

1 *Leiothecium cristatum* and *Aspergillus posadasensis*, two new species of Eurotiales
2 from rainforest soils in South America

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11

12 Running title: Two new species of Eurotiales

13

14 The GenBank accession numbers for the D1-D2, ITS, *Cct8*, *RPB1* and *RPB2* loci

15 sequences of the ex-type strain of *Leiothecium cristatum* sp. nov. are HG529487,

16 KF732838, HF954979, HF954982, and HF954976, respectively. The GenBank accession

17 numbers for the D1-D2, ITS, *Cct8*, *RPB1*, *RPB2*, *CAL* and *BT2* loci sequences of the ex-

18 type strain of *Aspergillus posadasensis* sp. nov. are HG529485, HG529483, HF954980,

19 HF954983, HF954977, HG529488 and HG529481, and for the strain of the same

20 species, FMR 12322, are HG529486, HG529484, HF954981, HF954984, HF954978,

21 HG529489, and HG529482, respectively. The GenBank accession number for the D1-D2

22 locus sequence of the ex-type strain of *Leiothecium ellipsoideum* is KF732839.

23 The MycoBank (<http://www.mycobank.org>) accession numbers of *Leiothecium*

24 *cristatum* and *Aspergillus posadasensis* are MB803513 and MB803514, respectively.

25

26 **Abstract**

27 We describe two new fungi isolated from soil samples collected in Northern Argentina
28 and belonging to the family Aspergillaceae of the order Eurotiales: *Leiothecium*
29 *cristatum* sp. nov. and *Aspergillus posadasensis* sp. nov. *Leiothecium cristatum*,
30 represented by the ex-type strain FMR 11998^T (= CBS 134260^T = NBRC 109843^T), is
31 distinguishable morphologically from the type species of the genus, *L. ellipsoideum*, by
32 the presence of irregular reticulate ascospores with two prominent equatorial crests,
33 and *A. posadasensis*, represented by the ex-type strain FMR 12168^T (= CBS 134259^T =
34 NBRC 109845^T) is differentiated from *Aspergillus acanthosporus*, the nearest species
35 phylogenetically, by its non-sclerotoid ascomata and a lack of an asexual stage on all
36 culture media tested. The taxonomic proposals are supported by the analysis of the
37 sequences of the internal transcribed spacer region, the D1-D2 domains of the 28S
38 rRNA gene and the fragments of the RNA polymerase II largest subunit, of the putative
39 chaperonin complex related to TCP-1, of β -tubulin and of calmodulin genes.

40

41 **Keywords.** Argentina, Aspergillaceae, *Aspergillus*, Eurotiales, *Leiothecium*, soil.

42

43 **INTRODUCTION**

44 The members of the order Eurotiales G.W. Martin ex Benny & Kimbr. (1980) are mainly
45 characterized by the production of spherical to ovoid, thin-walled evanescent
46 (prototunicate) asci, which arise free on the mycelium or are, more usually, produced
47 within globose, nonostiolate ascomata, and by one-celled, globose or lenticular,
48 smooth-walled or ornamented ascospores (spinulose, reticulate, tuberculate, etc.),

49 frequently with equatorial thickenings or crests. Their asexual stages are mostly
50 phialidic, but can also show a retrogressive conidiogenesis. Currently, the order
51 comprises three monophyletic families, Aspergillaceae, Thermoascaceae and
52 Trichocomaceae (Houbraken & Samson, 2011).

53 The genus *Aspergillus* is the most common and largest of the Aspergillaceae
54 and of the order Eurotiales. Gams et al. (1985) divided the genus into six subgenera
55 and 18 sections. However, Peterson (2008), using a multigene phylogeny based on
56 sequences of partial fragments of β -tubulin (*BT2*), calmodulin (*CAL*) and RNA
57 polymerase II (*RPB2*) genes, and ribosomal (ITS and LSU) genes, only accepted five
58 subgenera (*Aspergillus*, *Circumdati*, *Fumigati*, *Nidulantes* and *Ornati*). Most recently,
59 Houbraken & Samson (2011) also used the sequences of *RPB2* and other structural
60 genes (*RPB1*, the putative ribosome biogenesis protein (*Tsr1*) and the putative
61 chaperonin complex component TCP-1 (*Cct8*)), and they concluded that most of the
62 morphospecies traditionally belonging to the genus *Aspergillus* were included in the
63 *Aspergillus s. str.* clade, which was divided into four subgenera and 17 sections.
64 *Cristaspora* has a single species that lacks an anamorph stage (Fort & Guarro, 1984);
65 *Phialosimplex* has conidiogenous cells consisting of simple phialides, sometimes
66 proliferating to form a second opening (Sigler et al., 2010); and *Polypaecilum* has
67 conidiogenous cells that are polyphialides (Smith, 1961). All three of these genera are
68 morphologically very dissimilar to the typical *Aspergillus* and were surprisingly also
69 placed in the mentioned *Aspergillus s. str.* clade (Houbraken & Samson, 2011).

70 During a survey on soil-borne ascomycetes from Northern Argentina, two fungi
71 apparently related with some members of the Eurotiales were isolated in pure culture.

72 These fungi were phenotypically and molecularly characterized and are proposed here
73 as new species.

74

75 **METHODS**

76 **Soil sampling and fungal isolation.** Soil samples were collected in Misiones Province,
77 Argentina, at two locations: the Iguazú National Park (-25° 41' 28.5", -54° 26' 54.9594")
78 and the Alberto Roth botanical garden (-27° 24' 28.6092", -55° 53' 48.1158"). Both
79 locations are included in the Paranaense phytogeographical province of the Amazonian
80 domain at the neotropical region. They have a hot, wet climate with an average annual
81 temperature of 21 °C, an average maximum temperature of about 32 °C and an
82 average minimum temperature of about 10 °C. The total annual rainfall is about 1900
83 mm. The Iguazú National Park is situated in the boundaries of the Iguazú River, and has
84 an area of around 550 square kilometres. The soil is acidic, red and lateritic. The park
85 has more than 300 species of plants, including trees, ferns, shrubs, lianas, epiphytes
86 and herbs. The Alberto Roth botanical garden is on the south side of the city of
87 Posadas, and has an area of 11 ha. The altitude ranges from 75 to 100 m, and the
88 terrain is mostly basaltic. This location also has a broad diversity of trees, shrubs and
89 herbs, of which 109 are native species.

90 To carry out the isolation of the soil-borne ascomycetes, we followed a
91 previously described protocol (Stchigel et al., 2001). Approximately 1 g of each soil
92 sample was suspended in 5 mL of 5% v/v acetic acid, shaken vigorously for 5 min and
93 left for 5 min. The liquid layer was decanted and the residual soil was resuspended in 9
94 ml of sterile water and plated onto three Petri dishes of 9 cm diam. Melted potato
95 carrot agar (PCA: grated potatoes, 20 g; grated carrot, 20 g; agar-agar, 20 g; L-

96 chloramphenicol, 100 mg; 1% w/v dieldrin™ in dimethyl-ketone, 20 drops; tap water, 1
97 L) at 50-55 °C was placed on top of the soil suspension and mixed by hand. All cultures
98 were incubated at 15, 25 and 35 °C. The ascomata of the taxonomically interesting
99 fungi were transferred using a sterile needle to two 5 cm diam Petri dishes containing
100 oatmeal agar (OA: oatmeal flakes, 30 g; agar-agar, 20 g; tap water, 1 L) and incubated
101 under the same conditions as described above.

102

103 **Phenotypic study.** For cultural characterization, the isolates were grown for up to 30
104 days on OA, PCA, potato dextrose agar (PDA; Pronadisa, Madrid, Spain), *Czapek yeast*
105 *extract agar* (CYA: sucrose, 30 g; sodium nitrate, 3 g; yeast extract, 5 g; potassium
106 phosphate, 1 g; potassium chloride, 0.5 g; magnesium sulphate, 0.5 g; iron sulphate,
107 0.01 g; agar, 15 g; tap water, 1 L) and malt extract agar (MEA: bacteriological peptone,
108 1 g; glucose, 20 g; malt extract, 20 g; agar, 15 g; tap water, 1 L) at 25 °C. Colour
109 notations in parentheses are from Kornerup & Wanscher (1984). To induce the
110 production of asexual reproductive structures, the isolates were grown on MEA + 40%
111 of sucrose (Samson et al., 2007) at 25 and at 37 °C. In order to determine the minimum
112 and maximum temperatures of growth of the isolates, a 5 °C increment from 5 to 40
113 °C, and 2 °C increment from 40 to 50 °C, were used. Fertile fungal structures were
114 mounted and measured in water and in lactic acid. Photomicrographs of the structures
115 were taken with a Zeiss Axio Imager M1 light microscope. The scanning electron
116 microscope (SEM) techniques used were described previously by Figueras & Guarro
117 (1988). SEM micrographs were taken with a Jeol JSM 840 at 15 keV.

118

119 **BLAST search and phylogenetic study.** The DNA of the isolates of interest (see
120 Supplementary Table S1 in IJSEM online) was extracted and purified directly from
121 fungal colonies according to the Fast DNA Kit protocol (MP Biomedicals, Solon, Ohio).
122 D1-D2, ITS, *RPB1*, *RPB2* and *Cct8* genes were amplified for all isolates, and *BT2* and *CAL*
123 genes also for the isolates FMR 12168^T and FMR 12322, according to Cano et al. (2004)
124 (D1-D2 and ITS), Houbraken & Samson (2011) (*RPB1*, *RPB2* and *Cct8*), Glass &
125 Donaldson (1995) (*BT2*) and Hong et al. (2005) (*CAL*). The sequences of these
126 amplicons were obtained using the protocol of the Taq Dye-Deoxy Terminator Cycle
127 Sequencing Kit. PCR products were purified and sequenced at Macrogen Europe
128 (Amsterdam, The Netherlands) with a 3730XL DNA analyser (Applied Biosystems).
129 Consensus sequences were obtained using SeqMan (version 7.0.0; DNASTAR, Madison,
130 WI, USA) and they were aligned using Clustal X (version 1.83) (Thompson et al., 1997)
131 followed by manual adjustments with a text editor. Sequences retrieved from GenBank
132 and included in this analysis are also given in Table S1. ITS, D1-D2 and *CAL* BLAST
133 searches were carried out in order to corroborate the previous taxonomical placement
134 of our isolates. The phylogenetic analyses of the combined data set (*RPB1*, *RPB2* and
135 *Cct8*) of our isolates and selected members of the families Aspergillaceae and
136 Thermoascaceae were carried out using MEGA v. 5.05 (Tamura et al., 2011). The
137 combined data set was tested for incongruence with the partition homogeneity test
138 (PHT) as implemented in PAUP* (Swofford, 2002). Maximum Likelihood (ML) method
139 using Tamura-Nei model with gamma distribution, was carried out for the phylogenetic
140 analyses of *RPB1*, *RPB2* and *Cct8*, and Kimura 2-parameter model with invariable sites
141 for the ML phylogenetic analysis of *BT2* sequences, both with the pair-wise deletion of
142 gaps option. The robustness of branches was assessed by bootstrap analysis with 1000

143 replicates. The sequences generated in this study are deposited in GenBank (see
144 Supplementary Table S1 in IJSEM online) and the alignments used in the phylogenetic
145 analyses are deposited in TreeBASE: (www.treebase.org, accession
146 URL:<http://purl.org/phylo/treebase/phyloids/study/TB2:S14135>).

147

148 **RESULTS**

149 **Phenotypic study**

150 The isolate FMR 11998^T, from a soil sample of the Iguazú National Park (see
151 Supplementary Table S1 in IJSEM online), was identified as belonging to the genus
152 *Leiothecium* based on the presence of typical morphological features, such as
153 spherical, glabrous, dark brown, non-ostiolate ascomata with a peridium of *textura*
154 *angularis*; one-celled, hyaline, ellipsoidal, reticulate ascospores; and absence of an
155 asexual stage. Two other isolates, FMR 12168^T and FMR 12322, from two soil samples
156 of the Alberto Roth botanical garden were classified as belonging to the genus
157 *Cristaspora*. They were characterized by the production of orange, spherical, non-
158 ostiolate ascomata covered by a dense mass of aerial hyphae; hyaline to subhyaline
159 ascospores with two equatorial crests and a convex surface verruculose to echinulate
160 and the absence of an asexual stage on all culture media tested.

161

162 **BLAST search**

163 The BLAST search with the D1-D2 sequence of the isolate FMR 11998^T (HG529487)
164 showed 97% of similarity with the sequence of the type strain of *L. ellipsoideum*
165 (FJ358285) whereas the isolates FMR 12168^T (HG529485) and FMR 12322 (HG529486)
166 showed 99% similarity with *Aspergillus clavatus* (JN938924) and the type strain of *A.*

167 *acanthosporus* (EF669992). The most related member of the Eurotiales in the ITS
168 BLAST search of FMR 11998^T (KF732838) showed a similarity of less than 90%
169 (*Aspergillus fischerianus*), but the similarity between the sequence of the former with
170 that of the type strain of *L. ellipsoideum*, sequenced by us (KF732839), was 92.76%.
171 The BLAST search of ITS sequences of FMR 12168^T (HG529483) and FMR 12322
172 (HG529484) showed 98.19% and 98.43% similarity with the type strain *A. clavatus*,
173 respectively, and the same percentage for the two isolates (98.42%) with the ITS
174 sequence of the type strain of *A. acanthosporus* (EF669992). The BLAST search with
175 the *CAL* sequences of FMR 12168^T (HG529488) and FMR 12322 (HG529489) showed
176 93% and 93.2% similarity with the type strain of *A. clavatus* (EU078665), respectively,
177 and 90.87% with the type strain of *A. acanthosporus* (EU078676) for both strains.

178

179 **Phylogenetic study**

180 The lengths of the fragments of the three genes used in the combined data set were:
181 646 bp (*Cct8*), 708 bp (*RPB1*) and 957 bp (*RPB2*), from which 271, 302 and 396 bp were
182 parsimony informative, respectively. The length of the final alignment was 2311 bp.
183 The result of the partition homogeneity test showed that the datasets for the three
184 loci were congruent (P = 0.29) and could be combined.

185 Fig. 1 shows the tree inferred from an ML analysis of the combined dataset. A
186 main clade with a bootstrap support (bs) of 100% grouped the members of the family
187 Aspergillaceae, including our isolates. The isolate FMR 11998^T grouped in a terminal
188 clade with the type strain of *L. ellipsoideum* (89% bs) whereas the isolates FMR 12168^T
189 and FMR 12322 grouped with the type strain of *A. acanthosporus* and *A. clavatus*

190 (100% bs), despite these two isolates first being morphologically identified as
191 belonging to the genus *Cristaspora*.

192 A phylogenetic analysis of the ITS region (415 bp), of the *CAL* (367 bp), and of the *BT2*
193 (381 bp) was carried out in order to assess the genetic relatedness of the isolates FMR
194 12168^T and FMR 12322 with other members of the sect. *Clavati* of the genus
195 *Aspergillus*. The ITS and *CAL* ML trees showed the same topology that we observed in
196 the *BT2* ML tree. We only included results of the last locus (see Supplementary Fig. S1
197 in IJSEM online) because *BT2* was the most phylogenetically informative and
198 sequences of all the species of this section were available in the GenBank. The tree
199 revealed two main clades (with bs of 89% and 93%, respectively). The first one
200 encompassed three sister clades, all of them with 100% bs, corresponding to four
201 isolates of *A. clavatus* the first, our two isolates (FMR 12168^T and 12322) the second,
202 and four strains of *A. acanthosporus* the third, respectively. In the second clade (93%
203 bs) other species of this section were located, i.e. *A. rhizopodus*, *A. clavaticus*, *A.*
204 *longivesica* and *A. giganteus*.

205

206 **TAXONOMY**

207 The previous data demonstrated that our isolate FMR 11998^T belongs to the genus
208 *Leiothecium* but is distinguishable molecularly from the only species of this genus *L.*
209 *ellipsoideum* and also morphologically mainly by the presence of irregular reticulate
210 ascospores with two prominent equatorial crests in our isolate; our studies also
211 provide evidence that the isolates FMR 12168^T and FMR 12322 are molecularly and
212 morphologically different from *A. acanthosporus* and *A. clavatus*, the nearest
213 phylogenetic species, by the production of non-sclerotoid ascomata and the absence

214 of an anamorphic stage in our isolates. Therefore, we propose the following new
215 species:

216 **Description of *Leiothecium cristatum* Y. Marín, Stchigel & Cano, sp. nov. (Fig. 2)**

217 *Leiothecium cristatum* (cris.ta'tum. L. neut. adj. *cristatum*, referring to the equatorial
218 crests of the ascospores).

219 Colonies on PDA attaining a diam of 71–73 mm after 7 days at 25 °C, cottony, white,
220 margins fringed; reverse yellowish-white to pale yellow (M. 3A2 to 3A3). Hyphae thick-
221 and smooth-walled, hyaline to pale brown, septate, 3–9 µm wide. Ascomata initials
222 arising on aerial and submerged hyphae as lateral branches, consisting of single coils.
223 Ascomata superficial and immersed on the medium, spherical, glabrous, dark brown,
224 non-ostiolate, 100–220 µm diam; peridium brown, 3-layered, 15–20 µm thick, *textura*
225 *angularis*, composed of polyhedric flattened cells of 10–20 µm diam. Asci 8-spored,
226 broadly clavate to spherical, non-catenulate, 12–16 x 10–14 µm, evanescent.
227 Ascospores one-celled, hyaline, ellipsoidal, 6–8.5 x 4.5–5.5 µm, irregularly reticulated
228 due to the anastomosing low ridges, with two prominent crests of 0.5–1 µm.
229 Chlamydospores mostly terminal, sometimes intercalary, hyaline, subspherical to
230 ellipsoidal, smooth- and thick-walled, 12–19 x 13–18.5 µm. Anamorph not observed.
231 Colonies on MEA similar to those on PDA. After 7 days at 25 °C, colonies on OA and
232 PCA of 34–36 and 61–64 mm diam, respectively. Minimum and maximum temperature
233 of growth 15 and 35 °C, respectively.

234 Holotype is CBS-H 21130, a dried culture; isotype FMR 11998^T.

235 Mycobank accession no. MB803513.

236 The ex-type culture is FMR 11998^T (= CBS 134260^T = NBRC 109843^T), isolated from
237 rainforest soil sample, in Iguazú National Park, Misiones province, Argentina, -25° 41'
238 28.5", -54° 26' 54.9594", 2 Aug 1997, M. Calduch, J. Guarro and A. M. Stchigel.

239

240 **Description of *Aspergillus posadasensis* Y. Marín, Stchigel & Cano, sp. nov. (Fig. 3)**

241 *Aspergillus posadasensis* (po.sa.das.en'sis. N.L. masc. adj. posadasensis, belonging to
242 Posadas, capital city of the Misiones province, Argentina).

243 Colonies on PDA attaining 52–58 mm in diam in 14 days at 25 °C , velvety, white,
244 irregularly folded and with margins fringed; reverse yellowish-white to pale yellow (M.
245 3A2 to 3A3). Ascomata superficial, spherical, tomentose, orange to brown at maturity,
246 non-ostiolate, 330–720 µm diam; peridium 20–30 µm thick, composed of an outer
247 layer of orange-brown moniliform hyphae, and 3–5 inner layers of flattened, prismatic,
248 brown cells 6–12 µm in diam. Asci 8-spored, globose to subglobose, 9–12.5 x 8.5–10
249 µm, evanescent at maturity. Ascospores one-celled, hyaline to subhyaline, globose to
250 subglobose, 3.5–4.5 x 3–4 µm, with two equatorial crests, 0.5–1 µm wide; convex
251 surface of ascospores ornamented with triangular projections, long ridge lines and
252 microtubercles. Anamorph not observed in any of the culture media tested, including
253 MEA + 40% sucrose. Colonies on PCA attaining a diameter of 52–58 mm in 14 days at
254 25 °C, velvety to cottony, with margins fringed, white; reverse white to yellowish-
255 white (M. 2A2). Colonies on MEA attaining 18–20 mm in diam after 14 days at 25 °C ,
256 velvety, white, with orange grey to brownish-grey (M. 5B2 to 5C2) margins, fimbriate;
257 reverse brownish-orange to yellowish-brown (M. 5C4 to 5E4), white to yellowish-white
258 (M. 4A1 to 4A2) at the margins; ascomata produced. Colonies on CYA attaining 16–20
259 mm in diam after 14 days at 25 °C, flattened, mycelium mostly submerged, yellowish-

260 white (M. 2A2); reverse yellowish-white (M. 2A2); ascomata not formed. Minimum and
261 maximum temperature of growth 15 and 42 °C, respectively.

262 Holotype is CBS-H 21131, a dried culture; isotype FMR 12168^T.

263 Mycobank accession no. MB803514.

264 The ex-type culture is FMR 12168^T (=CBS 134259^T =NBRC 109845^T), isolated from a soil
265 sample, in Alberto Roth botanical garden, Misiones province, Argentina, -27° 24'
266 28.6092", -55° 53' 48.1158", 2 Aug 1997, M. Calduch, J. Guarro and A.M. Stchigel.

267 Other specimen examined: FMR 12322 (from the same origin and source).

268

269 **DISCUSSION**

270 The genus *Leiothecium* was erected by Samson & Mouchacca (1975) to include an
271 ascomycete isolated from soil in Greece. Later, this fungus was also reported from soil
272 in South America, Asia and Europe, and from seeds of the capsicum and nest material
273 of a ground-nesting solitary bee in North America, in areas of temperate climate. This
274 fungus shows some similarities with *Ascorhiza* and *Hapsidospora* (Samson &
275 Mouchacca, 1975) because of the presence of cleistothecial ascomata and reticulate
276 ascospores. They also mentioned the possible relationship of *Leiothecium* with
277 *Monascus*, but they remarked on the differences among them (ascomata with a very
278 thin, plectenchymatous peridial wall in *Monascus* vs. prosenchymatous and thickness
279 in *Leiothecium*; smooth-walled ascospores in *Monascus* vs. reticulate in *Leiothecium*;
280 and the presence of an anamorph with retrogressive ontogeny in *Monascus*, which is
281 absent in *Leiothecium*). Despite *Hapsidospora* and *Leiothecium* producing dark
282 coloured, closed ascomata, *Leiothecium* can be differentiated morphologically from
283 *Hapsidospora* because the latter produces dark, globose ascospores of 5–7.5 µm diam

284 (Guarro et al., 2012), which are hyaline and ellipsoidal, of 7–8.5 x 4.5–5.5 µm in
285 *Leiothecium*. *Ascorhiza* lacks of original type material, and has a poor description
286 (Lechtova-Trnka, 1931) lacking of any illustrations, therefore it cannot be compared
287 with *Leiothecium*, and its validity as a taxon is doubtful.

288 A recent phylogenetic study carried out by Houbraken & Samson (2011), based on the
289 nucleotidic sequences of *Cct8*, *RPB1*, *RPB2* and *Tsr1* genes demonstrated that
290 *Leiothecium* belongs to the family Aspergillaceae, while in a previous molecular study,
291 based on the analysis of SSU and LSU rRNA gene sequences (Suh & Blackwell, 1999),
292 *Hapsidospora* had been placed in the Hypocreales. Our molecular analysis, using three
293 of those genes, demonstrates that the isolate FMR 11998^T represents a new species of
294 *Leiothecium*. This fungus is morphologically distinguishable from *L. ellipsoideum* by the
295 presence of two prominent equatorial crests (absent in *L. ellipsoideum*) and an
296 irregular pattern in its ascospore wall ornamentation (which is more regularly
297 reticulate in *L. ellipsoideum*).

298 The molecular study of the isolates FMR 12168^T and FMR 12322 shows that they are
299 related to *A. acanthosporus* and *A. clavatus*. The type strain of *A. acanthosporus* was
300 isolated from a soil sample in Solomon Islands, Papua-New Guinea (Udagawa &
301 Takada, 1971), along with another three isolates from the same source of the same
302 country. Houbraken & Samson (2011) placed *A. acanthosporus* into the section *Clavati*
303 of *Aspergillus* subg. *Fumigati*. *Aspergillus posadasensis* is easily distinguishable from *A.*
304 *acanthosporus* by the non-sclerotoid nature of its ascomata and the absence of an
305 anamorph. Other taxa are morphologically similar to the new species and belong to
306 *Aspergillus* subgenus *Fumigati* are *A. aureola* and *A. spinosus*. They also produce
307 ascospores with two equatorial crests and a similar ornamentation to that of *A.*

308 *posadasensis*; however, their ascomata are white or very pale yellow, and both
309 produce an anamorph. There are other *Aspergillus* of which no conidiophores
310 structures have been described. Conidiophore structures in *Aspergillus monodii*, which
311 is accommodated in *Aspergillus* section *Usti*, are also not known. However, *A. monodii*
312 has different ascospores and produces Hülle cells and ascomata in stromata.

313

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372

373 **Fig. 1.** Maximum Likelihood (ML) rooted tree obtained from the combined DNA
374 sequence data from three loci (*Cct8*, *RPB1* and *RPB2*) of our isolates and 11 selected
375 species belonging to the family Aspergillaceae, chosen because of their molecular or
376 morphological similarity to our isolates. *Rasamsonia byssochlamydoides* (CBS 413.71^T)
377 and *Talaromyces flavus* (CBS 310.38^T) (family Trichocomaceae) were used as outgroup.
378 Bootstrap support values above 70% are indicated at the nodes. Branch lengths are
379 proportional to distance. Type and Neotype strains of the different species are
380 indicated with ^T and ^{NT} respectively. New species proposed in this study are indicated in
381 bold font.

382

383 **Fig. 2.** *Leiothecium cristatum* (FMR 11998^T). (a), (b) Ascoma; (c) detail of the peridium;
384 (d) asci and terminal chlamydospores; (e), (f) ascus; (g) ascospores (SEM). Bars: (a), 50
385 μm; (b), 25 μm; (c), 20 μm; (d), (e), 10 μm; (f), 5 μm; (g), 5 μm.

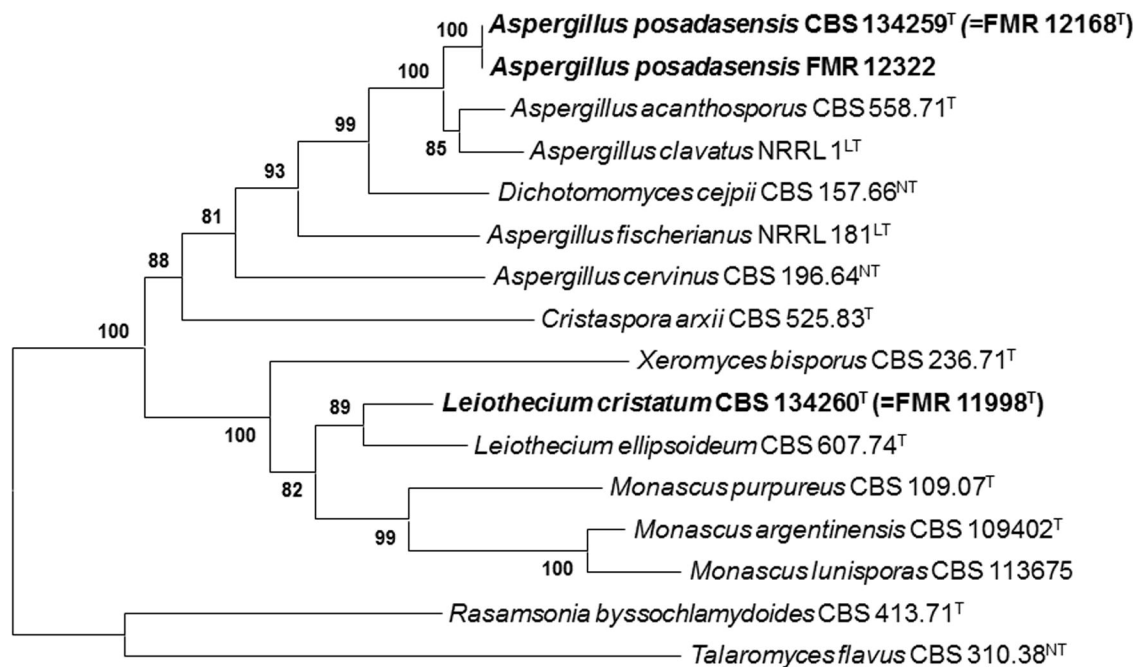
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387 **Fig. 3.** *Aspergillus posadasensis* (FMR 12168^T). (a) Ascoma; (b) detail of the peridium;

388 (c), (d) asci; (e), (f) ascospores. Bars: (a), 100 μm ; (b), 20 μm ; (c), 10 μm ; (d), 10 μm ; (e),

389 5 μm ; (f), 2.5 μm .

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0.05

