

Three new species in the *Biatoropsis usnearum* complex

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Abstract: MILLANES, A. M., DIEDERICH, P., WESTBERG, M. & WEDIN, M. 2016. Three new species in the *Biatoropsis usnearum* complex. – *Herzogia* 29: 337–354.

Three new species of *Biatoropsis* are formally described based on our previous molecular studies, and on additional molecular, morphological, and ecological data. *Biatoropsis protousneae* sp. nov. is confined to *Protousnea dusenii*. *Biatoropsis minuta* sp. nov. is characterized by the small and brown to black basidiomatal galls, and by growing on *Usnea barbata* and *U. lapponica*. *Biatoropsis hafellneri* sp. nov. is distinguished by 2-celled basidia with cells that elongate laterally at maturity, and by growing on species of the *Usnea fragilesceus* aggregate. A fourth *Biatoropsis* species is left unnamed, and two other lineages are not described, all waiting for the study of additional material.

Zusammenfassung: MILLANES, A. M., DIEDERICH, P., WESTBERG, M. & WEDIN, M. 2016. Drei neue Arten aus dem *Biatoropsis usnearum*-Komplex. – *Herzogia* 29: 337–354.

Drei neue *Biatoropsis*-Arten werden auf Basis unserer früheren molekularen Studien, sowie auf zusätzlichen molekularen, morphologischen und ökologischen Daten beschrieben. *Biatoropsis protousneae* sp. nov. kommt ausschließlich auf *Protousnea dusenii* vor. *Biatoropsis minuta* sp. nov. ist charakterisiert durch kleine, braune bis dunkle Basidiomata-Gallen und durch das Vorkommen auf *Usnea barbata* und *U. lapponica*. *Biatoropsis hafellneri* sp. nov. unterscheidet sich durch zweizellige Basidien mit Zellen die sich lateral deutlich verlängern, und durch das Vorkommen auf Arten aus dem *Usnea fragilesceus*-Aggregat. Eine vierte *Biatoropsis*-Art wird nicht formal beschrieben, und zwei weitere Abstammungslinien werden nicht behandelt, da zusätzliches Material untersucht werden sollte.

Kew words: Basidia, galls, host selection, phylogenetics, *Protousnea*, Tremellales, Tremellomycetes, *Usnea*.

Introduction

Lichen-inhabiting fungi are common within the Tremellales (DIEDERICH 1986, 1996, 2003, 2007, DIEDERICH & MARSON 1988, SÉRUSIAUX et al. 2003, ZAMORA et al. 2011, 2016, MILLANES et al. 2012, 2014a, 2015, DIEDERICH et al. 2014, ARIYAWANSA et al. 2015). Among them, the monotypic genus *Biatoropsis* is probably one of the most common and widespread lichenicolous taxa, growing on different hosts of the genera *Usnea* and *Protousnea*. *Biatoropsis usnearum* was first described as an ascomycete by RÄSÄNEN (1934), but it was later revealed as a basidiomycete with auricularioid basidia (DIEDERICH 1990, DIEDERICH & CHRISTIANSEN 1994). MILLANES et al. (2011) confirmed its placement within the Tremellales using molecular methods. As many other taxa in the group, *Biatoropsis* induces the formation of galls on the host thallus, which, in the case of this genus, show a remarkable variation in morphology. This led DIEDERICH & CHRISTIANSEN (1994) to suggest that *B. usnearum* could in reality represent several species. MILLANES et al. (2014b) proved later, based on several lines of evidence and using a multigene approach, the existence of several independently evolving lineages within

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the genus. The purpose of this manuscript is to formally describe three new species within the *Biatoropsis usnearum* species complex, based on a combination of molecular, morphological and ecological data. The presence of additional lineages in the complex, which we refrain from formally describing at this moment, is also discussed.

Material and Methods

Morphological studies

Herbarium specimens are deposited in ASU, GZU, H, MA-Fungi, MAF-Lich, O, S, SEY, UBC and UPS, and in the private collections of F. Berger and P. Diederich. External morphology of herbarium specimens was examined and measured using an Olympus SZX16 or a Leica MZ 7.5 dissecting microscope. Macroscopic photographs were either taken using an Olympus DP11 camera on an Olympus SZX16 dissecting microscope, or using a Canon 40D camera with a Canon MP-E 65 macro lens or a Nikon BD Plan 10× microscope objective, StackShot (Cognisys) and Helicon Focus (HeliconSoft) for increasing the depth of field. Microscopical structures were studied using hand-cut sections stained with phloxine (1% in water) after pretreatment with KOH (5%), following the methods of DIEDERICH (1996), and observed with an Olympus CX40 microscope or a Leica DMLB. Drawings were performed by direct observation. Microscopic photographs were prepared using an Olympus BX53 microscope fitted with differential interference contrast (DIC), and an Olympus DP11 camera; or a Leica DMLB microscope with DIC, using a Leica EC3 camera, and Helicon Focus. The apiculus was not included in basidiospore measurements. Basidiospore length/width ratio is expressed as Q. Sizes in parentheses represent minimum and maximum observed values.

Molecular studies

Choice of additional taxa and outgroup: In addition to 38 *Biatoropsis* specimens studied in MILLANES et al. (2014b), 29 additional specimens were included in the molecular study (Table 1). *Tremella cetrariicola* was used as outgroup.

DNA extraction and amplification: DNA was extracted directly from the specimens examined (Table 1). Three to ten basidiomata were selected from each specimen for DNA extraction. Total DNA was extracted using the Qiagen DNeasy Plant MiniKit, according to the manufacturer's instructions, but using 50 µl of water in each of the last two steps of final elution.

For PCR amplification, the primers ITS1F (GARDES & BRUNS 1993) and BasidLSU3-3 (MILLANES et al. 2011) were used to amplify the internal transcribed spacer I, the 5.8 rDNA gene, the internal transcribed spacer II and a fragment of c. 400 bp in the nuLSU rDNA gene. PCR amplifications were performed using Illustra™ Hot Start PCR beads, according to the manufacturer's instructions, with the following settings: we used initial denaturing at 95 °C for 3 min, four cycles (95 °C for 40 s, 53 °C for 40 s and 72 °C for 90 s), four cycles (95 °C for 30 s, 50 °C for 30 s and 72 °C for 90 s), and finally 32 cycles (95 °C for 30 s, 47 °C for 30 s and 72 °C for 90 s) with a final extension at 72 °C for 480 s.

The PCR-products were sequenced using the DYEnamic ET terminator cycle sequencing kit (Amersham Biosciences, Freiburg, Germany), with the following settings: 25 cycles (95 °C for 20 s, 50 °C for 15 s, and 60 °C for 60 s). Post-reaction clean-up was carried out following DYEnamic ET terminator cycle sequencing kit protocols (Amersham Biosciences). The purified samples were run on an automated sequencer (ABI Prism 377) located in the Molecular

Systematic Laboratory at the Swedish Museum of Natural History, or on an automated sequencer (ABI Prism 3100-Avant) in the Genomic Unit at Rey Juan Carlos University (Madrid).

Sequence alignment and phylogenetic analyses: Sequences were aligned using the Q-INS-i algorithm (KATOH & TOH 2008a) of the multiple sequence alignment software MAFFT version 6.611 (KATOH et al. 2002, KATOH & TOH 2008b). Ambiguous regions were identified and eliminated with Gblocks version 0.91b (CASTRESANA 2000). Bayesian analyses were performed by Markov chain Monte Carlo (MCMC) sampling as implemented in the software MrBayes 3.2.4 (RONQUIST et al. 2012). We selected the substitution model using the Akaike Information Criterion (AIC) as implemented in jModeltest (GUINDON & GASCUEL 2003, POSADA 2008). We used full likelihood optimization, six discrete gamma categories, and selected only among the 24 models implemented in MrBayes, and a SYM+ Γ model was selected. Maximum likelihood analyses were performed in RAXMLGUI 1.3 (SILVESTRO & MICHALAK 2012), a graphical front-end for RAXML (STAMATAKIS 2006), using the GTRGAMMAI model of nucleotide substitution applied to all partitions. We performed a thorough ML search and assessed node support by thorough bootstrap using 1000 bootstrap pseudoreplicates.

Results and Discussion

We generated 29 new sequences including the ITS and a fragment of ca. 400 pb in the nuLSU. Only the sequenced fragments corresponding to the ITS were used in the alignment, together with ITS sequences already available in GenBank (Table 1). We excluded the fragment corresponding to the nuLSU, because the quality of this portion was not good in some of the newly generated sequences. In this way we produced a homogeneous data matrix, including 408 characters and no missing data. The best tree obtained from the ML analysis had a ln-likelihood value of -2684.4092 . The Bayesian analysis halted after 3,700,000 generations, at which time the average standard deviation of split frequencies across runs was 0.0098, which indicates that the three runs had converged (< 0.01). A majority-rule consensus tree was constructed from the 18,500 trees of the stationary tree sample. Since the topologies of the ML and the Bayesian trees were congruent, only the best tree of the ML analysis is shown in Fig. 1.

The phylogenetic analyses recover six monophyletic lineages, coinciding with the clades A2, B, C, D, E and F, already distinguished by MILLANES et al. (2014b) (Fig. 1). From these, three new species corresponding to clades A2, B and D, are formally described below (Figs 2–7). Clade F is not formally described, and left unnamed, since we have not been able to study enough material to identify characters, other than molecular data, that allow distinguishing it from other species. However, the main morphological and ecological characteristics observed so far are summarized and discussed also below (Fig. 10). Clade E is based on a single specimen and we refrain from describing this as a species until more material can be studied. In addition, group A1 (MILLANES et al. 2014b) is not supported, and was revealed to comprise several lineages, which probably represent several species. Therefore, the taxonomic treatment of group A1 is also postponed until additional available material and sequence data will allow a detailed study and the identification of supported independent evolving lineages. An identification key is therefore not presented at the moment, and will be prepared at a later stage, when it is possible to include the currently undescribed species that belong in the complex.

Table 1: Sequences newly produced for the present study (bold), or downloaded from GenBank. Type specimens are indicated by (T).

Taxon name-DNA extraction number	Specimen data	ITS
<i>Biatoropsis hafellneri</i> -AM299	Azores, Diederich 17087b (S F264687)	KJ404880
<i>Biatoropsis hafellneri</i> -AM463	Madeira, Diederich 17788 (hb. Diederich)	KX687722
<i>Biatoropsis hafellneri</i> -AM462	Madeira, Diederich 17791 (S F291448)	KX687723
<i>Biatoropsis hafellneri</i> -AM461	Madeira, Diederich 17793 (hb. Diederich)	KX687724
<i>Biatoropsis hafellneri</i> -AM464a	Madeira, Diederich 17796, pale galls (S F291456)	KX687725
<i>Biatoropsis hafellneri</i> -AM464b	Madeira, Diederich 17796, dark galls (S F291456)	KX687726
<i>Biatoropsis hafellneri</i> -AM459	Madeira, Diederich 17800 (hb. Diederich)	KX687727
<i>Biatoropsis hafellneri</i> -AM398	Norway, Haugan 7969 (O L157359)	KX687728
<i>Biatoropsis hafellneri</i> -AM216	UK, 2010, Millanes s.n. (S F291458)	KX687729
<i>Biatoropsis hafellneri</i> -AM110	UK, Wedin 7306 (S F92121, UPS F766825)	KJ404881
<i>Biatoropsis hafellneri</i> -AA10 (T)	UK, Wedin 7308 (UPS F766824 – holotype; GZU, S F102403 – isolotypes)	JN053489
<i>Biatoropsis minuta</i> -AM172	Canada, Diederich 17269. (S F102406, hb. Diederich)	KJ404866
<i>Biatoropsis minuta</i> -AM229	India, Divakar s.n. (MAF-Lich)	KJ404868
<i>Biatoropsis minuta</i> -AM137 (T)	Spain, Aragón s.n. (S F102398 – holotype; hb. Diederich, UPS F766826 – isolotypes)	KJ404869
<i>Biatoropsis minuta</i> -AM168	Spain, Burgaz & Martínez s.n. (MA-Fungi 48236)	KJ404870
<i>Biatoropsis minuta</i> -AM212	Spain, Merinero s.n. (S F264683)	KJ404871
<i>Biatoropsis minuta</i> -AA9	Sweden, Hagner s.n. (S F92134, (UPS F766831)	JN053486
<i>Biatoropsis minuta</i> -AM155	Sweden, Hagner s.n. (UPS 355785)	KJ404867
<i>Biatoropsis minuta</i> -CO294	Sweden, Wedin 7903 (S F102401, UPS F766830)	JN053487
<i>Biatoropsis minuta</i> -AM635	Sweden, 2015, Westberg s.n. (S F291459)	KX687730
<i>Biatoropsis protousneae</i> -AM215	Argentina, Wedin 8601 (S F264822)	KJ404861
<i>Biatoropsis protousneae</i> -AM214	Argentina, Wedin 8615 (S F264823)	KJ404865
<i>Biatoropsis protousneae</i> -AM139 (T)	Chile, Pérez-Ortega 207 (S F291460 – holotype; hb. Diederich – isolate)	KJ404862
<i>Biatoropsis protousneae</i> -AM138 (T)	Chile, Pérez-Ortega 207 (MAF-Lich – isolate)	KJ404863
<i>Biatoropsis protousneae</i> -AM142 (T)	Chile, Pérez-Ortega 207 (MAF-Lich – isolate)	KJ404864
<i>Biatoropsis protousneae</i> -AM141	Chile, Pérez-Ortega 216 (MAF-Lich)	KJ404860
<i>Biatoropsis usnearum</i> -AM196	Austria, Hafellner 49578 (GZU 02-99)	KJ404878
<i>Biatoropsis usnearum</i> -AM297	Azores, Diederich 17058 (S F264685)	KJ404873
<i>Biatoropsis usnearum</i> -AM298	Azores, Diederich 17087a (S F264684)	KJ404872
<i>Biatoropsis usnearum</i> -AM631	Canada, 2006, Goward s.n, pale galls (UBC)	KX687731
<i>Biatoropsis usnearum</i> -AM632	Canada, 2006, Goward s.n, brown galls (UBC)	KX687732
<i>Biatoropsis usnearum</i> -AM629	Canada, 2008, Goward s.n. (UBC)	KX687733
<i>Biatoropsis usnearum</i> -AM630	Canada, Björk 14689 (UBC)	KX687734
<i>Biatoropsis usnearum</i> -AM211	Canary islands, Amo de Paz s.n. (MAF-Lich)	KJ404874
<i>Biatoropsis usnearum</i> -AM478	Madeira, Diederich 17778 (hb. Diederich)	KX687735
<i>Biatoropsis usnearum</i> -AM465	Madeira, Diederich 17792 (hb. Diederich)	KX687736
<i>Biatoropsis usnearum</i> -AM460	Madeira, Diederich 17794 (S F291461)	KX687737
<i>Biatoropsis usnearum</i> -AM201	Sweden, Westberg 09-674 (S F264680)	KJ404877
<i>Biatoropsis usnearum</i> -AM203	Sweden, Westberg 09-675 (S F264682)	KJ404875
<i>Biatoropsis usnearum</i> -AM202	Sweden, Westberg 09-676 (S F264681)	KJ404876
<i>Biatoropsis usnearum</i> -AM171	UK, Coppins s.n. (S F102407)	KJ404879
<i>Biatoropsis usnearum</i> -AM218	UK, Millanes 507 (S F291462)	KX687738
<i>Biatoropsis usnearum</i> -AM222	UK, 2010, Westberg s.n. (S F291463)	KX687739

Taxon name-DNA extraction number	Specimen data	ITS
<i>Biatoropsis</i> sp. A1-AM112	Canary Islands, Diederich 16700 (S F102402)	KJ404856
<i>Biatoropsis</i> sp. A1-AM191	Chile, Etayo 22655 (MAF-Lich 15628)	KJ404852
<i>Biatoropsis</i> sp. A1-AM192	Chile, Etayo 23158 (MAF-Lich 15645)	KJ404851
<i>Biatoropsis</i> sp. A1-AM190	Chile, Etayo 24529 (MAF-Lich)	KJ404853
<i>Biatoropsis</i> sp. A1-AM140	Chile, Pérez-Ortega 215 (MAF-Lich)	KJ404855
<i>Biatoropsis</i> sp. A1-AM471	Madeira, Diederich 17770 (hb. Diederich)	KX687740
<i>Biatoropsis</i> sp. A1-AM479	Madeira, Diederich 17772 (hb. Diederich)	KX687741
<i>Biatoropsis</i> sp. A1-AM458a	Madeira, Diederich 17773 (S F291464)	KX687742
<i>Biatoropsis</i> sp. A1-AM476	Madeira, Diederich 17774 (hb. Diederich)	KX687743
<i>Biatoropsis</i> sp. A1-AM477	Madeira, Diederich 17779 (hb. Diederich)	KX687744
<i>Biatoropsis</i> sp. A1-AM475	Madeira, Diederich 17780 (hb. Diederich)	KX687745
<i>Biatoropsis</i> sp. A1-AM473	Madeira, Diederich 17782 (S F291457)	KX687746
<i>Biatoropsis</i> sp. A1-AM470	Madeira, Diederich 17797 (hb. Diederich)	KX687747
<i>Biatoropsis</i> sp. A1-AM307	New Zealand, Myles s.n. (S F186043)	KJ404859
<i>Biatoropsis</i> sp. A1-AM296	New Zealand, Wedin 9033 (S F180874)	KJ404854
<i>Biatoropsis</i> sp. A1-AM143	Spain, Pérez-Ortega s.n. a (MAF-Lich)	KJ404857
<i>Biatoropsis</i> sp. A1-AM144	Spain, Pérez-Ortega s.n. b (MAF-Lich)	KJ404858
<i>Biatoropsis</i> sp. A1-AM217	UK, Millanes 574 (S F291465)	KX687748
<i>Biatoropsis</i> sp. E-AM213	Argentina, Wedin 8742 (S F264824)	KJ404882
<i>Biatoropsis</i> sp. F-AM567	France, Diederich 18149 (hb. Diederich)	KX687749
<i>Biatoropsis</i> sp. F-AM10	New Zealand, Berger 16609 (S F92784)	KJ404884
<i>Biatoropsis</i> sp. F-295	New Zealand, Wedin 9429 (S F181399)	KJ404883
<i>Biatoropsis</i> sp. F-AM576	Seychelles, Diederich 18087 (S, SEY, hb. Diederich)	KX687750
<i>Biatoropsis</i> sp. F-AM166	USA, Kocourková s.n. (S F264679)	KJ404885
<i>Tremella cetrariicola</i> AM111	Finland, Suija s.n. (S F102413)	JN053490

The species

Biatoropsis hafellneri Millanes, Diederich, M. Westb. & Wedin **sp. nov.** [Mycobank 817903] (Figs 2–3)

Type: United Kingdom, Cornwall, Lamorna Cove, Lamorna Valley, 50°03'N, 05°32'E, on *Usnea cornuta*, 11.IV.2004, M. Wedin 7308 (holotype: UPS F766824; isotypes: GZU and S F102403).

Etymology: It is with great pleasure that we dedicate the new species to Prof. Josef Hafellner (Austria), in recognition and gratitude to his contributions to lichenology in general, and to the study of lichenicolous fungi in particular.

Description: **Basidiomata** inducing the formation of galls that are initially regularly convex to subglobose, later constricted at the base, sometimes with a central depression and/or tuberculate when mature, very pale to orangish, occasionally brown, waxy gelatinous when wet 0.1–1.3 mm diam. **Galls** often developing on broken fibrils. **Context hyphae** thin-walled, 2–3 µm diam., clamp connections occasionally present. **Haustorial branches** frequent, mother cell spherical to subspherical, (2–)3–4 × 3–4(–5) µm, haustorial filament 1 µm diam., up to 5 µm long. **Hymenium** hyaline, containing numerous probasidia, probasidial initials clavate, often developing from a basal clamp, which is not observed in mature basidia. **Basidia**, when mature, 2-celled (exceptionally 3-celled), with one transverse septum 20–46 × 4–8 µm, the two cells laterally much elongate at maturity, sometimes giving the appearance of two immature independent basidia, individual basidial cells up to 40 µm long, 4–8 µm wide. **Basidiospores** globose to subglobose, 5–8.5 × 5–9 µm, Q = 0.7–1, with a distinct apiculus, c. 1 µm diam. **Conidia** catenulate, hyaline, individual cells 3–8 × 3–5 µm.

Ecology and host: The species grows on the thallus of species of the *Usnea fragiliscens* aggregate (CLERC 1987), most frequently on *U. cornuta*. It occurs in oceanic and humid forests where the hosts are present, usually on trees but also on acidic rocks.



Fig. 1: Phylogram based on ITS sequences, corresponding to the best tree recovered in the maximum likelihood analysis (implemented in RAxMLGUI 1.3), with information on the Bayesian posterior probability values added. Black dots indicate nodes supported both by ML and Bayesian analyses. A white dot indicates a node supported only in the Bayesian analyses. Type specimens are indicated by (T).

Distribution: Known from the Azores, the Canary Islands, France, Madeira, Norway, Spain, and the U.K.

Notes: *Biatoropsis hafellneri* corresponds to lineage D distinguished by MILLANES et al. (2014b) using multigene analyses, and recovered also in this study (Fig. 1). This is the only *Biatoropsis* species known so far with a very characteristic basidium morphology, consisting of two cells that elongate laterally before the formation of epibasidia. Similar basidia are found in *Tremella christiansenii*, *T. diderichiana* and *T. hypocenomycis*, although none of these species grow on *Usnea*. *Tremella christiansenii* differs also from the new species in the darker basidiomata, and the size of basidia and basidiospores. *Tremella diderichiana* has 2- or 4-celled basidia with longitudinal septa, and much bigger basidiospores. The basidiomata of *Tremella hypocenomycis* are blackish, do not induce the formation of galls, and this species has smaller basidia and basidiospores. *Biatoropsis hafellneri* is closely related to *Biatoropsis usnearum* (Fig. 1) from which it is clearly distinguished by the basidium morphology (four celled basidia in *B. usnearum*) and the host selection. Moreover, although it is not constant in all samples studied, the basidiomata of *B. hafellneri* have a marked tendency to develop on decorticated areas remaining after fibrils of the host have broken (Fig. 3C–D), the galls induced are usually smaller, and its inner part appears to be formed by white host hyphae only.

Additional specimens examined (all on species of the *Usnea fragilesceus* aggregate): **Azores. Pico**, between Lajes do Pico and Sao Roque do Pico, Bosque da Junqueira, 38°27.93'N, 28°17.95'W, on *U. cornuta*, 26.VII.2010, P. Diederich 17087b (S F264687, hb. Diederich). – **Canary Islands. Tenerife**, Monte Aguirre, on *U. cornuta*,

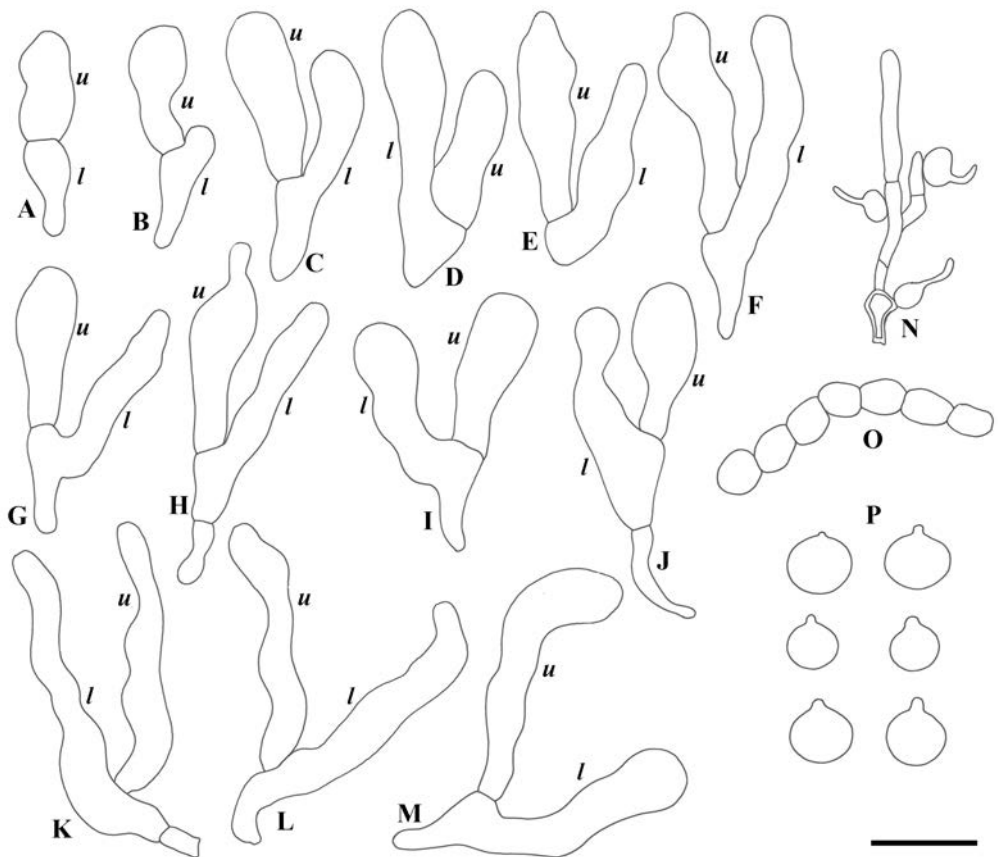


Fig. 2. *Biatoropsis hafellneri*. A–M – Development of basidia (l: lower and u: upper cell of basidium). N – Hyphae with haustorial branches. O – Catenulate conidia. P – Basidiospores. [All based on type material]. – Scale = 10 µm.

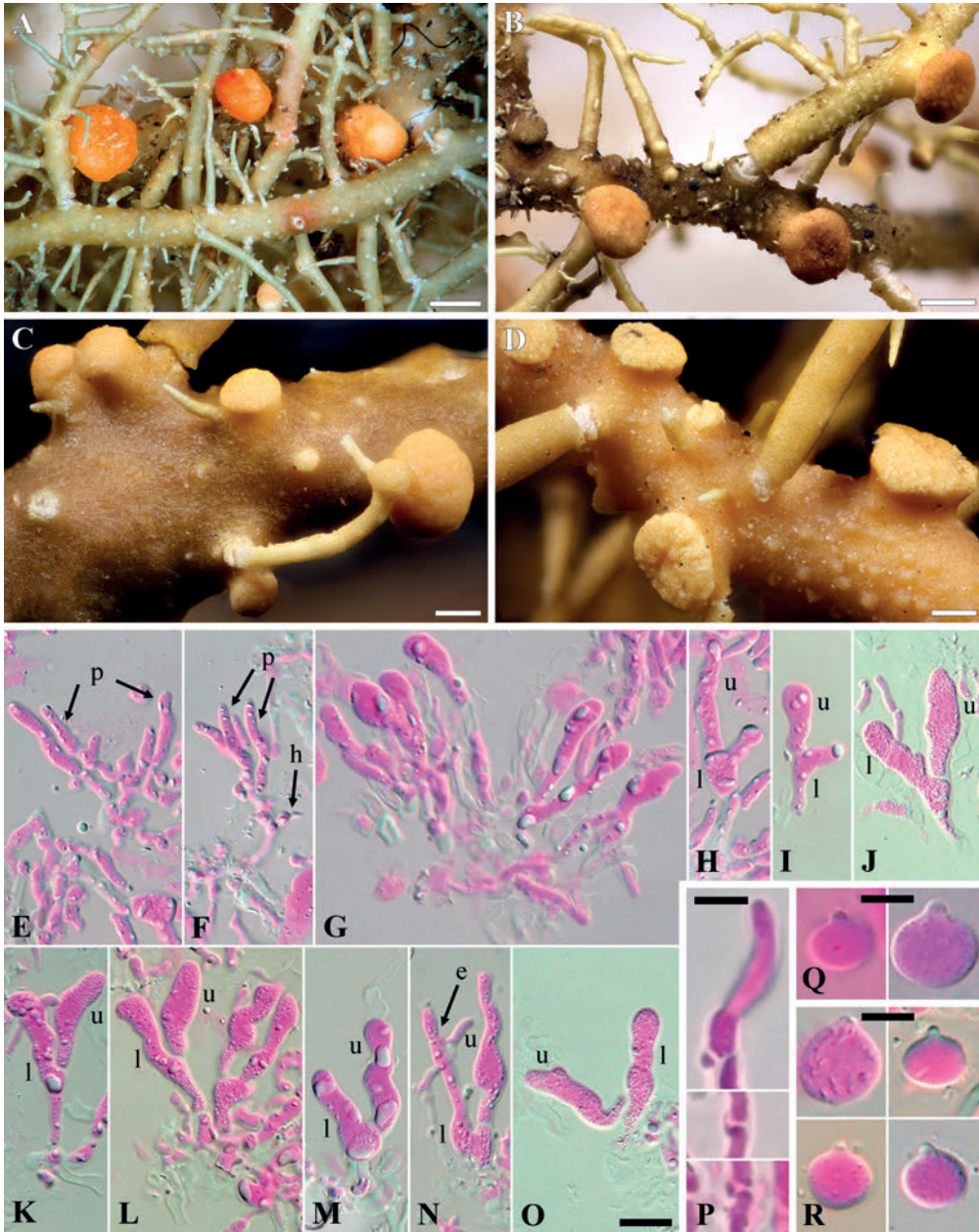


Fig. 3: *Biatoropsis hafellneri*. **A–B** – Basidiomata on the thallus of *Usnea cornuta*. **C–D** – Young basidiomata developing from broken fibrils of the host (on *U. sp.* in the *U. fragiliscens* aggregate). **E–O** – Development of basidia (p: probasidia; l: lower and u: upper cell of mature basidium). **P** – Probasidia with basal clamps. **Q–R** – Basidiospores. [A, E, H, Q: Wedin 7308 (holotype); B: Diederich 17087b; C, P: Diederich 14614; D: Diederich 4854; F, N, R: Diederich 17800, G: Wedin 7306; I–M, O: Diederich 17788]. – Scales: A–B = 500 µm; C–D = 200 µm; E–O = 10 µm; P–R = 5 µm.

1990, U. Becker (hb. Diederich). – **France. Finistère**, à l'E du Faou, forêt de Cranou, on *U. cornuta*, 18.VII.2000, P. Diederich 14614 (hb. Diederich). – **Madeira**. S of Santana, Queimadas, 7.IV.1992, P. Diederich 4854 (hb. Diederich); Rabaçal, along Levada das 25 Fontes, 32°45.81'N, 17°8.03'W, 30.VIII.2014, P. Diederich 17791, 17796 (S F291448, F291456), 17788, 17793, 17796 (S, hb. Diederich); S of São Vicente, 500 m N of Encumeada, 32°45.48'N, 17°1.13'W, 27.VIII.2014, P. Diederich 17800 (hb. Diederich). – **Norway. Hordaland**, Bjørnåsen, 60°08.32'N, 5°26.40'E, on *U. cornuta*, 22.V.2008, R. Haugan 7969 (O L157359). – **Spain. Navarra**, 50 km N of Pamplona, Oronoz-Mugaire, Señorío de Bértiz, on *U. cornuta*, 22.VII.1991, P. Diederich 9738 (hb. Diederich). – **U.K.** Lamorna Cove, Lamorna Valley, 50°03'N, 5°32'E, on *U. cornuta*, 11.IV.2004, M. Wedin 7306 (S F92121, UPS F766825); Devon, Dartmoore National Park, South Brent, Shipley Bridge, 50°27.06'N, 3°51.64'W, 25.VIII.2010, A. Millanes s.n. (S – F291458).

Biatoropsis minuta Millanes, Diederich, M. Westb. & Wedin **sp. nov.** [Mycobank 817904] (Figs 4–5)

Type: SPAIN, Palencia, Piedrasluengas, Piedrasluengas mountain pass, 43°2.95'N, 4°27.19'W, on *Usnea barbata*, 5.IV.2010, G. Aragón s.n. (holotype: S F102398; isotypes: UPS F766826 and hb. Diederich).

Etymology: In reference to the size of the induced galls, always smaller than 1 mm.

Description: **Basidiomata** inducing the formation of convex, basally constricted galls, sometimes with a central depression when mature, waxy gelatinous when wet, brown to dark brown or black, 0.1–0.8 mm diam. **Context hyphae** thin-walled, 2–3 µm diam., clamp connections not observed.

Haustorial branches frequent, mother cell spherical to subspherical, (2–)3–4 × 3–4 µm, haustorial filament 1 µm diam., up to 15 µm long. **Hymenium** hyaline, containing numerous probasidia; proba-

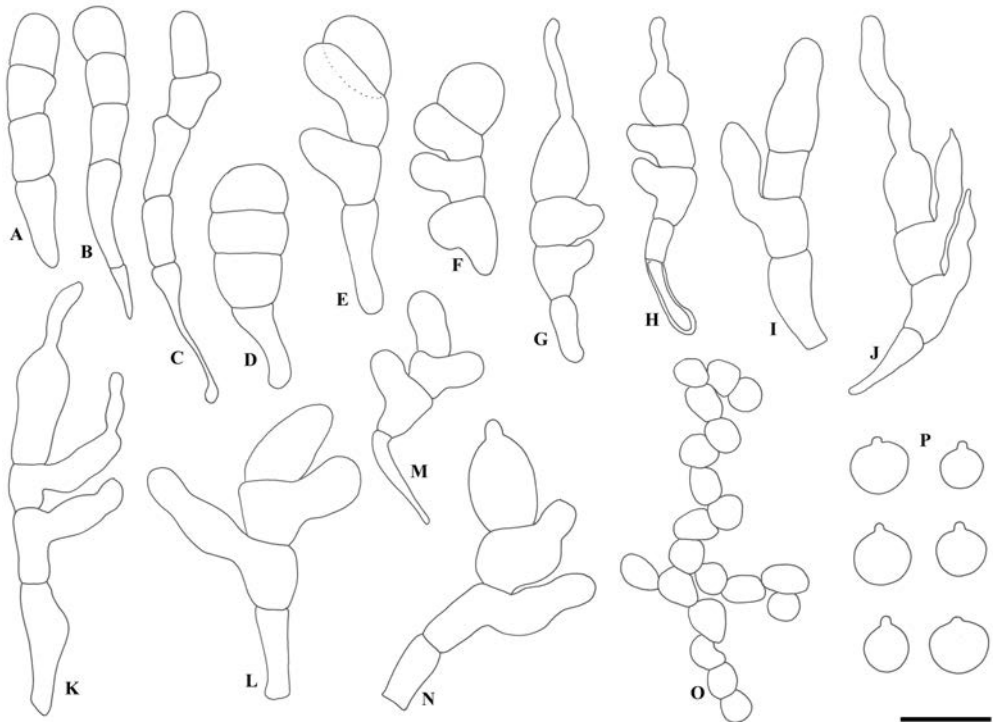


Fig. 4: *Biatoropsis minuta*. A–N – Development of basidia. O – Catenulate conidia. P – Basidiospores [All based on type material]. – Scale = 10 µm.

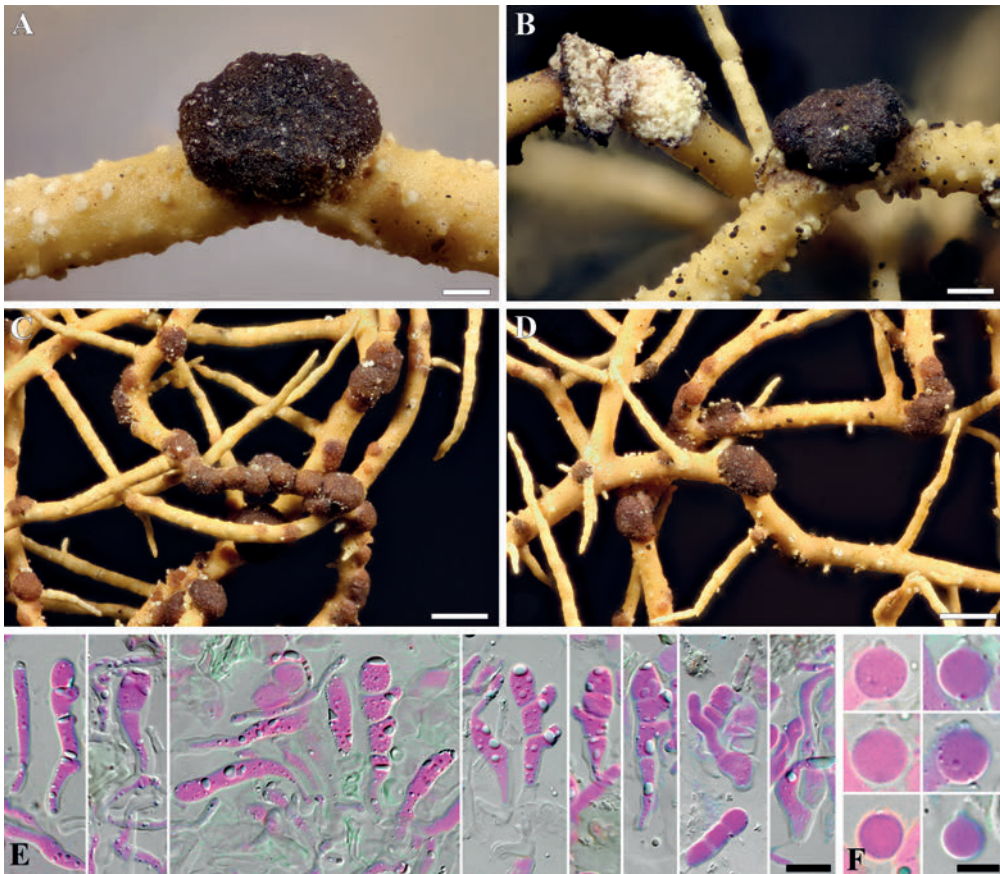


Fig. 5: *Biatoropsis minuta*. **A–D** – Basidiomata on the thallus of *Usnea lapponica*. **E** – Basidia. **F** – Basidiospores. [A, E–F: Aragón s.n. (holotype); B: Goward 91-16; C–D: Goward 91-13]. – Scales: A–B = 200 μ m; C–D = 500 μ m; E = 10 μ m; F = 5 μ m.

sidial initials clavate, proliferations occurring from a basal hypha. **Basidia**, when mature, 4-celled, with three transverse septa, often constricted at the septa, the lower cell with an attenuated stalk-like base, often longer than the upper cells, 20–46 (–50) \times 4–10 μ m (incl. stalk-like base, excl. epibasidia), lower part of the stalk-like base 2–4 μ m diam. **Basidiospores** globose to subglobose, 5–9.5 \times 5–8 μ m, $Q = 0.83$ –1.3, with a distinct apiculus, c. 1 μ m diam. **Conidia** catenulate, hyaline, individual cells 2–6 \times 2–4 μ m.

Ecology and hosts: Known only from two closely related species of *Usnea*, *U. barbata* and *U. lapponica* (thallus).

Distribution: Known from Canada, India, Spain, Sweden, Norway, and the U.S.A.

Notes: *Biatoropsis minuta* corresponds to clade ‘B’ distinguished by MILLANES et al. (2014b), and obtained also in this study (Fig. 1). The phylogenetic position of this clade within the whole *Biatoropsis usnearum* complex is not supported, although the topology recovered place the new species closely related to *B. protousneae* and group ‘A1’, and not closely related to other *Biatoropsis* species. It is a very widespread taxon, clearly distinguished from other known *Biatoropsis* species by the morphology of the induced galls, which are always smaller than 1 mm and constantly brown or dark. This colour is due to inner pigments, and not to the presence of hyperparasites intermixed with *Biatoropsis*. There

are, to our knowledge, no clear micro-morphological features that could serve to characterize this species, as the microscopical characters are undistinguishable from those of other *Biatoropsis* species, except *B. hafellneri*.

Additional specimens examined (all on *Usnea lapponica*, unless otherwise specified): **Canada. British Columbia**, Edgewood Blue, 26 km N of Clearwater on Wells Gray Rd., 51°45'N, 120°02'W, 9.II.1991, T. Goward 91-13, 91-16 (UBC, hb. Diederich); Wells Gray Provincial Park, Clearwater valley, 26 km N of Clearwater, near Kingfishers Wood Cottages, 22.VII.2008, P. Diederich 17269 (S F102406, hb. Diederich). – **India**, Uttarakhand [=Uttarakhand], Joshimath, Nanda Devi National Park, 10 km before Gamshali, on the way to Niti, 30°42.66'N, 79°50.62'E, 20.VIII.2007, P. Divakar s.n. (MAF-Lich). – **Norway. Buskerud**, Todalen, E side of Rudåsen, 17.IX.2000, G. Gundersen, H. Gundersen, T.H. Hofton, B.P. Løfall, E. Timdal LL111/14 (O L55896). **Hedmark**, Alvdal, N of Jutulhogget, 62°N, 10°55'E, 2.VI.1998, R. Haugan 7102 (O L118308). **Nord-Trøndelag**, Lierne, Storåa, west of Gambrennseteren, 9.IX.2009, J.T. Klepsland JK09-L570 (O L165113), Østfold, Marker, Måstadsetra, 18.III.2000, B.P. Løfall bpl-L6558 (O L77293). **Sogn of Fjordane**, Urnes, 12.VI.1976, R. Bryhn (O); c. 500 m S of Lisete 13.VI.1976, H. østhaugen 3540 (O). – **Spain. Lleida**, Montellà i Martinet, Serra del Cadí, on the way to Prat d'Aguiló, on *U. cf. barbata*, 42°19.68'N, 1°42.09'E, 2.VII.1996, A.R. Burgaz & I. Martínez s.n. (MA-Fungi 48236); **Madrid**, Montejo de la Sierra, on *U. cf. barbata*, 2010, S. Merinero s.n. (S F264683). – **Sweden. Jämtland**, Alanäs par., Lidsjöberg, Prästberget, 28.III.2002, Å. Hagner s.n., (S F92134, UPS 766831); Alanäs par., Lidsjöberg, close to the village at Hembäcken 30.XII.2003, Å. Hagner s.n., (UPS L160312); Stugun par., Nybodberget, 63°9.54'N, 15°45.31'E, 12.IX.2015, M. Westberg s.n. (S-F291459). **Lule Lappmark**: Jokkmok par., Lilla Luleäven river, Lake Saggat, Kvikkjokk, along river Kamajokk, 66°57'N, 17° 42'E, 20.VI.2005, M. Wedin 7903 (S F102401, UPS F766830). – **U.S.A. Arizona**, Coconino Co., Grand Canyon National Park, North Rim, 6 km from State Highway 67 on the road to Cape Royal, 36°15'N, 112°01'W, 3.VIII.1991, M. Boykin 2410 (ASU).

Biatoropsis protousneae Millanes, Diederich, M. Westb. & Wedin **sp. nov.**

(Figs 6–7)

[Mycobank 817905]

Type: Chile, XII Region of Magallanes and Chilean Antarctica, Puerto Natales, along the road to Lake Balmaceda, 51°56.48'S, 72°23.28'W, on *Protousnea dusenii*, 14.I.2008, S. Pérez-Ortega 207 (holotype: S F291460; isotypes: MAF-Lich and hb. Diederich).

Etymology: In reference to the host, *Protousnea dusenii*.

Description: **Basidiomata** inducing the formation of convex, basally constricted galls, tuberculate and often with a central depression when mature, waxy gelatinous when wet, pale to light brown, dark brown, or black, 0.1–2.4 mm diam. **Context hyphae** thin-walled, 2–3 µm diam., clamp connections not observed. **Haustorial branches** frequent, mother cell spherical to subspherical, 3–4 × 3–5 µm, haustorial filament 1 µm diam., up to 14 µm long. **Hymenium** hyaline, containing numerous probasidia, probasidial initials clavate, proliferations occurring from a basal hypha. **Basidia**, when mature, 4-celled, with three transverse septa, often constricted at the septa, the lower cell with an attenuated stalk-like base, often longer than the upper cells, 22–60 × 4–10 µm (incl. stalk-like base, excl. epibasidia), lower part of the stalk-like base 2–4 µm diam., epibasidia frequent, 2–4 µm wide, up to 45 µm long. **Basidiospores** globose to subglobose, 5–8 × 5–9 µm, Q = 0.7–1.3, with a distinct apiculus, c. 1 µm diam. **Conidia** catenulate, hyaline, individual cells 3–7 × 3–6 µm.

Ecology and host: Lichenicolous on *Protousnea dusenii* (thallus).

Distribution: Known from Argentina and Chile.

Notes: This species corresponds to clade A2 distinguished by MILLANES et al. (2014b) using multigene analyses, and obtained also in this study (Fig 1). The species is micro- and macromorphologically similar to samples belonging to group A1, to which it is phylogenetically closely related. *Biatoropsis protousneae* is, however, distinguished from that taxon by its host selection. The species can also be confused with *Biatoropsis usnearum* s. str. except for the distinct host. The brown colour of the basidiomata of *B. protousneae* seems to be due to inner pigments, and not to the presence of hyperparasites, as it is often the case in *B. usnearum*. However, these two circumstances (presence or absence of hyperparasites in brown galls) have been observed in both species and the character is therefore not suitable for distinguishing them. Orange galls have not been found in *B. protousneae*.

Additional specimens examined (all on *Protousnea dusenii*): **Argentina. Río Negro**, Nahuel Huapi National Park, ca. 13 km S Bariloche, along the road to Refugio Neumeyer, 41°15.48'S, 71°17.16'W, 14.II.2010, M. Wedin 8601

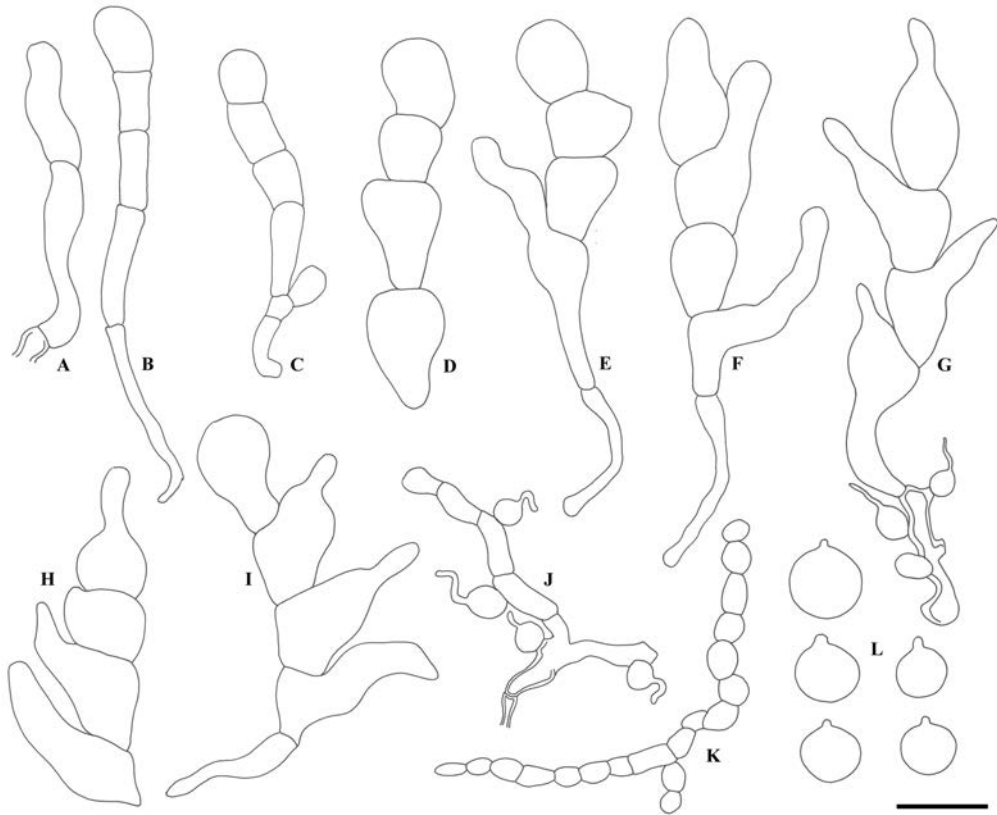


Fig. 6: *Biatoropsis protousneae*. **A–I** – Development of basidia. **J** – Hyphae with haustorial branches. **K** – Catenulate conidia. **L** – Basidiospores. [All based on type material]. – Scale = 10 μ m.

(S F264822); Parque Nacional Nahuel Huapi, c. 5 km W Bariloche, Cerro Otto, 41°8.68'S, 71°22.1'W, 19.II.2010, M. Wedin 8615 (S F264823). – **Chile. XII Region of Magallanes and Chilean Antarctica**, Isla Navarino, Puerto Williams, Cerro Bandera, 55°0.08'S, 67°41.53'W, 19.I.2008, S. Pérez-Ortega 216 (MAF-Lich).

Biatoropsis usnearum Räsänen s.str. [Mycobank 281203]

(Figs 8–9)

Ann. bot. Soc. Zool.-bot. fenn. 'Vanamo' **5(9)**: 8 (1934)

Type: Finland, Ostrobothnia borealis, Simo Kuusella, on '*Usnea comosa*', 1.VII.1915, V. Räsänen (lectotype: H[!]; isotype: IMI, fide Hawksworth).

Description: **Basidiomata** extremely variable in form, size and colour, generally subspherical and convex with a constricted base, often with lobate margins, sometimes flattened or with a concave central part, rarely effuse and covering larger areas around the branches of the host, surface smooth, rarely tuberculate, cartilaginous, pale pinkish, reddish brown, dark brown or black, 0.2–3 mm diam.

Context hyphae 2–3 μ m wide, mostly uniform, walls not markedly thickened, clamps not observed.

Haustorial branches frequent, mother cell subspherical or sometimes elongate, 2.5–5 μ m diam., haustorial filament 0.5–1 μ m thick, up to 11 μ m long. **Hymenium** containing numerous probasidia, developing from a basal clamp that is not observed in mature basidia. **Basidia**, when mature, clavate to subcylindrical, 4-celled, with 3 transverse septa, 20–44 \times 3–6.5 μ m, epibasidia 2–3 μ m thick, up to 85 μ m long.

Basidiospores subglobose to ellipsoid, 4.5–8 \times 4–7.5 μ m, with a distinct apiculus.

Conidia catenulate, hyaline, individual cells 3–5 \times 2–3.5 μ m.

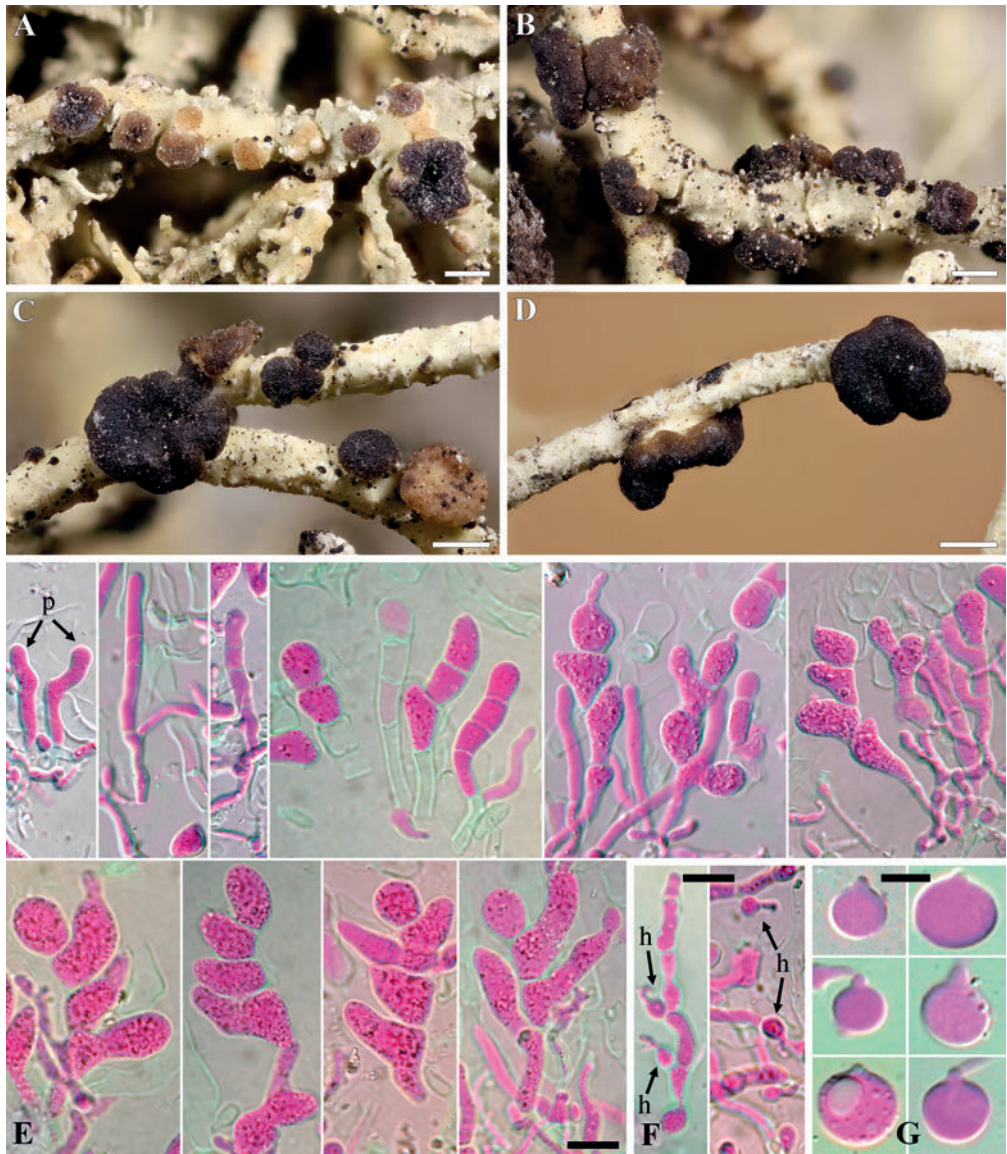


Fig. 7: *Biatoropsis protousneae*. **A–D** – Basidiomata on the thallus of *Protousnea dusenii*. **E** – Probasidia (p) and mature basidia. **F** – Haustorial branches (h). **G** – Basidiospores. [All based on type material]. – Scales: A–D = 500 μ m; E–F = 10 μ m; G = 5 μ m.

Ecology and hosts: Lichenicolous on the thallus of *Usnea* hosts (neither on *Protousnea* nor on the subgenus *Neuropogon*), including *U. flavocardia*, *U. florida/subfloridana*, *U. fragileszens*, *U. glabrescens*, *U. cf. krogiana* and *U. macaronesica*. The European specimens (excluding Macaronesia) appear to grow exclusively on hosts of the *U. florida/subfloridana* group. Samples from other geographical regions are however more diverse regarding host selection, and we expect many more host species to occur when more non-European specimens have been sequenced.

Distribution: Known from Austria, the Azores, Canada, the Canary Islands, Madeira, Sweden, and the U.K.

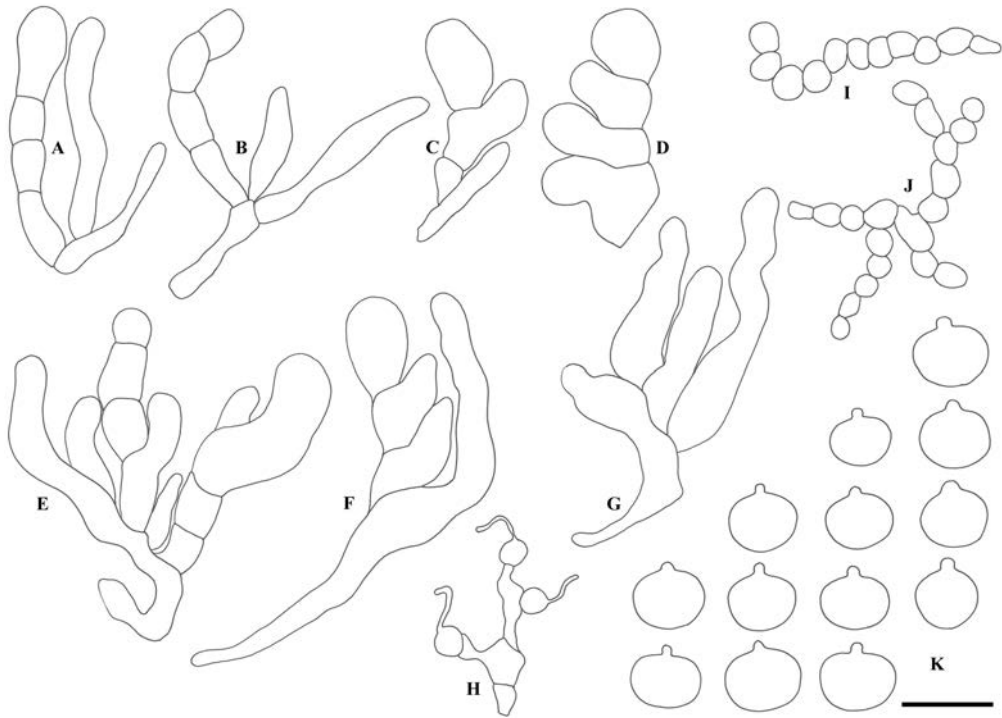


Fig. 8: *Biatoropsis usnearum* s.str. **A–G** – Development of basidia. **H** – Hyphae with haustorial branches. **I–J** – Catenulate conidia. **K** – Basidiospores. [A–E: Millanes 507; F–K: Björk 14689]. – Scale = 10 μ m.

Notes: The description above refers to *Biatoropsis usnearum* s. str. and is based on the descriptions by DIEDERICH & CHRISTIANSEN (1994) and DIEDERICH (1996), with a few modifications based on recently studied material, and on the exclusion of the newly described species. The circumscription of *Biatoropsis usnearum* s. str. will still need to be modified in the future when there is a better knowledge of the whole species complex. It is remarkable that both *Biatoropsis hafellneri* and *Biatoropsis usnearum* s. str., which are closely related to each other, share the presence of a basal clamp at the base of young basidia. This character had previously been overlooked in *Biatoropsis* and should be carefully checked in future morphological studies.

Additional specimens examined (on *Usnea* sp., unless specified): **Austria. Steiermark**, Steirisches Randgebirge, 47°24.08'N, 15°32.08'E, on *U. cf. florida*, 24.X.1999, J. Hafellner s.n. (GZU 02-99). – **Azores. Pico**, between Lajes do Pico and Sao Roque do Pico, Bosque da Junqueira, 38°27.93'N, 28°17.95'W, on *U. macaronesica*, 26.VII.2010, P. Diederich 17087a (S F264684); Pico, S of Sao Roque do Pico, 38°29.9'N, 28°18.58'W, on *U. macaronesica*, 24.VI.2010, P. Diederich 17058 (S F264685, hb. Diederich). – **Canada. Alberta**, Boreal Plains, on *U. glabrescens*, 22.VII.2006, T. Goward s.n. (UBC); *ibid.*, on *U. glabrescens*, 23.VI.2008, T. Goward s.n. (UBC). – **British Columbia**, Homathko Valley, 51°2'N, 124°58'W, on *Usnea* sp., 11.VIII.2007, C. Björk 14689 (UBC). – **Canary Islands. La Palma**, El Paso, Refugio El Pilar, 28°36'N/17°50'W, 1452 m, VIII.2009, G. Amo s.n. (MAF-Lich). – **Madeira**, S of Santana, West of Casa de Abrigo das Queimadas, along Levada do Caldeirão Verde, 32°47.09'N, 16°54.49'W, on *U. cf. krogiana*, 28.VIII.2014, P. Diederich 17778 (hb. Diederich); Rabaçal, along Levada das 25 Fontes, 32°45.81'N, 17°8.03'W, on *U. flavocardia*, 30.VIII.2014, P. Diederich 17792 (S, hb. Diederich); *ibid.*, on *U. fragilescens*, 17794 (S F29146). – **Sweden. Småland**, Kalmar, Hallingeberg par., Borgberget, c. 2.6 km S of Ankarstrum church, 57°40.65'N, 16°20.45'E, on *U. subfloridana*, 3.X.2009, M. Westberg 09-674 (S F264680), M. Westberg 09-676 (S F264681); *ibid.*, on *U. florida*, M. Westberg 09-675 (S F264682). – **U.K. Scotland**, East Lothian (v.c. 82), on *U. subfloridana*, 6.VII.2008, B. Coppins s.n. (S F102407); West Ross, Duirinish, west side of Port Bàn, on *Usnea cf. florida*, 25.VII.2010, A. Millanes 507 (S F291462); West Ross, Loch Carron, Glen Attadale, North side of River Attadale, west of Strathan, on *Usnea subfloridana*, 27.VII.2010, M. Westberg s.n. (S F219463).

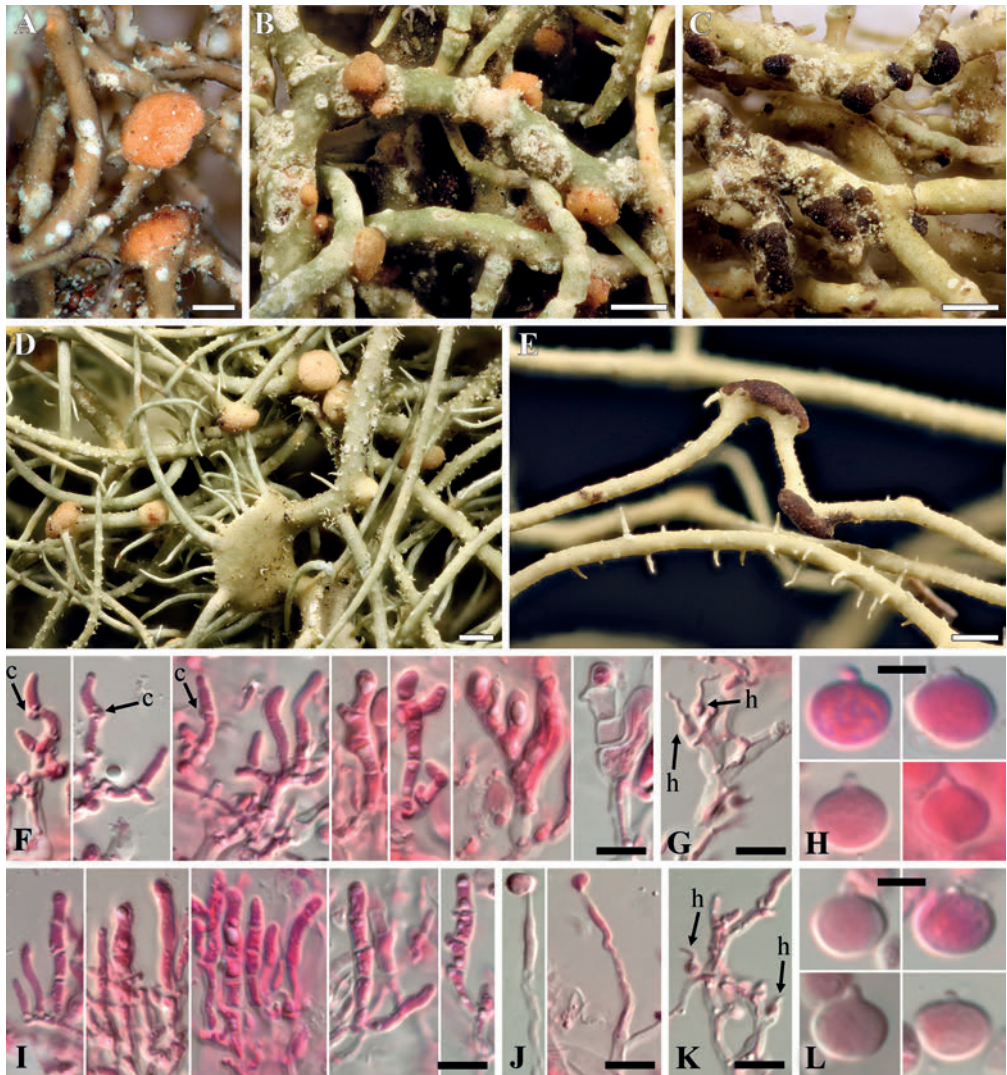


Fig. 9: *Biatoropsis usnearum* s.str. **A–E** – Basidiomata on the thallus of *Usnea* spp. [A, D, on *U. subfloridana*; B–C, on *U. flavocardia*; E, on *Usnea* sp.]. **F, I** – Probasidia with basal clamps (c) and mature basidia. **G, K** – Haustorial branches (h). **J** – Epibasidia with basidiospores. **H, L** – Basidiospores. [A: Räsänen s.n. (lectotype); B–C: Amo s.n.; D, I–L: Millanes 507; E, F–H: Björk 14689]. – Scales: A = 200 μ m; B–E = 500 μ m; F–G, I–K = 10 μ m; H, L = 5 μ m.

Biatoropsis sp. F

(Fig. 10)

Description: **Basidiomata** inducing the formation of convex, basally constricted galls, sometimes tuberculate with a central depression when mature, waxy gelatinous when wet, pale brown to dark brown or black, often with a reddish tinge, sometimes bright orange, 0.18–2.2 mm diam. **Context hyphae** thin-walled, 2–3 μ m diam. clamp connections not observed. **Haustorial branches** frequent, mother cell spherical to subspherical, (2–)3–4 \times 2–3 μ m, haustorial filament 1 μ m diam., up to 10 μ m long. **Hymenium** hyaline, containing numerous probasidia; probasidial initials clavate, occasionally with a basal clamp. **Basidia**, when mature, 4-celled, with three transverse septa, often constricted at the septa, the lower cell with an attenuated stalk-like base, often longer than the upper cells, 21–72 \times 4–10 μ m

(incl. stalk-like base, excl. epibasidia), lower part of the stalk-like base 2–4 µm diam. **Basidiospores** globose to subglobose, (4–)5–8 × (5.5–)6–9(–11) µm, Q = 0.6–1, with a distinct apiculus, c. 1 µm diam. **Asexual morph:** Catenulate hyaline conidia, individual conidia 2–6 × 2–4 µm.

Ecology and hosts: Lichenicolous on *Usnea* spp., including *U. ceratina*, *U. exasperata* s. l., *U. hirta* and *U. rubicunda* (thallus).

Distribution: Known from France, New Zealand, Papua New Guinea, Seychelles, and the U.S.A.

Notes: This species corresponds to clade ‘F’ distinguished by MILLANES et al. (2014b) using multigene analyses, which is also recovered in this study (Fig. 1). Although *Biatoropsis* sp. F clearly constitu-



Fig. 10: *Biatoropsis* sp. ‘F’. **A–G** – Basidiomata on the thallus of *Usnea* spp. **A–C** – On *U. exasperata* s. lat. **D–G** – On *U. rubicunda*. **H–I** – Hymenium and basidia. **J** – Haustorial branches (h). **K** – Basidiospores. [A–C, H: Diederich 18087B; D, K: Diederich 18149; E: Wedin 9429; F–G, I–J: Diederich 10800]. – Scales: A–D = 500 µm; E = 200 µm; F–G = 1 mm; H–K = 10 µm.

tes an independent evolving lineage based on our molecular results, we have not been able to identify any morphological or ecological characters that could clearly distinguish specimens in this clade from *Biatoropsis usnearum* s. str. We thus wait with the formal description of this taxon, until more material allow us to get a better understanding of its morphology and ecology.

Specimens examined: **France. Pyrénées-Atlantiques**, 15 km SSE of Saint-Jean-de-Luz, S of Sare, forêt communale de Sare, along road D306 to col de Lizarieta, on *Usnea rubicunda*, 26.VIII.2015, P. Diederich 18149 (hb. Diederich). – **New Zealand. North Island**, Rotorua, Lake Tarawera, north coast, 1 km E of Humphreys Bay, 13.XI.2001, F. Berger 16609 (S F92784, hb. Berger); Wellington, ca. 25 km N Masterton, 40°43.23'S, 175°38.4'E, on *U. rubicunda*, 28.XII.2010, M. Wedin 9429 (S F181399). – **Papua New Guinea. Madang Province**, Huon Peninsula, Finisterre range, Yupna valley, Teptep village, 5°57'S, 146°33'E, on *U. rubicunda*, 30.VII.1992, P. Diederich 10800 (hb. Diederich). – **Seychelles. Praslin**, Praslin National Park, SE of Vallée de Mai, along trail to Glacis Noir and fire tower, 4°20.23'S, 55°44.58'E, on *U. exasperata* s. l., 5.VIII.2015, Diederich 18087B (S, SEY, hb. Diederich). – **U.S.A. Arizona**, Cochise Co., Chiricahua Mountains, lower portion of Morse Canyon, 31°51'N, 109°19.5'W, on *U. hirta*, 3.IX.1983, T. H. Nash III 20992 (ASU); Pima Co., road to Mount Lemon, NE of Tucson, 32°25'N, 110°43'W, on *U. hirta*, III.1985, B. D. Ryan 12533 (ASU); Gila County, Tonto National Forest, Col. Devin Trail no 290, above Washington Park, along Mail Creek, 34°26'N, 11°16.33'W, on *U. hirta*, 11.VII.1997, T.H. Nash 39494 (ASU, S F102404). **California**, Mendocino Co., Sky Trail, on *U. ceratina*, 11.VII.2008, Kocourková s.n. (S F264679).

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