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

30 The systematic position of three yeast strains isolated from a plant cell culture, a piece of termite

31 nest, and as a foliar endophyte of *Coffea arabica*, respectively, is evaluated using morphological,

32 physiological, and phylogenetical analyses. In culture, all three isolates produced white, pale

33 orange to pink colored colonies of cylindrical cells with monopolar budding and pseudohyphae.

34 Standard phenotypic, biochemical, physiological characterization and phylogenetic analyses of

35 the combined 26S rRNA gene (D1/D2 domains) and ITS region sequences showed the

36 conspecificity of these isolates and suggest their placement within the Exobasidiales

37 (Ustilaginomycotina) as a sister lineage of the sampled and sequenced *Graphiola* species. Here,

38 we describe this species as *Graphiola fimbriata* sp. nov. MycoBank MB 825077 (holotype:

39 PC1^T; ex-type cultures: IBRC-M 30158^T = CBS 13945^T = DSM 104832^T). This is the first

40 anamorphic saprobic species described in the genus *Graphiola*. The description of the genus

41 *Graphiola* is therefore emended to allow species known only from a saprobic state.

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INTRODUCTION

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

The anamorphic saprobic members of Ustilaginomycotina have previously been classified mainly in the polyphyletic genera *Pseudozyma* and *Tilletiopsis* (Begerow et al. 2000, Sampaio 2004, Kurtzman et al. 2011, 2015), and later in several monophyletic genera *Acaromyces*, *Farysizyma*, *Fereydounia*, *Jaminaea*, *Meira*, and *Sympodiomycopsis* (Sugiyama et al. 1991, Boekhout et al. 2003, Inácio et al. 2008, Sipiczki & Kajdacsi 2009, Nasr et al. 2014). Sexual and asexual morphs in Ustilaginomycotina were recently grouped together in order to unify the 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

MATERIALS AND METHODS

Sample collection and isolation

A plant cell culture of an unidentified plant species mixed with a yeast strain was obtained from

the plant bank section of the Iranian Biological Resource Center (IBRC) in 2013 (strain

designation: PC1). Another strain was isolated from a piece of *Nasutitermes corniger* nest

collected in Rémire-Montjoly, (Cayenne, French Guiana) in July 2011 (strain designation: SNB-

CN72; Nirma et al. 2013, 2015). A third strain was obtained from the CBS yeast collection of the

Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands (original strain designation:

IBL 03150). It was isolated as foliar endophyte of *Coffea Arabica* (Posada et al. 2007).

Designations and GenBank accession numbers of the yeast strains used in this study are given in

Table 1.

Cultures were maintained on YPG agar medium (0.5% yeast extract, 1% peptone, 2% glucose,

2% agar, w/v) at 25 °C during experiments.

To expand the ITS sequence sampling for molecular phylogenetic analyses a specimen of the

plant parasitic genus *Graphiola* was additionally used and newly sequenced: *Graphiola*

phoenicis (Moug. ex Fr.) Poit. on *Phoenix reclinata* Jacq., South Africa, Kwa Zulu-Natal,

Durban, Yellowwood Park, Kenneth Stainbank Nature Reserve, 14 Feb. 2012, leg. A.R. Wood

883, KR-M-0042315.

Morphological examination

Colony morphology images were taken using a stereo microscope coupled with the Nikon zoom

digital camera. In addition, scanning electron microscopy was performed on the isolate using a

5

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) × 29 (X)

8

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).

327

CONFLICTS OF INTEREST

329

The authors declare that there are no conflicts of interest.

331

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517   213

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

521

522

FIGURE LEGENDS

524

525 ⎯ 0.05 substitutions/site
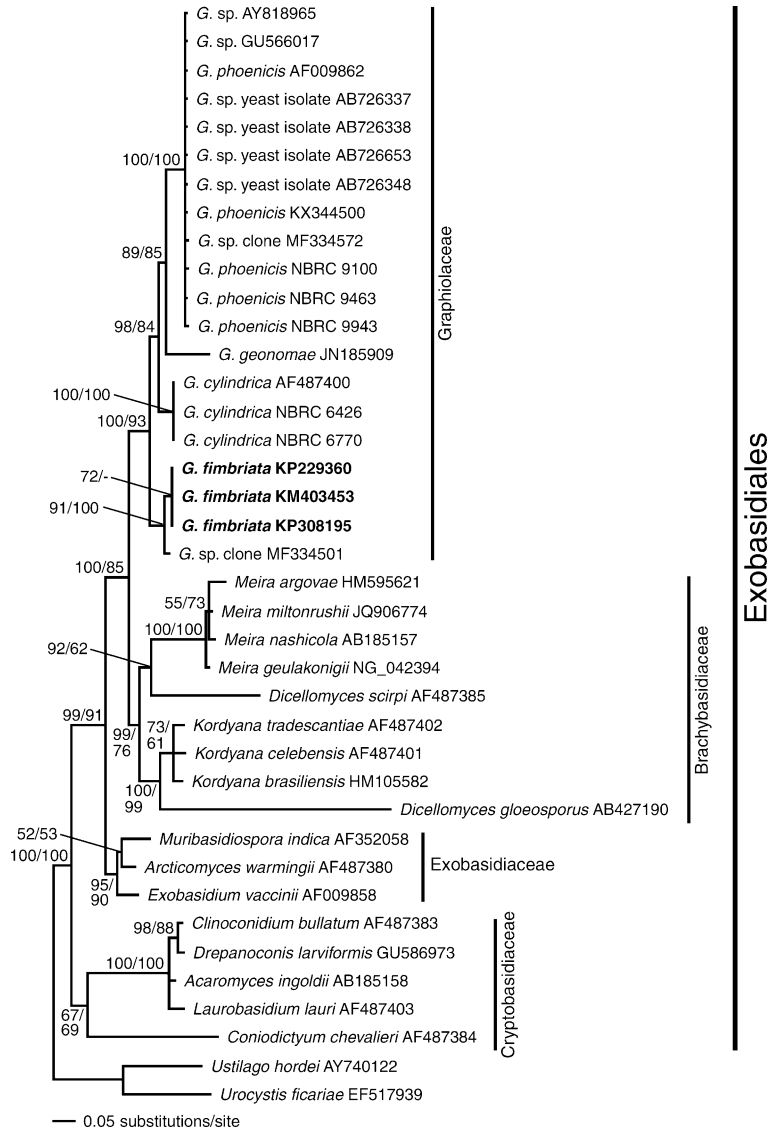
Fig. 1. Bayesian inference of phylogenetic relationships within the sampled Exobasidiales:

Markov chain Monte Carlo analysis of an alignment of 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

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
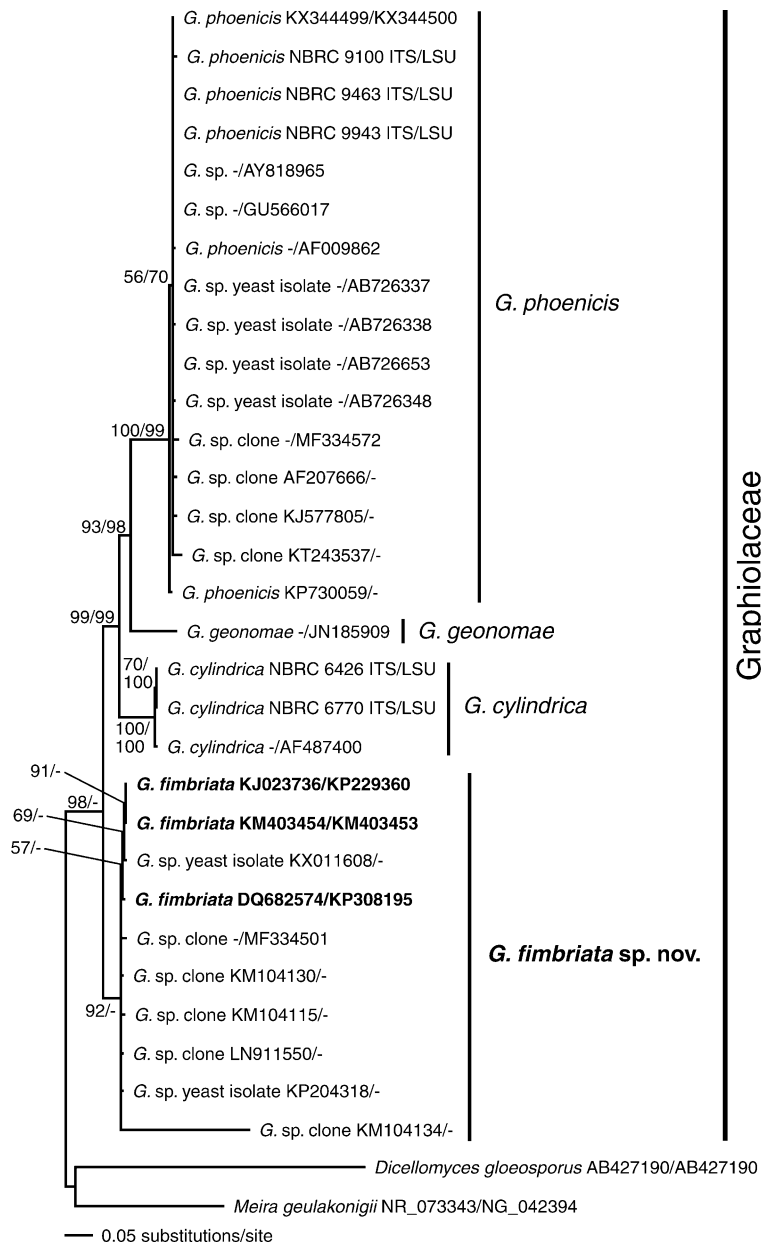
Fig. 2. Bayesian inference of phylogenetic relationships within the sampled Exobasidiales: Markov chain Monte Carlo analysis of an alignment of ITS+LSU base sequences using the GTR+I+G model of DNA substitution with gamma distributed substitution rates and estimation of invariant sites, random starting 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

26

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
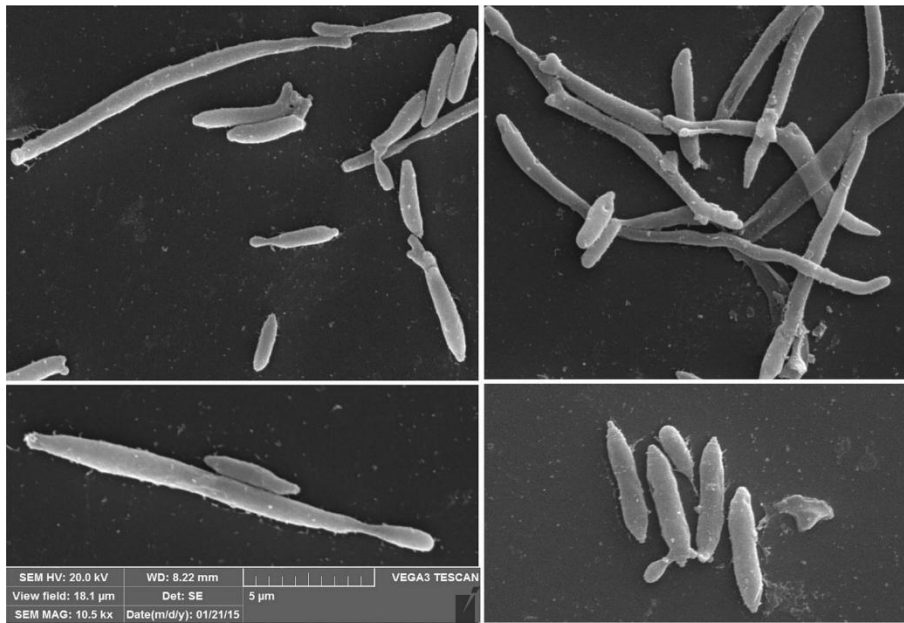
551

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