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1 **TITLE:** *Cyphobasidium* gen. nov., a new lichen-inhabiting lineage in the  
2 Cystobasidiomycetes (Pucciniomycotina, Basidiomycota, Fungi).

3

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27 **ABSTRACT**

28

29 Pucciniomycotina is a highly diverse group of fungi, showing a remarkably wide range  
30 of life-styles and ecologies. However, lichen-inhabiting fungi are only represented by a  
31 few species included in the genera *Chionosphaera* and *Cystobasidium*, and their  
32 phylogenetic position has never been investigated. Phylogenetic analyses using the  
33 nuclear SSU, ITS and LSU ribosomal DNA markers reveal that the lichenicolous  
34 members of *Cystobasidium* (*C. hypogymniicola*, *C. usneicola*) form a monophyletic  
35 group distinct from *Cystobasidium* and outside the Cystobasidiales. The new genus  
36 *Cyphobasidium* is consequently described to accommodate these lichen-inhabiting  
37 species. *Cyphobasidium* is characterized by producing conspicuous galls on the host  
38 lichen thalli, by having distinctive basidia that arise from a thick-walled, cup-like  
39 structure, the probasidium, that persists after the senescence of the actual basidium  
40 (meiosporangium), and by its lichenicolous habit on species of *Hypogymnia* and *Usnea*.  
41 *Cyphobasidium* is one of the few representatives of the Cystobasidiomycetes in which  
42 the sexual stage predominates in nature, whereas most species in the group are known  
43 only from an asexual yeast phase. This is the first time the position of lichen-inhabiting  
44 taxa within the Pucciniomycotina is investigated using molecular data.

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49 **Keywords:** Basidiomycota, Cystobasidiales, integrative taxonomy, lichenicolous fungi,  
50 mycoparasites.

51

## 52 1. INTRODUCTION

53 Lichenicolous fungi live in association with lichens with which they form assemblages  
54 of three, four, or even more organisms (Hawksworth 1988, Ihlen and Wedin 2008,  
55 Lawrey and Diederich 2003, Rambolt and Triebel 1992). Fungi with a lichenicolous  
56 life-style can be found in different lineages of the fungal tree of life, both within  
57 Ascomycota and Basidiomycota. Among the latter, phylogenetic studies have been  
58 conducted in the Agaricomycotina, focusing on representatives of the Agaricomycetes  
59 (Lawrey et al. 2007) and of the Tremellomycetes (Millanes et al. 2011), showing that  
60 the lichenicolous habit arose several times in the evolution of these groups. However,  
61 the phylogenetic position of the only lichen-inhabiting taxa known in Pucciniomycotina  
62 (*Chionosphaera* and *Cystobasidium*) remains uncertain.

63 Pucciniomycotina is one of the major clades of Basidiomycota. The Pucciniales  
64 (rust fungi) and the Microbotriales (false smuts) are parasitic fungi on vascular plants  
65 and altogether dominate the subclass in terms of diversity (Aime et al. 2006, Kelmer et  
66 al. 2006, Lutz et al. 2008, Vánky 2012). Other representatives show a great variety of  
67 lifestyles including saprotrophs (Pachnocybales), parasites specialized on plant roots  
68 (Helicobasidiales), fern parasites (Mixiomycetes), parasites growing on insects scales  
69 (Septobasidiales), or aquatic fungi of fresh water habitats (Classiculomycetes) (Aime et  
70 al. 2006). Fungal parasites are present in several lineages, such as the  
71 Agaricostilbomycetes, the Cryptomycocolacomycetes, the Cystobasidiomycetes, the  
72 Microbotryomycetes and the Pucciniomycetes. Among them, the lichenicolous  
73 representatives are comparatively rare. *Chionosphaera* is placed in the  
74 Agaricostilbomycetes (Aime et al. 2006, Kirschner et al. 2001), but the position of the  
75 two species growing exclusively on lichens, i.e. *Chionosphaera coppinsii* (Roberts  
76 1997) and *C. lichenicola* (Alstrup 1993), has not been investigated by molecular

77 methods. The Cystobasidiomycetes were established by Bauer et al. (2006) and include  
78 fungi without fucose in their cell walls, and spindle pole bodies that are intranuclear  
79 during the metaphase. Within Cystobasidiomycetes, the Cystobasidiales are  
80 characterized by producing tremelloid haustorial cells and septal pores with cystosomes,  
81 i.e., organelles that cover the pore channel (Bauer et al. 2006). Lichenicolous  
82 representatives of *Cystobasidium* were described by Diederich (1996), but only  
83 tentatively placed in the genus, based on the presence of a persistent thick-walled  
84 probasidium, from which a thin-walled transversely septate basidium develops.

85         Systematics of lichenicolous fungi has been largely hampered both by  
86 difficulties in obtaining molecular data, and by the scarcity of morphological characters.  
87 Nevertheless, in the past years, efforts to bring knowledge into the classification of  
88 different groups have been achieved (Baloch et al. 2013, Hawksworth et al. 2010,  
89 Lawrey et al. 2007, Millanes et al. 2011, 2014, Pérez-Ortega et al. 2014, Ruibal et al.  
90 2011).

91         The aim of this study is to resolve the phylogenetic position of the lichenicolous  
92 species of *Cystobasidium*, and to contribute to the knowledge on the evolution of the  
93 lichen-inhabiting life-style within the Pucciniomycotina. We will achieve this by using  
94 a dataset including the small subunit (nSSU), the internal transcribed spacer (ITS) and  
95 the large subunit (nLSU) nuclear ribosomal DNA repeats. This is the first time that the  
96 position of lichen-inhabiting species within the Pucciniomycotina is investigated using  
97 molecular data.

98

99

100 **2. MATERIAL AND METHODS**

101

102 **2.1 Taxon sampling**

103 Specimens included in the molecular study are listed in Tab. 1. The taxon sampling  
104 included species representative of nine classes in the Pucciniomycotina (i.e.,  
105 Agaricostilbomycetes, Atractiellomycetes, Classiculomycetes,  
106 Cryptomycocolacomycetes, Microbotryomycetes, Mixiomycetes, Pucciniomycetes,  
107 and Tritirachiomycetes (Kirk et al. 2008, Schell et al. 2011). To represent  
108 Cystobasidiomycetes we included (1) two specimens of *Cystobasidium hypogymniicola*  
109 and five of *C. usneicola*, (2) four representatives of the Cystobasidiales, including  
110 *Cystobasidium fimetarium* (syn. *Cystobasidium lasioboli*, type species of the genus  
111 *Cystobasidium*), (3) one representative of the Naohidelaes, (4) two representatives of  
112 the Erythrobasidiales, and (5) eight terminal taxa representing four different clades  
113 distinguished by Boekhout et al. 2011, and Pohl et al. 2011, i.e., the aurantiaca, marina,  
114 magnisporus, and Sakaguchia clades Two taxa of the Ustilaginomycotina, *Malassezia*  
115 *pachidematis* and *Ustilago tritici*, were used as outgroup.

116

117 **2.2 Morphological studies**

118 Herbarium specimens are deposited both in S and in the private collections of P.  
119 Diederich and P. van den Boom. Macroscopic photographs were done using a Canon  
120 40D camera with a Canon MP-E 65 mm lens or a Nikon BD Plan 10× microscope  
121 objective, StackShot (Cognisys). Microscopic structures were studied using handmade  
122 sections stained with Phloxin (1% in water) after pre-treatment with KOH (5%),  
123 following the methods of Diederich (1996), and observed with a Zeiss Axioscope-2  
124 microscope. Photographs were taken with a Nikon Coolpix 995 camera fitted to the

125 microscopes. Helicon Focus (HeliconSoft) was used to increase the depth of field in  
126 both macroscopic photographs and micrographs.

127

## 128 ***2.3 Molecular studies***

### 129 ***2.3.1. DNA extraction, amplification and sequencing***

130 DNA was extracted from either recently collected or dried herbarium specimens. ). The  
131 outer surface of the selected galls was sectioned and separated with a scalpel, in order to  
132 minimize the lichen tissue in the DNA extraction. Approximately 3 or 4 basidiomata  
133 were selected from each specimen for DNA extraction. Total DNA of the induced galls  
134 was extracted using the Qiagen DNeasy Plant miniKit, according to the manufacturer's  
135 instructions.

136         General fungal primers and newly-designed primers (Tab. 2) were combined to  
137 amplify the nSSU, the ITS and a fragment of ca. 600 pb of the nLSU in the nuclear  
138 ribosomal DNA.

139         PCR amplifications were performed using Illustra™ Hot Start PCR beads,  
140 according to the manufacturer's instructions. For the primer pairs ITS1F/CnLSU-447R,  
141 ITS1F/CnLSU-589R, CnLSU-70F/LR5, and CnLSU-511F/LR5 we used initial  
142 denaturation at 95°C for 5 min, four cycles (95°C for 40 s, 56°C for 40 s and 72°C for  
143 90 s), four cycles (95°C for 30 s, 53°C for 30 s and 72°C for 90 s), and finally 32 cycles  
144 (95°C for 30 s, 50°C for 30 s and 72°C for 90 s) with a final extension at 72°C for 420 s.  
145 For the primer pair NS1/CITS2-837R we used initial denaturation at 95°C for 5 min,  
146 followed by 35 cycles (95°C for 30 s, 50°C for 30 s and 72°C for 90 s) with a final  
147 extension at 72°C for 420 s. For the primer pair NS1/CITS2-831R we used initial  
148 denaturation at 95°C for 5 min, followed by 35 cycles (95°C for 30 s, 53°C for 30 s and  
149 72°C for 90 s) with a final extension at 72°C for 420 s. Before sequencing, the PCR

150 products were purified with Exo-sap-IT© (USB Corporation). The primers CnSSU-  
151 371F, CnSSU-1092R, and nuSSU1088Rb were used as internal sequencing primers.  
152

### 153 ***2.3.2. Multiple alignment and phylogenetic analyses***

154 For phylogenetic analyses, sequences were aligned using MAFFT version 7 (Kato et  
155 al. 2002; Kato and Toh 2008a; b). A G-INS-i algorithm was used for nSSU, a Q-INS-i  
156 algorithm was used for ITS and nLSU, and a L-INS-i algorithm was used for TEF1-  
157 alpha. The alignments were trimmed to exclude ambiguously aligned regions using  
158 GBlocks (Castresana 2000), following the relaxed conditions described by Talavera and  
159 Castresana (2007). Individual gene datasets were analysed individually by ML  
160 bootstrapping, to assess for conflicts – considered as contradicting clades recovered  
161 simultaneously with bootstrap support  $\geq 70\%$  (Hillis and Bull 1993). Data were further  
162 analysed as a three-gene concatenation.

163 Phylogenetic relationships were reconstructed using maximum likelihood (ML)  
164 and Bayesian approaches. In both cases we considered five independent partitions,  
165 namely: nSSU, ITS1, 5.8S, ITS2, nLSU. Maximum likelihood analyses were done in  
166 RAxMLGUI 1.3 (Silvestro & Michalak, 2012), a graphical front-end for RAxML  
167 (Stamatakis, 2006). The GTRGAMMA model of nucleotide substitution applied to all  
168 partitions because of constraints of the software RAxML. We performed a thorough ML  
169 search with a total of 10 runs and assessed node support by thorough bootstrap using  
170 1000 bootstrap pseudo-replicates. Bayesian analyses were performed by Markov Chain  
171 Monte Carlo (MCMC) sampling as implemented in the software MrBayes 3.2.4  
172 (Ronquist et al. 2012). We selected substitution models for each of the regions, using  
173 the Akaike Information Criterion (AIC) as implemented in jModeltest (Guindon and  
174 Gascuel 2003, Posada 2008). We used full likelihood optimization, 6 discrete gamma



175 categories, and selected only among the 24 models implemented in MrBayes. A GTR+  
176 I+ $\Gamma$  was selected for the nuclear SSU rDNA, a SYM+ $\Gamma$  for the ITS1 and the 5.8S, a  
177 HKY+ $\Gamma$  for the ITS2, and a GTR+I+  $\Gamma$  for the nuclear LSU rDNA. The combined  
178 analyses treated the different regions as separate partitions with topology linked across  
179 partitions but separate model parameter values and proportional rates across partitions.  
180 The number of discrete gamma categories was set to six. The analyses were diagnosed  
181 for convergence every 100 000 generations, and were set to halt automatically when the  
182 average standard deviation of splits across runs in the last half of the analysis descended  
183 below 0.01. Every 100th tree was saved. The first halves of each run were discarded as  
184 burn-in.

185

186

187

188 **3. RESULTS**

189 **3.1 Molecular studies**

190 We generated 14 new sequences (2 nSSU, 5 ITS and 7 nLSU rDNA), which were  
191 aligned together with sequences already available in GenBank (Tab. 1). Four data  
192 matrices were produced including nSSU, ITS, and nLSU rDNA. The combined matrix  
193 contained 2447 aligned characters (nLSU: 1–521; ITS1: 522–576; 5.8S: 577–724; ITS2:  
194 725–809; nSSU: 810–2447).

195 We tried to design specific primers to selectively amplify the DNA of the new  
196 genus, avoiding that of the lichenized host. Suitable priming sites were identified by  
197 aligning available sequences of representatives of the Cystobasidiomycetes against  
198 sequences of *Hypogymnia* and *Usnea* (Lecanorales, Ascomycota) selecting conserved  
199 fragments differing markedly between the Cystobasidiomycetes and the lichenized  
200 hosts. However, *Biatoropsis* and *Tremella* were sometimes amplified when present in  
201 the same gall. Further studies will require the design of more specific primers being able  
202 to discriminate the DNA of Cystobasidiomycetes from that of Tremellomycetes.

203 The best tree obtained from the ML analysis had a ln-likelihood value of --  
204 16558.491932. The Bayesian analysis halted after 1 900 000 generations, when the  
205 average standard deviation of split frequencies across runs was 0.008, which indicated  
206 that the three runs had converged (<0.01). A majority rule consensus tree was  
207 constructed from the 9 500 trees of the stationary tree sample. There was no  
208 incongruence between the ML and Bayesian trees. Therefore, only the 50% majority  
209 rule consensus tree from the Bayesian analysis is shown in Fig. 2. Our analyses revealed  
210 a distinct group including both *Cystobasidium hypogymniicola* and *C. usneicola*, which  
211 is clearly separated from the Cystobasidiales. The new genus *Cyphobasidium* is hereby  
212 described to accommodate these lichen-inhabiting taxa.

213

214 **3.2 Taxonomy**

215

216 **Cyphobasidium** Millanes, Diederich & Wedin **gen. nov.**

217 Mycobank no. XXXX

218 **Etymology:** From Latin *cyphus* (= bowl, cup), in reference to the probasidium, a thick-  
219 walled cup-like structure from which the meiosporangium arises.

220 **Description:** *Basidiomata* inducing conspicuous galls on the host lichen thalli. Galls  
221 convex, with a constricted base, often becoming irregular in form. *Context hyphae* with  
222 or without clamps; haustorial branches unknown. *Basidia*, when mature, consisting of a  
223 thick-walled, ellipsoid to elongate lower part (the probasidium) and a thin-walled,  
224 cylindrical, often bent, transversely 3-septate, upper part (the meiosporangium);  
225 epibasidia short, more or less perpendicular to the basidium. *Basidiospores* ellipsoid to  
226 broadly fusiform, sometimes slightly curved, with a distinct refractive apiculus at the  
227 lower end and a rounded upper end. *Asexual stage* unknown.

228 **Type species:** *Cyphobasidium hypogymniicola* (Diederich & Ahti) Millanes, Diederich  
229 & Wedin

230 **Ecology:** Lichenicolous, growing on *Hypogymnia* and *Usnea* (Parmeliaceae,  
231 Lecanorales, Ascomycota), gall-inducing.

232

233 **Cyphobasidium hypogymniicola** (Diederich & Ahti) Millanes, Diederich & Wedin

234 **comb. nov.**

235 Mycobank no. XXXX

236 Basionym: *Cystobasidium hypogymniicola* Diederich & Ahti, *Bibliotheca*

237 *Lichenologica* **61**: 21 (1996). Type: xxxxxxx, holotype

238 A detailed description of this species is provided by Diederich (1996). *Cyphobasidium*  
239 *hypogymniicola* has been reported from Canada, Finland, Norway, Russia and the  
240 U.S.A., growing most commonly on *Hypogymnia physodes*, more rarely on *H. hultenii*,  
241 *H. imshaugii*, *H. incurvoides*, *H. krogiae* and *H. vittata* (Diederich 1996, 2003, 2007,  
242 Hodkinson et al. 2009, Holien 2001, Urbanavichene and Urbanavichus 2005).  
243 Specimens examined: U.S.A.: Maine, 45°3.75' N, 67°3.75' W, on *H. physodes*, 2000,  
244 Cole 9092 (herb. Diederich); Maine, Washington Co., 44°27.59' N, 67°55.97' W, on *H.*  
245 *krogiae* on *Abies balsamifera*, 2008, Hawksworth s.n (S-F264671).

246

247 ***Cyphobasidium usneicola*** (Diederich & Alstrup) Millanes, Diederich & Wedin **comb.**  
248 **nov.**

249 Mycobank no. XXXX

250 Basionym: *Cystobasidium usneicola* Diederich & Alstrup, *Bibliotheca Lichenologica*  
251 **61**: 25 (1996).

252 A detailed description of this species is provided by Diederich (1996). *Cyphobasidium*  
253 *usneicola* was first described from Vancouver Island in Canada (British Columbia),  
254 growing on *Usnea* spp. (including *U. subfloridana*), and later reported from Colombia,  
255 Costa Rica, Mexico and the U.S.A. (Diederich 1996, 2003). We report it here as new to  
256 Ecuador (incl. Galapagos), Peru, and Macaronesia (Canary Islands, Madeira), always on  
257 the thalli of *Usnea* spp. From the specimen cited below, sequences of *Cystobasidium*  
258 *usneicola* have been obtained, although microscopically only *Tremella nashii* (Nash  
259 35351) could be observed.

260 Specimens examined: **ECUADOR**: Loja, 4°6.72' S, 79°10.32' W, on *U. cf. praetervis*,  
261 2007, Truong 279 (S-F264672); Galapagos 00°51.43' S, 89°27.58' W, on *U.*  
262 *galapagona*, 2008, Clerc 08/334 (S-F264673). **PERU**: Cusco, 13°15.63' S, 72°27.65'

263 W, on *U. silesiaca*, 2007, Truong 1953 (S-F264674). *PORTUGAL*: Madeira, NE of  
264 Funchal, Santo da Serra, botanical garden Quinta do Santo da Serra, 32°43.51' N,  
265 16°49.12' W, on *Usnea* sp. on *Prunus*, 2012, van den Boom 47648 (herb. van den  
266 Boom, sub *Biatoropsis usnearum*). *SPAIN*: Canary Islands, Gran Canaria, W of  
267 Artenara, along road to Cortijo de la Tirma, 15°41.36' N, 28°01.23' W, on *U. articulata*  
268 on *Cistus*, 2013, van den Boom 49225 (herb. Diederich). *U.S.A.*: Arizona, Coconino  
269 Co., upper part of the west fork of Oak Creek, canyon in the vicinity of West Buzzard  
270 Point, 35°1.25' N 111°50.25' W, on *U. hirta* on *Quercus gambelii*, 1994, Nash 35351  
271 (herb. Diederich, sub *Tremella nashii*); California, Mendocina Co., Jug Handle Creek,  
272 39°22.2' N, 123°48.48' W, on *U. subscabrosa*, 2008, Muggia 38882 (S-F264675).  
273

274 **4. DISCUSSION**

275 It is clear that the new genus *Cyphobasidium* represents a distinct lichen-inhabiting  
276 lineage in the Cystobasidiomycetes (Pucciniomycotina) that comprises only species  
277 growing on the lichen family Parmeliaceae. Host selection has shown to be an important  
278 factor characterizing monophyletic groups in other mycoparasitic basidiomycetes, like  
279 the Tremellomycetes (Millanes et al. 2011, 2014). Within them, the lichenicolous habit  
280 is present in several independent monophyletic groups that include lichenicolous  
281 species only. In the Pucciniomycotina, *Cyphobasidium* is the only taxa with a  
282 lichenicolous habit known so far, which belongs to the Cystobasidiomycetes.

283 The new genus is also characterized by its teleomorphic state. Life-cycles in the  
284 Pucciniomycotina are extremely variable, and many representatives are dimorphic fungi  
285 (i.e., fungi that have both a filamentous phase and a haploid yeast phase in their life-  
286 cycle). The Cystobasidiomycetes, for instance, include several dimorphic species, in  
287 which both the yeast stage and the basidia-producing mycelial phase are known in  
288 nature (e.g. *Nahoidea*, *Occultifur*; Oberwinkler 1990). In many yeast-forming species,  
289 however, basidia have never been observed (e.g. *Rhodotorula*, *Sporobolomyces*;  
290 Hamamoto et al. 2011, Sampaio 2011), or were only found in culture (e.g. *Sakaguchia*;  
291 Fell 2011). Other teleomorphs within the group are *Bannoa*, *Erythrobasidium* and  
292 *Sakaguchia* (Hamamoto et al. 1991, Hamamoto et al. 2002, Yamada et al. 1994),  
293 although the nature of the structures considered as basidia have been controversial in the  
294 literature, being interpreted as conidial stages by some authors (Aime et al. 2006, Bauer  
295 et al. 2006, Sampaio et al. 1999). A yeast phase has not yet been observed in  
296 *Cyphobasidium*, since none of the species have yet been obtained in culture.

297 The presence of tremelloid haustorial cells is one of the diagnostic characters of  
298 the order Cystobasidiales (Bauer et al. 2006), and the absence of haustorial cells in

299 those representatives of *Cystobasidium* now transferred to *Cyphobasidium* was already  
300 mentioned by Diederich (1996). However, the study of other potentially important  
301 physiological and ultrastructural diagnostic characters (e.g. the coenzyme Q system, and  
302 the presence of cystosomes in the septal pores) is again hindered by the lack of cultures.  
303 One additional complication, particularly in the case of *Cyphobasidium usneicola*, is the  
304 fact that it occasionally coexists with *Biatoropsis usnearum* or *Tremella nashii* within  
305 the same, morphologically indistinguishable, galls. Diederich (1996) already  
306 hypothesized on the possibility of *Cyphobasidium usneicola* being, in reality, a parasite  
307 of *Biatoropsis*. Most probably, *Biatoropsis*-like galls on *Usnea* (Figs. 1C – E)  
308 sometimes contain a mixture of several species, some of which can be detected  
309 microscopically, while others can only be detected by molecular methods. The galls  
310 induced by *Cyphobasidium hypogymniicola*, on the contrary, differ morphologically  
311 from the galls formed by another tremellalean parasite on *Hypogymnia*, *Tremella*  
312 *hypogymniae*, in that they develop into much larger bullate galls (Figs. 1A – B) that are  
313 concolorous with the thallus when young. Moreover, there is so far no morphological or  
314 molecular evidence that both species can coexist in the same gall.

315 Diederich (1996) considered several possible allies of *Cyphobasidium*, based on  
316 the presence of a thick-walled probasidium, which included *Cystobasidium*, *Platycarpa*,  
317 and *Septobasidium*. *Cyphobasidium* produces transversely septate basidia in nature (Fig.  
318 1J), arising from a thick-walled probasidium (Figs. 1H – I) that persists beyond the  
319 actual basidium. The type species of *Cystobasidium* (*C. lasioboli* syn. *C. fimetarium*)  
320 differs considerably from *Cyphobasidium* in the arachnoid basidiomata and the absence  
321 of a proper hymenium (Diederich 1996). *Cystobasidium proliferans* lacks basidiomata  
322 and produces almost spherical proliferating probasidia (Olive 1952). On the contrary,  
323 *Cystobasidium sebaceum*, a species described from decaying wood in Columbia, largely

324 resembles the macro- and micro-morphology of *Cyphobasidium hypogymniicola*  
325 (Martin 1939, Diederich 1996). The phylogenetic position of these last two species is  
326 not resolved yet, however, and therefore not possible to draw conclusions on their  
327 relationship to *Cyphobasidium*. Recently, several yeast species originally described in  
328 *Rhodotorula* have been transferred to *Cystobasidium* and two other anamorphic species  
329 have been described, but a teleomorphic stage is not known in any of them (Yurkov et  
330 al. 2015). Species of *Platycarpa polypodii* produces basidia similar to those of  
331 *Cyphobasidium*, although they differ by spores being bent-ellipsoid (Couch 1949), and  
332 by parasitism on fern sporangia (Oberwinkler & Bandoni 1984). *Platycarpa* belongs to  
333 the order Platyglloeales (Bauer et al. 2006). Our molecular data have confirmed that the  
334 new genus is not closely related to *Septobasidium* either, a genus of scale insect  
335 parasites within the Pucciniomycetes (Aime et al. 2006, Bauer et al. 2006, Henk and  
336 Vilgalys 2007). Other taxa that morphologically resemble *Cyphobasidium* are the  
337 monotypic genera *Ptechetelium* (Oberwinkler & Bandoni 1984) and *Cystogloea*  
338 (Roberts 2006). *Ptechetelium cyatheae* differs by growing on living leaves of ferns, and  
339 by having stalked probasidia with a thick wall that is multilayered after development of  
340 successive probasidia. *Cystogloea oelandica* produces multiple thin-walled probasidia  
341 by disarticulation of hyphae in compartments. The phylogenetic position of these two  
342 taxa is not known either.

343         Our results show that species circumscription and diversity within the new genus  
344 deserve further studies. At least two species are included within *Cyphobasidium*  
345 *usneicola*, represented as *C. usneicola* clade 1 and *C. usneicola* clade 2 in Fig. 2.  
346 *Cyphobasidium usneicola* clade 1 is the sister group to *C. hypogymniicola*, whilst *C.*  
347 *usneicola* clade 2 appears as the sister group to the clade formed by *C. hypogymniicola*  
348 and *C. usneicola* clade 1. However, with the data at hand, the two clades cannot be



349 distinguished morphologically or by host specificity. Also, as no sequences could be  
350 obtained from the type specimen of *C. usneicola*, it is not known which of the two  
351 clades represents *C. usneicola* s. str. *Cyphobasidium hypogymniicola* seems to be a  
352 better delimited taxon, but still our sampling is very limited to draw conclusions on  
353 species delimitation. We therefore prefer to tentatively avoid assigning species rank to  
354 the two clades of *C. usneicola*, and increase our sampling for future studies dealing with  
355 species delineation within the new genus.

356           Integrative taxonomy –which combines several lines of evidence to delimit taxa  
357 – is a particularly useful approach in groups with a challenging taxonomy due to  
358 scarcity of characters, homoplastic characters, or both (Damm et al. 2010; DeSalle et al.  
359 2005; Pires and Marinoni 2010). This applies not only to species delimitation, but is  
360 also useful at the generic rank (Dohrmann et al. 2012, Vicente et al. 2013). In the case  
361 of *Cyphobasidium*, combining molecular and ecological evidences has allowed  
362 establishing a new genus within Pucciniomycotina, and to re-interpret morphological  
363 characters (e.g., presence of a thick-walled probasidium) previously considered as  
364 potential synapomorphies.

365

366

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379

380 **REFERENCES**

- 381 Aime MC, Matheny PB, Henk DA, Frieders EM, Nilsson RH, Piepenbring M,  
382 McLaughlin DJ, Szabo LJ, Begerow D, Sampaio JP, Bauer R, Weiß M,  
383 Oberwinkler F, Hibbett DS, 2006. An overview of the higher-level classification  
384 of Pucciniomycotina based on combined analyses of nuclear large and small  
385 subunit rDNA sequences. *Mycologia* **98**: 896-905.
- 386 Alstrup V, 1993. News on lichens and lichenicolous fungi from the Nordic countries.  
387 *Graphis Scripta* **5**: 96-104
- 388 Baloch E, Gilenstam G, Wedin M, 2013. The relationships of *Odontotrema*  
389 (Odontotremataceae) and the resurrected *Sphaeropezia* (Stictidaceae) – new  
390 combinations and three new *Sphaeropezia* species. *Mycologia* **105**: 384-397.
- 391 Bauer R, Begerow D, Sampaio JP, Weiß M, Oberwinkler F, 2006. The simple-septate  
392 basidiomycetes: a synopsis. *Mycological Progress* **5**: 41-66.
- 393 Boekhout T, Fonseca A, Sampaio JP, Bandoni RJ, Kwon-Chung KJ, 2011. Discussion  
394 of teleomorphic and anamorphic basidiomycetous yeasts. In: Kurtzman CP, Fell  
395 JW, Boekhout T (eds.), *The Yeasts: A Taxonomic Study*. Elsevier, London, pp.  
396 1339-1372.
- 397 Castresana J, 2000. Selection of conserved blocks from multiple alignments for their  
398 use in phylogenetic analysis. *Molecular Biology and Evolution* **17**: 540-552.
- 399 Couch JN, 1949. The taxonomy of *Septobasidium polypodii* and *S. album*. *Mycologia*  
400 **41**: 427-441.
- 401 Damm S, Schierwater B, Hadrys H, 2010. An integrative approach to species discovery  
402 in odonates: from character-based DNA barcoding to ecology. *Molecular Ecology*  
403 **19**: 3881-3893.

404 DeSalle R, Egan MG, Siddall M, 2005. The unholy trinity: taxonomy, species  
405 delimitation and DNA barcoding. *Philosophical Transactions of the Royal Society*  
406 *of London B* **360**:1905-1916.

407 Diederich P, 1996. The lichenicolous heterobasidiomycetes. *Bibliotheca Lichenologica*  
408 **61**: 1-198.

409 Diederich P, 2003. New species and new records of American lichenicolous fungi.  
410 *Herzogia* **16**: 41-90.

411 Diederich P, 2007. New or interesting lichenicolous heterobasidiomycetes. *Opuscula*  
412 *Philolichenum* **4**: 11-22.

413 Diederich P, Millanes AM, Wedin M, 2014. *Tremella umbilicariae* (Tremellomycetes,  
414 Basidiomycota), a new lichenicolous species on *Umbilicaria* from Peru. *Bulletin*  
415 *de la Société des naturalistes luxembourgeois* **115**: 167-172.

416 Dohrmann M, Göcke C, Reed J, Janusen D, 2012 Integrative taxonomy justifies a new  
417 genus, *Nodastrella* gen. nov., for North Atlantic "Rossella" species (Porifera:  
418 Hexactinellida: Rossellidae). *Zootaxa* **3383**: 1-13.

419 Fell JW, 2011. *Sakaguchia* Y. Yamada, Maeda and Mikata (1994). In: Kurtzman CP,  
420 Fell JW, Boekhout T (eds), *The Yeasts: a Taxonomic Study*, 5<sup>th</sup> edn. Elsevier, San  
421 Diego, pp. 1541-1544.

422 Gardes M, Bruns TD, 1993. ITS primers with enhanced specificity for basidiomycetes,  
423 application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**:  
424 113-118.

425 Guindon S, Gascuel O, 2003. A simple, fast, and accurate algorithm to estimate large  
426 phylogenies by maximum likelihood. *Systematic Biology* **52**: 696-704.

- 427 Hamamoto M, 2011. *Erythrobasidium* Hamamoto, Sugiyama and Komagata (1991). In:  
428 Kurtzman CP, Fell JW, Boekhout T (eds), *The Yeasts: a Taxonomic Study*, 5<sup>th</sup>  
429 edn. Elsevier, San Diego, pp. 1433-1435.
- 430 Hamamoto M, Boekhout T, Nakase T, 2011. *Sporobolomyces* Kluver and van Niel  
431 (1924). In: Kurtzman CP, Fell JW, Boekhout T (eds), *The Yeasts: a Taxonomic*  
432 *Study*, 5<sup>th</sup> edn. Elsevier, San Diego, pp. 1929-1990.
- 433 Hamamoto M, Sugiyama J, Komagata K, 1991. Nomenclature of the basidiomycetous  
434 yeast species *Erythrobasidium hasegawae*. *Journal of General and Applied*  
435 *Microbiology* **37**: 131-132.
- 436 Hamamoto M, Thanh VN, Nakase T, 2002. *Bannoa hahajimensis* gen. nov., sp. nov.,  
437 and three related anamorphs, *Sporobolomyces bischofia* sp. nov.,  
438 *Sporobolomyces ogasawarensis* sp. nov. and *Sporobolomyces syzygii* sp. nov.,  
439 yeasts isolated from plants in Japan. *International Journal of Systematic and*  
440 *Evolutionary Microbiology* **52**: 1023-1032.
- 441 Hawksworth, DL, 1988. The variety of fungal-algal symbioses, their evolutionary  
442 significance, and the nature of lichens. *Botanical Journal of the Linnean Society*  
443 **96**: 3-20.
- 444 Hawksworth DL, Millanes AM, Wedin M, 2010. *Roselliniella* revealed as an  
445 overlooked genus of Hypocreales, with the description of a second species on  
446 parmelioid lichens. *Persoonia* **24**: 12-17.
- 447 Henk DA, Vilgalys R, 2007. Molecular phylogeny suggests a single origin of insect  
448 symbiosis in the Pucciniomycetes with support for some relationships within the  
449 genus *Septobasidium*. *American Journal of Botany* **94**: 1515-1526.

- 450 Hillis DM, Bull JJ, 1993. An empirical test of bootstrapping as a method for assessing  
451 confidence in phylogenetic analyses. *Systematic Biology* **42**: 182-192.
- 452 Hodkinson BP, Harris RC, Case MA, 2009. A checklist of Virginia lichens. *Evansia* **26**:  
453 64-88.
- 454 Holien H, 2001. Additions to the Norwegian flora of lichens and lichenicolous fungi II -  
455 with some further distributional notes on Norwegian Caliciales. *Graphis Scripta*  
456 **12**: 51-58.
- 457 Ihlen PG, Wedin M, 2008. An annotated key to the lichenicolous Ascomycota  
458 (including mitosporic morphs) of Sweden. *Nova Hedwigia* **86**: 275-365.
- 459 Katoh K, Misawa K, Kuma K, Miyata T, 2002. MAFFT, a novel method for rapid  
460 multiple sequence alignment based on fast Fourier transform. *Nucleic Acids*  
461 *Research* **30**: 3059-3066.
- 462 Katoh K., Toh H, 2008a. Improved accuracy of multiple ncRNA alignment by  
463 incorporating structural information into a MAFFT-based framework. *BMC*  
464 *Bioinformatics* **9**: 212.
- 465 Katoh K, Toh H, 2008b. Recent developments in the MAFFT multiple sequence  
466 alignment program. *Briefings in Bioinformatics* **9**: 286-298.
- 467 Kemler M, Göker M, Oberwinkler F, Begerow D, 2006. Implications of molecular  
468 characters for phylogeny of the Microbotryaceae (Basidiomycota:  
469 Urediniomycetes). *BMC Evolutionary Biology* **6**: 35.
- 470 Kirk PM, Cannon PF, Minter DW, Stalpers JA, 2008. *Ainsworth and Bisby's Dictionary*  
471 *of the Fungi*, 10th ed. CAB International, Wallingford.
- 472 Kirschner R, Begerow D, Oberwinkler F, 2001. A new *Chionosphaera* species  
473 associated with conifer inhabiting bark beetles. *Mycological Research* **105**: 1403-

474 1408.

475 Lawrey JD, Binder M, Diederich P, Molina MC, Sikaroodi M, Ertz D, 2007.

476 Phylogenetic diversity of lichen-associated homobasidiomycetes. *Molecular*

477 *Phylogenetics and Evolution* **44**: 788-789.

478 Lawrey JD, Diederich P, 2003. Lichenicolous fungi: interactions, evolution, and

479 biodiversity. *Bryologist* **106**: 81-120.

480 Lutz M, Piątek M, Kemler M, Chlebicki A, Oberwinkler F, 2008. Anther smuts of

481 Caryophyllaceae: molecular analyses reveal further new species. *Mycological*

482 *Research* **112**: 1280-1296.

483 Martin GW, 1939. New or noteworthy fungi from Panama and Colombia. IV.

484 *Mycologia* **31**: 507-518.

485 Millanes AM, Diederich P, Ekman S, Wedin M, 2011. Phylogeny and character

486 evolution in the jelly fungi (Tremellomycetes, Basidiomycota, Fungi). *Molecular*

487 *Phylogenetics and Evolution* **61**:12-28.

488 Millanes AM, Truong C, Westberg M, Diederich P, Wedin M, 2014. Host switching

489 promotes diversity in host-specialized mycoparasitic fungi: uncoupled evolution

490 in the *Biatoropsis-Usnea* system. *Evolution* **68**: 1576-1593.

491 Oberwinkler F, 1990. New genera of auricularioid heterobasidiomycetes. *Reports of the*

492 *Tottori Mycological Institute* **28**: 113-127.

493 Oberwinkler F, Bandoni R, 1984. *Herpobasidium* and allied genera. *Transactions of the*

494 *British Mycological Society* **83**: 639-658.

495 Olive LS, 1952. A new species of *Cystobasidium* from New Jersey. *Mycologia* **44**: 564-

496 569.

497 Pérez-Ortega S, Suija A, Crespo A, de los Ríos A, 2014. Lichenicolous fungi of the  
498 genus *Abrothallus* (Dothideomycetes: Abrothallales ordo nov.) are sister to the  
499 predominantly aquatic Jahnulales. *Fungal Diversity* **64**: 295-304.

500 Pires AC, Marinoni L, 2010. DNA barcoding and traditional taxonomy unified through  
501 Integrative Taxonomy: a view that challenges the debate questioning both  
502 methodologies. *Biota Neotropica*. **10**: 339-346.

503 Posada D, 2008. JModelTest: Phylogenetic Model Averaging. *Molecular Biology and*  
504 *Evolution* **25**: 1253-1256.

505 Pohl CH, Smit MS, Albertyn J, 2011. *Rhodotorula bloemfonteinensis* sp. nov.,  
506 *Rhodotorula eucalyptica* sp. nov., *Rhodotorula orientis* sp. nov. and *Rhodotorula*  
507 *pini* sp. nov., yeasts isolated from monoterpene-rich environments. *International*  
508 *Journal of Systematic and Evolutionary Microbiology*. 61: 2320-2327.

509 Rambolt G, Triebel D, 1992. The interlecanoralean associations. *Bibliotheca*  
510 *Lichenologica* **48**: 1-201.

511 Roberts P, 1997. New heterobasidiomycetes from Great Britain. *Mycotaxon* **63**: 195-  
512 216.

513 Roberts P, 2006. *Cystogloea oelandica*: an unusual new auricularioid species from  
514 Sweden. *Acta Mycologica* **41**: 25-28.

515 Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu  
516 L, Suchard MA, Huelsenbeck JP, 2012. MrBayes 3.2: Efficient Bayesian  
517 Phylogenetic Inference and Model Choice across a Large Model Space.  
518 *Systematic Biology* **61**: 1-4.

519 Ruibal C, Millanes AM, Hawksworth DL, 2011. Molecular phylogenetic studies on the  
520 lichenicolous *Xanthoriicola physciae* reveal Antarctic rock-inhabiting fungi and



521 *Piedraia* species among closest relatives in the Teratosphaeriaceae. *IMA Fungus*  
522 **2**: 97-103.

523 Sampaio JP, 2011. *Rhodotorula* Harrison (1928). In: Kurtzman CP, Fell JW, Boekhout  
524 T (eds), *The Yeasts: a Taxonomic Study*, 5<sup>th</sup> edn. Elsevier, San Diego, pp. 1873-  
525 1927.

526 Sampaio JP, Bauer R, Begerow D, Oberwinkler F, 1999. *Occultifur externus* sp. nov., a  
527 new species of simple-septate auricularioid heterobasidiomycete from plant litter  
528 in Portugal. *Mycologia* **91**: 1094-1101.

529 Schell WA, Lee AG, Aime MC, 2011. A new lineage in Pucciniomycotina: class  
530 Tritirachiomycetes, order Tritirachiales, family Tritirachiaceae. *Mycologia* **103**:  
531 1331-1340.

532 Silvestro D, Michalak I, 2012. RaxmlGUI: a graphical front-end for RAxML.  
533 *Organisms Diversity and Evolution* **12**: 335-337.

534 Stamatakis A, 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic  
535 analyses with thousands of taxa and mixed models. *Bioinformatics* **22**: 2688-  
536 2690.

537 Takashima M, Hamamoto M, Nakase T, 2000. Taxonomic significance of fucose in the  
538 class Urediniomycetes: distribution of fucose in cell wall and phylogeny of  
539 urediniomycetous yeasts. *Systematic and Applied Microbiology* **23**: 63-70.

540 Talavera G, Castresana J, 2007. Improvement of phylogenies after removing divergent  
541 and ambiguously aligned blocks from protein sequence alignments. *Systematic*  
542 *Biology* **56**: 564-577.

543 Urbanavichene IN, Urbanavichus GP, 2005. [Southern lichen floristic elements in  
544 spruce forests of the White Sea coast of Murmansk Region]. In: M.A.

545 Bondartseva et al. [eds]. Fungi in natural and anthropogenic ecosystems, St.-  
546 Petersburg, 24-28 April 2005. Vol. 2. Pp. 240-244. Komarov Botanical Institute,  
547 St.-Petersburg. (In Russian)

548 Vánky K, 2012. *Smut Fungi of the World*. American Phytopathological Society, St Paul,  
549 Minnesota, USA.

550 Vicente F, Fontoura P, Cesari M, Rebecchi L, Guidetti R, Serrano A, Bertolani R, 2013.  
551 Integrative taxonomy allows the identification of synonymous species and the  
552 erection of a new genus of *Echiniscidae* (Tardigrada, Heterotardigrada). *Zootaxa*  
553 **3613**: 557-572.

554 Vilgalys R, Hester M, 1990. Rapid genetic identification and mapping of enzymatically  
555 amplified ribosomal DNA from several *Cryptococcus* species. *Journal of*  
556 *Bacteriology* **172**: 4238-4246.

557 White TJ, Bruns T, Lee S, Taylor J, 1990. Amplification and direct sequencing of  
558 fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH,  
559 Sninsky JJ, White, TJ (eds), *PCR Protocols*. Academic Press, San Diego, pp. 315-  
560 322.

561 Yamada Y, Maeda K, Mikata K, 1994. The phylogenetic relationships of  
562 *Rhodosporidium dacryoidum* Fell, Hunter et Tallman based on the partial  
563 sequences of 18S and 26S ribosomal RNAs: the proposal of *Sakaguchia* gen.  
564 nov., a heterobasidiomycetous yeast genus. *Bioscience, Biotechnology, and*  
565 *Biochemistry* **58**: 99-103.

566 Yurkov AM, Kachalkin AV, Daniel HM, Groenewald M, Libkind D, de Garcia V, Zalar  
567 P, Gouliamova DE, Boekhout T, Begerow D, 2015. Two yeast species  
568 *Cystobasidium psychroaquaticum* f.a. sp. nov. and *Cystobasidium rietchieii* f.a.  
569 sp. nov. isolated from natural environments, and the transfer of *Rhodotorula*

570 *minuta* clade members to the genus *Cystobasidium*. *Antonie van Leeuwenhoek*

571 **107**: 173-185.

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575 **FIGURE CAPTIONS**

576

577 **Fig. 1.** Photographs showing morphological characters of *Cyphobasidium*. **A–B:**  
578 Macroscopical variation within *Cyphobasidium hypogymniicola* (A: Canada, on  
579 *Hypogymnia physodes*, isotype, herb. Diederich; B: Norway, on *H. hultenii*, Tønsberg  
580 30510, BG). **C–E:** Macroscopical variation within *Cyphobasidium usneicola* (C–D:  
581 Canada, on *Usnea*, isotype, herb. Diederich; E: Costa Rica, on *Usnea*, Bandoni 12367,  
582 herb. Diederich). **F–J:** Microscopical characters of *Cyphobasidium usneicola* (isotype,  
583 herb. Diederich). **F:** Basidiospore with refractive apiculus (arrow). **G:** Context hypha  
584 with clamp connection (arrow). **H–I:** Immature basidia arising from a thick-walled  
585 probasidium (arrows show the place where the basidium protrudes from the  
586 probasidium). **J:** Mature septate basidium (= meiosporangium) (arrow points to upper  
587 septum), showing one epibasidium (arrowhead). Scale bars: 1 mm (A), 200  $\mu\text{m}$  (B–C),  
588 500  $\mu\text{m}$  (E) and 5  $\mu\text{m}$  (F–J).

589

590 **Fig. 2.** Fifty percent majority rule Bayesian consensus tree from the combined analysis  
591 including nSSU, ITS, and nSLU. Black dots represent branches supported by both  
592 Bayesian and ML analyses. White dots indicate branches supported only by the  
593 Bayesian analysis. Branch lengths are scaled to the expected number of substitutions per  
594 site. *Cyphobasidium* representatives and *Cystobasidium fimetarium* (syn. of  
595 *Cystobasidium lasioboli*, the type species of *Cystobasidium*) are highlighted in bold  
596 font. Genera and particular clades within the Cystobasidiomycetes, as well as  
597 suprageneric taxa, are indicated in the right margin.

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