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High diversity of *Trichonectria* (*Hypocreales*) inhabiting *Usnea* in Bolivia

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Abstract. The Neotropics, particularly mountain cloud forests, are characterized by a high diversity of microfungi that inhabit lichens. However, based on our field studies, many of these microfungi remain undescribed, and their phylogenetic relationships are poorly understood. This study focuses on Bolivian lichenicolous *Trichonectria* inhabiting the genus *Usnea* (*Parmeliaceae*), a common lichen host in the tropical Andean forests. Here, we present 14 species of which eight are described as new to science: *Trichonectria abortispora* sp. nov., *T. biglobospora* sp. nov., *T. boliviana* sp. nov., *T. citrispora* sp. nov., *T. cylindrospora* sp. nov., *T. gigaspora* sp. nov., *T. microsporusneae* sp. nov. and *T. toensbergiana* sp. nov. The five-locus phylogenetic analyses show that the anamorphic genus *Cylindromonium* and the teleomorphic genus *Trichonectria* cluster together in a well-supported clade within the order *Hypocreales*, but we have not yet reached a taxonomic conclusion. Phylogenetic placements of five lichenicolous species of the genus *Trichonectria* are reported here for the first time, including *T. vinosa* comb. nov.

Key words: biodiversity hotspots, cloud forests, eight new species, lichenicolous fungi, mycoparasites

Introduction

This paper is a part of our long-term study aimed at identifying biodiversity and phylogenetic placements of Bolivian lichenicolous fungi (e.g., Flakus & Kukwa 2012a, b; Kukwa et al. 2012; Etayo et al. 2013, 2015, 2018; Flakus et al. 2014, 2019a, b; Crous et al. 2023a, b; Darmostuk & Flakus 2024). Despite Bolivia being a country with a relatively small area in South America, it preserves the most diverse vegetation on the continent scale, including the hyperdiverse biodiversity hotspot in the tropical Andes (Myers et al. 2000; Josse et al. 2003). The extraordinarily wide variety of potential habitats results in a high diversity of lichens and associated microbiota (including lichenicolous fungi) which, however, is still in need of further investigations.

The genus *Trichonectria* was placed in the family *Bionectriaceae* by Samuels & Rossman within *Hypocreales* (Rossman et al. 1999). The material of the generic type *T. aculeata* was destroyed and as a consequence, *T. hirta* was selected by Hawksworth (1978) as the neotype. Unfortunately, *T. hirta* does not seem to be a common species in Europe and we have not had the opportunity to study it. A short diagnosis of the species was given by Rossman et al. (1999).

Thirty-one taxa of *Trichonectria* were known before this paper: 20 grow on lichens and 11 on other substrates (e.g., decaying algae, mosses and non-lichenized fungi). Those growing on lichens are mainly parasitic on members of *Parmeliaceae* (10 species; including 5 on *Usnea*), on various corticolous lichens without strict host preferences (2 species) and others on *Calopadia*, *Cladonia*, *Leptogium*, *Lobariella*, *Megalospora*, *Pertusaria* and *Ramalina* (Hawksworth 1978; Alstrup & Svane 1998; Rossman et al. 1999; Sérusiaux et al. 1999; Etayo 2001, 2002, 2017; Etayo & van den Boom 2005; Etayo & Sancho 2008; Gardiennet & Lechat 2011; Brackel 2014; Farkas & Flakus 2016; Crous et al. 2023b; Haldeman & Darmostuk 2023; Etayo & López de Silanes 2024). Species within the genus *Trichonectria*, like most lichen-inhabiting fungi, are considered obligate mycoparasites and associated with specific lichen hosts (Samuels 1988; Rossman et al. 1999).

Trichonectria species, including those growing on members of the genus *Usnea*, are characterized by having nonstromatic, small, yellowish to orangish, orange-brown or almost black perithecia, which frequently are

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covered by thick-walled setae arising from the surface of the perithecial wall and (0–)1-multiseptate ascospores (e.g., Rossman 1983; Samuels 1988; Rossman et al. 1999; Etayo 2017). However, in numerous samples, these setae may be absent due to their extreme fragility. Therefore, the presence or absence of setae as a taxonomic character should be carefully assessed based on large samples containing ascomata at various stages of development. However, even without setae, the ascomatal walls in this genus are very characteristic. Two main wall types can be distinguished: (i) with elongated cells densely covering the outermost portion of the exciple and masking its cellular structure (*setadpressa*-like wall; Fig. 1A–C) or (ii) without hyphal coverage, revealing the anatomical structure of perithecial wall (*gigaspora*-like; Fig. 1D). Most *Trichonectria* species develop *gigaspora*-like wall in ascomata with naked paraplectenchymatic wall composed of more or less isodiametric cells (see also figure of *T. usneicola* in Etayo 2002). The cells develop irregular widenings of the wall which gives them a sinuose shape, sometimes similar to textura epidermoidea (epidermoid tissue consisting of puzzle-like cells connected by fine pores according to Lowen 1995). Some species, however, have the last row of cells covered by a more or less dense net of cylindrical hyphae concolourous with the commented wall. This structure was first mentioned for *Trichonectria setadpressa* (Etayo 2002).

Before our study, four species of lichenicolous *Trichonectria* were reported from *Usnea* in South America, from Columbia (Etayo 2002), Chile (Etayo & Sancho 2008) and Ecuador (including *Nectriopsis vinosa* here considered a member of *Trichonectria*; Etayo 2017). Furthermore, only *Trichonectria pyrenaica* was described on *Usnea* sp. from Europe (Gardiennet & Lechat 2011). In this paper, we increased the number of species inhabiting *Usnea* in South America to 12, including eight species new to science. Our results suggest that *Usnea* is an important host for *Trichonectria* in Bolivia, especially in tropical montane forests.

Material and methods

Taxon sampling and morphological studies

Our results are based mainly on fresh material collected by the authors from Bolivia and deposited at KRAM, LPB, UGDA, as well as in the private herbarium of J. Etayo (Pamplona). Morphological and anatomical characters were examined using standard stereo- and compound microscopes (Nikon SMZ 800, Nikon Eclipse 80i DIC, Tokyo, Japan). Sections were prepared manually using a razor blade or a Thermo Fisher Scientific HM430 microtome (Walldorf, Germany) combined with Physitemp Instruments Inc. BFS-3MP freezing stage (Clifton, NJ, USA). Sections and squash mounts were

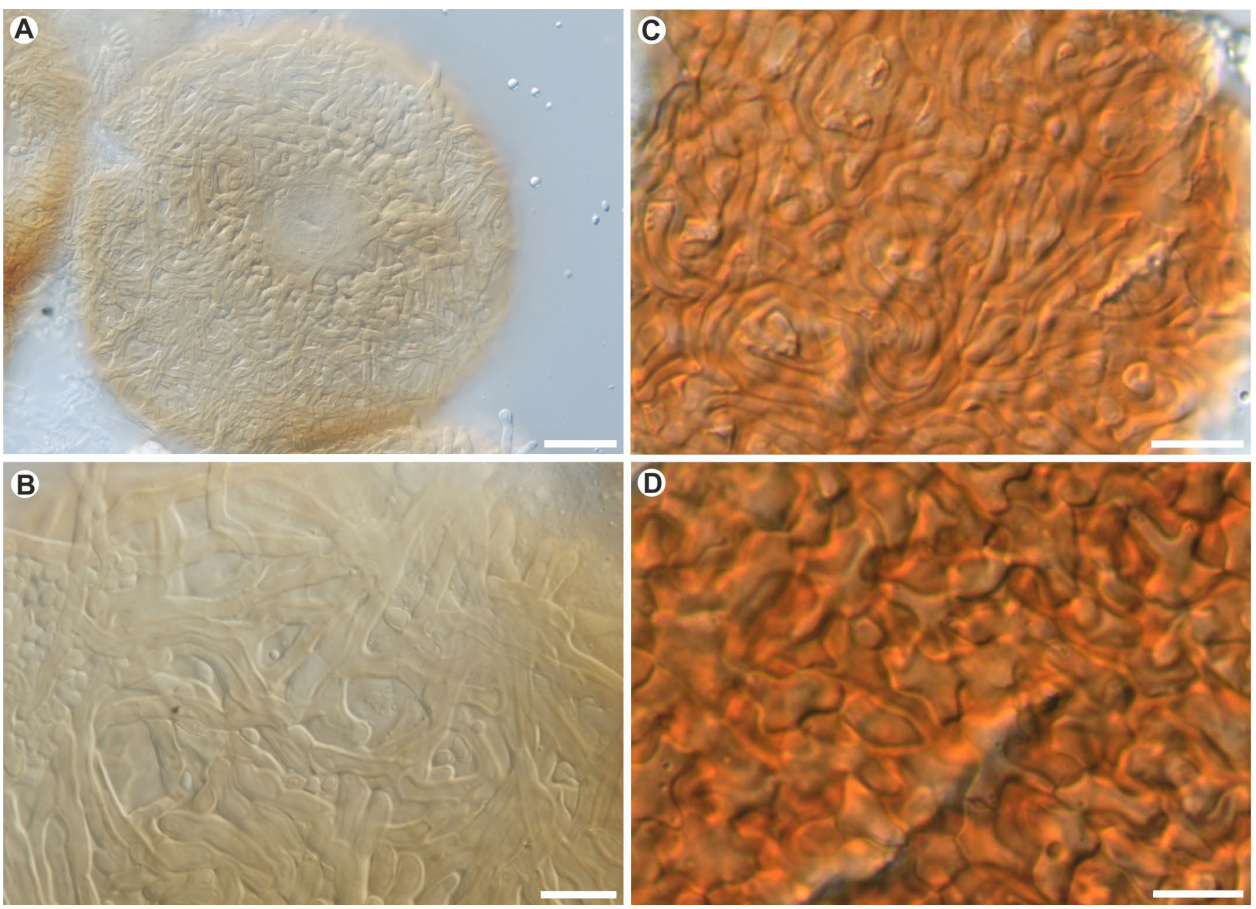

Figure 1. Anatomy of *Trichonectria* ascomata in external view. A, B – *T. setadpressa*, elongated cells covering the outermost portion of the exciple (*setadpressa*-like wall; A – squashed ascoma; B – elongated cells); C – *T. microsporusneae*, with *setadpressa*-like wall covered by elongated cells; D – *T. gigaspora*, with *gigaspora*-like wall without hyphae coverage. Scales: A = 25 µm; B, C = 10 µm.

examined in distilled water, 10% KOH (K), Lactophenol Cotton Blue (LPCB; Fluka, no. 61335-100ML), or Congo Red. All photomicrographs showing anatomical characters were made using transmitted differential interference contrast (DIC) microscopy with Nikon Digital Sight DS-Fi1 microscope c-mount camera and have been taken in water except where another reaction is mentioned. Amyloid reactions of anatomical structures were tested using Lugol's solution (I) (Fluka, no. 62650-1L-F), or with Lugol's solution preceded by a 10% KOH treatment (K/I). The solubility of crystals was tested using a water solution of potassium hydroxide (K) and a 50% nitric acid solution (N). All measurements were made in distilled water or LPCB and are presented as intervals with maximum and minimum sizes between the brackets. In the case of ascospores, the number of measurements is also recorded at the end.

DNA extraction, PCR amplification, and DNA sequencing

Ascomata of lichenicolous fungi, still on its lichen host thalli, were stored at –20°C. They were removed from the host thallus and carefully cleaned in double distilled water on a microscope slide under sterile conditions to remove host tissues and other visible impurities using ultra-thin tweezers and a razor blade. DNA was extracted from 4 to 10 clean ascomata or hymenia, depending on each specimen, using the QIAamp DNA Investigator Kit (Qiagen, Germany) following the manufacturer's instructions. We amplified and sequenced small subunit nuc rDNA (SSU) using the primer pair NS24 and NS1 (White et al. 1990; Gargas & Taylor 1992), nuc rDNA internal transcriber spacers (ITS = ITS1 + 5.8S + ITS2) and nuc rDNA large subunit (LSU) with primers ITS1F and LR5 (White et al. 1990; Gardes & Bruns 1993), translation elongation factor 1-alpha (*TEF1*) with primers EF1-2228R and EF1-1983F (Rehner & Samuels 1995) and RNA polymerase largest subunit (*RPB1*) with primers RPB1cf and RPB1Af (Stiller & Hall 1997). The amplification parameters and additional detailed information on PCR, visualization of amplicons, and preparation of samples can be found in Rodriguez-Flakus & Printzen (2014) and Flakus et al. (2019b). PCR amplicons were sequenced by Macrogen (Amsterdam, the Netherlands). The newly generated sequences were carefully checked, assembled, and edited manually using Geneious Pro 8.0. (Biomatters Ltd) and deposited in GenBank. Accession numbers for all sequences used in this study are provided in Table 1.

Phylogenetic analyses and taxon selection

All sequences generated were checked by BLAST (Altschul et al. 1990) to verify potential contaminations by any unrelated fungi. We aligned our newly generated *Trichonectria* sequences with other available sequences of the genus *Trichonectria*, as well as *Cylindromonium*. The tree was rooted by using *Nectria cinnabarina* (*Nectriaceae*) as the outgroup. Alignments were generated for each region using MAFFT (Katoh et al. 2005; Katoh & Standley 2013)) as implemented on the GUIDANCE2 Web server (Penn et al. 2010). We used the default cut-off score of 0.93 in all single gene alignments. PartitionFinder2 was used to select the best partition for our data and substitution models (Lanfear et al. 2016). A single substitution model was selected for each region (GTR+G for ITS1 and ITS2, TRNEF+I+G for 5.8S and LSU, JC for SSU, F81+I+G for the first and second codon position of *TEF1*, TVM+G for the third codon position of *TEF1*, TIM+I for the first codon position of *RPB1*, K81UF+I the second codon position of *RPB1* and HKY+I+G for the third codon position of *RPB1*) under a greedy search algorithm and the Akaike Information Criterion (AIC) (Lanfear et al. 2012). Subsequently, the analyses were performed in the CIPRES Scientific gateway portal (www.phylo.org/portal2) (Miller et al. 2010). Maximum likelihood (ML) and Bayesian inference (BI) analyses were performed on the concatenated dataset (ITS+LSU+SSU+*TEF1*+*RPB1*) using substitution models obtained from PartitionFinder2. Maximum likelihood analyses were carried out using a heuristic search as implemented in IQ-TREE 2.1.2 on XSEDE and 1000 ultrafast bootstrap replicates to estimate branch support (Nguyen et al. 2015; Minh et al. 2020). Bayesian inference of the phylogenetic relationships was calculated using the Markov chain Monte Carlo (MCMC) approach as implemented in MrBayes 3.2.6 on XSEDE (Ronquist et al. 2012) using the partitions and substitution models obtained by PartitionFinder 2. Two independent parallel runs were started each with four incrementally heated (0.15) chains. This MCMC was allowed to run for 100 million generations, sampling every 1,000th tree and discarding the first 50% of the sampled tree as a burn-in factor. The analysis was stopped after 1 million generations when the standard deviation of split frequencies had dropped below 0.01. The resulting ML and BI phylogenetic trees were visualized in FigTree 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) and Inkscape 1.3.2 (https://inkscape.org/). The alignment was deposited at figshare.com (10.6084/m9.figshare.26869912).

Results

Phylogenetic placement of *Trichonectria* species growing on *Usnea*

In total, 13 sequences of the ITS region, 13 sequences of LSU, 13 sequences of SSU, 13 sequences of *TEF1*, and 9 sequences of *RPB1* of *Trichonectria* species were generated during this study. The final concatenated alignment included sequences from 32 specimens (together with the outgroup; in this, it contains five usneicolous species with three species described here) with a total of 4,562 characters, among them 517 parsimony-informative sites, 255 singleton sites, and 3,790 constant sites. The phylogenetic trees based on ML and BI analyses yielded similar topologies. Therefore, we present the tree with the topology recovered from the BI analysis (Fig. 2).

In the resulting phylogeny, specimens of the anamorphic genus *Cylindromonium* and the teleomorphic genus *Trichonectria* clustered together in a well-supported clade (PP = 1, BS = 100). The generic type of *Cylindromonium* (*C. eugeniicola*) showed a sister relationship to the remaining taxa *Cylindromonium* and *Trichonectria*.

Table. GenBank and voucher accession numbers of sequences included in the phylogenetic analyses. Newly generated sequences are marked in bold.

Species name	Voucher data	Host/isolation source	Type	nSSU	ITS	LSU	*TEF1*	*RPB1*
Cylindromonium alloxyli	Australia, B.A. Summerell (CBS 146806)	mycophilic on *Meliola*	T	–	MW175339	MW175379	MW173133	–
Cylindromonium dirinariae	Japan, A. Ohmaki et al. (TNS–L–131533)	*Dirinaria applanata*	T	–	LC731273	LC731274	LC731275	–
Cylindromonium dirinariae	Japan, A. Ohmaki et al. (TNS–L–131534)	*Dirinaria applanata*	–	–	LC731276	LC744402	LC744396	–
Cylindromonium dirinariae	Japan, A. Ohmaki (TNS–L–131535)	*Dirinaria applanata*	–	–	LC731277	LC744401	LC744395	–
Cylindromonium eugeniicola	South Africa, M.J. Wingfield (CBS146075)	leaf litter of *Eugenia capensis*	T	–	MN562142	MN567649	OQ470832	–
Cylindromonium everniae	Netherlands, J. Boers (CBS 148255)	*Evernia prunastri*	T	–	OK664736	OK663775	OK651192	–
Cylindromonium lichenicola	Germany, W. Gams 643 (CBS 188.70)	bark with lichens	–	–	MH859549	LC744399	LC744398	–
Cylindromonium lichenicola	Germany, W. Gams 1119 (CBS 303.70)	*Alnus glutinosa*, bark	–	–	MH859675	MH871429	OQ470833	–
Cylindromonium lichenicola	Germany, W. Gams s.n. (CBS 415.70A)	*Desmococcus* sp., on bark	–	–	MH859774	MH871536	LC744397	–
Cylindromonium lichenicola	Germany, W. Gams 562 (CBS 776.69)	*Bulgaria inquinans*	–	–	–	MH871194	–	–
Cylindromonium rhabdosporum	Austria, W. Gams 561 (CBS 438.66)	old *Cladonia furcata*	T	–	MH858850	HQ232120	OQ470835	–
Nectria cinnabarina	France, C. Lechat CLL 7152 (CBS 125165)	*Aesculus* sp.	T	–	HM484548	KM231715	HM484527	HM484577
Nectria mariae	France, C. Lechat (CBS 125294)	*Buxus sempervirens*	T	–	JF832629	JF832684	JF832542	JF832789
Trichonectria abortispora	Bolivia, J. Etayo 2983 (LPB)	*Usnea* sp.	T	**OR510619**	**OR511511**	**OR510606**	**OR525306**	**OR529401**
Trichonectria anisospora	Norway, V. Darmostuk 1277 (KRAM-L-74392)	*Hypogymnia physodes*	–	–	OR511514	OR510609	–	–
Trichonectria anisospora	Poland, V. Darmostuk 1235 (KRAM-L-74391)	*Hypogymnia physodes*	–	–	OR511515	OR510610	OR525303	–
Trichonectria citrispora	Bolivia, A. Flakus 26300 (KRAM-L-74385)	*Usnea* sp.	–	**OR510613**	**OR511507**	**OR510600**	**OR525312**	**OR529396**
Trichonectria pertusariae	Bolivia, A. Flakus 27679 (KRAM-L-74380)	*Pertusaria* sp.	–	OR510616	OR511508	OR510603	OR525309	OR529398
Trichonectria pertusariae	Bolivia, J. Etayo 30-80 (LPB)	*Pertusaria* sp.	–	OR510620	OR511512	OR510607	OR525305	OR529402
Trichonectria pyrenaica	Portugal, O. Sira 1407 (KRAM-L-74393)	*Usnea* sp.	–	–	**OR511516**	**OR510611**	–	–
Trichonectria rectipila	USA, C.T. Rogerson s.n (CBS 132.87)	*Diatrype stigma*	T	–	MH862058	MH873746	–	–
Trichonectria rubefaciens	Spain, J. Etayo 29085.1 (hb. Etayo)	*Parmelina tiliacea*	–	OR510614	OR511505	OR510601	OR525310	OR529397
Trichonectria rubefaciens	Spain, J. Etayo 29085.2 (hb. Etayo)	*Parmelina tiliacea*	–	OR510615	OR511506	OR510602	OR525311	–
Trichonectria rubefaciens	Poland, V. Darmostuk 1084 (KRAM-L-74390)	*Parmelia sulcata*	–	–	OR511517	OR510612	OR525302	–
Trichonectria setadpressa	Bolivia, A. Flakus 28886 (KRAM-L)	*Lobariella pallida*	–	OR510622	MT153965	MT154012	OR525301	–
Trichonectria setadpressa	Bolivia, A. Flakus 29612-1 (KRAM-L)	*Lobariella pallida*	–	OR510623	MT153966	MT154013	–	–
Trichonectria setadpressa	Bolivia, A. Flakus 29612-2 (KRAM-L)	*Lobariella pallida*	–	OR510624	MT153967	MT154014	OR525300	–
Trichonectria setadpressa	Bolivia, A. Flakus 29617 (KRAM-L)	*Lobariella pallida*	–	OR510625	MT153968	MT154015	OR525299	OR529404
Trichonectria setadpressa	Bolivia, J. Etayo 20-13 (LPB)	*Lobariella pallida*	–	–	MT153969	MT154016	–	–
Trichonectria toensbergiana	Bolivia, A. Flakus 27081 (KRAM-L-74384)	*Usnea* sp.	T	**OR510618**	**OR511510**	**OR510605**	**OR525307**	**OR529400**
Trichonectria usneicola	Bolivia, A. Flakus 25230 (KRAM-L-74383)	*Usnea* sp.	–	**OR510617**	**OR511509**	**OR510604**	**OR525308**	**OR529399**
Trichonectria vinosa	Bolivia, A. Flakus 27452.1 (KRAM-L-74382)	*Usnea* sp.	–	**OR510621**	**OR511513**	**OR510608**	**OR525304**	**OR529403**

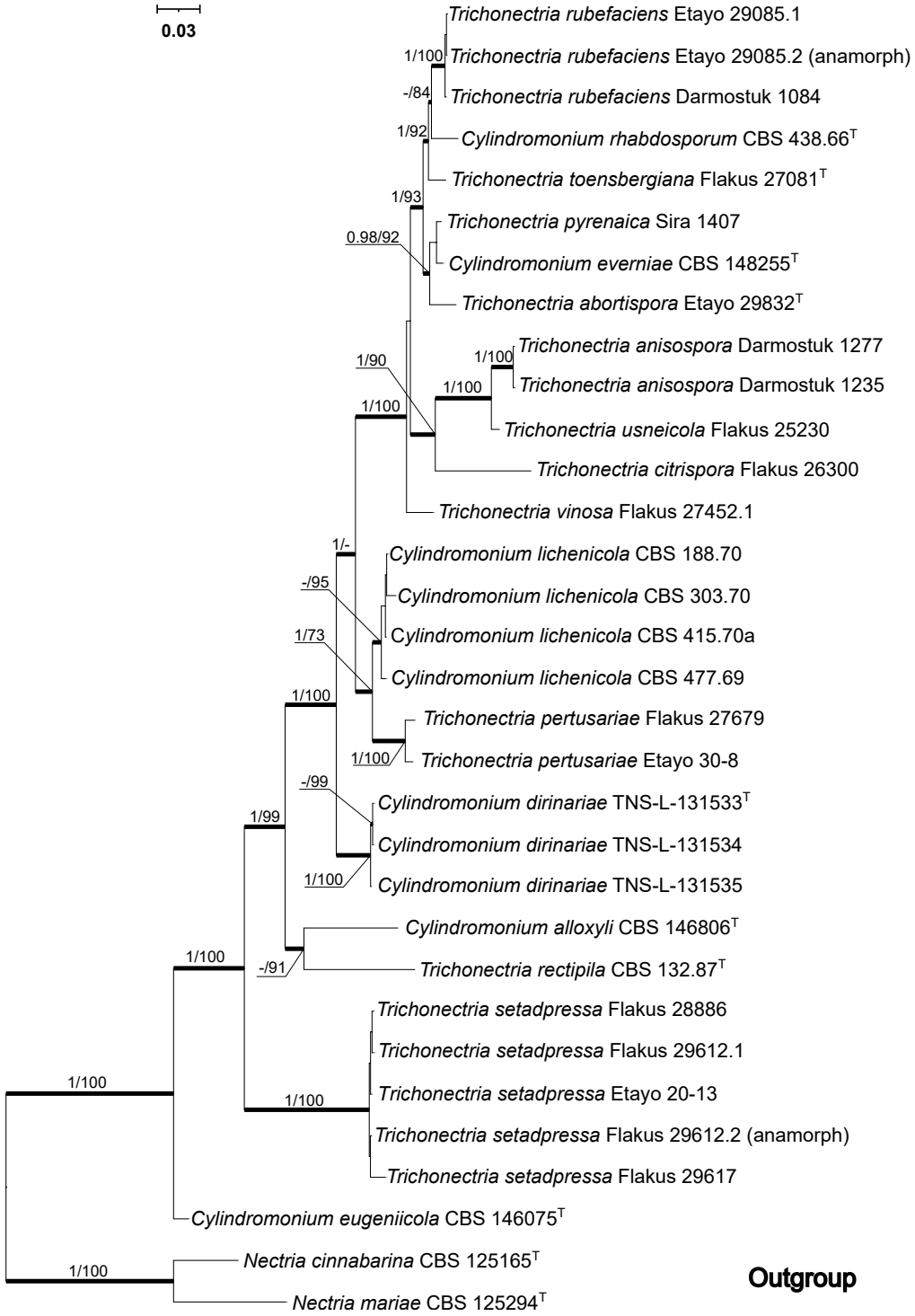

Figure 2. Phylogenetic placement of the selected species of *Trichonectria* inhabiting *Usnea* inferred from BI analyses of combined ITS, LSU, SSU, *TEF1* and *RPB1* data set. *Nectria cinnabarina* and *N. mariae* were used as the outgroup. Bold branches represent either bootstrap values ≥70 and/or Bayesian posterior probabilities ≥0.95.

Other species of *Cylindromonium* demonstrate a sister relationship to *Trichonectria* species rather than *C. eugeniicola*. The fungicolous *C. alloxyli* clustered together with another fungicolous species, *Trichonectria rectipila*, although without strong statistical support. *Cylindromonium lichenicola*, represented by four specimens, forms a well-supported clade (PP = 1, BS = 95) and shows a sister relationship to the two specimens of *Trichonectria pertusariae*. A single specimen of *Cylindromonium rhabdosporum* showed a well-supported sister relationship to the *Trichonectria rubefaciens* clade. *Cylindromonium everniae* clustered together with *Trichonectria pyrenaica* in a well-supported clade (PP = 1, BS = 100). However, the molecular data for the generic type of *Trichonectria* (*T. hirta*) is unavailable, and therefore, we are not able to clarify the relationship between *Cylindromonium* and *Trichonectia*.

The species of the genus *Trichonectria* growing on *Usnea* are clustered into several clades. The newly described *Trichonectria toensbergiana* showed a sister

relationship (PP = 1, BS = 92) to the clade containing *Trichonectria rubefaciens* and *Cylindromonium rhabdosporum*. Another new species, *Trichonectria abortispora*, showed a strongly supported sister relationship (PP = 0.98, BS = 92) to the clade comprised of *T. pyrenaica* and *Cylindromonium everniae*. Newly described *Trichonectria citrispora* together with *T. anisospora* and *T. usneicola* formed a separate, well-supported clade (PP = 1, BS = 90). The single specimen of *Nectriopsis vinosa* clustered together with *Trichonectria* species rather than *Nectriopsis* s.str. with the type *Nectriopsis violacea* (phylogenetic tree not shown). Therefore, we transfer this species to *Trichonectria* and a new combination is proposed.

Taxonomic results

Trichonectria abortispora Etayo, sp. nov. (Fig. 3)

MycoBank MB 855864

Diagnosis: It differs from *Trichonectria usneicola* in that it has 2–6-spored mature asci and ascospores that are tapered to the apex and measure 13–16(–19) × (4–)5–6 µm in size.

Type: Bolivia. Dept. Tarija, Prov. Aniceto Arce, Reserva Natural de Flora y Fauna Tariquía, between La Cumbre and Campamento Los Alisos, close to guard camp, 22°01′18.9″S, 64°34′22.5″W, 1951 m, on a small *Usnea* thallus growing on a twig, 24 July 2015, J. Etayo 29832 (LPB – holotype; hb. Etayo – isotype).

Description. Ascomata perithecioid, superficial, orange-brown to dark brown, cupuliform or discoidal when dry, aggregated, 100–150 µm diam, with a central ostiole. Ascomatal wall composed of several rows of cells, the external surface K–, without crystals, shows a net of branched, septate, 4–8 µm wide, concolorous hyphae (*setadpressa*-like). Setae abundant only around the ostiole, typically curved, hyaline, septate, with thick wall, 20–40 × 3.5–5 µm. Periphyses present around the ostiole, 12–18 × 1–2 µm. Hamathecium absent in mature ascomata and without oil droplets, I–, K/I–. Asci clavate with an obtuse end and thickened in the upper part with a thin tube visible in K, 2–6(–8)-spored, 40–45 × 7–12 µm. Ascospores hyaline, ellipsoidal, (0–)1-septate, slightly to strongly constricted at the septum, with a large oil droplet inside each cell, apex tapering, 13–16(–19) × (4–)5–6 µm (n = 15).

Etymology. The epithet emphasizes that the development of a portion of the ascospores remains incomplete, leading to a reduced final ascospore number within the ascus.

Notes. *Trichonectria abortispora* can be confused with *T. usneicola* in the ascospore shape and size, but it differs in several characters: the ascomatal wall is covered by a net of external elongated hyphae, mature asci produce less than 8 ascospores and ascospores are not widened towards the apex as in *T. usneicola* (Etayo 2002). The number of ascospores lower than 8 per ascus is rather an uncommon character in usneicolous *Trichonectria*, except for *T. intermedia* which produces constantly 4-spored asci. Asci in *T. abortispora* are at first 6–8-spored, but soon fully developed mature asci contain usually 2–4 ascospores.

This species is only known from the type locality where it grew on a small non-identified epiphytic *Usnea* species. As we have extensively collected poorly developed *Usnea* in Bolivia, we assume that this is rather a rare species.

Trichonectria apiculata Etayo

Notes. This species has been recently described from Ecuador (Etayo 2017) and is characterized by obpyriform, relatively large perithecia, (125–)200–225 µm diam., with brown exciple with the K– pigment (*gigaspora*-like wall), lack of setae, 8-spored asci measuring 42–54 × 14–20 µm., and large (16–24 × 6–8 µm), apiculate ascospores

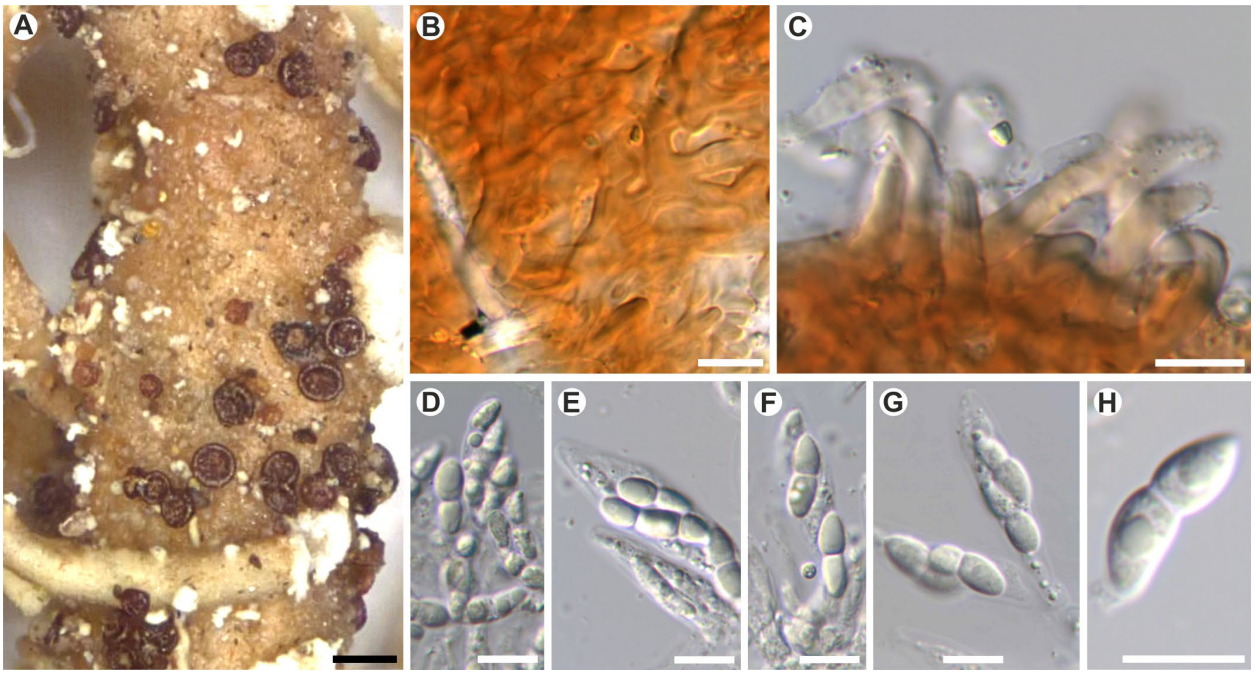

Figure 3. *Trichonectria abortispora* (Etayo 29832). A – habit; B – ascomatal wall covered by a net of elongated, septate hyphae; C – setae around ostiole; D–G – asci with 6, 4 and 2 ascospores (from left to right); H – ascospore. Scales: A = 100 µm; B–H = 10 µm.

constricted at the septa. *Trichonectria apiculata* is known from three localities in Ecuador and two in Bolivia. Some Bolivian samples have slightly larger perithecia (up to 280 µm in diam.) and ascospores (20–31 × 7–9 µm).

Specimens examined. BOLIVIA. Dept. Chuquisaca, Prov. Zudañez, Área Natural de Manejo Integrado El Palmar, segunda villa de presto, Lomán, Salviatójo, 18°45′53″S, 64°49′57″W, 2875 m, Boliviano-Tucumano forest with *Podocarpus* and shrubs, on corticolous *Usnea* sp. growing on tree, 14 July 2015, A. Flakus 26434 (KRAM L-74387, LPB); Dept. Cochabamba, Prov. Carrasco, Parque Nacional Carrasco, Koricaza, 17°33′21″S, 65°16′29″W, 2950 m, Páramo Yungueño, on *Usnea* sp. growing on tree, 18 Aug. 2012, J. Etayo 29326 (LPB, hb. Etayo); Dept. La Paz, Prov. Franz Tamayo, Área Natural de Manejo Integrado Nacional Apolobamba, near Rio Pelechuco, below Pelechuco close to the new road to Apolo, 14°46′39″S, 69°00′35″W, 2550 m, lower montane Yungas cloud forest, on *Usnea* sp. growing on tree, 16 Nov. 2014, M. Kukwa 14763a (LPB); Área Natural de Manejo Integrado Nacional Apolobamba, near Rio Pelechuco, below Pelechuco close to new road to Apolo, 14°47′28″S, 69°01′32″W, 2860 m, open area with scattered trees, on *Usnea* sp. growing on tree, 16 Nov. 2014, M. Kukwa 14823 (LPB, UGDA); Prov. Nor Yungas, near Nogalani village, on the road Coroico-La Paz (casa azul de Alejo), 16°12′57″S, 67°49′15″W, 2168 m, Yungas secondary cloud forest, on *Usnea* sp. growing on tree, 23 May 2011, J. Etayo 28002 (LPB, hb. Etayo).

Trichonectria australis Etayo s.lat.

Notes. This species was described from southern South America (Etayo & Sancho 2008). It usually grows on reddish lacinia of *Usnea* species, color probably produced by the fungal infection, and is characterized by yellowish to orange perithecia with a K– pigment, of size (70–)100–170 µm, with a *gigaspora*-like ascomatal wall, composed of irregular cells, hyaline setae, cylindrical to tapering or clavate asci with obtuse ends and without apical apparatus (35–43 × 5–7 µm), and small straight to curved ascospores. The type specimen is characterized by 8–11 × 2–3 µm ascospores (Etayo & Sancho 2008). Most of the presented here samples have ascospores measuring 7–10 × 2–3.5 µm (n = 70) and asci measuring 27–35 × 5–6 µm (n = 20). Some of our specimens develop larger ascospores (the largest found in Etayo 27304; 11–16.5 × 3–4 µm), perithecia (Etayo 26962, 170–215 µm, but ascospores in the same size as in the diagnosis of *T. australis*) or asci (Etayo 33212, 50–59 × 5–6 µm) which suggest that the material may represent a complex of species which need to be studied in the future on larger sampling.

The species with ascospores size similar to *T. australis*, [(9–)11–14(–15) × 3–4.5 µm] is the European *T. pyrenaica* (Gardiennet & Lechat 2011). However, molecular data for *T. australis* are not available, leaving the question of its conspecificity with *T. pyrenaica* unresolved.

Specimens examined. BOLIVIA. Dept. Cochabamba, Prov. Carrasco, Parque Nacional Carrasco, Korikaza close to Monte Punku, lower montane Yungas cloud forest, 17°33′27.5″S, 65°16′28.5″W, 2855 m, on *Usnea* sp. growing on twigs, 27 Nov. 2014, J. Etayo 33212 (LPB, hb. Etayo); near Río Lopez Mendoza, lower montane Yungas cloud forest, 17°30′23.5″S, 65°16′51″W, 2250 m, on *Usnea* sp. growing on tree, 27 Nov. 2014, J. Etayo 33970 (LPB); Wayra Mayu close to Monte Punku, lower montane Yungas cloud forest, 17°32′04.7″S, 65°16′12.6″W, 2520 m, on *Usnea* sp. growing on tree, 28 Nov. 2014, J. Etayo 33870 (LPB); 17°33′30″S, 65°16′08″W, 2750 m, on small *Usnea* sp. growing on twigs, 28 Nov. 2014, J. Etayo 34306 & 34308 (LPB, hb. Etayo); Dept. La Paz, Prov. Franz Tamayo, Area Natural de Manejo Integrado Apolobamba, near Río Pelechuco, below Pelechuco close to new road to Apolo, lower montane Yungas cloud forest, 14°46′39″S, 69°00′35″W, 2550 m, on *Usnea* sp. growing on twig, 16 Nov. 2014, J. Etayo 33876, 33887 (LPB, hb. Etayo); Prov. Larecaja, vicinity of Jocollone village, 15°37′35″S, 68°41′21″W, 3545 m, Páramo Yungeño vegetation, open area with *Berberis*, NE oriented slope, on *Usnea* growing on twigs, 14 May 2011, J. Etayo 27304 (LPB, hb. Etayo); Prov. Murillo, Sainani, Valle del Zongo, open area with shrubs and scattered trees, 16°07′01″S, 68°04′49″W, 2100 m, on *Usnea* sp. growing on tree, 7 Dec. 2014, J. Etayo 34352 (LPB); Prov. Nor Yungas, Parque Nacional y Area Natural de Manejo Integrado Cotapata, between Tunkini and Chairo villages, above Tunkini, even Biologic station, 16°11′00″S, 67°52′00″W, 2005 m, Yungas montane forest, on *Usnea* sp. growing on tree, 22 May 2011, J. Etayo 27751 (LPB, hb. Etayo); 16°11′12″S, 67°52′07″W, 1936 m, Yungas montane forest, on *Usnea* sp. growing on tree, 22 May 2011, J. Etayo 27383 (LPB, hb. Etayo); Parque Nacional y Area Natural de Manejo Integrado Cotapata, 30′ of Unduavi by Sillu Tincara pre-Columbian route, 16°17′22″S, 67°53′29″W, 3489 m, transition Páramo Yungeño – Yungas montane cloud forest, on *Usnea* sp. growing on tree, 25 May 2011, J. Etayo 28081 (LPB, hb. Etayo); below Unduavi village, on the road La Paz – Chulumani, near the river, slope E, secondary Yungas cloud forest, on *Usnea* sp. growing on twigs, 3135 m, S16°18′50″, W67°54′35″, 31 May 2011, J. Etayo 26962 (LPB, hb. Etayo);

Trichonectria biglobospora Etayo & Flakus, sp. nov. (Fig. 4)

MycoBank MB 855865

Diagnosis: *Trichonectria biglobospora* differs from other species of *Trichonectria* in having an ascus with obtuse and relatively thick apex and ascospores composed of two similar in size subspherical cells.

Type: Bolivia. Dept. La Paz, Prov. Franz Tamayo, Área Natural de Manejo Integrado Nacional Apolobamba, near Rio Pelechuco, below Pelechuco close to the new road to Apologranjita by Pelechuco river, open area with scattered trees, 14°47′25.1″S, 69°01′32.6″W, 2880 m, on *Usnea* sp. growing on twigs, 16 Nov. 2014, J. Etayo 33111 (LPB – holotype, hb. Etayo – isotype).

Description. Ascomata perithecia, lichenicolous on *Usnea*, superficial, cupuliform when dry, dispersed, dark brown to almost black, 120–170(–200) µm diam, with a central ostiole, always observed without setae. Ascomatal wall orange-brown, K–, formed by several layers, ~20 µm thick in the lateral wall, inner portion hyaline and the outer layer orange with *gigaspora*-like texture, composed of irregular cells, without crystals. Setae absent. Periphyses present around the ostiole, 15–20 × 1–1.5 µm. Hamathecium soon gelatinized I–, K/I–, without oil droplets. Asci clavate, with obtuse apex, slightly thickened at the apex, 8-spored, 35–40 × 11–12 µm. Ascospores biseriate in the ascus, hyaline, 1-septate, strongly constricted at the septum, composed of two subspherical cells, 8.5–12 × 5–7 µm (n = 25).

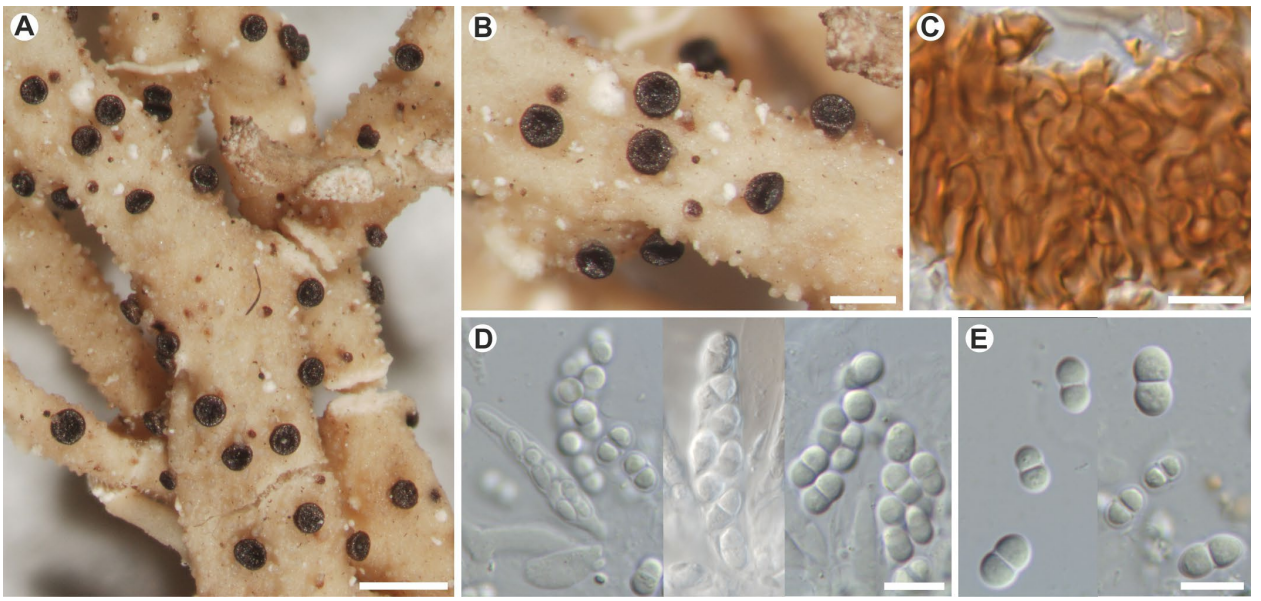

Figure 4. *Trichonectria biglobospora* (Etayo 33111). A, B – habit; C – ascomatal wall with irregularly thickened cells (*gigaspora*-like); D – asci; E – ascospores. Scales: A = 500 µm; B = 250 µm; C–E = 10 µm.

Etymology. The epithet refers to ascospores that are composed of two semi-globose cells.

Notes. *Trichonectria biglobospora* differs from other species of *Trichonectria* in its characteristic ascus with obtuse apex of relatively thick wall, and ascospores formed by two subspherical cells. No other species growing on *Usnea* have these features. Among the species growing on *Usnea*, there is only one with similar wide ascospore cells, *T. usneicola* (Etayo 2002). It differs in its usually vivid orange and setose ascomata, and larger, 14–16.5 × 4.5–5.5 µm, strongly constricted ascospores, never formed by subspherical cells.

No other species of *Trichonectria* can be confused with *T. biglobospora*, however, *Pronectria biglobosa* growing on *Hypotrachyna* have ascospores similar in shape (Etayo 2017). This species can be differentiated by its immersed ascomata and verruculose (at young stage) and longer (11–14.5 × (5–)6–6.5(–7) µm) ascospores.

Specimens examined. BOLIVIA. Dept. Cochabamba, Prov. Chapare, Parque Nacional Carrasco, near Lago Corani, cose to Villa Tunari-Cochabamba road, open area with shrubs, 17°13′24″S, 65°53′31″W, 3270 m, on *Usnea* growing on twigs, 2 Dec. 2014, J. Etayo 34868 (LPB, hb. Etayo); Dept. La Paz, Prov. Franz Tamayo, Parque Nacional y Área Natural de Manejo Integrado Madidi, near Keara Bajo, open area with shrubs and scattered trees, 14°41′51.8″S, 69°05′06.9″W, 3300 m, on *Usnea* growing on twigs, 17 Nov. 2014, J. Etayo 33906 (LPB, hb. Etayo).

Trichonectria boliviana Etayo & Flakus, sp. nov.
(Fig. 5)

MycoBank MB 855866

Diagnosis: Differs from *T. usneicola* in having slightly larger perithecia measuring 120–200 µm diam., scarce setae around ostiole, larger asci 55–67(–75) × 12–25 µm, and larger ascospores tapering to the apex (16–)18–24(–29) × (6–)7–9(–10) µm.

Type: Bolivia. Dept. Tarija, Prov. Burnet O'Connor, close to Entre Ríos, new road between Tarija and Entre Ríos, 21°30′47″S, 64°11′49″W, 1338 m, disturbed Tucumano-Boliviano forest with shrubs and *Tillandsia*, on *Usnea* growing on twigs, 28 July 2015, J. Etayo 30641 (LPB – holotype, hb. Etayo – isotype).

Description. Ascomata perithecioid, lichenicolous on *Usnea*, superficial, dispersed or in small groups of 3–4, first yellowish to orange then brown when mature, K–, subsphaerical, but constricted vertically or horizontally, with a central ostiole, 120–200 µm diam. Ascomatal wall orange, K–, with yellow crystals (disappearing in K), 10–15 µm diam., composed of several layers; the external layer of *gigaspora*-like texture, with irregularly thickened cells, 8–12 µm in diam. Setae scarce, restricted to a zone around the ostiole, septate, cylindrical, with obtuse apex and thickened walls, 18–35 × 3–6 µm. Periphyses around ostiole, simple, with very thin wall, ~20 × 1.5–2 µm. Hamathecium gelatinized, sometimes with large, hyaline oil droplets, I–, KI–. Asci widely clavate, with obtuse ends, without visible apical apparatus, (4–6)–8-spored, 55–75 × 12–25 µm. Ascospores biseriate, ellipsoid, hyaline, 1-septate, strongly constricted at the septa, rarely breaking in half, with wider upper cell, tapering to the apex, straight to slightly curved, (16–)18–24(–30) × (6–)7–9(–10) µm (n = 89).

Etymology. This new species is named after the country where it has been found.

Notes. Macroscopically, the new species is similar to *T. usneicola*, a common parasite of *Usnea* in Bolivia, however, *T. usneicola* can be separated by its almost twice smaller ascospores (14–16.5 × 4.5–5.5 µm), characteristically widened to the apex (usually of polyhedral shape; see drawing in Etayo 2002) and slightly smaller perithecia (150–170 µm in diam.) (Etayo 2002). We observed that *T. boliviana* produces strongly constricted ascospores in an overmature state with a tendency to break into half. The size of the ascospores is variable within observed specimens which may suggest strong intraspecific variation that requires future research.

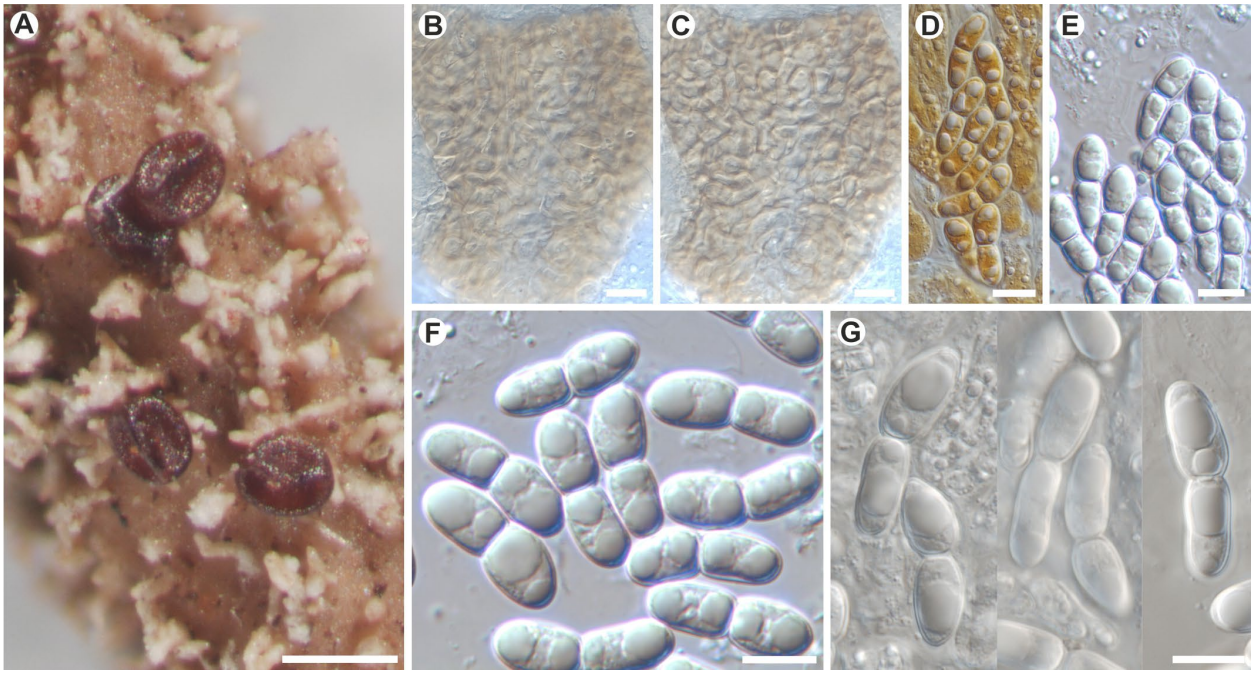

Figure 5. *Trichonectria boliviana* (Etayo 30641). A – subpyramidal dry ascomata; B, C – external view of exciple (B – with few elongated cells covering the outermost portion of exciple; C – without hyphae coverage); D, E – 8-spored asci with obtuse apex (D – mounted in Congo Red; E – in water); F, G – ascospores. Scales: A = 200 µm; B–G = 10 µm.

Additional specimens examined. BOLIVIA. Dept. Chuquisaca, Prov. Zudañez, Área Natural de Manejo Integrado El Palmar, segunda villa de presto, Lomán, Salviatójo, 18°45′53″S, 64°49′57″W, 2875 m, Boliviano-Tucumano forest with *Podocarpus* and shrubs, on *Usnea* sp. growing on twigs, 14 July 2015, J. Etayo 30385 (LPB, hb. Etayo); Dept. Cochabamba, Prov. Carrasco, Parque Nacional Carrasco, near Río López Mendoza, 17°30′23.5″S, 65°16′51″W, 2250 m, lower montane Yungas cloud forest, on unhealthy reddish *Usnea* growing on twigs, 27 Nov. 2014, J. Etayo 33939 (LPB, hb. Etayo); Dept. La Paz, Prov. Larecaja, Jocollone village and 1 km further, PáramoYungeño vegetation, open anthropogenic area, much *Berberis*, NE oriented slope, 15°37′35″S, 68°41′21″W, 3545 m, on *Usnea* sp. growing on tree, 14 May 2011, J. Etayo 27353 (LPB, hb. Etayo); Prov. Murillo, Valle de Zongo, bosque nublado, near metal bridge, on *Usnea* sp. growing on branches, 2450 m, 16°07′41″S, 68°05′55″W, 29 May 2011, J. Etayo 26729, (LPB, hb. Etayo); Prov. Nor Yungas, below Unduavi village, near Río Unduavi, 16°18′50″S, 67°54′35″W, 3135 m, Yungas cloud forest, on *Usnea* growing on trunk, 31 May 2011, J. Etayo 26961 (LPB, hb. Etayo); Dept. Tarija, Prov. Burnet O'Connor, Sandiego Sur, top of the hill on old road between Tarija and Entre Ríos, 21°27′04″S, 64°13′59″W, 1812 m, Boliviano-Tucumano forest, 30 July 2015, on *Usnea* sp. growing on twigs, J. Etayo 32631 (LPB); close to Los Pinos, old road between Entre Ríos and Tarija, 21°24′50″S, 64°18′33″W, 2149 m, Boliviano-Tucumano forest dominated by shrubs, with *Alnus acuminata*, *Podocarpus* and *Ericaceae*, on apothecial margin of *Usnea* sp. growing on twigs, 29 July 2015, J. Etayo 32799 (LPB, hb. Etayo).

Trichonectria citrispora Etayo, Darmostuk & Flakus, sp. nov. (Fig. 6)

MycoBank MB 855867

Diagnosis: Differs from species of *Trichonectria* growing on *Usnea* in having lemon-shaped, usually simple ascospores, measuring (14.5–)16–19(–22) × (5.5–)6–8(–8.5) µm.

Type: Bolivia. Dept. La Paz, Prov. Franz Tamayo, Parque Nacional y Área Natural de Manejo Integrado Madidi, near Keara Bajo, 14°41′51.8″S, 69°05′06.9″W, 3300 m, open area with shrubs and scattered trees, on small *Usnea* growing on twigs, 17 Nov. 2014, J. Etayo 33921 (LPB – holotype, hb. Etayo – isotype).

Description. Ascomata perithecioid, lichenicolous on *Usnea*, superficial, solitary, subglobose, cupuliform when dry, with smooth surface, orange-brown to dark brown, K–, without crystals, apparently always without setae, with a central ostiole, (120–)150–250 µm diam. Ascomatal wall 10–13 µm thick in section, composed of several layers, externally of *gigaspora*-like texture, orange. Periphyses around ostiole abundant, 20–30 × 1–2 µm, surrounded by a gelatin coat. Hamathecium gelatinized, I–, K/I– and without oil droplets. Asci widely clavate when filled with mature ascospores, with obtuse apex without apical apparatus, 8-spored, 52–70 × 12–18 µm. Ascospores biseriate, ellipsoidal to citriform, straight, rarely slightly curved, sometimes with a mucron in each apex, hyaline, with many small oil droplets, simple, rarely 1-septate, smooth-walled, (13–)16–19(–22) × (5–)6–8(–8.5) µm (n = 62).

Etymology. The epithet refers to lemon-shaped ascospores.

Notes. This is the only known species of *Trichonectria* inhabiting *Usnea* with mostly simple ascospores which make it a unique and easily distinguishable species. It seems to be a homogeneous species in terms of ascospore size and number of ascospores in asci; only in one sample being in a bad stage (Etayo 34534) we found some asci with 1–2 ascospores. We have found it in several localities always growing on reddish moribund *Usnea* thalli.

Additional specimens examined. BOLIVIA. Dept. Cochabamba, Prov. Chapare, near Lago Corani, 17°13′44″S, 65°53′39″W, 3260 m, open area with shrubs, on *Usnea* sp. growing on shrubs, 4 Dec. 2014, A. Flakus 26300 (KRAM L-74385,

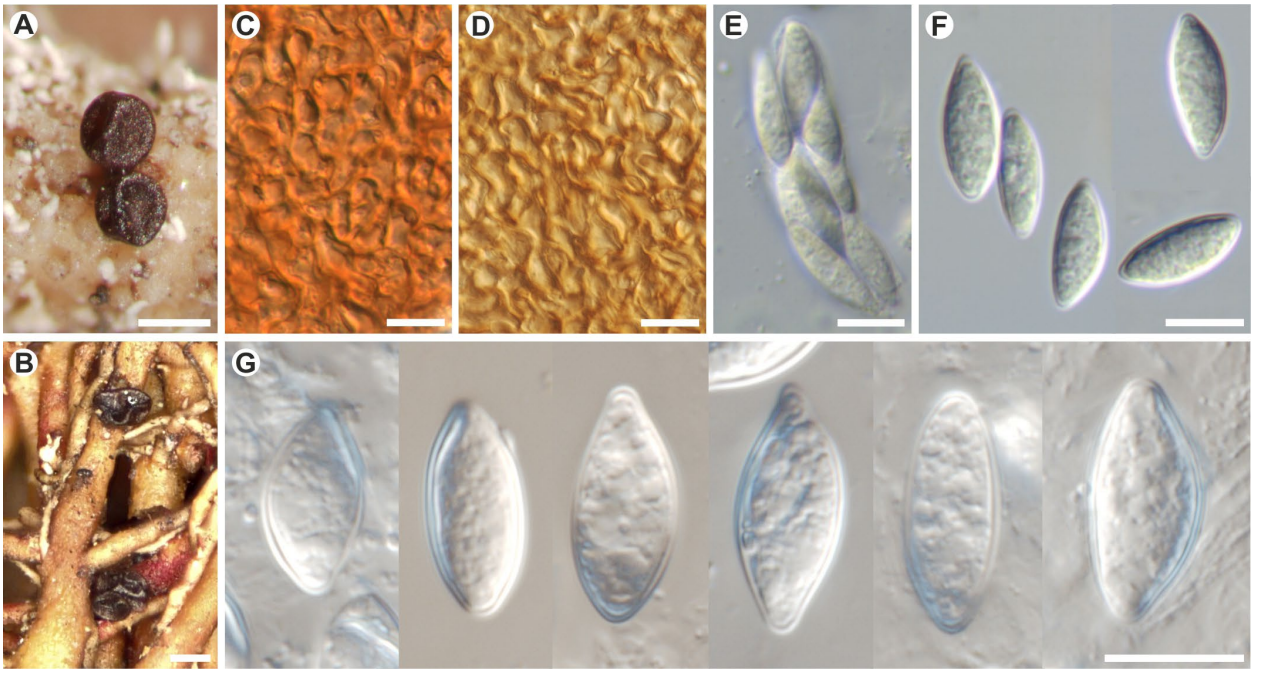

Figure 6. *Trichonectria citrispora*. A, B – subpyramidal dry ascomata (A – Flakus 26300; B – Etayo 33921); C, D – ascomatal wall (*gigaspora*-like) (C – Etayo 33921; D – Flakus 23600); E – ascus with obtuse apex (Etayo 33252); F, G – ascospores (F – Etayo 33252; G – Flakus 26300). Scales: A, B = 200 µm; C–G = 10 µm.

LPB); close to Villa Tunari-Cochabamba road, open area with shrubs, 17°13′24″S, 65°53′31″W, 3270 m, on *Usnea* sp. growing on tree, 2 Dec. 2014, J. Etayo 34866 (LPB, hb. Etayo); Prov. Tiraque, Parque Nacional Carrasco, Camino de los Nubes, Antenas Sillar-Villa Tunari old road, upper montane Youngas cloud forest, 17°12′32″S, 65°41′52″W, 3520 m, on *Usnea* sp. growing on twigs, 29 Nov. 2014, J. Etayo 33252 (LPB, hb. Etayo); old guard's camp, 17°18′23″S, 65°45′60″W, 3360 m, open area with shrubs, on unhealthy reddish *Usnea* sp. growing on twigs, 1 Dec. 2014, J. Etayo 34534 (LPB, hb. Etayo); Dept. La Paz, Prov. Franz Tamayo, Parque Nacional y Área Natural de Manejo Integrado Madidi, below Keara Bajo, 14°41′67″S, 65°04′76″W, 3030 m, open area with shrubs and scattered trees, on *Usnea* spp. growing on twigs and tree trunk, 18 Nov. 2014, J. Etayo 34092 & 34100 (LPB, hb. Etayo); Dept. Tarija, Prov. Aniceto Arce, Reserva Natural Flora y Fauna de Tariquía, between la Cumbre and camamento los Alisos, 22°00′41″S, 64°36′02″W, 2560, Boliviano-Tucumano forest with *Alnus acuminata* and *Polylepis*, on *Usnea* sp. growing on twigs, 22 July 2016, J. Etayo 32857 (LPB, hb. Etayo).

Trichonectria cylindrospora Etayo & Flakus, sp. nov. (Fig. 7)

MycoBank MB 855867

Diagnosis: Differs from *T. rubefaciens* in having 6-spored asci, larger ascospores, (25–)30–37(–40) × 4–5 µm, arranged in a row inside the ascus and larger perithecia, 150–200 µm diam.

Type: Bolivia. Dept. Tarija, Prov. Aniceto Arce, close to Coyambuyo, between Padcaya and Bermejo, 22°17′23″S, 64°28′50″W, 940 m, Sub-Andean Tucumano-Boliviano forest with bryophytes, *Lauraceae* and *Meristomataceae*, on dying *Usnea* sp. growing on branches, 26 July 2015, J. Etayo 30697 (LPB – holotype; herb. Etayo – isotype).

Description. Ascomata perithecioid, lichenicolous on *Usnea*, superficial, cupuliform when dry, sunken in ostiolar region, dark brown to blackish, K–, with a central ostiole, always without setae, 150–200 µm diam. Ascomatal wall 10–18 µm thick, composed of several rows of cells, the inner rows hyaline with thin walls and small oil droplets, the external portion of *gigaspora*-like textura with irregularly widened walls, orange and without crystals. Periphyses around ostiole, 20–25 × ~1 µm, wider at the base (to 2.5–3 µm). Hamathecium usually absent, but in some perithecia with abundant filaments, with large cells, up to 15 µm wide and without oil droplets, I–, K/I–. Asci thin-walled, clavate to ellipsoid, with obtuse end, mainly 6-spored, 40–50 × 12–15 µm. Ascospores arranged in bundles inside ascus, hyaline, long cylindrical to fusiform, straight to rarely curved, with obtuse ends, 1-septate, not breaking, not or slightly constricted at the septum, (25–)30–37(–40) × 4–5 µm (n = 23).

Etymology. The epithet refers to the ascospores, which are cylindrical in shape.

Notes. The shape of ascospores of the new species reminds this of *T. rubefaciens* s.str., a species not growing on *Usnea*, but ascospores are much smaller, (12–)14–18(–20) × 2.5–3(–4) µm, in the latter species. *T. rubefaciens* also differs in having 8-spored asci and much smaller perithecia (100–160 µm) (Lowen 1995). *Trichonectria boliviana* can easily be separated from *T. cylindrospora* by having broadly ellipsoid ascospores, that are strongly constricted at septa and wider (6–10 µm) than those of *T. cylindrospora* (4–5 µm).

After intensive sampling of unhealthy *Usnea* it was collected only at the type locality, and we assume that it is a rare species.

Trichonectria gigaspora Etayo & Flakus, sp. nov. (Fig. 8)

MycoBank MB 855868

Diagnosis: Differs from the other species of *Trichonectria* growing on *Usnea* by having vermiform, 3-septate and very long ascospores, (51–)60–70(–77) × (4–)4.5–5.5(–6) µm.

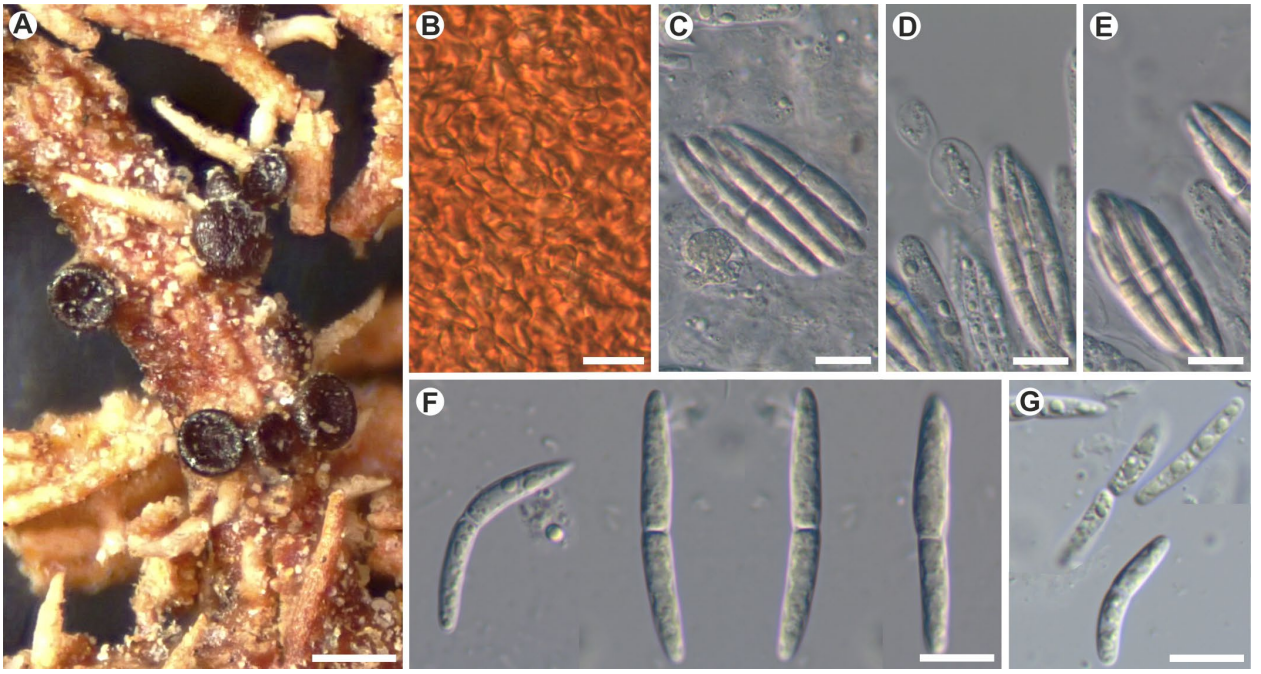

Figure 7. *Trichonectria cylindrospora* (Etayo 30697). A – ascomata on host thallus; B – external view of exciple; C–E – widely clavate asci with obtuse apex; F – ascospores; G – ascospores of *T.* aff. *rubefaciens* on *Usnea* (Etayo 33949). Scales: A = 250 µm; B–G = 10 µm.

Type: Bolivia. Dept. Chuquisaca, Prov. Luis Calvo, Parque Nacional y Área Natural de Manejo Integrado Serranía del Iñao, Serranía del Iñao, close to Ticucha, between Tranqua and Monte Agudo, 19°39′50″S, 63°49′14″W, 1022 m, disturbed area with shrubs in Sub-Andean Tucumano-Boliviano forest, on *Usnea* sp. growing on twigs, 18 July 2015, J. Etayo 32686 (LPB – holotype; herb. Etayo – isotype).

Description. Ascomata lichenicolous on *Usnea*, dispersed, superficial, cupuliform when dry, sometimes with a small umbo around the ostiole in the centre, brown, K–, smooth, always without setae, 200–250 µm diam. Ascomatal wall composed of several layers, hyaline with thin wall cells in the internal region and orange brownish to brown, K–, without crystals, in the external layer, of *gigaspora*-like textura with polygonal cells, cells 7–17 µm diam. Periphyses abundant around ostiole, cylindrical, hyaline, 20–25 × 1.5–2 µm thick. Hamathecium gelatinized, I–, K/I– and without oil droplets. Asci ellipsoidal, with an obtuse and thin-walled apex (when young with a thicker wall, up to 2 µm thick in K), 6–8-spored, 77–95 × (15–)17–20(–24) µm. Ascospores arranged in bundles, parallel to spirally twisted in asci, straight to slightly vermiform when released, hyaline, 3-septate, with obtuse apex and many small oil droplets inside, (51–)60–70(–77) × (4–)4.5–5.5(–6) µm (n = 15).

Etymology. The epithet refers to the size of the ascospores, which are very large compared to most species of the genus and especially to usneicolous *Trichonectria*.

Notes. It is a species of usneicolous *Trichonectria* characterized by the largest ascospores. This unusual size of ascospores together with the development of 3 septa differentiates it well from all other species growing on *Usnea*. *Trichonectria gigaspora* has been found only once

Figure 8. *Trichonectria gigaspora* (Etayo 32686). A – ascomata on host thallus; B – apical part of asci; C, D – ascospores. Scales: A = 100 µm; B–D = 10 µm.

during our extensive fieldwork in Bolivia which may suggest that it is rather a rare species.

Trichonectria cf. ***intermedia*** Etayo

Description. Ascomata orange-brown to dark brown, constricted laterally, superficial, small, 70–100 µm diam., dispersed or grouped on fibrils of *Usnea*, always observed without setae. Setae cylindrical and slightly curved. Ascomatal wall with *gigaspora*-like textura with large cells, 7–12 µm diam, without crystals. Hymenium without paraphyses in mature perithecia, but with periphyses around the ostiole, 12–15 × 1–1.5 µm. Asci clavate, apex obtuse without apical apparatus, 4-spored, 29–40 × 7–9 µm. Ascospores subcylindrical, straight to more habitually curved, not constricted at septum and with obtuse apex, (14–)15–19 × 3–4 µm (n = 25).

Notes. *Trichonectria intermedia* was described on *Parmotrema* from Ecuador (Etayo 2017) and is characterized by small, orange ascomata, 4-spored asci, and 1-septate ascospores measuring (12–)15.5–17(–19) × 3–4 µm. Bolivian material presented here, in general, fits the concept of *T. intermedia*, but the latter species differs in some details, e.g., slightly wider ascomata (70–120 µm diam.) which first are immersed and then erumpent, and different host selection (*Parmotrema*; Etayo 2017). Further, larger sampling and molecular studies are needed to establish the systematic position of such specimens.

Specimens examined. BOLIVIA. Dept. Tarija, Prov. Burnet O'Connor, old road between Tarija and Entre Ríos, 21°27′35″S, 64°13′28″W, 1917 m, Boliviano-Tucumano forest with *Podocarpus*, on *Usnea* sp. growing on twigs, 30 July 2015, J. Etayo 32471 (LPB, hb. Etayo).

Trichonectria microsporusneae Etayo & Flakus, sp. nov. (Fig. 9)

MycoBank MB 855869

Diagnosis: It differs from *T. australis* in having ascomatal wall covered by a net of cylindrical cells (*setadpressa*-like) and smaller ascospores 6.5–10(–11) × 1.5–2(–2.5) µm.

Type: Bolivia. Dept. Cochabamba, Prov. Carrasco, Parque Nacional Carrasco, near Río Batea Mayu close to Monte Punku, lower montane Yungas cloud forest, 17°31′33″S, 65°16′21″W, 2430 m, on *Usnea* sp. growing on twigs, 28 Nov. 2014, J. Etayo 34203 (LPB – holotype, hb. Etayo – isotype).

Description. Ascomata perithecioid, lichenicolous on *Usnea*, superficial, dispersed to grouped, orange-brown to dark brown, K–, cupuliform when dry, with a central ostiole, 70–120 µm diam. Ascomatal wall very thin, with a small number of hyaline inner cells surrounded by an orange colored (K–) layer, external surface of the perithecial wall fully covered by concolorous hyphae (*setadpressa*-like), without crystals. Setae sparse, near the ostiole, simple to branched, 10–25 × 3–3.5 µm, orangish in the lower part and hyaline at the top, with thick walls. Periphyses abundant near ostiole, 10–15 × 0.8–1 µm. Hamathecium gelatinized I–, K/I–, without oil droplets. Asci clavate, 8-spored, with truncate apex and apical apparatus, 23–35 × 4–6 µm. Ascospores biseriate, cylindrical to narrowly ellipsoidal, straight to slightly curved, 1-septate, with two small oil droplets by cell, 6.5–10(–11) × 1.5–2(–2.5) µm (n = 25).

Etymology. The epithet refers to the ascospores, which are the smaller of the usneicolous *Trichonectria*.

Notes. Our first impression was that the specimens recorded here could be part of *T. australis*, however, the latter species has paler and slightly bigger ascomata (100–170 µm) and ascospores (8–11 × 2–3 µm), different

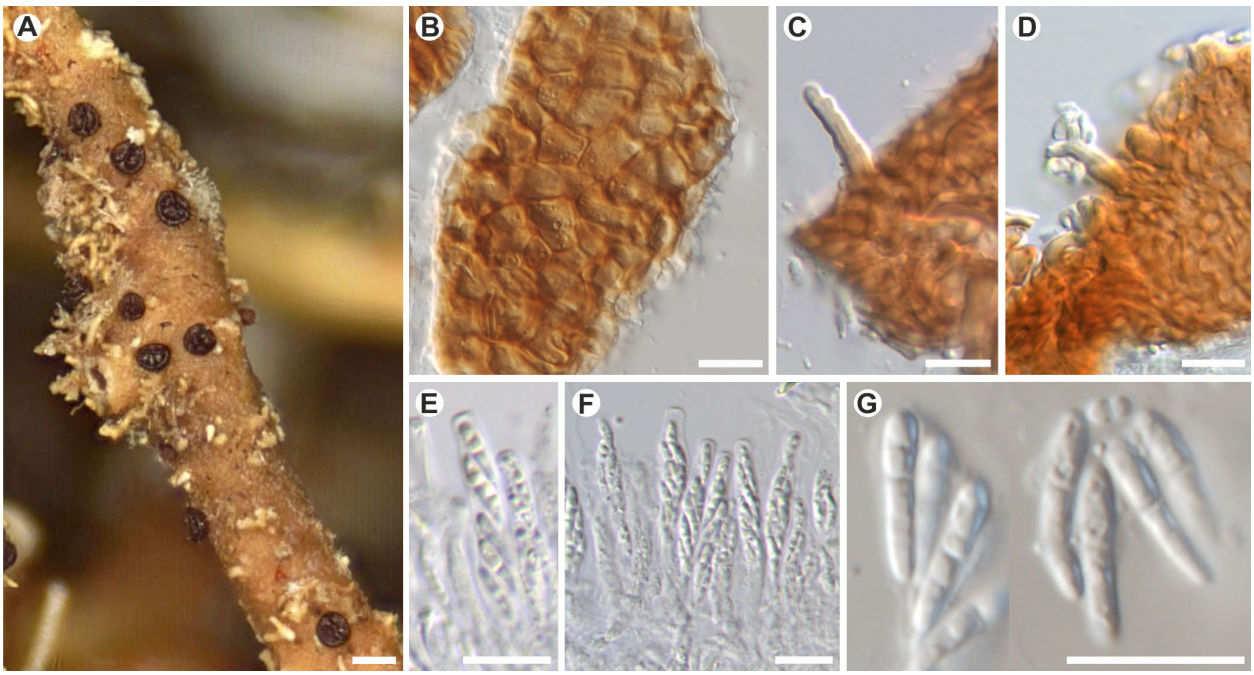

Figure 9. *Trichonectria microsporusneae* (Etayo 34203). A – habit of ascomata on *Usnea*; B – external view of exciple cells; C, D – setae and ascomatal wall covered by a net of hyphae (*setadpressa*-type); E, F – 8-spored asci with truncate apex; G – ascospores. Scales: A = 200 µm; B–G = 10 µm.

ascomatal external wall and lacks the net of cylindrical cells surrounding the perithecia.

We observed an abundant population of the species on the type locality and in three additional localities.

Additional specimens examined. BOLIVIA. Dept. Chuquisaca, Prov. Zudañez, Área Natural de Manejo Integrado El Palmar, segunda villa de presto, Lomán, Salviatójo, 18°45′53″S, 64°49′57″W, 2875 m, Boliviano-Tucumano forest with *Podocarpus* and shrubs, on *Usnea* growing on tree, 14 July 2015, A. Flakus 26434 (KRAM L-74387, LPB); Dept. Cochabamba, Prov. Carrasco, Parque Nacional Carrasco, near Río Batea Mayu close to Monte Punku, lower montane Yungas cloud forest, 17°31′33″S, 65°16′21″W, 2430 m, on *Usnea* sp. growing on tree, J. Etayo 34170 & 34174 (LPB, hb. Etayo); Dept. La Paz, Prov. Franz Tamayo, Área Natural de Manejo Integrado Nacional Apolobamba, near Rio Pelechuco, below Pelechuco close to new road to Apolo, 14°46′39″S, 69°00′35″W, 2550 m, lower montane Yungas cloud forest, on *Usnea* sp. growing on tree, 16 Nov. 2014, M. Kukwa 14763[a] (LPB).

Trichonectria aff. ***rubefaciens*** (Ellis & Everh.) Diederich & Schroers

Notes. *Trichonectria rubefaciens* is a relatively well-known species described from Europe and known from South America (Etayo & Sancho 2008; Etayo 2017) that has been reported so far on foliose *Parmeliaceae* (see a list of genera in Brackel 2014). This species has characteristic clavate setae (Lowen 1995; Etayo & Sancho 2008). The sample we found growing on *Usnea* fits rather well with that description, including shape of setae, but has smaller ascomata (100–120 µm diam. vs 80–160 µm diam.) and larger ascospores [(15–)17–22(–24) × 3–4 µm vs. 12–18 × 3–4 µm] (Lowen 1995). Because there is no molecular data available, the presence of *T. rubefaciens* in tropical South America (especially on *Usnea*) requires further confirmation.

This species is not easily found in the field because of its small size. The only specimen growing on *Usnea* had ascospores that remind those of *T. australis*, but differs fundamentally in being much larger (8–11 × 2–3 µm in the type of *T. australis*). However, we have found slightly larger spores in the Bolivian material we have named *T. australis* (11–16.5 × 3–4 µm) nearest to those of *T. rubefaciens*.

Specimens examined. BOLIVIA. Dept. Cochabamba, Prov. Carrasco, Parque Nacional Carrasco, near Río Lopez Mendoza, lower montane Yungas cloud forest, 17°30′23.5″S, 65°16′51″W, 2250 m, on *Usnea* sp. growing on tree, 27 Nov. 2014, J. Etayo 33949 (LPB).

Trichonectria toensbergiana Flakus, Etayo, Darmostuk & Kukwa, sp. nov. (Fig. 10)

MycoBank MB 855870

Diagnosis: Differs from *Trichonectria australis* by having dark brown to almost black ascomata, slightly bigger ascospores (9–)10–12.5(–13) × 3–4 µm, and lacks of setae.

Type: Bolivia. Dept. Tarija, Prov. Aniceto Arce, Reserva Nacional de Flora y Fauna Tariquía, close to campamento los Alisos, 22°01′25″S, 64°34′06″W, 1900 m, disturbed Boliviano-Tucumano forest with *Alnus acuminata*, *Podocarpus* and *Solanaceae*, on *Usnea* sp. growing on tree, 24 July 2015, A. Flakus 27081 (KRAM L-74384 – holotype; LPB – isotype).

Description. Ascomata perithecioid, lichenicolous on *Usnea*, superficial, situated on the lower side of apothecia and on the host thallus, solitary or forming small groups, dark brown to almost black, K–, matt to shiny, subglobose, collapsing when dry, ostiole central, always without setae, (100–)130–180(–200) µm diam. Ascomatal wall 15–35 µm thick, uniformly orange-brown (K–), without crystals, composed of several rows of thin-walled to slightly thickened, more or less isodiametric cells, 3–13 × 2–6 µm, with thin internal portion, 3–5 µm wide, composed of compressed yellowish to hyaline cells, superficially *gigaspora*-like; rarely surrounded with some interwoven hyphae; periphyses around ostiole poorly developed, 8–9 × 0.5–1.5 µm; hamathecium gelatinized, without oil droplets, I–, K/I–. Asci subcylindrical to narrowly clavate with obtuse to slightly tapering apical part, 28–34 × 6–8 µm, 8-spored. Ascospores biseriate, ellipsoid to narrowly ellipsoid, smooth, straight to slightly curved, with round ends, variable in shape, usually with broader upper cell and larger lower cell, 1-septate, not constricted at the septum, (9–)10–12.5(–13) × 3–4 µm (n = 30).

Etymology. The new species is named in honor of Tor Tønsberg (Bergen, Norway), the prominent lichenologist who recently celebrated his 75th birthday.

Notes. The diagnostic features of the new species are the dark brown to almost black perithecia always lacking setae, and small narrowly ellipsoid ascospores, with the upper cells slightly wider. The most similar species is *T. australis* described from Argentina and Chile (Etayo & Sancho 2008). However, the latter species can be separated by orange, setose ascomata, and slightly smaller ascospores (8–11 × 2–3 µm).

Additional specimen examined. BOLIVIA. Dept. Chuquisaca, Prov. Luis Calvo, Parque Nacional y Área Natural de Manejo Integrado Serranía del Iñao, close to Ticucha, between Tranqua and Monte Agudo, 19°39′50″S, 63°49′14″W, elev. 1022 m, disturbed area with shrubs, on *Usnea* sp. growing on tree, 18 July 2015, M. Kukwa 16425 (LPB, UGDA).

Trichonectria usneicola Etayo

Notes. This species was described from Columbia (Etayo 2002) and it is characterized by ascospores measuring 14–16.5 × 4.5–5.5 µm, which are slightly constricted in the centre and characteristically widened to the apex. Furthermore, ascomata are 150–170 µm diam., vivid orange, with setae around ostiole and surface of ascomatal wall *gigaspora*-like, and clavate asci 45–52 × 8–14 µm with obtuse apex. In Ecuador, some specimens having slightly larger (12–19 × 5–5.5 µm) ascospores with obtuse ends were included in the concept of this species (Etayo 2017), but we are not completely sure of this decision. More material from similar samples is needed to get a conclusion.

Specimens examined. BOLIVIA. Dept. Cochabamba, Prov. Caballero, cerca Siberia, 17°45′54″S, 64°48′47″W, 2570 m, open Yungas cloud forest, on unhealthy reddish *Usnea* sp. growing on tree, 17 Aug. 2012, J. Etayo 28823 (LPB, hb. Etayo); Prov. Chapare, Parque Nacional Carrasco, Incachaca, 17°14′11″S, 65°49′02″W, 2280 m, vegetation with *Pinus* sp. near Yungas cloud forest, on unhealthy reddish *Usnea* sp. growing on tree,

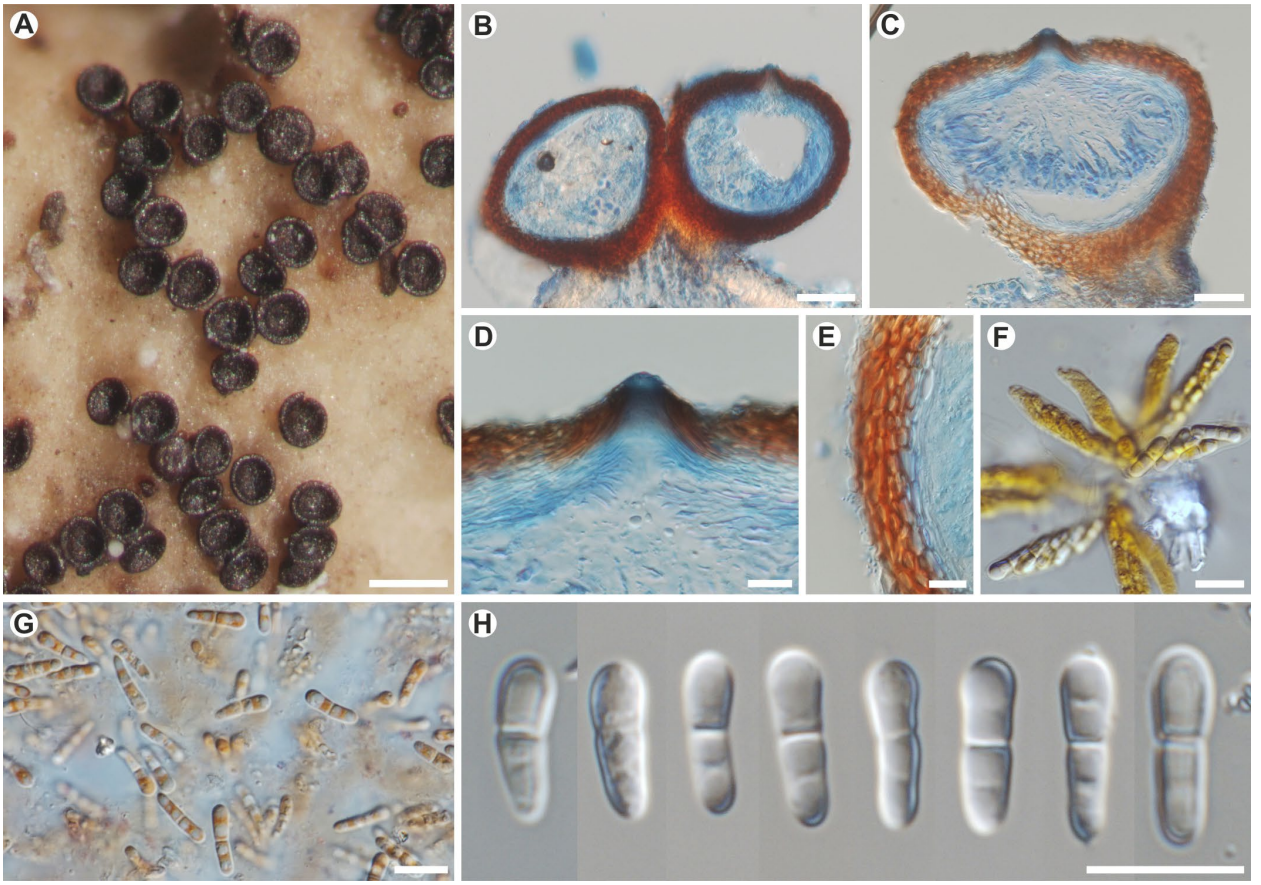

Figure 10. *Trichonectria toensbergiana* (Flakus 27081). A – habit of ascomata on the host; B, C – cross-section trough ascomata (mounted in LPCB); D – section of ascomata showing ostiole and peryphyses (mounted in LPCB); E – section of exciple (mounted in LPCB); F – asci (mounted in Congo Red); G, H – ascospores mounted in Congo Red (G) and water (H). Scales: A = 200 µm; B = 50 µm; C = 25 µm; D–H = 10 µm.

19 Aug. 2012, J. Etayo 28830 (LPB, hb. Etayo); Dept. La Paz, Prov. Franz Tamayo, Parque Nacional y Área Natural de Manejo Integrado Madidi, Chuñuna above Keara, *Polylepis pepei* forest, 14°41′11″S, 69°05′30″W, 4053 m, on *Usnea* sp. growing on twigs of *Polylepis*, 19 Nov. 2014, J. Etayo 33132 (LPB, hb. Etayo); Parque Nacional y Área Natural de Manejo Integrado Madidi, near Keara Bajo, 14°41′59″S, 69°04′34″W, 3290 m, open area with shrubs and scattered trees, on *Usnea* sp. growing on tree, 17 Nov. 2014, A. Flakus 25230 (KRAM L-74383, LPB); Chuñuna above Keara, 14°41′11″S, 69°05′30″W, elev. 4053 m, *Polylepis pepei* forest, on *Usnea* sp. growing on tree, 19 Nov. 2014, M. Kukwa 14955d (LPB); Dept Tarija, Prov. Burnet O'Connor, la Cumbre close to Entre Ríos, old road between Entre Ríos and Tarija, 21°27′48″S, 64°13′24″W, 1943 m, Boliviano-Tucumano forest with *Podocarpus* and *Prumnopitys exigua*, on dying laciniae of *Usnea* sp. growing on twigs, 28 July 2015, J. Etayo 32777 (LPB).

Trichonectria vinosa (Etayo) Etayo, Darmostuk & Flakus, comb. nov.

MycoBank MB 855871

Basionym: *Nectriopsis vinosa* Etayo, Opera Lilloana 50: 301. 2017.

Description. For the detailed description see Etayo (2017).

Notes. This species grows on *Usnea* and was described by Etayo (2017) in the genus *Nectriopsis*. It is characterized by blackish to purple-black, cupuliform when dry, and small ascomata. The inspersed pigment covers a paraplectenchymatic dark violet exciple composed of polyhedric cells, 4–11 µm diam., and dissolves in K+ dark purple. Ascospores are small, 9–10 × 3–3.5 µm with a wider and shorter upper cell. Specimens from Bolivia are similar to the original description, but we have seen in one young ascomata hamathecial hyphae with large ovoid to ellipsoid cells, 8–11 µm wide, similar to those found in *Xenonectriella* species.

This is a morphologically distinct taxon easy to differentiate from other *Trichonectria* species, which have usually orange to brown and K– ascomata. However, phylogenetic analyses revealed this species in *Trichonectria* clade.

Trichonectria vinosa has been found in five localities on brown, dead lacinia of *Usnea* spp.

Specimens examined. BOLIVIA. Dept. Chuquisaca, Prov. Zudáñez, Área Natural de Manejo Integrado El Palmar, La Cascada bajo de El Palmar, 18°41′23″S, 64°54′26″W, 2740 m, Boliviano-Tucumano forest with *Podocarpus*, *Lauraceae* and palms, on *Usnea* sp., growing on branches, 15 July 2015, J. Etayo 32670 (LPB, hb. Etayo); Dept. La Paz, Prov. Larecaja, near Achiquiri village and Charapampa, 18 km from Mapiri on the road to Apolo, 15°13′31″S, 68°13′49″W, 1071 m, Yungas inferior secondary forest, on *Usnea* sp. growing on trees, 16 May 2011, J. Etayo 27232 (LPB, hb. Etayo); Prov. Saavedra, on the road from Apolo to Charazani villages, 20 km from Charazani, 15°12′35″S, 68°51′30″W, 2259 m, transition Yungas montane forest-cloud forest, on *Usnea* sp. growing on tree, 18 May 2011, J. Etayo 27425, (LPB, hb. Etayo); Dept. Tarija, Prov. Aniceto Arce, Papachacra, 21°41′41″S, 64°29′35″W, 2330–2400 m, Tucumano-Boliviano montano forest, with *Alnus acuminata*

and *Podocarpus* sp., on *Usnea* sp. growing on tree, 7 Aug. 2012, J. Etayo *28192* (LPB, hb. Etayo); Prov. Burnet O'Connor, close to los Pinos, 21°25′30″S, 64°19′07″W, 2265 m, Boliviano-Tucumano forest, on *Usnea* sp. growing on tree, 29 July 2015, M. Kukwa 16850e (LPB); close to los Pinos, old road between Entre Ríos and Tarija, 21°25′07″S, 64°18′50″W, 2190 m, Boliviano-Tucumano forest dominated by shrubs, with *Alnus acuminata*, *Podocarpus* and *Ericaceae*, Andino Montano belt, on *Usnea* sp. growing on branches, 29 July 2015, J. Etayo 30721 & A. Flakus 27452-2 (KRAM L-74382, LPB, hb. Etayo).

Key to lichenicolous *Trichonectria* growing on *Usnea* in Bolivia

1 Ascomata brown to black with purple tinge, K+ purple . . . . . ***T. vinosa***
Ascomata yellowish, orange to dark brown or almost black, without purple tinge, K– 2

2(1) Ascospores mostly simple and citriform . . ***T. citrispora***
Ascospores 1-septate to pluriseptate 3

3(2) Ascospores 3-septate, fusiform-vermiform, (51–)60–70(–77) × (4–)4.5–5.5(–6) µm . . . . . ***T. gigaspora***
Ascospores 1-septate, ellipsoid, cylindrical to fusiform, shorter than 50 µm 4

4(3) Ascomata covered by a net of long hyphae, wall *setadpressa*-like 5
Ascomata without an external hyphae coverage, wall *gigaspora*-like. 6

5(4) Asci 8-spored; ascospores 6.5–10(–11) × 1.5–2(–2.5) µm . . . . . ***T. microsporusneae***
Asci 2–4(–6)-spored; ascospores 13–16(–19) × (4–)5–6 µm . . . . . ***T. abortispora***

6(4) Asci 4-spored; ascospores (12–)15.5–17(–19) × 3–4 µm . . . . . ***T.*** cf. ***intermedia***
Asci 6–8-spored 7

7(6) Ascospores composed of two subglobose cells, 8.5–12 × 5–7 µm . . . . . ***T. biglobospora***
Ascospores composed of cylindrical to ellipsoid cells . 8

8(7) Ascospores 25–40 × 4–5 µm, narrow cylindrical . . . . . ***T. cylindrospora***
Ascospores shorter, subcylindrical, ellipsoid to fusiform 9

9(8) Ascospores subcylindrical, (15–)17–22(–24) × 3–4 µm . . . . . ***T.*** aff. ***rubefaciens***
Ascospores ellipsoid to fusiform 10

10(9) Ascospores fusiform with pointed ends, 16–31 × 6–9 µm . . . . . ***T. apiculata***
Ascospores ellipsoid with round to obtuse ends, shorter 11

11(10) Ascospores ≥4.5 µm wide 12
Ascospores ≤ 4 µm wide 13

12(11) Ascospores 14–16.5(–19) × 4.5–6 µm, obtuse at the apex . . . . . ***T. usneicola***
Ascospores 18–24 × 7–9 µm, tapering to the apex . . . . . ***T. boliviana***

13(11) Ascomata dark brown to black, without setae, ascospores (9–)10–12.5(–13) × 3–4 µm. . . . . ***T. toensbergiana***
Ascomata orange, setose 14

14(13) Ascospores 8–11 × 2–3 µm . . . . . ***T. australis*** s.str.
Ascospores 11–16.5 × 3–4 µm. . . . . ***T. australis*** s.lat.

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