Graphiola fimbriata: the first species of Graphiolaceae (Exobasidiales, Basidiomycota) described only based on its yeast stage

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Graphiola fimbriata sp. nov. is the first anamorphic species of Graphiolaceae (Exobasidiales, Basidiomycota)

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Abstract

The systematic position of three yeast strains isolated from a plant cell culture, a piece of termite nest, and as a foliar endophyte of *Coffea arabica*, respectively, is evaluated using morphological, physiological, and phylogenetical analyses. In culture, all three isolates produced white, pale orange to pink colored colonies of cylindrical cells with monopolar budding and pseudohyphae. Standard phenotypic, biochemical, physiological characterization and phylogenetic analyses of the combined 26S rRNA gene (D1/D2 domains) and ITS region sequences showed the conspecificity of these isolates and suggest their placement within the Exobasidiales (Ustilaginomycotina) as a sister lineage of the sampled and sequenced *Graphiola* species. Here, we describe this species as *Graphiola fimbriata* sp. nov. MycoBank MB 825077 (holotype: PC1^{T}; ex-type cultures: IBRC-M 30158^{T} = CBS 13945^{T} = DSM 104832^{T}). This is the first anamorphic saprobic species described in the genus *Graphiola*. The description of the genus *Graphiola* is therefore emended to allow species known only from a saprobic state.

Key words: 1 new taxon, *Graphiola fimbriata* sp. nov., Asexual state, Morphological characterization, Phylogenetic analyses, Yeast

53 ## INTRODUCTION

54 Fungi with yeast and yeast-like morphology are present within all three main lineages of
55 Basidiomycota, namely the subphyla Agaricomycotina, Pucciniomycotina, and
56 Ustilaginomycotina (Sampaio 2004, Boekhout et al. 2011, Kurtzman & Boekhout 2017). Yeast
57 and filamentous taxa are often intermixed as revealed by recent phylogenetic studies (Liu et al.
58 2015a, 2015b, Wang et al. 2015a, 2015b, 2015c). With a few exceptions, a transition from
59 filamentous to yeast stage is often associated with the change from a parasitic to saprobic life
60 style (Begerow et al. 2017). The subphylum Ustilaginomycotina comprises a highly diverse
61 assemblage of fungi, including teliosporic plant pathogens, non-teliosporic plant pathogens,
62 anamorphic plant pathogens, as well as endophytic, lipophilic, saprobic, and zoophilic yeasts
63 (Begerow et al. 2014). The orders Malasseziales, Monilielalles, and Violaceomycetales include
64 exclusively yeast species of the genera *Malassezia*, *Moniliella*, and *Violaceomyces*, respectively
65 (Wang et al. 2014, Albu et al. 2015), while the remaining yeasts and yeast-like fungi are
66 scattered amongst the remaining orders of Ustilaginomycotina, with the exception of the
67 members of Doassansiales, Tilletiales and Uleiellales for which no yeasts have been discovered
68 so far.

69 The anamorphic saprobic members of Ustilaginomycotina have previously been classified
70 mainly in the polyphyletic genera *Pseudozyma* and *Tilletiopsis* (Begerow et al. 2000, Sampaio
71 2004, Kurtzman et al. 2011, 2015), and later in several monophyletic genera *Acaromyces*,
72 *Farysizyma*, *Fereydounia*, *Jaminaea*, *Meira*, and *Sympodiomycopsis* (Sugiyama et al. 1991,
73 Boekhout et al. 2003, Inácio et al. 2008, Sipiczki & Kajdacsi 2009, Nasr et al. 2014). Sexual and
74 asexual morphs in Ustilaginomycotina were recently grouped together in order to unify the
75 taxonomy of plant parasites and species known only from their yeast states (Piątek et al. 2015,

76 Wang et al. 2015a, Kijpornyongpan & Aime 2016). As the result, several monophyletic genera
77 (*Dirkmeia*, *Golubevia*, *Kalmanozyma*, and *Robbauera*) were erected to accommodate species
78 previously classified in the genera *Pseudozyma* and *Tilletiopsis* (Wang et al. 2015a). The current
79 classification scheme recommends that each novel lineage not linked with a teleomorphic genus
80 or recognized anamorphic genus should be assigned to a new genus. The subphylum
81 Ustilaginomycotina comprises the least number of yeast and yeast-like species (71) compared to
82 the two other subphyla of Basidiomycota (Wang et al. 2015a). However, studies that involved
83 environmental sequencing suggest a much greater, unexplored diversity of fungi in this group
84 (e.g., Richards et al. 2012, Nasanit et al. 2015, Dunthorn et al. 2017, Jimu et al. 2017).
85 In the course of independent studies two yeast strains have been isolated. The first culture (PC1)
86 was isolated in the Iranian Biological Resource Center (IBRC) from a contaminated plant
87 culture, and the second isolate (SNB-CN72) was obtained from a nest of the termite species
88 *Nasutitermes corniger* harvested in Rémire-Montjoly, French Guiana. Phylogenetic analyses of
89 the ITS region and the D1/D2 domains of the LSU rRNA gene placed the strains within
90 Graphiolaceae (Exobasidiomycetes, Exobasidiales) close to plant parasites of the genus
91 *Graphiola,* and revealed relatedness to a third yeast isolate (IBL 03150) reported by Posada et al.
92 (2007) and deposited in the CBS yeast collection of the Westerdijk Fungal Biodiversity Institute,
93 Utrecht, The Netherlands. Species in the plant pathogenic genus *Graphiola* are traditionally
94 circumscribed based on their host spectrum and symptoms, and nucleotide sequence data for
95 these fungi are rare (Wang et al. 2015a).
96 The aim of the study was to characterise the yeast-like isolates and determine their phylogenetic
97 placement, incorporating morphological, physiological, and molecular data yet available for
98 members of the genus *Graphiola*.

99

100 ## MATERIALS AND METHODS

101 ### Sample collection and isolation

102 A plant cell culture of an unidentified plant species mixed with a yeast strain was obtained from
103 the plant bank section of the Iranian Biological Resource Center (IBRC) in 2013 (strain
104 designation: PC1). Another strain was isolated from a piece of *Nasutitermes corniger* nest
105 collected in Rémire-Montjoly, (Cayenne, French Guiana) in July 2011 (strain designation: SNB-
106 CN72; Nirma et al. 2013, 2015). A third strain was obtained from the CBS yeast collection of the
107 Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands (original strain designation:
108 IBL 03150). It was isolated as foliar endophyte of *Coffea Arabica* (Posada et al. 2007).
109 Designations and GenBank accession numbers of the yeast strains used in this study are given in
110 Table 1.

111 Cultures were maintained on YPG agar medium (0.5% yeast extract, 1% peptone, 2% glucose,
112 2% agar, w/v) at 25 °C during experiments.

113 To expand the ITS sequence sampling for molecular phylogenetic analyses a specimen of the
114 plant parasitic genus *Graphiola* was additionally used and newly sequenced: *Graphiola*
115 *phoenicis* (Moug. ex Fr.) Poit. on *Phoenix reclinata* Jacq., South Africa, Kwa Zulu-Natal,
116 Durban, Yellowwood Park, Kenneth Stainbank Nature Reserve, 14 Feb. 2012, leg. A.R. Wood
117 883, KR-M-0042315.

118

119 ### Morphological examination

120 Colony morphology images were taken using a stereo microscope coupled with the Nikon zoom
121 digital camera. In addition, scanning electron microscopy was performed on the isolate using a

122 VEGA3-TESCAN SEM instrument (Van Wyk & Wingfield 1994). Briefly, the cells were fixed
123 in 3.0%, 0.1 M, pH 7.0 sodium phosphate-buffered glutaraldehyde for 3 h at room temperature,
124 followed by 1 h fixation in 2% osmium tetroxide. The cells were dehydrated by increasing
125 ethanol concentrations (30%, 50%, 70%, 90%, and 96%) for 30 min and two 30 min washes in
126 100% ethanol. The standard characterization of the yeast isolates was performed according to
127 methods described earlier (Barnett et al. 2000, Kurtzman et al. 2011). Assimilation of carbon and
128 nitrogen sources was carried out on solid and in liquid media, respectively.

129

130 **DNA extraction, PCR, and sequencing**

131 Nuclear DNA was extracted by the method of Hanna & Xio (2006). For the *Graphiola phoenicis*
132 specimen, the genomic DNA was isolated directly from the herbarium specimen. The 5'-end
133 (D1/D2 region) of the nuclear large subunit ribosomal DNA (LSU) and the ITS 1 and ITS 2
134 regions of the nuclear rDNA including the 5.8S rDNA (ITS) were amplified and sequenced using
135 primer pairs NL1 (5'GCATATCAATAAGCGGAGGAAAAG-3') and NL4 (5'-
136 GGTCCGTGTTTCAAGACGG3') (Kurtzman & Robnett 1998) and ITS1 (5'-
137 TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et
138 al. 1990), respectively. DNA sequences determined for this study were deposited in GenBank
139 (accession numbers are given in Figs 1 & 2 and in Table 1. Additional sequences of *Graphiola*
140 *cylindrica* and *G. phoenicis* were obtained from GenBank and the public catalogue of the NITE
141 Biological Resource Center collection (NBRC), Japan.

142

143 **Phylogenetic analyses**

144 To elucidate the phylogenetic position of the isolated strains their sequences were analysed
145 within a LSU and a concatenated ITS + LSU dataset. Since preliminary analyses and Blast
146 searches (Altschul et al. 1997) revealed an affinity of the stains to the order Exobasidiales (class
147 Exobasidiomycetes), the LSU dataset was reduced to members of the Exobasidiales and some
148 representatives of the Ustilaginomycetes were used as an outgroup. Isolates and clones, for
149 which only ITS sequences were available, were analysed in the LSU + ITS dataset. If present in
150 GenBank sequences of the respective type species were used. Additionally sequences of all
151 available brachybasidiaceous species and all available sequences clustering within the
152 Graphiolaceae including sequences from the Biological Resource Center, NITE, Japan were
153 added.
154 GenBank accession numbers of the sequences used for both the LSU and ITS+LSU dataset
155 (Begerow et al. 1997, 2001, 2002, Guo et al. 2001, Boekhout et al. 2003, Castlebury et al. 2005,
156 Stoll et al. 2005, Yasuda et al. 2005, 2006, Posada et al. 2007, Tanaka et al. 2008, Piepenbring et
157 al. 2010, 2012, Yuan et al. 2011, Jusino et al. 2015, Urbina et al. 2016) are given in Figs 1 & 2.
158 Sequence alignment was obtained independently for both the LSU dataset and the ITS and LSU
159 part of the ITS+LSU dataset using MAFFT 7.313 (Katoh & Standley 2013) using the L-INS-i
160 option. To obtain reproducible results, manipulation of the alignments by hand as well as manual
161 exclusion of ambiguous sites were avoided as suggested by Gatesy et al. (1993) and Giribet and
162 Wheeler (1999), respectively. Instead, highly divergent portions of the alignments were omitted
163 using GBlocks 0.91b (Castresana 2000) with the following options for the LSU dataset:
164 'Minimum Number of Sequences for a Conserved Position': 20, 'Minimum Number of
165 Sequences for a Flank Position': 20, 'Maximum Number of Contiguous Non-conserved
166 Positions': 8, 'Minimum Length of a Block': 5, and 'Allowed Gap Positions' to 'With half', for

167 the ITS part of the ITS+LSU dataset: 11/11/8/5/'With half' and for the LSU part of the ITS+LSU
168 dataset: 12/12/8/5/'With half'. After alignment the ITS and LSU part of the ITS+LSU dataset
169 were concatenated. The resulting alignments [LSU dataset: new number of positions: 591 (32%
170 of the original 1801 positions), number of variable sites: 279; ITS+LSU dataset: new number of
171 positions: 1121 (34% of the original 3264 positions), number of variable sites: 468] were used
172 for phylogenetic analyses using a Maximum Likelihood (ML) and a Bayesian Approach (BA)
173 following Vasighzadeh et al. (2014).
174 For the LSU dataset trees were rooted with the ustilaginomycetous species *Urocystis ficariae* and
175 *Ustilago hordei,* for the ITS+LSU dataset trees were rooted with the brachybasidiaceous species
176 *Dicellomyces gloeosporus* and *Meira geulakonigii*.
177 Metabolite profiling analysis was carried out for the three yeast strains and the results are
178 presented as supplementary data.
179
180 **RESULTS**
181 **Morphological examination**
182 Morphology of the strains IBL 03150, PC1, and SNB-CN72 showed no significant differences,
183 results are included in the species description.
184 Metabolite profiling with UHPLC-MS was carried out for the three yeast strains and the results
185 are provided in supplementary data. In the absence of metabolic data from closely related
186 species, the analysis of UHPLC-MS profiles was restricted to the tree strains. We observed that
187 the culture medium impacted more significantly on the profiles than the nature of the strain
188 (Supplementary data). Hierarchical clustering analyses (HCA) conducted on the 3 (N) $\times$ 29 (X)

189 matrix of merged profiles showed that strains PC1 and IBL 03150 are more alike than SNB-
190 CN72 (Supplementary data).
191
192 **Phylogenetic analyses**
193 The different runs of the BA that were performed and the ML analyses yielded consistent
194 topologies. To illustrate the results the consensus trees of one run of the BA of the LSU and the
195 concatenated ITS+LSU dataset are presented (Figs 1 & 2). Using the ustilaginomycetous species
196 as outgroup in the LSU analysis, the clades in the phylogenetic tree were congruent to the
197 families discussed in Begerow et al. (2014). In all analyses the sequences of the yeast isolates
198 clustered within the Exobasidiales together with the sequence MF334501 from an uncultured
199 fungus clone forming the sister lineage of the sampled *Graphiola* spp. of which the *G. phoenicis*
200 cluster included several sequences of yeast isolates and uncultured fungus clones, respectively.
201 The clade comprising *Graphiola cylindrica, G. geonomae, G. phoenicis*, and the yeast isolates
202 received good statistical support (BA: 100%, ML: 93%). The yeast isolates branched first in the
203 *Graphiola* clade and were placed sister to *G. cylindrica, G. geonomae,* and *G. phoenicis*.
204 Combined ITS+LSU analyses (Fig. 2) revealed the same groups and relations including more
205 sequences from *Graphiola* spp. as well as from the studied yeast isolates and uncultured fungus
206 clones. The three strains shared identical LSU sequences and showed 5 variable positions (2
207 substitutions and 3 indels) in the ITS region.
208
209 **TAXONOMY**
210 ***Graphiola* Poit., Ann. Sci. Nat. (Paris): 473 (1824) emend. "**S. Nasr, M. Lutz, D. Stien & A.
211 Yurkov**"**

212 According to the current genus concept of *Graphiola* (Piepenbring et al. 2012), the genus
213 comprises plant pathogens on palms (Arecaceae). Tubaki & Yokohama (1971) and Oberwinkler
214 et al. (1982) obtained and studied *Graphiola phoenicis* in culture. They provided also the
215 diagnosis of this species, including morphological and physiological properties observed in
216 culture. Emendation of the diagnosis of the genus *Graphiola* is proposed to allow classification
217 of asexual, known only from a saprobic state yeast species in the genus.
218 Sexual reproduction is observed in some species. Fungi are dimorphic with a filamentous sexual
219 form parasitizing on plants and free-living saprobic yeast states. On malt extract, colonies are
220 white, pale orange to pink in color. Budding cells are present in culture. Colonies are white, pale
221 orange to pink in color. Ballistoconidia are not produced. Pseudohyphae may be present in
222 cultures. Fermentation is absent. DBB reaction is positive. Urease activity is positive.
223
224 ***Graphiola fimbriata*** S. Nasr, M. Lutz, D. Stien & A. Yurkov, sp. nov. (Fig. 3)
225 MycoBank MB 825077
226 *Etymology*: Referring to the margin of the colonies.
227
228 *Description:* After three days on YPG agar at 25 ºC, the cells are cylindrical, 1-2.5 × 4-8.5 µm,
229 and occur single or in small chains. Budding is monopolar. After five days, the colony is white,
230 convex, and the margin is filiform. Pellicles are formed on liquid media. After one week at 25 ºC
231 on MEA and Cornmeal agar, the slide culture undifferentiated pseudohyphae are formed.
232 Fermentation is negative. The following compounds are assimilated: glucose, sucrose, maltose,
233 trehalose, melezitose, D-xylose, raffinose, L-arabinose, D-mannitol, myo-inositol, D-ribose and
234 arbutin; assimilation of salicin, glycerol and cellobiose is weak. No growth occurs on galactose,

235 lactose, L-rhamnose, melibiose, inulin, D-arabinose, soluble starch, ethanol, methanol, DL-
236 lactate, succinate, citrate and n-hexadecane. Sodium nitrate, potassium nitrate, L-lysine,
237 Ethylamine hydrochloride (variable) and cadaverine dihydrochloride (variable) are assimilated.
238 No growth occurs on glucosamine, imidazole, creatine or creatinine. Growth in vitamin-free
239 medium is positive. Growth at 15 °C, 25 °C, 30 °C, and 34 °C is positive but not at 4 °C, 37 °C
240 and 40 °C. Growth occurs on YM agar supplemented with 5% (w/v) NaCl, 10 % (w/v) NaCl but
241 not on YM agar with 16% (w/v) NaCl. Starch-like compounds are not produced. No growth
242 occurs on media supplemented with 0.01% and 0.1% cycloheximide, and 1% acetic acid. The
243 diazonium blue B reaction is weakly positive. Urease activity is positive.
244 Molecular characteristics: nucleotide sequences of ITS and LSU (D1/D2 domains) rDNA
245 sequences are deposited in GenBank (Table 1.)
246 Deposits: holotype, PC1^T (= IBRC-M 30158^T) isolated as a contaminant of an unidentified plant
247 culture, preserved in a metabolically inactive state at the Iranian Biological Resource Centre,
248 Teheran (holotype), Iran. Ex-type cultures are deposited in the CBS yeast collection of the
249 Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands (= CBS 13945^T) and in the
250 German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany (DSM
251 104832^T).
252 Strain examined: PC1^T, SNB-CN72 (= DSM 104833), IBL 03150
253
254 **DISCUSSION**

255 Morphological and physiological characteristics of the three yeast isolates examined in this study
256 are uniform, differences in ITS and LSU sequences are low all that suggesting assignment to a
257 single species. Molecular phylogenetic analyses reveal the three yeast isolates within the

258 Graphiolaceae as a sister lineage of *Graphiola cylindrica, G. geonomae,* and *G. phoenicis.* Thus,
259 we propose *Graphiola fimbriata* sp. nov. to accommodate the three yeast strains. On the basis of
260 the pair-wise sequence similarities (ITS and D1/D2 regions) and subsequence phylogenetic
261 analyses (Figs 1 & 2), the novel species differs from all other *Graphiola* species.

262 We also emend the description of the genus *Graphiola* to allow the inclusion of species known
263 from saprobic state only (see Taxonomy section).

264 The ecology and distribution range of the novel species is unknown. All described *Graphiola*
265 species are plant pathogens on palms (Arecaceae). We hypothesize that the novel species is
266 probably associated with plants, as also suggested by the origin of the studied isolates and
267 closely related culture Exobasidiomycetidae sp. AUMC 10262 (KX011608) from the palm
268 weevil *Rhynchophorus ferrugineus* that infests palms, which is a primary host of *Graphiola*.
269 Although the novel species is known from its asexual state, it is most likely that it has a host like
270 all other *Graphiola* species. Widespread transcontinental transfer of plants results in a global
271 dissemination of plant pathogens and crop pests (Bebber 2015, Hurley et al. 2016). Among them,
272 fungal pathogens currently lead the global invasion of agriculture, despite their more restricted
273 host range (Bebber et al. 2014, Wingfield et al. 2017). In our study, a new species of the plant
274 pathogenic genus *Graphiola* was identified over a broad geographic range that includes the Old
275 World (Iran and Egypt), tropical Asia and the Americas (French Guiana and USA). It is very
276 likely that the ongoing transport of plants and pests promoted dissemination of *Graphiola*
277 *fimbriata* between continents, localities, and habitats.

278 It cannot be precluded that *Graphiola fimbriata* represents the anamorphic stage of a palm
279 pathogen of which no sequence data are available. Of the 12 *Graphiola* species described
280 (Piepenbring et al. 2012) only three are represented by sequence data in GenBank. Moreover

281 species diversity seems underestimated in the genus *Graphiola.* Assuming that its host
282 specificity developed to the same degree shown for other plant pathogens (Piątek et al. 2013,
283 Savchenko et al. 2014, Vasighzadeh et al. 2014, Choi & Thines 2015, Scholler et al. 2016), the
284 number of 12 *Graphiola* species on 38 palm species from 21 plant genera (Farr & Rossman n.d.,
285 Piepenbring et al. 2012) is lower than in other known plant-parasite systems. Intensive sampling
286 and molecular analyses were used successfully in other groups to link anamorph and teleomorph
287 stages (Sampaio 2004, Boekhout et al. 2006, Inácio et al. 2008, Wang et al. 2015a, Kruse et al.
288 2017, Piątek et al. 2017). However, both sexual and asexual species are yet strongly under
289 sampled in many lineages (Liu et al. 2015a, Wang et al. 2015a, Wang et al. 2015b). Although
290 these studies unified systematics of plant parasites and yeast taxa, the results strongly suggest
291 that the available genetic data is insufficient to resolve anamorph-teleomorph relationships in
292 sister lineages (e.g., Boekhout et al. 2006, Kruse et al. 2017).

293 The lack of sequence data of sexual species also complicates biodiversity assessments and new
294 species discovery. With this study we provide the first overview on *Graphiola* diversity from
295 sequence data available in public databases. We propose new species *Graphiola fimbriata* to
296 accommodate yeasts from several localities and habitats. Our results show that sequences related
297 to *Graphiola* species are rapidly accumulating in public databases as taxonomically unassigned
298 isolates and clones. In our opinion, it is important to describe *Graphiola fimbriata* to provide a
299 proper name for these isolates to communicate it in the future. We agree that taxonomic
300 redundancy cannot be ruled out in the genus *Graphiola* considering a very few sequenced
301 species. Rediscovery and description of already known species as new may happen when stable
302 and informative morphological characters for species differentiation are limited, both in plant
303 material and in culture. Thus, future studies should be addressed on resampling and sequencing

304 of new and already existing material in order to better understand genetic diversity and taxonomy
305 in this group of fungi.

306 There is little known about physiological properties of dimorphic plant parasites, whereas fungi
307 traditionally recognized as "yeasts" were intensively studied in this respect. Undersampling and
308 the lack of genetic (including housekeeping genes) and physiological data do not allow
309 delimitation of many genera (e.g., Wang et al. 2015a). In this study, we decided not to propose a
310 new genus for the novel fungus, but to accommodate it in the genus *Graphiola* in order to reduce
311 the taxonomic complexity in this group. Neither phylogenetic analyses nor the analysis of
312 physiological data revealed a basis to distinguish the new species from other *Graphiola* species.
313 Therefore, we modified the description of the genus *Graphiola* to include information about its
314 asexual form considering own results and previous reports by Tubaki & Yokohama (1971) and
315 Oberwinkler et al. (1982). Despite limited genetic data, our study identified several fungal
316 isolates and metabarcoding clones as *Graphiola fimbriata* and *G. phoenicis* (Figs. 1 & 2). These
317 fungi were found in Indomalayan and Neotropical realms (e.g., Takashima et al. 2012, Urbina et
318 al. 2016), which are the regions where palms are widely distributed (Kissling et al. 2012).

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327

328 **CONFLICTS OF INTEREST**

329

330 The authors declare that there are no conflicts of interest.

331

332 **LITERATURE CITED**

333

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518

519 **TABLES**

520 **Table 1.** Designations and GenBank accession numbers of the yeast strains used in this study.

Yeast strains	Strain designation		GenBank accession numbers	
	IBRC-M	CBS	LSU	ITS
PC1	30158[T]	13945[T]	KM403453	KM403454
SNB-CN72	30163	–	KP229360	KJ023736
IBL 03150	-	14052	KP308195	DQ682574

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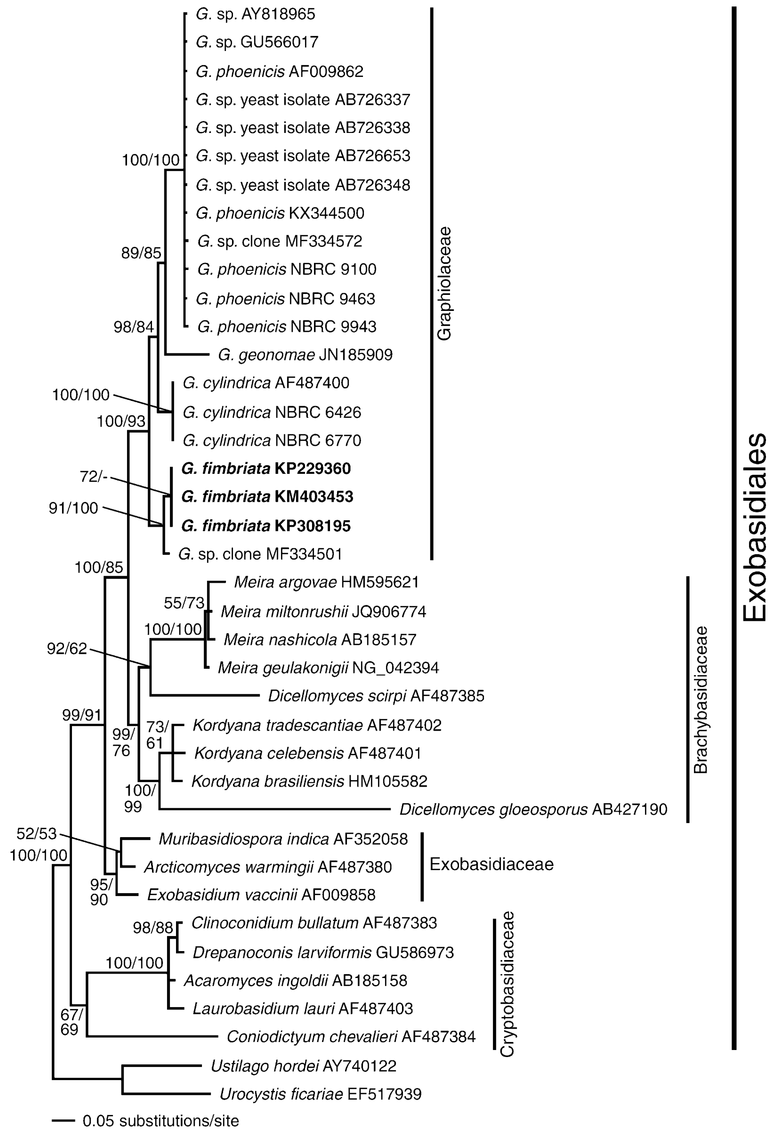
522

523 **FIGURE LEGENDS**

524



525

526 **Fig. 1.** Bayesian inference of phylogenetic relationships within the sampled Exobasidiales:
527 Markov chain Monte Carlo analysis of an alignment of LSU base sequences using the GTR+I+G
528 model of DNA substitution with gamma distributed substitution rates and estimation of invariant
529 sites, random starting trees, and default starting parameters of the DNA substitution model. A
530 50% majority-rule consensus tree is shown computed from 75 000 trees that were sampled after

531 the process had become stationary. The topology was rooted with the ustilaginomycetous species
532 *Urocystis ficariae* and *Ustilago hordei*. Numbers on branches before slashes are estimates for a
533 posteriori probabilities; numbers on branches after slashes are ML bootstrap support values.
534 Branch lengths were averaged over the sampled trees. They are scaled in terms of expected
535 numbers of nucleotide substitutions per site. The taxonomical concept applied corresponds to
536 Begerow et al. (2014).
537

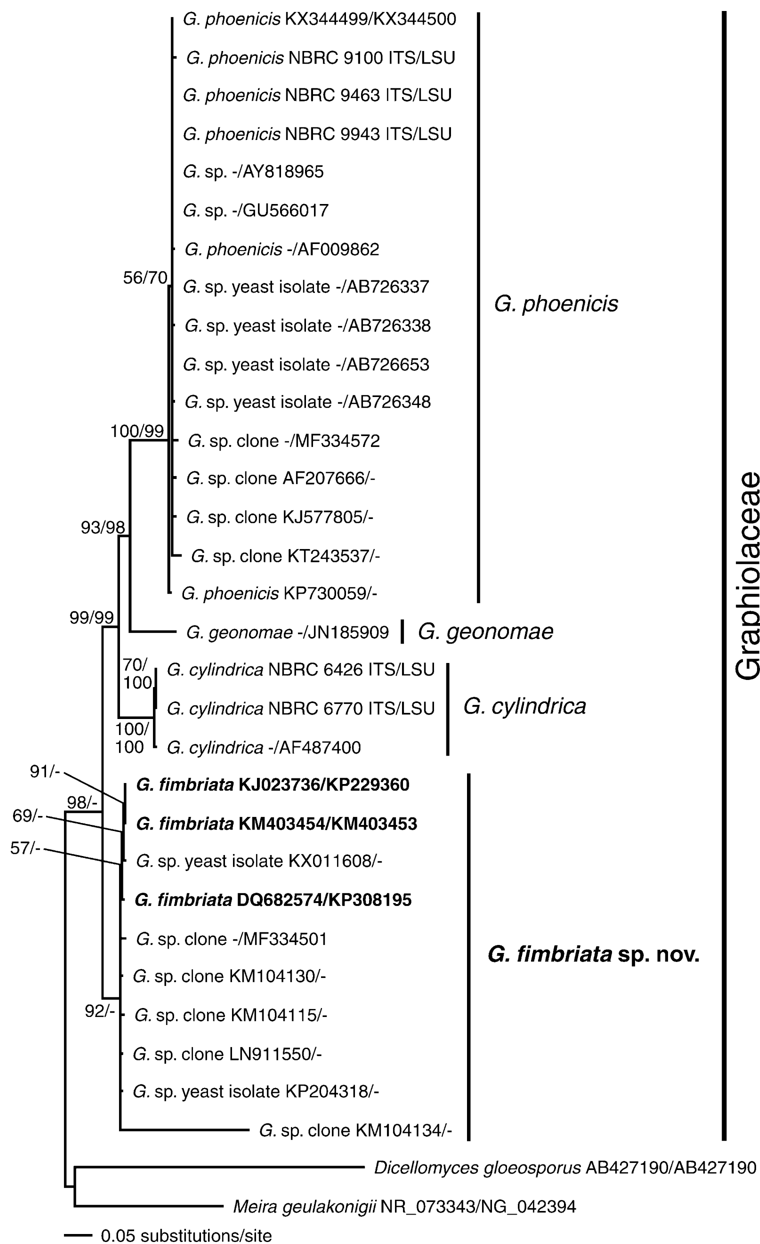
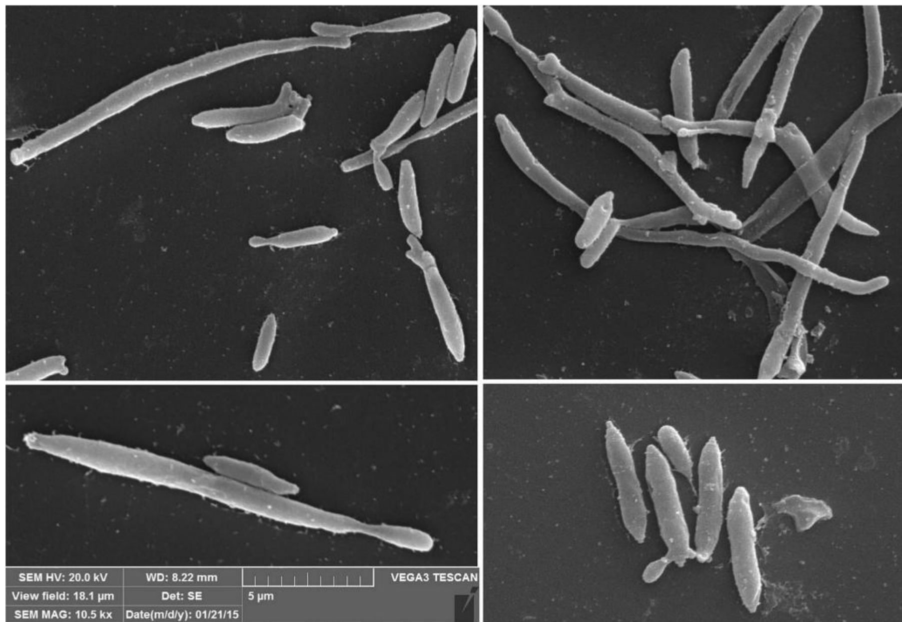


Fig. 2. Bayesian inference of phylogenetic relationships within the sampled Exobasidiales: Markov chain Monte Carlo analysis of an alignment of ITS+LSU base sequences using the GTR+I+G model of DNA substitution with gamma distributed substitution rates and estimation of invariant sites, random starting trees, and default starting parameters of the DNA substitution model. A 75% majority-rule consensus tree is shown computed from 75 000 trees that were sampled after the process had become stationary. The topology was rooted with the

545 cryptobasidiaceous species *Acaromyces ingoldii* and *Clinoconidium* sp. Numbers on branches
546 before slashes are estimates for a posteriori probabilities; numbers on branches after slashes are
547 ML bootstrap support values. Branch lengths were averaged over the sampled trees. They are
548 scaled in terms of expected numbers of nucleotide substitutions per site. The taxonomical
549 concept applied corresponds to Begerow et al. (2014).

550

551 

552 **Fig. 3.** Scanning electron micrographs of the strain IBRC-M 30158^{T} showing budding cells and
553 bud scars. Scale bars: 5 µm.

554