# Two taxa of the genus Sticta (Peltigerales, Ascomycota), S. andina and S. scabrosa subsp. scabrosa, new to Bolivia confirmed by molecular data

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Abstract. The first records of *Sticta andina* and *S. scabrosa* subsp. scabrosa from Bolivia are presented. All records are confirmed by molecular data. Sticta andina has flattened, marginal isidia and phyllidia with abundant, submarginal apothecia, while tomentum is dark brown to black, whereas, S. scabrosa subsp. scabrosa has marginal and laminal phyllidia and pale to dark, grey tomentum. Two Bolivian specimens of the latter possess sparse, marginal to laminal apothecia, which previously have not been observed in this species. The haplotype network indicates that the S. andina specimens from Bolivia have three haplotypes, two of which are different from those previously reported. In the case of S. scabrosa subsp. scabrosa, two haplotypes were found, of which one is newly reported. A distribution map of Sticta species confirmed for Bolivia is presented.

Key words: lichens, lichenized fungi, Peltigeraceae, Lobarioideae, nucITS rDNA, distribution, haplotype network

# Introduction

The subfamily Lobarioideae within the family Peltigeraceae consists of macrolichens with foliose to fruticose thalli (Cannon & Kirk 2007; Kirk et al. 2008; Moncada et al. 2013a; Lücking et al. 2021a). It comprises about 800 taxa, of which almost 400 belong to the genus Sticta (Galloway 1994, 2007; Kirk et al. 2008; Moncada & Lücking 2012; Moncada et al. 2013a, b, 2020, 2021a, b; Mercado-Díaz et al. 2020; Lücking et al. 2021a).

So far several Sticta species were listed from Bolivia by Rodriguez-Flakus et al. (2016), but the occurrences of only two taxa, S. isidiokunthii (Moncada & Lücking 2012) and S. weigelii (Acharius 1810), were confirmed by the results of phylogenetic analyses (Moncada & Lücking 2012; Ossowska 2021). Most of the records listed by Rodriguez-Flakus et al. (2016) require re-examination as they are historical and were made before many taxa were recognized (e.g., Nylander 1859, 1861; Rusby 1896; Herzog 1922, 1923). In addition, the correct identification of Sticta taxa requires a molecular approach due to the high level of homoplasy between different species (Moncada et al. 2021a, b). This treatment is particularly important in the case of morphodemes, i.e., species that are morphologically similar, but phylogenetically distant (Lücking et al.

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2021b). In recent years, several S. weigelii morphodemes have been described, among them, S. andina (Moncada et al. 2021a, b) and S. scabrosa (Moncada et al. 2020, 2021a, b). According to literature data (Moncada 2012; Mercado-Díaz et al. 2020; Moncada et al. 2021a, b), both species are common representatives of the genus Sticta, their occurrences having been confirmed from several countries in South and North America. Conversely, they have not been reported so far from Bolivia. The records we present here contribute to the increasing knowledge of the diversity of Sticta in Bolivia. Indeed, it is presumed that diversity may be as high in Bolivia as it was shown for neighboring Colombia (Moncada 2012; Moncada & Lücking 2012; Moncada et al. 2013b, 2014) and many species may occur there including taxa new to science (Ossowska et al. 2022). A map of known localities of recently reported Sticta species from Bolivia is presented in Fig. 1.

Sticta andina and S. scabrosa have been characterised in detail in previous papers (Moncada 2012; Moncada et al. 2020, 2021a, b; Mercado-Díaz et al. 2020), but their full variability is still not well understood. Specifically, so far apothecia have not been observed in S. scabrosa specimens across its distribution range (Mercado-Díaz et al. 2020; Moncada et al. 2021a), whereas we found them in our specimens from Bolivia.

In this paper we report the first localities of *Sticta* andina and S. scabrosa from Bolivia. The identity of both is supported by the results of phylogenetic analyses.

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Figure 1. Distribution map of Sticta species recently confirmed from Bolivia.

In addition, their morphological and anatomical characteristics are presented, together with the description of apothecia in *S. scabrosa* subsp. *scabrosa*, previously not observed in this species, distribution map of all *Sticta* reported from Bolivia and haplotype network results.

# Material and methods

#### Material

The studied material is deposited in LPB, KRAM and UGDA herbaria. Morphology and anatomy were examined using a Nikon SMZ 800N stereomicroscope. In addition, the color reaction with K (water solution of potassium hydroxide) was checked (Orange et al. 2001). The distribution map was prepared using ArcGis Pro 2.8.0. software (ESRI, Redlonds, California, USA).

DNA extraction, PCR amplification and DNA sequencing

DNA extractions were undertaken using the Sherlock AX Kit (A&A Biotechnology, Poland) and the Plant & Fungi DNA Purification Kit (Eurx, Poland) following the manufacturer's protocol. Fungal nucITS rDNA, which is used as a universal barcode marker for fungi (Schoch et al. 2012), was amplified using the primers ITS1F (Gardes & Bruns 1993) and ITS4A (White et al. 1990). In PCR amplification, Start-Warm HS-PCR Mix (A&A Biotechnology, Poland) was used with the following parameters: an initial denaturation 94°C for 3 min and 33 cycles of 94°C for 30 sec; annealing at 52° for 45 sec; extension at 72°C for 1 min and final extension at 72°C for 10 min. The PCR products were purified with



Figure 2. Majority-rule consensus tree from Bayesian analysis of Sticta based on nucITS rDNA data set with posterior probabilities and bootstrap support values from IQ-TREE analysis presented near the branches. For each record, GenBank accession no. or voucher no. (for newly sequenced samples) are followed with species name. Newly sequenced records from Bolivia are marked in bold.

0.80/

n.93/9<u>6</u>

AF129284 Lobaria pulmonaria

0.02



Figure 3. Haplotype network based on nucITS rDNA sequences of Sticta andina including S. "squamifera" and S. "phyllidiata".

Clean-Up Kit (A&A Biotechnology, Poland) according to the instructions. Sequencing was performed in Macrogen (http://www.macrogen.com).

## Sequence alignment and phylogenetic analyses

The newly generated nucITS rDNA sequences were compared with all Sticta sequences available in GenBank database using BLAST search (Altschul et al. 1997). The phylogenetic analyses were performed based on the alignment reduced to selected Sticta related to the species treated in this paper. The final alignment consisted of 89 Sticta sequences and 561 characters. A sequence of Lobaria pulmonaria was used as an outgroup. Sequence alignments of the nucITS rDNA were performed using MAFFT v.7.475 (Katoh et al. 2002) as implemented in UGENE v.44.0 (Okonechnikov et al. 2012). Phylogenetic relationships were inferred with Bayesian Inference (BI) carried out in MrBayes v.3.2.2 (Huelsenbeck & Ronquist 2001). Two parallel MCMC runs were performed, using four independent heated chains and 5 million generations, sampling every 1000th tree. The initial 1250 trees of each

run (25%) were discarded as burn-in, and posterior probabilities were estimated by constructing a majority-rule consensus tree of all sampled post-burn-in trees. Maximum likelihood (ML) analyses were performed using the edge-linked partition model in IQ-TREE (Nguyen et al. 2015; Chernomor et al. 2016) with 10,000 bootstrap replications on the CIPRES Science Gateway (Miller et al. 2010). The best-fit substitution model was selected based on AIC (Akaike Information Criterion) as implemented in MrModeltest 2.0 (Nylander 2004); GTR+I+G was selected for both datasets. The tree topology obtained using the ML method did not contradict the Bayesian tree; therefore, only the Bayesian tree is shown (Fig. 2). The consensus trees were visualized using FigTree v.1.4.275 (Rambaut 2006–2014). Branches with bootstrap support  $\geq$  90% and posterior probabilities  $\geq$  0.99 were considered to be supported. New sequences of S. andina and S. scabrosa subsp. scabrosa have been deposited in GenBank (Supplementary Material Table S1).

To incorporate new records of *Sticta andina* and *S. scabrosa* subsp. *scabrosa* within available biogeographical data, we performed nucITS rDNA haplotype networks on the separate species alignments of these two species,



Figure 4. Haplotype network based on nucITS rDNA sequences of Sticta scabrosa including S. "pseudobeauvoisii".

following Moncada et al. (2020) (Supplementary Material Table S2). We excluded several previously obtained sequences of *Sticta scabrosa* subsp. *scabrosa* and one new sequence (specimen 15051) due to too short nucITS rDNA fragments. For both datasets, TCS networks (Clement et al. 2002) were executed with 95% connection limit and gaps treated as missing as implemented in PopART (Leigh & Bryant 2015).

#### **Results and discussion**

Eight new nucITS rDNA sequences were generated for this study (Supplementary Material Table S1). Based on the nucITS rDNA dataset, specimens 17210, 15051, 9257 and 22002 belonged to Sticta andina and were placed in well-supported subclades with sequences from GenBank forming a larger clade together with the sequence of the not yet formally described S. "squamifera" (Moncada et al. 2014; Moncada et al. 2020) (Fig. 2, Supplementary Material Table S1). Specimens 19712, 11229, 16336 and 18470 represented Sticta scabrosa subsp. scabrosa and in our tree form a well-supported clade with other sequences downloaded from GenBank and these obtained from S. scabrosa subsp. hawaiiensis (Moncada et al. 2021a, b). Based on TCS networks, we observed three haplotypes of S. andina, of which two were different from those previously reported (Moncada et al. 2021a) (Fig. 3). The nucITS rDNA obtained from specimen 22002 represented the same haplotype that was found in Ecuador (Moncada et al. 2021c). In the case of *S. scabrosa* subsp. *scabrosa*, we observed two haplotypes (Fig. 4). The H6 haplotype was distinct from remaining haplotypes, differing from H4 and H7 by only one position. The H4 haplotype was present in three Bolivian specimens and seems to be the most common *S. scabrosa* haplotype, also observed in 47 specimens from the Galapagos Islands (25), Colombia (11), Puerto Rico (7), Mexico (2), Costa Rica (1) and the Dominican Republic (1) (McDonald et al. 2003; Widhelm et al. 2018; Mercado-Díaz et al. 2020; Moncada et al. 2021c), and in Bolivia (3) (Fig. 4).

Initially, due to the presence of apothecia, the specimens presented here as *S. scabrosa* subsp. *scabrosa* were described as *S.* aff. *scabrosa* on the basis of other morphological features, yet the results of molecular studied allowed their classification as *S. scabrosa* subsp. *scabrosa*. This example shows that, firstly, individual *Sticta* specimens in different regions may be morphologically different. Secondly, phylogenetic analyses are an absolutely essential approach for several groups in this genus.

*Sticta andina* and *S. scabrosa* subsp. *scabrosa* were found in different Departments in Bolivia, Cochabamba and La Paz in Yungas forests, and Chuquisaca and Tarija in Tucumano-Boliviano forests, respectively. According to Moncada et al. (2021a), these species have a wider



Figure 5. Morphology of *Sticta andina*. A & C – morphology of upper surface (A – Kukwa 9257; C – Kukwa 15051); B – upper surface with isidia and phyllidia forming in the thallus cracks (arrow) (Kukwa 9257); morphology of lower surface (Kukwa 15051). Scale = 1 mm.

geographical distribution than *S. weigelii*, the species so far considered a cosmopolitan representative of the genus (Ossowska 2021 and literature cited therein). Thus, it can be assumed that also in Bolivia the distributions of *S. andina* and *S. scabrosa* subsp. *scabrosa* are wider than presented here, since the diversity of the genus in this country is not yet fully explored. Unfortunately, the inaccessibility of some regions of Bolivia, e.g., due to the lack of access roads, will make the distribution of many species of the genus not well understood in the near future.

*Sticta andina* B. Moncada, Lücking & Sérus., in Moncada et al., Willdenowia 51(1): 38. 2021. (Fig. 5)

**Description**. Photobiont cyanobacteria. Stipe absent. Thallus irregular to suborbicular, forming rosettes, up to 10 cm wide, moderately branched. Lobes 5–15 mm long and 3–20 mm wide, elongate to flabellate, with rounded apices; margins entire to sinuous, not thickened. Upper surface yellowish brown to dark brown with marginal line in the same color, smooth to rugose or scrobiculate, shiny, without papillae but with irregular, cream maculae. Cilia rare or absent, but tomentum sometimes projects laterally beyond lobes margins and resemble cilia, dark to brown, fasciculate to agglutinated, up to 0.5 mm long. Apothecia abundant to rare, mainly submarginal, dispersed, subpedicellate, disc orange to brownish red, up to 5 mm

in diam., margins dark brown, verrucous to crenate. Vegetative propagules present in all Bolivian specimens (but sometimes can be absent), in the form of isidia and phyllidia, isidia mainly marginal (or present along cracks on the upper surface, Fig. 5C), richly branched, aggregated, palmate to coralloid, with terminal parts dorsiventrally flattened, up to 1 mm long and 0.5 mm broad, phyllidia darker or of the same color as thallus. Medulla white with light yellow patches, K+ yellow. Lower surface uneven to undulate, brown to black; primary tomentum dense to the margin, thick and thinner towards the margin, spongy and fasciculate, dark brown to blackish; secondary tomentum present, arachnoid. Rhizines abundant, developed centrally to submarginally, fasciculate to squarrose, up to 3 mm long, dark brown. Cyphellae dispersed, abundant, 1–20 per cm<sup>2</sup> toward the center and 61–100 per cm<sup>2</sup> toward the margins, rounded, cupuliform to urceolate with wide pore, erumpent to prominent, with erect margins, without tomentum, margin black, basal membrane white, K+ yellow.

Upper cortex paraplectenchymatous, up to 45  $\mu$ m thick, differentiated into two cell layers; the upper of single cell layer, with cells up to 5  $\mu$ m diam. Photobiont layer up to 75  $\mu$ m thick, cells up to c. 15  $\mu$ m diam. Medulla up to 170  $\mu$ m thick with hyphae 2.5  $\mu$ m wide, with yellow-orange crystals. Lower cortex paraplectenchymatous 17–35  $\mu$ m thick, composed of 2–3 cell layers, cells up

to 15  $\mu$ m in diam., with a wall 0.6–2.5  $\mu$ m thick. Hairs of primary tomentum up to 1000  $\mu$ m long, in fascicles of more to 15, hyphae branched; hairs of secondary tomentum up to 35  $\mu$ m long, moniliform. Cyphellae cavity up to 300  $\mu$ m high; without papillae. Apothecia biatorine, up to 500  $\mu$ m high; epithecium 2.5–5  $\mu$ m thick, orangebrown. Ascospores 1–3 septate, 27–38 × 9–13  $\mu$ m (based on own observations and Moncada et al. 2021a).

**Distribution and ecology**. The records of *S. andina* presented here are the first from Bolivia. The species was found on bark of trees in Andean Yungas forests at 1989–3082 m a.s.l. in Cochabamba and La Paz Departments (Fig. 1). Beside Bolivia, the species is known from the Azores, Brazil, Colombia, Costa Rica, Ecuador, Hawaii, Mexico and USA (Moncada et al. 2021a, b). According to Moncada et al. (2021a), *S. andina* is more common than *S. weigelii*.

**Notes.** At first the material belonging at present to *Sticta andina* was divided into three morphologically distinct entities, differing in the type of vegetative propagules and the distribution of apothecia (Moncada 2012; Moncada et al. 2014): *S. andina* s.str. characterized by the absence of propagules and submarginal apothecia, *S. "colombiana*" with phyllidia and laminal apothecia and *S. "paramuna*" lacking vegetative propagules and with marginal apothecia (Moncada 2012; Moncada et al. 2014, 2021a). Despite these differences, the topology of the phylogenetic tree did not validate the recognition of the subclades 'colombiana', 'andina' and 'paramuna' (Moncada et al. 2021a).

Sticta andina belongs to the S. weigelii morphodeme, but is the only taxon within that group characterised by the presence of often flattened to dorsiventral isidia and phyllidia (but some specimens may lack vegetative propagules; see Moncada et al. 2014, 2021a), which are generally darker than the thallus. Moreover, it differs also in the color of the tomentum and often the presence of abundant apothecia (and then vegetive propagules may be absent) (Moncada et al. 2014, 2021a, b). The type of vegetative propagules is a feature that distinguishes S. andina from two isidiate species, S. isidiokunthii and S. weigelii, recently confirmed from Bolivia (Moncada & Lücking 2012; Ossowska 2021). Both of those species are isidiate, and isidia are branched to coralloid in S. isidiokunthii and coralloid to cylindric in S. weigelii. Furthermore, the apothecia are less abundant than in S. andina. Moreover, S. isidiokunthii has the beige tomentum and the lower surface is beige to brown towards the center, whereas in S. weigelii the tomentum is brown to black and the lower surface is beige to reddish brown towards the center (Moncada 2012; Moncada & Lücking 2012; Moncada et al. 2020, 2021a, b; Ossowska 2021). Concerning the phyllidiate S. scabrosa subsp. scabrosa, this species has narrow, easily detached phyllida and the tomentum is light to dark grey-brown, while the cilia are golden brown (cilia often absent in S. andina, but the tomentum can extend beyond the edge of lobes and may resemble cilia) (Moncada et al. 2020, 2021a).

Other species of the *S. weigelii* morphodeme (species with quotation marks – not yet formally described), *S. beauvoisii* (Delise 1825), *S. carolinensis* (McDonald et al. 2003), *S. "hypoglabra*", *S. "luteocyphellata*" (Moncada 2012), *S. rhizinata*, *S. tunjensis* (Moncada & Lücking 2012), and *S. waikamoi* (Moncada et al. 2021b), produce typical isidia, whereas *S. harrisii* (Mercado-Díaz et al. 2020), *S. fragilinata* (McDonald et al. 2003), *S. "laselvae*", *S. "lobulata*" (Moncada 2012), *S. phyllidiokunthii* (Moncada et al. 2013c), *S. pseudobeauvoisii* (Moncada 2012), *S. scabrosa*, develop phyllidia or lobules (see also Moncada et al. 2013b, 2014, 2021a, b; Widhelm et al. 2018; Ossowska 2021).

**Specimens examined**. BOLIVIA. Dept. Cochabamba, Prov. Carrasco, Parque Nacional Carrasco, between Muruvia and Monte Punku, elev. 3082 m, 17°34′43″S, 65°15′25″W, *Podocarpus* forest, Ceja de Monte Inferior (Altimontano), corticolous, 26 Nov 2014, M. Kukwa 15051 (LPB, UGDA); Dept. La Paz, Prov. Nor Yungas, Parque Nacional y Área Natural de Manejo Integrado Cotapata, near Urpuma colony, elev. 1989 m, 16°13′20″S, 67°52′34″W, Yungas montane forest, corticolous, 30 June 2010, A. Flakus 17210 & P. Rodriguez (KRAM, LPB); Prov. Nor Yungas, near Siniary colony, between Coroico and La Paz, elev. 2190 m, 16°13′20″S, 67°50′37″W, Yungas secondary cloud forest, corticolous, 24 May 2011, M. Kukwa 9257 (LPB, UGDA); Prov. Murillo, Valle del Zongo, elev. 2850 m, 16°08′38″S, 68°06′59″W, Yungas cloud forest, corticolous, 29 May 2011, A. Flakus 22002 & O. Plata (KRAM, LPB).

Sticta scabrosaB. Moncada, Merc.-Díaz & Bungartzsubsp. scabrosa, in Moncada et al., Willdenowia 51(1):41. 2021.(Fig. 6)

Description. Photobiont cyanobacteria. Stipe absent. Thallus suborbicular to irregular, up to 15 cm in diam., strongly branched. Lobes 5-10 mm long and 4-10 mm broad, ligulate to flabellate, imbricate, undulate, with rounded tips, margins entire to crenate, not thickened. Upper surface uneven to scrobiculate, yellow brown, with lobe margins in the same color; maculae cream. Medulla white to light cream; K+ ochraceous. Cilia scarce to absent, up to 0.5 mm long, pale to golden brown, fasciculate. Apothecia scarce, marginal to submarginal or laminal, dispersed to aggregated, subpedicellate to pedicelled, without pronounced invagination on lower side, up to 2 mm diam.; disc orange brown to chocolate brown (mature), matt to shiny; margin entire to very rarely shallowly crenate, with very short and very rarely hyaline hairs on some apothecia, margin at the same color as thallus, light to dark brown. Phyllidia marginal and some also laminal, branched and coralloid, aggregate, 0.5 mm long and 0.3 mm broad, of the same colour as thallus or darker. Lower surface undulate, brown yellow to brownish yellow; primary tomentum dense, but sparse towards the margins, thin or thick, spongy to arachnoid, pale grey to darker grey in the centrum; secondary tomentum present, appressed, arachnoid, pale. Rhizines sparse, irregularly dispersed, fasciculate, grey brown. Cyphellae abundant, 25–40 per  $cm^2$  towards center of thallus and 45–60 per cm<sup>2</sup> towards the margins, dispersed, rounded to irregular, urceolate with wide pore, prominent, below the level of tomentum, with the margin raised and involute, cream



Figure 6. Morphology of *Sticta scabrosa*. A & C – morphology of upper surface; arrows point to apothecia (A – Kukwa 11229; C – Flakus 19712); B – close up of apothecia (Kukwa 11229); D – morphology of lower surface (Flakus 19712). Scale = 1 mm.

yellow to light brown, with tomentum; pore 0.3-1.4 mm in diam; basal membrane yellow to cream, K+ ochraceous.

Upper cortex paraplectenchymatous, up to 30 µm thick, homogenous, cells diam. up to 11 µm and their wall up to 2 µm thick. Photobiont layer up to 70 µm thick, cells diam. up to 15 µm. Medulla up to 160 µm thick with hyphae 2.5 µm wide, without crystals. Lower cortex paraplectenchymatous, up to 30 µm thick, cells diam. up to 12 µm with a wall up to 2.5 µm thick. Hairs of primary tomentum up to 1000 µm long, composed of fascicles of 6-12 agglutinate hyphae; hairs of secondary tomentum up to 25 µm long, moniliform. Cyphellae cavity up to 200 µm high; without papillae. Apothecia biatorine, up to 400 µm high, without distinct stipe; excipulum up to 100 µm broad. Hymenium up to 75 µm high; epihymenium up to 12 µm high, orange brown, K-, with gelatinous upper layer. Ascospores (0-)1-3-septate, usually constricted in middle septa, but sometimes constricted in all septa,  $23-27.5 \times 5-6 \mu m$  (based on own observations and Moncada et al. 2021a).

**Distribution and ecology**. *Sticta scabrosa* subsp. *scabrosa* is new for Bolivia and it is reported from Tarija and Chuquisaca Departments (Fig. 1). It was found growing on the bark of trees in Tucumano-Boliviano forests at an altitude 1040–1400 m a.s.l. Previously, it has been reported from Argentina, Brazil, Colombia, Costa Rica,

Dominican Republic, Ecuador, Mexico, and Puerto Rico. *Sticta scabrosa* subsp. *hawaiiensis* is known only from Hawaii (Moncada 2012; Mercado-Díaz et al. 2020; Moncada et al. 2021a, b).

**Notes.** Two specimens of *S. scabrosa* subsp. *scabrosa* from Bolivia (11229, 19712) have scarce, marginal to laminal apothecia (Fig. 6). So far, fertile material has not been known in this species (see Moncada 2012; Moncada et al. 2021a, b) and here we presented the first description of apothecia.

Within *S. scabrosa* Moncada et al. (2021a, b), two subspecies were distinguished differing in in two positions in the nucITS rDNA, *S. scabrosa* subsp. *hawaiiensis* (Moncada et al. 2020) and *S. scabrosa* subsp. *scabrosa*. All specimens from Bolivia represent *S. scabrosa* subsp. *scabrosa* (Fig. 2).

Sticta scabrosa, like S. andina, belongs to the S. weigelii morphodeme (Moncada et al. 2021a) and produces phyllidia. It differs from other phyllidiate taxa, such as S. harrisii, S. "pseudobeauvoisii" or S. fragilinata in the color of the tomentum, which is at least partly brown (Moncada 2012; Moncada et al. 2021a; Ossowska 2021). Moreover, the lobe margins in S. harrisii are covered by abundant, white true cilia (Moncada 2012; Mercado-Díaz et al. 2020). On the other hand, S. scabrosa differs from other taxa to the S. weigelii morphodeme with grey tomentum, such as *S. "hypoglabra*" or *S. "luteocyphellata*" in type and morphology of vegetative propagules as those species produce cylindrical to coralloid, sometimes flattened isidia (Moncada 2012; Ossowska 2021). A table comparing all species was presented by Ossowska (2021).

**Specimens examined**. BOLIVIA. Dept. Chuquisaca, Prov. Luis Calvo, Parque Nacional y Área Natural de Manejo Integrado Serranía de Iñao, close to Ticucha, elev. 1040 m, 19°37'26"S, 63°50'50"W, disturbed Sub-Andean Tucumano-Boliviano forest with *Acacia*, corticolous, 18 July 2015, M. Kukwa 16336 (LPB, UGDA); Dept. Tarija, Prov. Burnet O'Connor, RN de Flora y Fauna Tariquía near Salinas, elev. 1400 m, 21°49'15"S, 64°12'44"W, Tucumano-Boliviano montano forest, 10 Aug 2012, M. Kukwa 11229 (LPB, UGDA); Prov. Aniceto Arce, RN de Flora y Fauna Tariquía Le Hierba, elev. 1070 m, 22°08'46"S, 64°31'36"W, Tucumano-Boliviano submontane forest, near river, 25 Nov 2010, A. Flakus 19712 & J. Quisbert (KRAM, LPB); Prov. Aniceto Arce, Filo de Sidras, elev. 1064 m, 22°14'50"S, 64°33'28"W, Tucumano-Boliviano submontane forest, 22 Nov 2010, A. Flakus 18470 (KRAM, LPB).

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#### Supplementary electronic materials

 Table S1. Sequences of nucITS rDNA of references downloaded from

 GenBank for phylogenetic inference. Download file

 Table S2. Sequences of nucITS rDNA of references downloaded from

 GenBank and used in this study in haplotype networks. Newly sequenced

 records from Bolivia are marked in bold. Download file

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