolution of complex symbiotic relationships in a morphologically derived family of lichen–forming fungi. **New Phytologist 208**: 1217–1226.

Divakar, P.K., Crespo, A., Kraichak, E., Leavitt, S.D., Singh, G., Schmitt, I. & Lumbsch, H.T. 2017. Using a temporal phylogenetic method to harmonize family- and genus-level classification in the largest clade of lichen-forming fungi. **Fungal Diversity 84:** 101–117.

Elix, J.A. 1996. Biochemistry and secondary metabolites. In: **Lichen Biology**, 1st Ed. (T. H. Nash, III, eds.): 155–180. Cambridge: Cambridge University Press.

Elix, J.A. 2014. **A catalogue of standardized chromatographic data and biosynthetic relationships for lichen substances**, 3rd edn. Published by the author, Canberra.

Fernandez, E., Quilhot, W., Rubio, C., Hidalgo, M.E., Diaz, R. & Ojeda, J. 2006. Effects of UV radiation on usnic acid in *Xanthoparmelia microspora* (Müll. Arg.) Hale. **Photochemistry and Photobiology 82:** 1065–1068.

Fleig, M. 1988. Liquens da Estação Ecológica do Taim, Rio Grande, RS, Brasil. **Napaea 6:** 916.

Fleig, M. 1990. Líquens saxícolas, corticícolas e terrícolas do Morro Santana, Rio Grande do Sul. II. Espécies e novas ocorrências. **Pesquisas, Botânica 41:** 33–50.

Fleig, M. 1995. Lichens from "Casa de Pedra" and surroundings, Bagé, Rio Grande do Sul, Brazil. In: **Contributions to lichenology in Honour of Gerhard Follmann** (F.J.A. Daniëls, M. Schulz, J. Peine, eds.): 415–426. Flechten Follmann. Geobotanical and Phytotaxonomical Study Group, Botanical Institute, University of Cologne, Cologne.

Fleig, M. & Grüninger, W. 2008. **Liquens da Floresta com Araucária no Rio Grande do Sul / Flechten des Araukarienwaldes von Rio Grande do Sul / Lichens of the Araucaria Forest of Rio Grande do Sul**. Pro-Mata: Guia de Campo no 3 / Naturführer Nr. 3 / Field Guide No. 3. University of Tübingen, Germany. 217 pp.

Forzza, R.C., Baumgratz, J.F.A., Bicudo, C.E.M., Canhos, D.A.L., Carvalho, A.A., Coelho, M.A.N., Costa, A.F., Costa, D.P., Hopkins, M.G., Leitman, P.M., Lohmann, L.G., Lughadha, E.N., Maia, L.C., Martinelli, G., Menezes, M., Morim, M.P., Peixoto, A.L., Pirani, J.R., Prado, J., Queiroz, L.P., Souza, S., Souza, V.C., Stehmann, J.R., Sylvestre, L.S., Walter, B.M.T. & Zappi, D.C. 2012. New Brazilian Floristic List highlights Conservation Challenges. **BioScience 62**: 39–45.

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

Galloway, D. 2007. **Flora of New Zealand Lichens**. Revised Second Edition Including Lichen-Forming and Lichenicolous Fungi. Volumes 1 and 2. Manaaki Whenua Press, Lincoln, New Zealand. 2261 pp.

Gerlach, A.C.L., Clerc, P. & da Silveira, R.M.B. 2017. Taxonomy of the corticolous, shrubby, esorediate, neotropical species of *Usnea* Adans. (Parmeliaceae) with an emphasis on Southern Brazil. **Lichenologist 49**: 199–238.

Grube, M. & Wedin, M. 2016. Lichenized fungi and the evolution of symbiotic organization. **Microbiology spectrum 4(6):** FUNK-0011-2016.

Gumboski, E.L. & Eliasaro, S. 2011. Checklist of lichenized fungi of Santa Catarina State (Brazil). **Mycotaxon 115**: 535–535

Hahn, M.W. & Nakhleh, L. 2016. Irrational exuberance for resolved species trees. **Evolution 70:** 7–17.

Hale, M.E. Jr. 1979. **How to Know the Lichens**, 2nd edition. Dubuque, Iowa: William C. Brown.

Hale, M.E. 1983. **The Biology of Lichens**. Third Edition. Edward Arnold, London. 190 pp.

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

Halonen, P., Myllys, L., Ahti, T. & Petrova, O.V. 1999. The lichen genus *Usnea* in East Fennoscandia. III. The shrubby species. **Annales Botanici Fennici 36**: 235–256.

Halonen, P. 2000. **Studies on the lichen genus *Usnea* in East Fennoscandia and Pacific North America**. PhD thesis. Oulu: University of Oulu.

Hawksworth, D.L. 1976. Lichen chemotaxonomy. In: **Lichenology: Progress and Problems.** (D.H. Brown, D.L. Hawksworth & R.H. Bailey, eds.): 139–184. London: Academic Press.

Herbário Alexandre Leal Costa (ALCB), *Arizona State University Lichen Herbarium* (ASU–Lichen), *Herbário da Universidade Federal de Minas Gerais* (BHCB), *Botanical Collections* (BM), *Herbário da Embrapa Recursos Genéticos e Biotecnologia* (CEN), *Herbário do Centro de Pesquisas do Cacau* (CEPEC), *Herbário Leopoldo Krieger* (CESJ), *Herbário da Fundação Universidade Federal de Mato Grosso do Sul* (CGMS), *Herbário da Universidade Federal de Mato Grosso do Sul, Campus Pantanal* (COR), *Herbário CPAP da Embrapa Pantanal* (CPAP), *Royal Botanic Garden Edinburgh Herbarium* (E), *Herbário Escola de Florestas Curitiba* (EFC), *Herbário da Escola Superior de Agricultura Luiz de Queiroz* (ESA), *Herbário Friburguense* (FCAB), *Herbário do Departamento de Botânica da Universidade Federal de Santa Catarina* (FLOR), *Geneva Herbaria Catalogue with species Brazil* (G), *Herbário Alarich Rudolf Holger Schultz* (HAS), *Herbário do Museu de Ciências Naturais da PUC–Minas* (HPUC–MG), *Herbário da Pontifícia Universidade Católica do Paraná* (HUCP), *Herbário Unisanta* (HUSC), *Herbário do Instituto Agronômico de Campinas* (IAC), *Herbário do Instituto de Ciências Naturais* (ICN), *Herbário – IPA Dárdano de Andrade*

Lima (IPA), *Herbário do Parque da Ciência Newton Freire Maia* (IRAI), *Herbário Lauro Pires Xavier* (JPB), *Herbário do Museu Botânico Municipal* (MBM), *Herbário Mello Leitão* (MBML–Herbario), *Herbário do Museu da Pontifícia Universidade Católica do Rio Grande do Sul* (MPUC), *OBIS Brasil* (OBIS_BR), *MNHN – Herbário Virtual A. de Saint–Hilaire* (P), *Herbarium Anchieta – Fungi Rickiani* (PACA–Fungi), *Herbário do Museu Nacional – Criptogamos* (R–Criptogamos), *Herbário de São José do Rio Preto* (SJRP), *Herbário de fungos de São José do Rio Preto* (SJRP–Fungi), *Maria Eneyda Pacheco Kauffmann Fidalgo* (SP–Fungi), *Herbário Dom Bento José Pickel* (SPSF), *Herbário da Universidade de Brasília* (UB), *Herbário da Universidade Federal de Goiás* (UFG), *Herbário UFP – Geraldo Mariz* (UFP), *Herbário da Universidade Estadual do Oeste do Paraná* (UNOP–Algae), *Herbário da Universidade Federal do Paraná* (UPCB), *NMNH Extant Specimen and Observation Records* (US), *Herbário Central da Universidade Federal do Espírito Santo – Coleção de Fungos* (VIES–Fungi), *Wisconsin State Herbarium* (WIS) disponível na **rede *species*Link** (http:www.splink.org.br) em **25 de Abril de 2017** às **08:28**.

Herrera-Campos, M.A., Clerc, P. & Nash, T.H., III. 1998. Pendulous species of *Usnea* from the temperate forests in Mexico. **Bryologist 101**: 303–329.

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

Herrera-Campos, M.A. 2016. *Usnea* in Mexico. **Bibliotheca Lichenologica 110**: 505–620.

Hinds, J.W. & Hinds, P.L. 2007. The Macrolichens of New England. In: **Memoirs of the New York Botanical Garden No. 96.** New York: New York Botanical Garden Press, Bronx, 584 pp.

Hodkinson, B.P. & Lendemer, J.C. 2011. Molecular analyses reveal semi–cryptic species in *Xanthoparmelia tasmanica.* **Bibliotheca Lichenologica 106**: 115–126.

Honda, N.K. & Vilegas, W. 1998. A química dos liquens. **Química Nova 21(6)**: 110–125.

Huelsenbeck, J.P., Bull, J.J. & Cunningham, C.W. 1996. Combining data in phylogenetic analysis. **Trends in ecology & evolution 11**:152–158.

Huneck, S. 1999. The significance of lichens and their metabolites. **Naturwissenschaften 86:** 559–570.

IBGE (Instituto Brasileiro de Geografia e Estatística). 2004. Mapa da vegetação do Brasil e Mapa de Biomas do Brasil. Available from: **http://www.ibge. gov.br**.

Iganci, J.R.V., Heiden, G., Miotto, S.T.S. & Pennington, R.T. 2011. Campos de Cima da Serra: the Brazilian Subtropical Highland Grasslands show an unexpected level of plant endemism. **Botanical Journal of the Linnean Society 167**: 378–393.

Kalb, K. 1982a. **Lichenes Neotropici** (K. Kalb, ed.) Neumarkt, OPf. Fascikel IV (No. 121-160). 12 pp.

Kalb, K. 1982b. **Lichenes Neotropici** (K. Kalb, ed.). Neumarkt, OPf. Fascikel V (No. 161-200). 12 pp.

Kalb, K. 1986. **Lichenes Neotropici**. (K. Kalb, ed.). Neumarkt, OPf. Fascikel IX (No. 351-400). 16 pp.

Kärnefelt, I. 1979. The brown fruticose species of *Cetraria*. **Opera Botanica 46:** 1–150.

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

Kirk, P.M., Cannon, P.F., David, J.C. & Stalpers, J.A. 2008. **Ainsworth & Bisby's Dictionary of the Fungi**, 10. ed. Wallingford, Oxon: CABI International, 771 pp.

Kraichak, E., Divakar, P.K., Crespo, A., Leavitt, S.D., Nelsen, M.P., Lücking, R. & Lumbsch, H.T. 2015. A tale of two hyper-diversities: diversification dynamics of the two largest families of lichenized fungi. **Scientific Reports 5**:10028.

Krempelhuber, A. 1868. Exotische Flechten aus dem Herbar des k. k. botanischen Hofkabinetes in Wien. **Verhandlungen der K. K. Zoologisch-botanischen Gesellschaft in Wien 18:** 303-330.

Krempelhuber, A. 1873. Lichenes brasiliensis. In: **Symbolae ad floram Brasiliae centralis cognoscendam. Particula XIV.** (Warming, E., ed.). Videnskabelige Meddelelser fra den naturhistorisk Forening i Kjöbenhavn 5: 1–35.

Krempelhuber, A. 1876. Lichenes Brasiliensis, collecti a D.A. Glaziou in provincia brasiliensi Rio Janeiro (continuatio). **Flora 59:** 56–63.

Krog, H. 1976. *Lethariella* and *Protousnea*, two new lichen genera in the Parmeliaceae. **Norwegian Journal of Botany 2**3: 83–106.

Krog, H. 1994. New observations on *Usnea* subgenus *Eumitria* in eastern and central Africa. In: **Proceedings of the XIII plenary meeting AETFAT Vol. 2** (J. Seyani & A. Chikuni, eds.): 813–821. Malawi: National Herbarium and Botanic Gardens.

Le Pogam, P. Herbette, G. & Boustie, J. 2015. Analysis of Lichen Metabolites, a Variety of Approaches. In: **Recent Advances in Lichenology** Vol 1 (D.K. Upreti, P.K. Divakar, V. Shukla, & R. Bajpai, eds): 229–261. Springer India.

Leavitt, S.D., Moreau, C.S. & Lumbsch, H.T. 2015. The dynamic discipline of species delimitation: progress toward effectively recognizing species boundaries in natural populations. In: **Recent Advances in Lichenology** Vol 2 (D.K. Upreti, P.K. Divakar, V. Shukla, & R. Bajpai, eds): 11–44. Springer India.

Leavitt, S.D., Esslinger, T.L., Pradeep, K.D., Crespo, A. & Lumbsch, H.T. 2016a. Hidden diversity before our eyes: Delimiting and describing cryptic lichen-forming fungal species in camouflage lichens (Parmeliaceae, Ascomycota). **Fungal Biology 120**: 1374–1391.

Leavitt, S.D., Pradeep, K.D., Crespo, A. & Lumbsch, H.T. 2016b. A matter of time – understanding the limits of the power of molecular data for delimiting species boundaries. **Herzogia 29**: 479–492.

Leliaert, F., Verbruggen, H., Vanormelingen, P., Steen, F., Lopez–Bautista, J.M., Zuccarello, G.C. & De Clerck, O. 2014. DNA-based species delimitation in algae. **European Journal of Phycology 49**: 179–196.

Lücking, R., Dal Forno, M., Sikaroodi, M., Gillevet, P.M., Bungartz, F., Moncada, B., Yánez–Ayabaca, A., Chaves, J.L., Coca, L.F., Lawrey, J.D. 2014, A single macrolichen constitutes hundreds of unrecognized species. **Proceedings of the National Academy of Sciences U.S.A. 111**: 11091–11096.

Lücking, R., Hodkinson, B.P. & Leavitt, S.D. 2016. The 2016 classification of lichenized fungi in the Ascomycota and Basidiomycota – Approaching one thousand genera. **Bryologist 119**: 361–416.

Lücking, R., Dal Forno, M., Moncada, B. *et al*. 2016. Turbo-taxonomy to assemble a megadiverse lichen genus: seventy new species of *Cora* (Basidiomycota: Agaricales: Hygrophoraceae), honouring David Leslie Hawksworth's seventieth birthday. **Fungal Diversity 84**: 139–207.

Lumbsch, H.T. & Wirtz, N. 2011. Phylogenetic relationships of the neuropogonoid core group in the genus *Usnea* (Ascomycota: Parmeliaceae). **Lichenologist 43**: 553–559.

Lumbsch, H.T. 1998. The use of metabolic data in lichenology at the species and subspecific levels. **Lichenologist**: 357–367.

Lumbsch, H.T. 2002. Analysis of phenolic products in lichens for identification and taxonomy. In: **Protocols in Lichenology. Culturing, Biochemistry, Ecophysiology and Use in Biomonitoring**. (I. Kranner, R.P. Beckett, R.P.Varma, A.K., eds): 281–295. Berlin: Springer-Verlag, Heidelberg.

Mark, K., Saag, L., Leavitt, S.D., Will-Wolf, S., Nelsen, M.P., Tõrra, T., Saag, A., Randlane, T. & Lumbsch, H.T. 2016. Evaluation of traditionally circumscribed species in the lichen-forming genus *Usnea*, section *Usnea* (Parmeliaceae, Ascomycota) using a six-locus dataset. **Organisms Diversity & Evolution 16**: 497–524.

Marcelli, M.P. 1998a. History and current knowledge of Brazilian lichenology. In: **Lichenology in Latin America: History, Current Knowledge and Applications. (**M.P. Marcelli & M.R.D. Seaward, eds.): 25–45. Brazil: Estado de Sao Paulo, São Paulo, Companhia de Tecnologia de Saneamento Ambiental, CETESB.

Marcelli, M.P. 1998b. Aspects on Vainio's Brazilian "Étude..." with keys to its species. In: **Recollecting Edvard August Vainio** (M.P. Marcelli & T. Ahti, eds.): 113–188. Brazil: Estado de Sao Paulo, São Paulo, Companhia de Tecnologia de Saneamento Ambiental, CETESB.

Martius, C.F.P. 1833. **Flora Brasiliensis seu enumeratio plantarum**. Vol. 1 (1). **Algae, lichenes, hepatiae**. Exposuerunt Martius, Eschweiler, Nees ab Esenbeck. Stuttgartiae et Tubingiae. 1–390.

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

Molnár, K. & Farkas, E. 2010. Current results on biological activities of lichen secondary metabolites: a review. **Zeitschrift für Naturforschung—Section C, Biosciences 65**: 157–173.

Moncada, B. & Lücking, R. 2012. Ten new species of *Sticta* and counting: Colombia as a hot spot for unrecognized diversification in a conspicuous macrolichen genus. **Phytotaxa 74**: 1–29.

Moncada, B., Lücking, R. & Suárez, A. 2014. Molecular phylogeny of the genus *Sticta* (lichenized Ascomycota: Lobariaceae) in Colombia. **Fungal Diversity 64**: 205–231.

Motyka, J. 1936. **Lichenum generis *Usnea* studium monographicum**. Pars systematica (Vol. 1). Leopoldi (privately printed).

Motyka, J. 1938. **Lichenum generis *Usnea* studium monographicum**. Pars systematica (Vol. 2). Leopoldi (privately printed).

Müller Argoviensis, J. 1881. Lichenologische Beiträge 12. **Flora 64(6):** 81–88.

Müller Argoviensis, J. 1887. Lichenologische Beiträge 25. **Flora 70(4):** 56–64.

Müller Argoviensis, J. 1891a. Lichenes Schenckiani. **Hedwigia 5**: 219–234.

Müller Argoviensis, J. 1891b. Lichenes Catharinenses. **Hedwigia 5**: 235–243.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B. & Kent, K. 2000. Biodiversity hotspots for conservation priorities. **Nature 403**: 853–858.

Naciri, Y. & Linder, H.P. 2015. Species delimitation and relationships: The dance of the seven veils. **Taxon 64**: 3–16.

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

Ohmura, Y. 2002. Phylogenetic evaluation of infrageneric groups of the genus *Usnea* based on ITS regions in rDNA. **Journal of the Hattori Botanical Laboratory 92**: 231–243.

Ohmura, Y. & Kanda, H. 2004. Taxonomic status of section *Neuropogon* in the genus *Usnea* elucidated by morphological comparisons and ITS rDNA sequences. **Lichenologist 36**: 217–225.

Ohmura, Y., Moon, K.-H. & Kashiwadani, H. 2008. Morphology and molecular phylogeny of *Ramalina pollinaria*, *R. sekika* and *R. yasudae* (Ramalinaceae, lichenized Ascomycotina). **Journal of Japanese Botany 83**: 156–164.

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

Ohmura, Y., Skirina, I. & Skirin, F. 2017. Contribution to the knowledge of the genus *Usnea* (Parmeliaceae, Ascomycota) in southern far East Russia. **Bulletin of the National Museum of Nature and Science, series B 43(1):** 1–10.

Oliveira–Filho, A.T., Budke, J.C., Jarenkow, J.A., Eisenlohr, P.V. & Neves, D.R.M. 2015. Delving into the variations in tree species composition and richness across South American subtropical Atlantic and Pampean forests. **Journal of Plant Ecology 8:** 242–260.

Osorio, H.S. & Fleig, M. 1986. Contribution to the lichen flora of Brazil XVIII. Lichens from Itaimbezinho, Rio Grande do Sul State. **Comunicaciones Botanicas del Museo de Historia Natural de Montevideo 4:** 1–8.

Osorio, H.S. & Fleig, M. 1988a. Contribution to the lichen flora of Brazil. XX. Additional records from Sao Francisco de Paula, Rio Grande do Sul State. **Comunicaciones Botanicas del Museo de Historia Natural de Montevideo 5:** 1–7.

Osorio, H.S. & Fleig, M. 1988b. Contribution to the lichen flora of Brazil. XXI. Lichens from Morro Santana, Rio Grande do Sul State. **Comunicaciones Botanicas del Museo deHistoria Natural de Montevideo 5:** 103.

Osorio, H.S. & Fleig, M. 1989. Contribution to the lichen flora of Brazil. XXII. Lichens from Canela, Rio Grande do Sul State. **Comunicaciones Botanicas del Museo de Historia Natural de Montevideo 5**: 1–4.

Osorio, H.S. & Fleig, M. 1990. Contribution to the lichen flora of Brazil. XXVI. Lichens from "Vale do Diabo", Rio Grande do Sul State. **Comunicaciones Botanicas del Museo de Historia Natural de Montevideo 5**: 1–6.

Osorio, H.S. & Fleig, M. 1991. Contribution to the lichen flora of Brazil. XXVIII. Lichens from northern Santa Maria, Rio Grande do Sul State. **Comunicaciones Botánicas del Museo de Historia Natural de Montevideo 5(96):** 1–7.

Osorio, H.S. & Fleig, M. 1994. Contribution to the lichen flora of Brazil. XXXI. Lichens from Julio de Castilhos, Rio Grande do Sul State. **Comunicaciones Botánicas del Museo de Historia Natural de Montevideo 5(101):** 1–7.

Overbeck, G.E., Müller, S.C., Fidelis, A.T., Pfadenhauer, J., Pillar, V.D., Blanco, C., Boldrini, I.I., Both, R. & Forneck, E.D. 2007. Brazil's neglected biome: the South Brazilian Campos. **Perspectives in Plant Ecology, Evolution and Systematics 9:** 101–116.

Pante, E., Puillandre, N., Viricel, A., Arnaud-Haond, S., Aurelle, D., Castelin, M., Chenuil, A., Destombe, C., Forcioli, D., Valero, M., Viard, F. & Samadi, S. 2015. Species are hypotheses: avoid connectivity assessments based on pillars of sand. **Molecular Ecology 24**: 525–544.

Pérez-Vargas, I., Padrón, C.H., Arroyo, R. & Seriñá, E. 2010. *Usnea brasiliensis* (Zahlbr.) Motyka (Parmeliaceae), a new amphi–Atlantic disjunct lichen species. **Bryologist 113:** 308–312.

Poelt, J. 1970. Das Konzept der Artenpaare bei den Flechten. **Deutsche Botanische Gesellschaft, neue Folge 4**: 187–198.

Poelt, J. 1972. Die taxonomische Behandlung von Artenpaare bei den Flechten. **Botanische Notiser 125**: 77–81.

Prateeksha, B.S.P., Bajpai, R., Jadaun, V., Kumar, J., Kumar, S., Upreti, D.K., Singh, B.R., Nayaka, S., Joshid, Y. & Singh, B.N. 2016. The genus *Usnea*: a potent phytomedicine with multifarious ethnobotany, phytochemistry and pharmacology. **Royal Society of Chemistry Advances 6**: 21672–21696.

Rannala, B. & Yang, Z. 2003. Bayes estimation of species divergence times and ancestral population sizes using DNA sequences from multiple loci. **Genetics 164:** 1645–1656.

Rizzini, C.T. 1952. Species Organenses generis lichenum *Usnea*e. (Omnes acidum usnicum praebentes). **Revista Brasileira de Biologia 12(4)**: 337–348.

Rizzini, C.T. 1956. Flora Organensis. Lichenes. **Revista Brasileira de Biologia 16(4)**: 387–402.

Rodriguez, J.M. 2011. **El género *Usnea* (Ascomycetes liquenizados) en Argentina: estudio sistemático y biogeográfico**. Tesis de doctorado en Ciencias Biologicas. Córdoba, Argentina. 217 pp.

Rodriguez, J.M., Estrabou, C., Truong, C. & Clerc, P. 2011. The saxicolous species of the genus *Usnea* subgenus *Usnea* (Parmeliaceae) in Argentina. **Bryologist 114**: 504–525.

Schmitt, I., Lumbsch, H.T. 2004. Molecular phylogeny of the Pertusariaceae supports secondary chemistry as an important systematic character set in lichen–forming ascomycetes. **Molecular Phylogenetics and Evolution 33**: 43–55.

Schmitt, I., Crespo, A., Divakar, P.K., Fankhauser, J.D., Herman-Sackett, E., Kalb, K., Nelsen , M.P., Nelson, N.A., Rivas-Plata, E., Shimp, A.D., Widhelm, T. & Lumbsch, H.T. 2009. New primers for promising single-copy genes in fungal phylogenetics and systematics. **Persoonia 23**: 35–40.

Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W.T. & Fungal Barcoding Consortium 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. **Proceedings of the National Academy of Sciences 109**: 6241–6246

Seymour, F.A., Crittenden, P.D. & Dyer, P.S. 2005. Sex in the extremes: lichen–forming fungi. **Mycologist 19(2)**: 51–58.

Singh, G., Dal Grande, F., Divakar, P.K., Otte, J., Leavitt, S.D., Szczepanska, K., Crespo, A., Rico, V.J., Aptroot, A., Cáceres, M.E., Lumbsch, H.T. & Schmitt, I. 2015. Coalescent-based species delimitation approach uncovers high cryptic diversity in the cosmopolitan lichen-forming fungal genus *Protoparmelia* (Lecanorales, Ascomycota). **PLoS ONE, 10(5)**, e0124625.

Spielmann, A.A. 2006. Checklist of lichens and lichenicolous fungi of Rio Grande do Sul (Brazil). **Caderno de Pesquisa Série Biologia 18(2)**: 7–125.

Spribille, T., Klug, B., Mayrhofer, H., 2011. A phylogenetic analysis of the boreal lichen *Mycoblastus sanguinarius* (Mycoblastaceae, lichenized Ascomycota) reveals cryptic clades correlated with fatty acid profiles. **Molecular Phylogenetics and Evolution 59**: 603–614.

Spribille, T., Tuovinen, V., Resl, P., Vanderpool, D., Wolinski, H., Aime, M.C., Schneider, K., Stabentheiner, E., Toome–Heller, M., Thor, G., Mayrhofer, H., Johannesson, H. & Cutcheon, J.P. 2016. Basidiomycete yeasts in the cortex of ascomycete macrolichens. **Science 353**: 488–492.

Stevens, G.N. 1992. Variation in thallus morphology in response to climatic and geographical distribution in an *Usnea* complex. **Lichenologist 24:** 229–248.

Stevens, G.N. 1999. A revision of the lichen family Usneaceae in Australia. **Bibliotheca Lichenologica 72**: 1–128.

Stevens, G.N. 2004. Usneaceae. In: **Flora of Australia** Vol. 56A, Lichens 4 (P. M. McCarthy & K. Mallett, eds.): 78–98 & 107–115. Melbourne: ABRS/CSIRO.

Stocker-Wörgötter, E. 2015. Biochemical diversity and ecology of lichen–forming Fungi: Lichen substances, chemosyndromic variation and origin of polyketide–type metabolites (biosynthetic pathways). In: **Recent Advances in Lichenology** Vol 2 (D.K. Upreti, P.K. Divakar, V. Shukla & R. Bajpai, eds.): 161–179. India: Springer, New Delhi.

Swinscow, T.D.V. & Krog, H. 1974. *Usnea* subgenus *Eumitria* in East Africa**. Norwegian Journal of Botany 21**: 165–185.

Swinscow, T.D.V. & Krog, H. 1976a. The *Usnea articulata* aggregate in East Africa. **Norwegian Journal of Botany 23**: 261–268.

Swinscow, T.D.V. & Krog, H. 1976b. The *Usnea bornmuelleri* aggregate in East Africa. **Norwegian Journal of Botany 23**: 23–31.

Swinscow, T.D.V. & Krog, H. 1978. Pendulous species of *Usnea* in East Africa. **Norwegian Journal of Botany 25**: 221–241.

Swinscow, T.D.V. & Krog, H. 1979. The fruticose species of *Usnea* subgenus *Usnea* in East Africa. **Lichenologist 11**: 207–252.

Swinscow, T.D.V. & Krog, H. 1988. **Macrolichens of East Africa**. British Museum (Natural History), London. 390 pp.

Sukumaran, J. & Knowles, L.L. 2016. Multispecies coalescent delimits structure, not species. **Proceedings of the National Academy of Sciences of the United States of America 114:** 1607–1612.

Tavares, I.I. & Sanders, W.B. 1998. Preliminary report on the short, apotheciate taxa of *Usnea* in the southwestern United States. In: **Lichenographia Thomsoniana: North American Lichenology in Honor of John W. Thomson** (M. G. Glenn, R. C. Harris, R. Dirig & M. S. Cole, eds): 171–185. Ithaca, New York: Mycotaxon Ltd.

Tehler, A. 1982. The species pair concept in lichenology. **Taxon 31:** 708–717.

Thell, A., Crespo, A., Divakar, P.K., Kärnefelt, I., Leavitt, S.D., Lumbsch, H.T. & Seaward, M.R.D. 2012. A review of the lichen family Parmeliaceae–history phylogeny and current taxonomy. **Nordic Journal of Botany 30**: 641–664.

Truong, C. 2012. **Systematics of the lichen genus *Usnea* in tropical South America**. PhD dissertation. University of Geneva, Faculty of Sciences. 286 pp.

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

Truong, C. & Clerc, P. 2012. The lichen genus *Usnea* (Parmeliaceae) in tropical South America: species with a pigmented medulla, reacting C+ yellow. **Lichenologist 44**: 625–637.

Truong, C. & Clerc, P. 2013. Eumitrioid *Usnea* species (Parmeliaceae, lichenized Ascomycota) in tropical South America and the Galapagos. **Lichenologist 45**: 383–395.

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

Truong, C., Rodriguez, J.M. & Clerc, P. 2013b. Pendulous *Usnea* species (Parmeliaceae, lichenized Ascomycota) in tropical South America and the Galapagos. **Lichenologist 45**: 505–543.

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

Van den Boom, P.P.G., Clerc, P., Ertz, D. 2015. New records of lichens and lichenicolous fungi from La Gomera (Canary Islands, Spain), including the new species: *Usnea boomiana* P. Clerc. **Candollea 70(2)**: 165–177.

Vandamme, A.M. 2009. Basic concepts of molecular evolution. In: **The Phylogenetic Handbook: A Practical Approach to Phylogenetic Analysis and Hypothesis Testing** (P. Lemey & M. Salemi, eds): 751 pp. Cambridge: Cambridge University Press.

Vainio, E.A. 1890. Étude sur la classification naturelle et la morphologie des lichens du Brésil, pars prima. **Acta Societatis pro Fauna et Flora Fennica 7(1)**: 1–247.

Velásquez, S.L.A. 2012. **Diversidad genética y delimitación de especies de *Usnea* (Parmeliaceae ; Ascomycetes liquenizados) en Bosques Templados de México.** Tesis para obtenir el Título de Bióloga. Universidad Nacional Autónoma de México, Mexico. 209 pp.

Vondrák, J., Ríha, P., Arup, U., Søchting, U., 2009. The taxonomy of the *Caloplaca citrina* group (Teloschistaceae) in the Black Sea region, with contributions to the cryptic species concept in lichenology. **Lichenologist 41**: 571–604.

Walker, F.J. 1985. The lichen genus *Usnea* subgenus *Neuropogon*. **Bulletin of the British Museum (Natural History), Botany 13**: 1–130.

Wei, X., McCune, B., Lumbsch, H.T., Li. H., Leavitt, S., Yamamoto, Y., Tchabanenko, S. & Wei, J. 2016. Limitations of species delimitation based on phylogenetic analyses: A case study in the *Hypogymnia hypotrypa* group (Parmeliaceae, Ascomycota). **PLoS ONE 11(11)**: e0163664.

Wilkins, J.S. 2011. Philosophically speaking, how many species concepts are there? **Zootaxa 2765:** 58–60.

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

Wirtz, N., Printzen, C. & Lumbsch, H.T. 2008. The delimitation of Antarctic and bipolar species of neuropogonoid *Usnea* (Ascomycota, Lecanorales): A cohesion approach of species recognition for the *Usnea perpusilla* complex. **Mycological Research 112:** 472–484.

Wirtz, N., Printzen, C. & Lumbsch, H.T. 2012. Using haplotype networks, estimation of gene flow and phenotypic characters to understand species delimitation in fungi of a predominantly Antarctic *Usnea* group (Ascomycota, Parmeliaceae). **Organisms Diversity & Evolution 12:** 17–37.

Wittmann, F., Marques, M.C.M., Damasceno G.J., Budke, J.C., Piedade, M.T.F., Wittmann, A. de O., Montero, J.C., de Assis, R.L., Targhetta, N., Parolin, P., Junk, W.J., & Householder, J.E. 2017. The Brazilian freshwater wetscape: Changes in tree community

diversity and composition on climatic and geographic gradients. **PLoS ONE 12(4):** e0175003.

Xi, Z., Liu, L., Rest, J.S. & Davis, C.C. 2014. Coalescent versus Concatenation Methods and the Placement of *Amborella* as Sister to Water Lilies. **Society of Systematic Biologists 63(6)**: 919–932.

Yasmín, L. V., Moncada, B., Lücking, R. 2014. Five new species of *Cora* and *Dictyonema* (Basidiomycota: Hygrophoraceae) from Colombia: chipping away at cataloging hundreds of unrecognized taxa. **Bryologist 117**: 368–378.

Zahlbruckner, A. 1902. Studien über brasilianische Flechten. **Sitzungsberichte der Akademie der Wissenschaften Wien, matematischnaturwissenschaftliche Classe, 111**: 357–432.

Zahlbruckner, A. 1904. Lichenes a cl. Damazio in montibus Serra do Ouro Preto Brasiliae lecti, in herb. Barbey-Boissier asservati. **Bulletin de L'Herbier Boissier, 2^{me} série, 4:** 134–136.

Zahlbruckner, A. 1905. Lichenes a cl. Damazio in Brasilia lecti II. **Bulletin de L'Herbier Boissier, 2^{me} série, 5:** 539–543.

Zahlbruckner, A. 1909. Lichenes (Flechten). In: **Ergebnisse der botanischen Expedition der kaiserlichen Akademie der Wissenschaften nach Südbrasilien, 1901, 2. Band. – \Denkschr.** (V. Schiffner, ed.). Kaiserl. Akad. der Wissensch. 83: 85–211.

APPENDICES

Appendix 1. Species described from Brazil (not included the ones proposed in this thesis). * incertae species (type not found); ** not found in this study. Species in bold are accepted species.

Species	**Syn.**	**Reference**
U. alata Motyka	–	**Chapter 2**
U. angulata var. rubiginosa Hillmann	U. sulcata	Motyka (1938: 478)
U. arthroclada Fée	U. sulcata	Motyka (1938: 478)
U. articulata var. intestiniformis Nyl.**	≠ U. articulata	**Chapter 2**
U. aspera (Eschw.) Vain.	–	**Chapter 6**
U. baileyi f. implexa Zahlbr.	U. inanis	Motyka (1936: 58)
U. barbata var. strigosa f. ferruginascens Müll. Arg.	=? U. tincta	
U. barbata var. dasopoga f. fuscorufa Müll. Arg.	U. subelegans	Motyka (1938: 520)
U. barbata var. florida stat. rubescens Müll. Arg.	U. florida	
U. bornmuelleri var. brasiliensis f. inactiva Zahlbr.	=? U. brasiliensis	**Chapter 5**
U. brasiliensis Zahlbr	–	**Chapters 3, 5**
U. ceratina var. reagens Zahlbr.	U. cladocarpa	Motyka (1938: 586)
U. ceratina f. pusilla Kremp.	U. aspera	Motyka (1938: 643)
U. cinchonarum var. inactiva Zahlbr.	–	
U. cladocarpa Fée	–	**Chapter 1**
U. complecta (Müll. Arg.) Motyka**	–	
U. concinna Stirt.	–	**Chapter 1**
U. disjuncta (Motyka) A. Gerlach & P. Clerc**	–	**Chapter 2**
U. duriuscula Motyka	U. mexicana	Herrera-Campos et al. (1998)
U. elongata f. sorediifera Rizzini*	–	
U. fallax Motyka	=? *U. dodgei*	**Chapter 2**
U. feeana Motyka**	≠ *U. dasaea*	
U. florida var. scabrosa Zahbr.	U. concinna	**Chapter 1**
U. firma Motyka**	–	
U. furfurosula (Zahlbr.) Motyka	*U. dasaea*	Clerc & Herrera-Campos (1998)
U. inanis Motyka	*U. baileyi*	Truong et al. (2013)
U. laevis(Eschw.) Nyl	–	**Chapter 6**
U. leioclada (Zahlbr.) Motyka**	–	–
U. ludicra Rizzini*	–	–
U. lunaria Motyka	–	**Chapter 1**
U. malmei Motyka	–	**Chapter 2**
U. meridionalis Zahlbr.	–	**Chapter 1**
U. papillata Motyka	–	**Chapter 2**
U. paradoxa Motyka	U. angulata	Truong et al. (2013)
U. poliothrix Kremp.	–	Appendices 2

Species	**Syn.**	**Reference**
U. radiata Stirt.	U. concinna	**Chapter 1**
U. regia (Kremp.) Motyka	–	**Chapter 2**
U. regnellii Motyka**	–	
U. repens Motyka	*U. malmei*	Truong et al. (2013)
U. santae-annae Motyka	*U. subscabrosa*	Clerc (1997)
U. sorediata (Zahlbr.) Motyka	*U. malmei*	Herrera-Campos et al. (1998)
U. spinulifera (Vain.) Motyka	*U. dasaea*	Clerc & Herrera-Campos (1997)
U. spinulosa (Müll. Arg.) Motyka	*U. regia*	Truong et al. (2013)
U. spinulosa var. scabra (Müll. Arg.) Motyka**	–	
U. steineri f. robusta Sambo	=? U. steineri	–
U. steineri Zahlbr.	–	**Chapter 1**
U. subcavata Motyka	U. perplectata	Truong et al. (2013)
U. subcomosa (Vain.) Vain.	U. ceratina	Halonen et al. (1999)
U. subelegans (Vain.) Räsänen	–	**Chapter 1**
U. sulcata Motyka	U. alata	Truong et al. (2013)
U. superba Motyka	U. arthroclada	Truong et al. (2013)
U. tincta (Zahlbr.) Motyka	=? U. subelegans	**Chapter 1**
U. trachyclada (Müll. Arg.) Zahlbr.**	–	
U. venusta Motyka	≠ U. articulata	**Chapter 2**

Appendix 2: List of species identified on this study

		species	**author**	**This thesis**
1	Usnea	aff. disjuncta		**chapter 2**
2	Usnea	alata	Motyka	**chapter 2**
3	Usnea	amblyoclada	(Müll. Arg.) Zahlbr.	(not treated)
4	Usnea	angulata	Ach.	**chapter 2**
5	**Usnea**	**arianae**	A. Gerlach & P. Clerc	**chapter 5**
6	Usnea	arthroclada	Fée	**chapter 2**
7	Usnea	aspera	(Eschw.) Vain.	(not treated)
8	**Usnea**	**aurantiaca-parvula**	A. Gerlach & P. Clerc	**chapter 1**
9	Usnea	baileyi	(Stirt.) Zahlbr.	(not treated)
10	Usnea	brasiliensis	(Zahlbr.) Motyka	**chapter 5**
11	**Usnea**	**catarinensis**	A. Gerlach & P. Clerc	**chapter 5**
12	Usnea	ceratina	Ach.	(not treated)
13	Usnea	cf. esperantiana	P. Clerc	(not treated)
14	Usnea	cf. flammea	Stirt.	(not treated)
15	Usnea	cf. krogiana	P. Clerc	(not treated)
16	Usnea	cf. moreliana	Motyka	**chapter 1**
17	Usnea	chilensis	Motyka	**chapter 2**
18	Usnea	cirrosa	Motyka	**chapter 1**
19	Usnea	cladocarpa	Fée	**chapter 1**
20	Usnea	concinna	Stirt.	**chapter 1**
21	Usnea	cornuta	Körb	**chapter 5**
22	Usnea	cristatula	Motyka	(not treated)
23	Usnea	crocata	Truong & P. Clerc	(not treated)
24	Usnea	dasaea	Stirt.	**chapters 2 and 5**
25	Usnea	densirostra	Taylor	(not treated)
26	Usnea	disjuncta	(Motyka) A. Gerlach & P. Clerc	**chapter 2**
27	Usnea	dodgei	Vain.	**chapter 2**
28	Usnea	entoviolata	Motyka	(not treated)
29	Usnea	erinacea s. l.	Vain.	**chapter 1**
30	Usnea	flavocardia	Räsänen	(not treated)
31	**Usnea**	**fleigiae**	A. Gerlach & P. Clerc	**chapter 1**
32	**Usnea**	**furnensis**	A. Gerlach & P. Clerc	**chapter 5**
33	Usnea	geissleriana	P. Clerc	**chapter 2**
34	Usnea	grandisora	Truong & P. Clerc	(not treated)
35	**Usnea**	**grandispora**	A. Gerlach & P. Clerc	**chapter 1**
36	**Usnea**	**isidiofibrillosa**	A. Gerlach & P. Clerc	**chapter 5**
37	**Usnea**	**kalbiana**	P. Clerc & A. Gerlach	**chapter 1**
38	**Usnea**	**kriegeriana**	A. Gerlach & P. Clerc	**chapter 5**

		species	author	This thesis
39	Usnea	laevis	(Eschw.) Nyl.	(not treated)
40	Usnea	lunaria	Motyka	**chapter 1**
41	Usnea	macaronesica	P. Clerc	(not treated)
42	Usnea	malmei	Motyka	**chapter 2**
43	Usnea	meridionalis	Zahlbr.	**chapter 1**
44	Usnea	merrillii	Motyka	**chapter 2**
45	Usnea	mexicana	Vain.	**chapter 2**
46	Usnea	moreliana	Motyka	(not treated)
47	**Usnea**	**oreophila**	A. Gerlach & P. Clerc	**Chapter 6**
48	Usnea	papillata	Motyka	**chapter 2**
49	Usnea	parvula	Motyka	**chapter 1**
50	Usnea	perhispidella	J. Steiner	**chapter 2**
51	Usnea	perplectata	Motyka	(not treated)
52	Usnea	poliotrix	Kremp.	(not treated)
53	**Usnea**	**pseudobrasiliensis**	A. Gerlach & P. Clerc	**chapter 5**
54	Usnea	regia	Motyka	**chapter 2**
55	Usnea	rubicunda	Stirt.	(not treated)
56	Usnea	sanctaeritae	P. Clerc & Herrera-Camp.	**chapter 2**
57	Usnea	spilota	Stirt.	(not treated)
58	Usnea	steineri	Zahlbr.	**chapter 1**
59	**Usnea**	**stipitata**	A. Gerlach & P. Clerc	**chapter 5**
60	Usnea	subdasaea	Truong & P. Clerc	(not treated)
61	Usnea	subelegans	(Vain.) Motyka	**chapter 1**
62	Usnea	subflammea s. lat.	P. Clerc	**chapter 2**
63	Usnea	subglabrata	Truong & P. Clerc	(not treated)
64	Usnea	subgracilis	Vain.	**chapter 2**
65	**Usnea**	**subparvula**	A. Gerlach & P. Clerc	**chapter 1**
66	Usnea	subrubicunda	P. Clerc	(not treated)
67	Usnea	subscabrosa	Nyl. ex Motyka	**chapter 2**
68	**Usnea**	**subsilesiaca**	P. Clerc & A. Gerlach	**chapter 2**
69	Usnea	transitoria	Motyka	**chapter 2**
70	Usnea	venusta	Motyka	**chapter 2**