

Aberystwyth University

A new anaerobic fungus (Oontomyces anksri gen. nov., sp. nov.) from the digestive tract of the Indian camel (Camelus dromedarius)

Dagar, Sumit S.; Kumar, Sanjay; Griffith, Gareth W.; Edwards, Joan E.; Callaghan, Tony M.; Singh, Rameshwar; Nagpal, Ashok K.; Puniya, Anil K.

Published in:
Fungal Biology

DOI:
[10.1016/j.funbio.2015.04.005](https://doi.org/10.1016/j.funbio.2015.04.005)

Publication date:
2015

Citation for published version (APA):

Dagar, S. S., Kumar, S., Griffith, G. W., Edwards, J. E., Callaghan, T. M., Singh, R., Nagpal, A. K., & Puniya, A. K. (2015). A new anaerobic fungus (*Oontomyces anksri* gen. nov., sp. nov.) from the digestive tract of the Indian camel (*Camelus dromedarius*). *Fungal Biology*, 119(8), 731-737. <https://doi.org/10.1016/j.funbio.2015.04.005>

General rights

Copyright and moral rights for the publications made accessible in the Aberystwyth Research Portal (the Institutional Repository) are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Aberystwyth Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Aberystwyth Research Portal

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

tel: +44 1970 62 2400
email: is@aber.ac.uk

Accepted Manuscript

A new anaerobic fungus (*Oontomyces anksri* gen. nov., sp. nov.) from the digestive tract of the Indian camel (*Camelus dromedarius*)

Sumit S. Dagar, Sanjay Kumar, Gareth W. Griffith, Joan E. Edwards, Tony M. Callaghan, Rameshwar Singh, Ashok K. Nagpal, Anil K. Puniya



PII: S1878-6146(15)00059-8

DOI: [10.1016/j.funbio.2015.04.005](https://doi.org/10.1016/j.funbio.2015.04.005)

Reference: FUNBIO 575

To appear in: *Fungal Biology*

Received Date: 31 October 2014

Revised Date: 13 April 2015

Accepted Date: 20 April 2015

Please cite this article as: Dagar, S.S, Kumar, S., Griffith, G.W, Edwards, J.E, Callaghan, T.M, Singh, R., Nagpal, A.K, Puniya, A.K, A new anaerobic fungus (*Oontomyces anksri* gen. nov., sp. nov.) from the digestive tract of the Indian camel (*Camelus dromedarius*), *Fungal Biology* (2015), doi: 10.1016/j.funbio.2015.04.005.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

A new anaerobic fungus (*Oontomyces anksri* gen. nov., sp. nov.) from the digestive tract of the Indian camel (*Camelus dromedarius*).

Dagar, Sumit S^{1*}, Sanjay Kumar^{1‡}, Gareth W Griffith², Joan E Edwards², Tony M Callaghan², Rameshwar Singh^{1#}, Ashok K Nagpal³ and Anil K Puniya^{1†}

¹Dairy Microbiology Division, ICAR-National Dairy Research Institute, Karnal - 132001, INDIA

²Institute of Biological, Environmental and Rural Sciences, Cledwyn Building, Aberystwyth University, Aberystwyth SY23 3DD, WALES UK

³ ICAR-National Research Centre on Camel, Bikaner - 334001, INDIA

Present address:

* Bioenergy Group, Agharkar Research Institute, Pune – 411004, INDIA

‡ College of Veterinary Medicine, University of Pennsylvania, USA

Directorate of Knowledge Management in Agriculture, Krishi Anusandhan Bhawan-I, Pusa, New Delhi 110012, INDIA

†Corresponding author: Tel: +91-184-2259176; E-mail: akpuniya@gmail.com, Anil.Puniya@icar.org.in

Email addresses of other authors:- SS Dagar (dagarsmit@gmail.com), S Kumar (sanjayvrm@gmail.com), GW Griffith (gwg@aber.ac.uk), JE Edwards (joanee2002@hotmail.com), TM Callaghan (toneocallaghan@hotmail.co.uk), R Singh (rsndri@gmail.com), AK Nagpal (aknagpal@scientist.com), and AK Puniya (Anil.Puniya@icar.org.in)

Short Title: *Oontomyces anksri* gen. nov., sp. nov. from camel

28 **ABSTRACT**

29 Two cultures of anaerobic fungi were isolated from the forestomach of an Indian camel
30 (*Camelus dromedarius* L.). Phylogenetic analysis using both the internal transcribed spacer
31 (ITS) and large-subunit (LSU) regions of the rRNA locus demonstrated that these isolates
32 were identical and formed a distinct clade within the anaerobic fungi (phylum
33 Neocallimastigomycota). Morphological examination showed that these fungi formed
34 monocentric thalli with filamentous rhizoids and uniflagellate zoospores, broadly similar to
35 members of the genus *Piromyces*. However, distinctive morphological features were
36 observed, notably the pinching of the cytoplasm in the sporangiophore and the formation of
37 intercalary rhizoidal swellings. Since genetic analyses demonstrated this fungus was only
38 distantly related to *Piromyces* spp. and closer to the polycentric *Anaeromyces* clade, we
39 have assigned it to a new genus and species *Oontomyces anksri* gen. nov., sp. nov.
40 Interrogation of the GenBank database identified several closely related ITS sequences,
41 which were all environmental sequences obtained from camels, raising the possibility that
42 this fungus may be specific to camelids.

43

44 **Key words:** Neocallimastigomycota; Indian camel; *Camelus dromedarius*; fungal taxonomy;
45 rumen fungi; host specificity; *Oontomyces anksri*

46 **Selected classifications:** Anaerobic fungi; Host specialization; Rumen fungi; Symbiosis;
47 Systematics

48

49 **1. INTRODUCTION**

50 Members of the phylum Neocallimastigomycota are a remarkable group of obligately
51 anaerobic fungi, which normally reside within the digestive tract of mammalian herbivores.
52 These fungi are important to the nutrition of their host, due to their significant role in the
53 degradation of ingested lignocellulosic plant material, which the host itself is incapable of

54 utilizing. The potent fibre-degrading enzymes of anaerobic fungi, in addition to their physical
55 disruption of the plant material, has led to recognition of their significant biotechnological
56 potential, for example in biofuel processing and biogas production (Gruninger et al. 2014;
57 Sirohi et al. 2013; Youssef et al.).

58

59 Since their belated recognition as Fungi (Orpin 1974), some 20 species have been reported
60 (Griffith et al. 2009; Sirohi et al. 2012) but the taxonomic status of some of these species is
61 uncertain (Eckart et al. 2010; Hibbett et al. 2007; Ho and Barr 1995; Ozkose et al. 2001).

62 Following revision of the broader taxonomy of kingdom Fungi, this group is now considered
63 as phylum Neocallimastigomycota, containing a single family, Neocallimastigaceae (in the
64 order Neocallimastigales) (Hibbett et al. 2007). However, the status of the anaerobic fungi as
65 a distinct phylum remains a matter of contention (Frey 2012; Powell and Letcher 2014).

66

67 The six genera within Neocallimastigomycota are divided into two groups based on their
68 growth patterns: monocentric (*Neocallimastix*, *Piromyces* and *Caecomyces*) or polycentric
69 (*Orpinomyces*, *Anaeromyces* and *Cyllamyces*), with the former growing as determinate thalli
70 with a single sporangium and the latter forming more complex thalli with multiple sporangia
71 (Griffith et al. 2009; Ho and Barr 1995). Two genera (*Neocallimastix* and *Orpinomyces*) form
72 zoospores with multiple (7-30) flagella, in contrast to the uniflagellate zoospores of all other
73 zoosporic fungi. Additionally, members of the genera *Caecomyces* and *Cyllamyces* are
74 unusual since they form a bulbous holdfast rather than filamentous rhizoids. The advent of
75 culture-independent methods for the study of these fungi has provided compelling evidence
76 that additional genera of anaerobic fungi, as yet uncultured or unrecognized exist (Griffith et
77 al. 2010; Kittelmann et al. 2012; Ligginstoffer et al. 2010; McGranaghan et al. 1999; Sirohi et
78 al. 2013), and that some of these undescribed taxa may exhibit distinct host specificity
79 (Ligginstoffer et al. 2010).

80

81 Here we present genetic and morphological data relating to a novel clade of anaerobic fungi
82 isolated from the forestomach of the Indian camel (*Camelus dromedarius*), which is
83 sufficiently distinct from the existing taxa of anaerobic fungi to merit its placement in a new
84 genus *Oontomyces*.

85

86 2. MATERIALS AND METHODS

87 Liquor samples were collected using a stomach pipe from single-humped camel calf
88 (Kutchchi breed male, 3 years-old, born domesticated), weighing 450 kg and maintained on a
89 concentrate (50%) / roughage (50%) diet at the ICAR-National Research Centre for Camels
90 (Bikaner, Thar Desert, Rajasthan, India; N28.001; E73.318; altitude 200 m). The strained
91 liquor was brought to the laboratory in pre-warmed and O₂-free (gassed with CO₂) thermos
92 flask. Isolations on cellobiose agar medium were performed at ICAR-NDRI, Karnal, as
93 described by Dagar *et al.* (2011), including roll tube purification (Joblin 1981) to avoid the
94 possibility of mixed cultures.

95

96 Taxonomic features were examined following growth on wheat straw medium for 3 days
97 (Dagar *et al.* 2011) using phase contrast microscopy, and images were recorded using a
98 Canon DS126191 digital camera. For genetic characterisation, the complete internal
99 transcribed spacer (ITS; partial 18S, complete ITS 1, 5.8S, ITS 2 and partial 28S) and D1/D2
100 domain at the 5' end of the large-subunit (LSU) ribosomal DNA were amplified, using the
101 primer pairs ITS1 (5'- TCC GTA GGT GAA CCT GCG G-3')/ITS4 (5'- TCC TCC GCT TAT
102 TGA TAT GC-3') and NL1 (5'-GCA TAT CAA TAA GCG GAG GAA AAG-3')/NL4 (5'-GGT
103 CCG TGT TTC AAG ACG G-3'), respectively (Dagar et al. 2011; Fliegerová et al. 2006).
104 Care was taken to delimit the different regions of the rRNA locus in a consistent manner, as
105 suggested by Hibbett et al. (1995), using the consensus sequences CATT/CAACTTCAG

(end of 18S/start of 5.8S) and GAGTGTCATTA/TTGACCTCAAT (end of 5.8S/start of 28S).

Phylogenetic reconstruction was conducted within the Geneious v6 bioinformatics package (Drummond et al. 2011), using MAFFT (v7.017 (Kato et al. 2002)) for sequence alignment (default settings) and Mr Bayes for phylogenetic analysis (default settings; (Huelsenbeck and Ronquist 2001)).

3. RESULTS

After three days of growth from the original isolation tubes, two representative fungal colonies were selected and purified by repeated subculturing. Both the isolates (SSD-CIB1 and SSD-CIB2) formed uniflagellate zoospores (Fig. 1A, 1B) and filamentous rhizoids (Fig. 1C). Sporangia were formed terminally and varied in shape from ellipsoid to elongate (Figs. 1C-1E) (see <http://www.forestphytophthoras.org/glossary/>), as has been reported for several other species of anaerobic fungus (Dagar 2011; Gleason et al. 2002). However, sporangia were never mucronate (pointed), as is the case for the related *Anaeromyces mucronatus*. The sporangiophore (sporangium stalk) was often 2-3 times longer than the sporangium and separated from the rhizomycelium by a distinct constriction (Figs. 1D, 1E). Intercalary rhizoidal swellings were frequently observed (Figs. 1F, 1G); these swelling bore some resemblance to the intercalary sporangia reported in *Orpinomyces intercalaris* (Dagar et al. 2011; Ho and Barr 1995) but none was ever seen to release or contain zoospores. Thus colony morphology was consistently monocentric (single sporangium per thallus) but confirmation using DAPI-staining and fluorescent microscopy that nuclei were restricted to sporangia (Ozkose et al. 2001) was not conducted.

Morphologically these new isolates conformed most closely to members of the genus *Piromyces*, in which nine species have been described (Ho and Barr 1995; Ho et al. 1993a, b; Kirk 2012). However, of these *Piromyces* species, none of the type specimens for these

132 species have been subject to both morphological and genetic analysis, except the rather
133 distinctive *P. polycephalus* (recently renamed as *Anaeromyces polycephalus* (Chen et al.
134 2002; Kirk 2012)). Apart from *Piromyces cryptodigmaticus*, an uncultured organism defined
135 by its ITS sequence alone (Kirk 2012), none of the type specimens or cultures are extant
136 (Prof. Ho Yin Wan and Dr. Brigitte Gaillard-Martinie, pers. comms.). However, the pinching of
137 the sporangiophore and highly variable sporangial shape (but not intercalary rhizoidal
138 swellings) have been reported for *P. rhizinflata* (Breton et al. 1991).

139

140 **Fig 1.** Morphology of *Oontomyces anksri*

141

142 DNA sequences obtained for the ITS region (ca.700 bp amplicon; GenBank JX017310-11) of
143 both isolates, and also for the D1/D2 domains of the LSU gene (ca. ≈780 bp amplicon;
144 GenBank JX017314-15), were identical. More detailed analysis of the LSU region (Fig. 2;
145 Suppdata 1) confirmed that these isolates were more closely related to *Anaeromyces* spp.
146 than the *Piromyces* spp., which it resembled morphologically. Whilst *Anaeromyces* spp. also
147 release uniflagellate zoospores, they form polycentric thalli with multiple sporangia.

148

149 **Fig. 2.** Bayesian backbone analysis of LSU sequences.

150 **Suppdata. 1.** ML analysis of LSU sequences.

151

152 Alignment of ITS sequences across the whole range of Neocallimastigomycota was
153 unsatisfactory due to very presence of many gaps in such alignments. Therefore, analysis
154 was restricted to only those genera forming uniflagellate zoospores (*Anaeromyces* /
155 *Caecomyces* / *Cyllamyces* / *Piromyces*), and excluding the genera *Neocallimastix* and
156 *Orpinomyces*, which formed a distinct clade in phylogenetic analysis of the LSU region (Fig.
157 3; Suppdata 2). The ITS sequences for Neocallimastigomycota lodged with GenBank

158 predominantly cover the ITS1 region, therefore, phylogenetic analysis was restricted to this
159 region (bounded by the conserved sequences CATTA [3' end of 18S region] and CAACTT [5'
160 end of 5.8S region), as suggested by Hibbett et al. (1995)). Following removal of duplicated
161 sequences, and inclusion of closely related environmental nucleic acid sequences (ENAS),
162 phylogenetic analysis was conducted on an alignment of 61 sequences (290 bp alignment).
163 As with LSU analysis, the *Oontomyces* clade was recovered as a sister clade to
164 *Anaeromyces* with high posterior probability support.

165

166 **Fig. 3.** Bayesian posterior probability analysis of ITS1 sequences.

167 **Suppdata 2.** ML analysis of ITS1 sequences.

168

169 4. DISCUSSION

170 The fact that the two isolates studied here form monocentric thalli and are thus clearly
171 distinct from the polycentric genus *Anaeromyces* spp., as defined by Breton et al. (1990),
172 indicates that the genus *Piromyces* (to which these fungi would have been consigned in the
173 absence of genetic evidence) is polyphyletic, as previously suggested by Fliegerová et al.
174 (2012). It is also apparent from Fig. 3 that several sequences lodged in GenBank and named
175 *Anaeromyces* are also only distantly related to *Anaeromyces sensu stricto* (for which isolate
176 JF1 [indicated in Figs. 2/3] is defined as the reference sequence [NCBI Reference
177 Sequence: NR_111156.1] in the RefSeq Targeted Loci (RTL) database (Schoch et al. 2014).
178 The most longstanding anomaly is *Anaeromyces* (formerly *Piromyces*) *polycephalus* (Chen
179 et al. 2002), which is both morphologically and genetically distinctive, and in need of
180 taxonomic reassessment, not least because it does not conform to the morphological
181 circumscription of the genus *Anaeromyces*. For the isolates studied here, we propose below
182 to assign these to a new genus, since they are similar in morphology to *Piromyces* spp. but
183 genetically distant. Their monocentric thallus morphology prevents their assignment to the

184 genus *Anaeromyces*, as do several other morphological features. They are genetically
185 distinct from *Anaeromyces sensu stricto*, being more closely related to *A. polycephalus* which
186 they do not resemble morphologically.

187

188 Intriguingly, the most closely related ITS1 sequences to *O. anksri*, and which clearly fall
189 within the *Oontomyces* clade, are part of a set of 155 ENAS sequences (JX944829-
190 JX944983; Huo,X., Zhang,Z., Wang,N. and Zeng,J., unpublished). These sequences are all
191 >89% identical across the ITS1 region, whereas the sister clades are <70% identical. These
192 also originated from camel 'psuedorumen' (Bactrian camel; *Camelus bactrianus*) from
193 Urumqi, Xinjiang, north-west China (N43.81; E87.58; altitude 830 m), some 2000 km north-
194 east of Rajasthan.

195

196 The fact that this novel clade, which we formally name below, is very close to other
197 sequences also isolated from camel raised the possibility that members of this clade exhibit
198 host specificity. By far the most extensive culture independent study of anaerobic fungi is that
199 of Ligginstoffer et al. (2010) (250,000 ITS1 GenBank sequences from a 454 NextGen
200 sequencing project), in which the faeces of diverse (>30 species) herbivores from Oklahoma
201 Zoo were studied. Several novel clades were discovered, some of which were apparently
202 host-specific in equids. The absence of any sequences similar to *Oontomyces* from this
203 dataset may relate to the fact that only one camelid host (*Lama glama*) was included, a
204 finding that is consistent with the possibility of host specificity. Although the primers used by
205 Ligginstoffer et al. (2010) are known not to be universal for all anaerobic fungi (Edwards et
206 al. 2008), these primer sites are conserved in *Oontomyces* and thus would have amplified
207 these sequences had they been present.

208

209 Camelids (family Camelidae; suborder Tylopoda) form a basal group within the class
210 Certartiodactyla (which also includes whales, hippos, ruminants and pigs), with a distinctive
211 gastrointestinal morphology, often described as pseudoruminant. The highly enlarged foregut
212 comprises three distinct regions, analogous to the four chamber of true ruminants (suborder
213 Ruminantia) and allows efficient digestion of plant lignocellulose via pre-gastric microbial
214 fermentation (Van Soest 1994; Wilson 1989). This difference in foregut morphology is also
215 associated with differences in protozoan populations, with several species (eg. *Entodinium*
216 *ovumrajae* and *Calascolex camelinus*) found to be specific to camels (Dogiel 1947; Imai et al.
217 2004) and others that are common in true ruminants (e.g. cows, sheep) being absent
218 (Kubesy and Dehority 2002).

219

220 **Diagnosis**

221 *Oontomyces* Dagar, Puniya & G.W. Griff. gen. nov.

222 Registration identifier: IF550795

223 Strictly anaerobic fungus with determinate, monocentric thallus with single terminal
224 sporangium, and uniflagellate zoospores. The clade is defined by the sequences JX017310
225 (ITS1, 5.8S, ITS2 complete) and JX017314 (LSU, partial sequence). The most genetically
226 similar genus is *Anaeromyces*, which is defined as forming a polycentric thallus (“Fungi
227 semper anaerobici, thallus polycentricus, zoosporangia mucronata, zoospora uniflagellata”)
228 (Breton et al. 1990), in contrast to the monocentric *Oontomyces*.

229 Registration identifier: IF550795.

230 Type species *Oontomyces anksri* Dagar, Puniya & G.W. Griff. sp. nov.

231 Etymology: “Oont” is from the Hindi, meaning “camel”.

232

233 *Oontomyces anksri* sp. nov. Dagar, Puniya & G.W. Griff. sp. nov.

234 Registration identifier: IF550796.

235 Holotype: SSD-CIB1 (ICAR-National Dairy Research Institute, Karnal, India)

236 Etymology: The specific name *anksri* is assigned in the honor of Dr. Anil Kumar Srivastava
237 (Director, NDRI, Karnal) by taking the first two, one and three letters of his first, middle and
238 surname (i.e. ANil Kumar SRivastava = ANKSRI), respectively, who always encouraged us
239 working in this under-explored area of microbiology.

240 Single terminal sporangium (70-100 µm long, 35-50 µm wide), ovoid to elongate, borne on a
241 long sporangiophore (150-200 µm) which bears a distinct constriction delimiting the rhizoid
242 from the sporangiophore. Ovoid to subovoid intercalary rhizoidal swelling are occasionally
243 found (50-70 µm long, 40-60 µm wide). Zoospores are uniflagellate, spherical 5-7 µm in
244 diameter, flagellum 24-30 µm in length (>3x longer than zoospore body). Obligate anaerobic
245 fungus, isolated from camel forestomach. The structures originally examined are no longer
246 extant nor are the pure cultures from which they were derived. The clade is defined by the
247 sequences JX017310 (ITS1, 5.8S, ITS2 complete) and JX017314 (LSU, partial sequence).
248 The type material for this species are the images contained in Figure 1 here and also a
249 sample of freeze-dried forestomach fluid from which the cultures SSD-CIB1 and SSD-CIB2
250 were originally isolated; isotype material deposited at the Aberystwyth Fungarium, Wales
251 (ABS) and Royal Botanic Gardens, Kew, UK (K).

252

253 5. ACKNOWLEDGEMENTS

254 Authors gratefully acknowledge Network projects on VTCC and NICRA for providing partial
255 financial support during this study, and also for the award of a Stapledon Memorial Trust
256 Travelling Fellowship (to SSD) and DBT-CREST Award Fellowships (to AKP), which
257 permitted research visits to IBERS, Aberystwyth University. The authors are also grateful to
258 SK Sirohi, RC Upadhyay, Parveen Malik, DN Kamra, NV Patil, RK Malik and SS Kundu for
259 their support in conducting the work. TMC is grateful to the Aberystwyth University
260 Postgraduate Studentship Scheme for funding. JE gratefully acknowledges funding from

261 BBSRC (Rumen Systems Biology; BBS/EW/10964A01). We are also grateful to Dr. Paul
262 Kirk (RBG Kew) for nomenclatural advice.

263

264 6. REFERENCES

- 265 Breton A, Bernalier A, Dusser M, Fonty G, Gaillard-Martinie B, Guillot J, 1990. *Anaeromyces*
266 *mucronatus* nov. gen., nov. sp. A new strictly anaerobic rumen fungus with polycentric
267 thallus. *FEMS Microbiology Letters* 58, 177-182.
- 268 Breton A, Dusser M, Gaillardmartinie B, Guillot J, Millet L, Prensier G, 1991. *Piromyces*
269 *rhizinflata* nov. sp., a strictly anaerobic fungus from feces of the Saharan ass - a
270 morphological, metabolic and ultrastructural study. *FEMS Microbiology Letters* 82, 1-8.
- 271 Chen Y-C, Hseu R-S, Chein C-Y, 2002. *Piromyces polycephalus* (Neocallimastigaceae), a
272 new rumen fungus. *Nova Hedwigia* 75, 409-414.
- 273 Dagar SS, 2011. Conjugated linoleic acid (CLA) producing potential and genetic
274 heterogeneity of rumen fungi., *PhD Thesis NDRI ICAR-National Dairy Research Institute,*
275 *Karnal, INDIA.*
- 276 Dagar SS, Kumar S, Mudgil P, Singh R, Puniya AK, 2011. D1/D2 domain of large-subunit
277 ribosomal DNA for differentiation of *Orpinomyces* spp. *Applied and environmental*
278 *microbiology* 77, 6722-6725.
- 279 Dogiel V, 1947. The Phylogeny of the stomach infusorians of Ruminants in the light of
280 palaeontological and parasitological data. *Quarterly Journal of Microscopical Science* 88,
281 337-343.
- 282 Drummond A, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearse
283 M, Markowitz S, Moir R, Stones-Havas S, Sturrock S, Thierer T, A W, 2011. Geneious
284 v5.4. Available from: <http://www.geneious.com/>.

- 285 Eckart M, Fliegerová K, Hoffmann K, Voigt K, 2010. Molecular Identification of Anaerobic
286 Rumen Fungi, in: Gherbawy Y, Voigt K (Eds), *Molecular Identification of Fungi*. Springer
287 Berlin Heidelberg, pp. 297-313.
- 288 Edwards J, Huws S, Kingston-Smith A, Jimenez H, Skøt K, Griffith GW, McEwan NR,
289 Theodorou MK, 2008. Dynamics of initial colonisation of non-conserved perennial
290 ryegrass by anaerobic fungi in the bovine rumen. *FEMS microbiology ecology* 66, 537-
291 546.
- 292 Fliegerová K, Mrazek J, Voigt K, 2006. Differentiation of anaerobic polycentric fungi by rDNA
293 PCR-RFLP. *Folia Microbiologica* 51, 273-277.
- 294 Fliegerová K, Voigt K, Kirk PM, 2012. *Caecomyces hurleyensis* (Theodorou & J. Webb)
295 Fliegerová, K. Voigt & P.M. Kirk, comb. nov. IF550013. *Index Fungorum* 1.
- 296 Frey W, 2012. Syllabus of plant families. Part 1/1: blue-green algae, myxomycetes and
297 myxomycete-like organisms, phytoparasitic protists, heterotrophic heterokontobionta and
298 fungi pp. No. Ed. 13. Gebrüder Borntraeger Verlagsbuchhandlung, 2012.
- 299 Gleason FH, Gordon GL, Phillips MW, 2002. Variation in morphology of rhizoids in Australian
300 isolates of *Caecomyces* (Chytridiomycetes). *Australasian Mycologist* 21, 94-101.
- 301 Griffith GW, Baker S, Fliegerova K, Ligginstoffer A, van der Giezen M, Voigt K, Beakes G,
302 2010. Anaerobic fungi: Neocallimastigomycota. *IMA Fungus* 1, 181-185.
- 303 Griffith GW, Ozkose E, Theodorou MK, Davies DR, 2009. Diversity of anaerobic fungal
304 populations in cattle revealed by selective enrichment culture using different carbon
305 sources. *Fungal Ecology* 2, 87-97.
- 306 Gruninger RJ, Puniya AK, Callaghan TM, Edwards JE, Youssef N, Dagar SS, Fliegerova K,
307 Griffith GW, Forster R, Tsang A, 2014. Anaerobic fungi (phylum Neocallimastigomycota):
308 advances in understanding their taxonomy, life cycle, ecology, role and biotechnological
309 potential. *FEMS microbiology ecology* DOI: 10.1111/1574-6941.12383.

- 310 Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson O-E, Huhndorf S,
311 James TY, Kirk PM, Lucking R, Lumbsch T, Lutzoni F, Matheny PB, McLaughlin DJ,
312 Powell MJ, Redhead S, Schoch CL, Spatafora JW, Stalpers JA, Vilgalys R, Aime MC,
313 Aptroot A, Bauer R, Begerow D, Benny GL, Castlebury LA, Crous PW, Dai Y-C, Gams W,
314 Geiser DM, Griffith GW, Gueidan C, Hawksworth DL, Hestmark G, Hosaka K, Humber
315 RA, Hyde K, Ironside JE, Koljalg U, Kurtzman CP, Larsson K-H, Lichwardt R, Longcore J,
316 Miadlikowska J, Miller A, Moncalvo J-M, Mozley-Standridge S, Oberwinkler F, Parmasto
317 E, Reeb V, Rogers JD, Roux C, Ryvarden L, Sampaio JP, Schussler A, Sugiyama J,
318 Thorn RG, Tibell L, Untereiner WA, Walker C, Wang Z, Weir A, Weiss M, White MM,
319 Winka K, Yao Y-J, Zhang N, 2007. A higher-level phylogenetic classification of the fungi.
320 *Mycological Research* 111, 509-547.
- 321 Hibbett DS, Tsuneda A, Fukumasa-Nakai Y, Donoghue MJ, 1995. Phylogenetic diversity in
322 shiitake inferred from nuclear ribosomal DNA sequences. *Mycologia* 87, 618-638.
- 323 Ho YW, Barr DJS, 1995. Classification of anaerobic gut fungi from herbivores with emphasis
324 on rumen fungi from Malaysia. *Mycologia* 87, 655-677.
- 325 Ho YW, Barr DJS, Abdullah N, Jalaludin S, Kudo H, 1993a. A New Species of *Piromyces*
326 from the Rumen of Deer in Malaysia. *Mycotaxon* 47, 285-293.
- 327 Ho YW, Barr DJS, Abdullah N, Jalaludin S, Kudo H, 1993b. *Piromyces spiralis*, a new
328 species of anaerobic fungus from the rumen of goat. *Mycotaxon* 48, 59-68.
- 329 Huelsenbeck JP, Ronquist F, 2001. MrBayes: Bayesian inference of phylogeny.
330 *Bioinformatics* 17.
- 331 Imai S, Shinno T, Ike K, Morita T, Selim HT, 2004. Fourteen Morphotypes of *Entodinium*
332 *ovumrajae* (Ophryoscolecidae, Entodiniomorphida) Found in the Dromedary Camel of
333 Egypt. *Journal of Eukaryotic Microbiology* 51, 594-597.
- 334 Joblin KN, 1981. Isolation, enumeration, and maintenance of rumen anaerobic fungi in roll
335 tubes. *Applied and environmental microbiology* 42, 1119-1122.

- 336 Katoh K, Misawa K, Kuma KÄ, Miyata T, 2002. MAFFT: a novel method for rapid multiple
337 sequence alignment based on fast Fourier transform. *Nucleic acids research* 30, 3059-
338 3066.
- 339 Kirk PM, 2012. *Piromyces cryptodigmaticus* Flieg., K. Voigt & P.M. Kirk. *Index Fungorum* 1,
340 1.
- 341 Kittelmann S, Naylor GE, Koolaard JP, Janssen PH, 2012. A proposed taxonomy of
342 anaerobic fungi (class Neocallimastigomycetes) suitable for large-scale sequence-based
343 community structure analysis. *PloS one* 7, e36866.
- 344 Kubesy AA, Dehority BA, 2002. Forestomach ciliate Protozoa in Egyptian dromedary camels
345 (*Camelus dromedarius*). *Zootaxa* 51, 1-12.
- 346 Liggenstoffer AS, Youssef NH, Couger MB, Elshahed MS, 2010. Phylogenetic diversity and
347 community structure of anaerobic gut fungi (phylum Neocallimastigomycota) in ruminant
348 and non-ruminant herbivores. *ISME Journal* 4, 1225-1235.
- 349 McGranaghan P, Davies JC, Griffith GW, Davies DR, Theodorou MK, 1999. The survival of
350 anaerobic fungi in cattle faeces. *FEMS microbiology ecology* 29, 293-300.
- 351 Orpin CG, 1974. Rumen flagellates *Callimastix frontalis* and *Monas communis* - Zoospores
352 of phycomycete fungi. *Journal of Applied Bacteriology* 37, R9-R10.
- 353 Ozkose E, Thomas BJ, Davies DR, Griffith GW, Theodorou MK, 2001. *Cyllamyces aberensis*
354 gen.nov sp.nov., a new anaerobic gut fungus with branched sporangiophores isolated
355 from cattle. *Canadian Journal of Botany* 79, 666-673.
- 356 Powell MJ, Letcher PM, 2014. Chytridiomycota, Monoblepharidomycota and
357 Neocallimastigomycota, in: Esser K (Ed), *The Mycota VII Systematics and Evolution*.
358 *PartA*. Springer, pp. 141-175.
- 359 Schoch CL, Robbertse B, Robert V, Vu D, Cardinali G, Irinyi L, Meyer W, Nilsson RH,
360 Hughes K, Miller AN, 2014. Finding needles in haystacks: linking scientific names,
361 reference specimens and molecular data for Fungi. *Database* bau061.

- 362 Sirohi SK, P.K. C, A.K. P, Singh D, Dagar SS, N. S, 2013. Ribosomal ITS1 sequence-based
363 diversity analysis of anaerobic rumen fungi in cattle fed on high fiber diet. *Annals of*
364 *Microbiology* 63, 1571-1577.
- 365 Sirohi SK, Singh N, Dagar SS, Puniya AK, 2012. Molecular tools for deciphering the
366 microbial community structure and diversity in rumen ecosystem. *Applied Microbiology*
367 *and Biotechnology* 95, 1135–1154.
- 368 Van Soest PJ, 1994. *Nutritional Ecology of the Ruminant*, 2nd ed. Cornell University Press,
369 Ithaca NY.
- 370 Wilson RT, 1989. The nutritional requirements of camel. *Options Méditerranéennes : Série A.*
371 *2*, 171-179.
- 372 Youssef NH, Couger MB, Struchtemeyer CG, Liggenstoffer AS, Prade RA, Najjar FZ, Atiyeh
373 HK, Wilkins MR, Elshahed MS, 2013. The genome of the anaerobic fungus *Orpinomyces*
374 sp. strain C1A reveals the unique evolutionary history of a remarkable plant biomass
375 degrader. *Applied and environmental microbiology* 79, 4620-4634.

376

377

378 **FIGURE LEGENDS**

379 **Fig 1.** Morphology of *Oontomyces anksri*. Zoospores (A, B) are uniflagellate, with the
380 flagellum ca. 4 times the length of the spore body. Thalli are monocentric with sporangia
381 normally being formed terminally (C-E). The shape of the sporangium was variable, ranging
382 from elongate (C) to ovoid (D, E) and the sporangiophore usually (D, E) 2-3 times the length
383 of the sporangium. A constriction is often visible at the base of the sporangiophore (arrowed,
384 D, E). Intercalary rhizoidal swellings were also observed on some thalli (F, G). Figs. 1A, 1E
385 are from isolate SSD-CIB1 and others from isolate SSD-CIB2. Scale bar indicates 10 μ m
386 (A,B) or 50 μ m (C-G).

387
388 **Fig. 2.** Bayesian backbone analysis of LSU sequences (750 bp alignment of D1/D2 variable
389 regions) of Neocallimastigomycota rooted with *Gromochytrium mamkaevae*
390 (Chytridiomycota, order Gromochytriales). Bayesian posterior probabilities ≥ 0.75 are shown
391 above the branches. The different genera of Neocallimastigomycota are shown in different
392 coloured font with the *Oontomyces* clade in blue (and boxed). The reference sequence for
393 *Anaeromyces* spp. is indicated by *. Scalebar indicates number of substitutions per site.

394
395 **Fig. 3.** Bayesian posterior probability analysis of ITS1 sequences of Neocallimastigomycota
396 (290 bp alignment), including the genera with uniflagellate zoospores. The *Oontomyces*
397 *anksri* clade is shown in blue font (*Anaeromyces* clade in red and the *P. polycephalus* clade
398 in green. Line thickness is proportional to Bayesian posterior probabilities (thin lines = <0.7 ;
399 thick lines >0.9) and PP probabilities are shown at salient nodes. * indicates the reference
400 sequences for the genus *Anaeromyces*. Scalebar indicates substitutions per site and the tree
401 is midpoint rooted.

402

403 **Suppdata1.** Maximum Likelihood tree of LSU sequences (750 bp alignment of D1/D2

404 variable regions; GTR substitution model) of Neocallimastigomycota rooted with
405 *Gromochytrium mamkaevae* (Chytridiomycota, order Gromochytriales). Salient bootstrap
406 values (as %; 1000 bootstrap replicates) are shown at nodes. Branches with >70% bootstrap
407 support are drawn with thick lines. * indicates the reference sequence for the genus
408 *Anaeromyces*. Scalebar indicates substitutions per site. The different genera of
409 Neocallimastigomycota are shown in different coloured font with the *Oontomyces* clade in
410 blue (and boxed). The reference sequence for *Anaeromyces* spp. is indicated by *. Scalebar
411 indicates number of substitutions per site.

412

413 **Suppdata 2.** Maximum Likelihood tree (GTR substitution model) of ITS1 sequences of
414 Neocallimastigomycota (290 bp alignment), including the genera with uniflagellate
415 zoospores. The *Oontomyces anksri* clade is shown in blue font (*Anaeromyces* clade in red
416 and the *P. polycephalus* clade in green. Salient bootstrap values (as %; 1000 bootstrap
417 replicates) are shown at nodes. Branches with >70% bootstrap support are drawn with thick
418 lines. * indicates the reference sequences for the genus *Anaeromyces*. Scalebar indicates
419 substitutions per site.

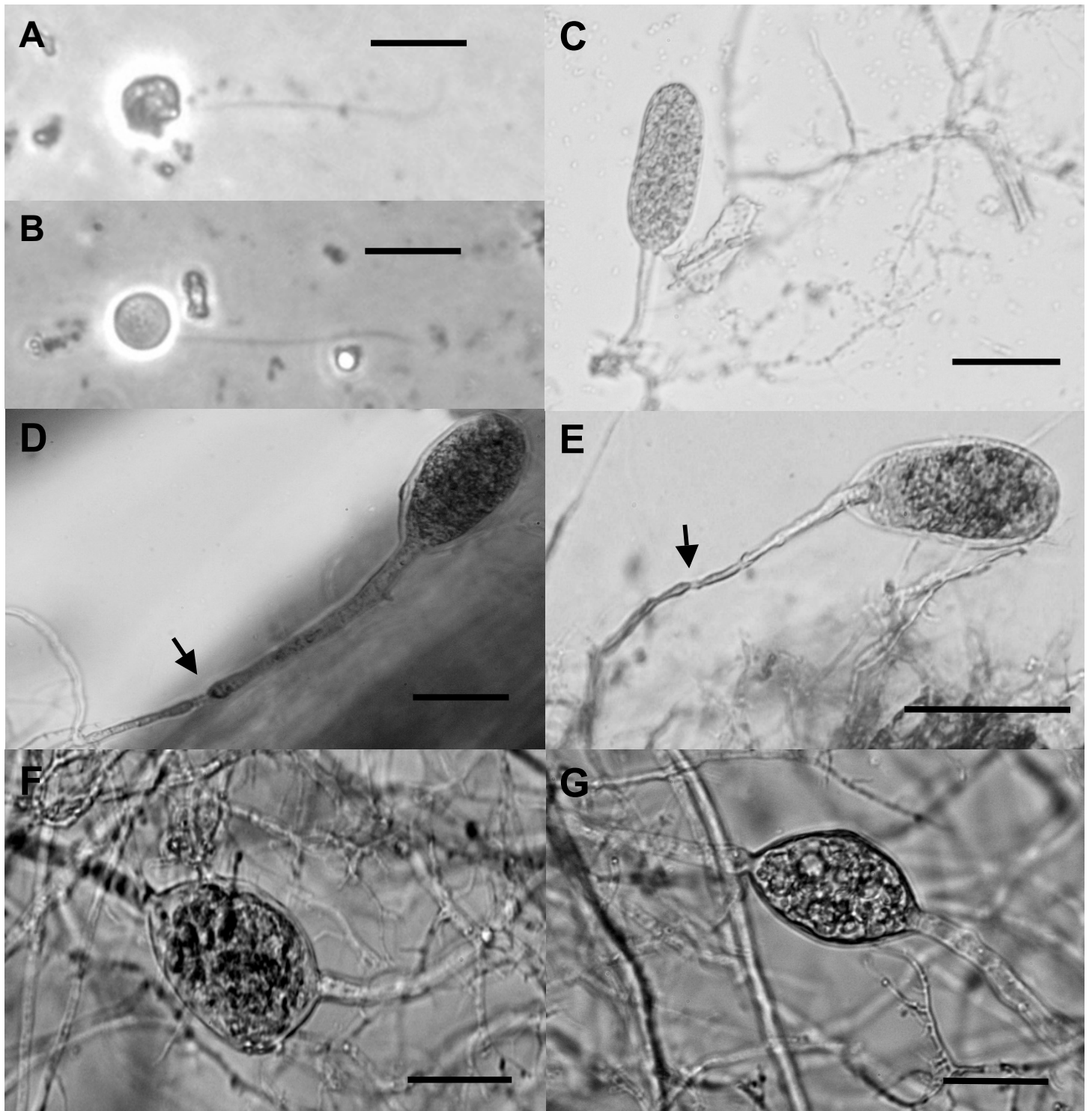


Fig 1. Morphology of *Oontomyces anksri*. Zoospores (A, B) are uniflagellate, with the flagellum ca. 4 times the length of the spore body. Thalli are monocentric with sporangia normally being formed terminally (C-E). The shape of the sporangium was variable, ranging from elongate (C) to ovoid (D, E) and the sporangiophore usually (D, E) 2-3 times the length of the sporangium. A constriction is often visible at the base of the sporangiophore (arrowed, D, E). Intercalary rhizoidal swellings were also observed on some thalli (F, G). Figs. 1A, 1E are from isolate SSD-CIB1 and others from isolate SSD-CIB2. Scale bar indicates 10 μm (A,B) or 50 μm (C-G).

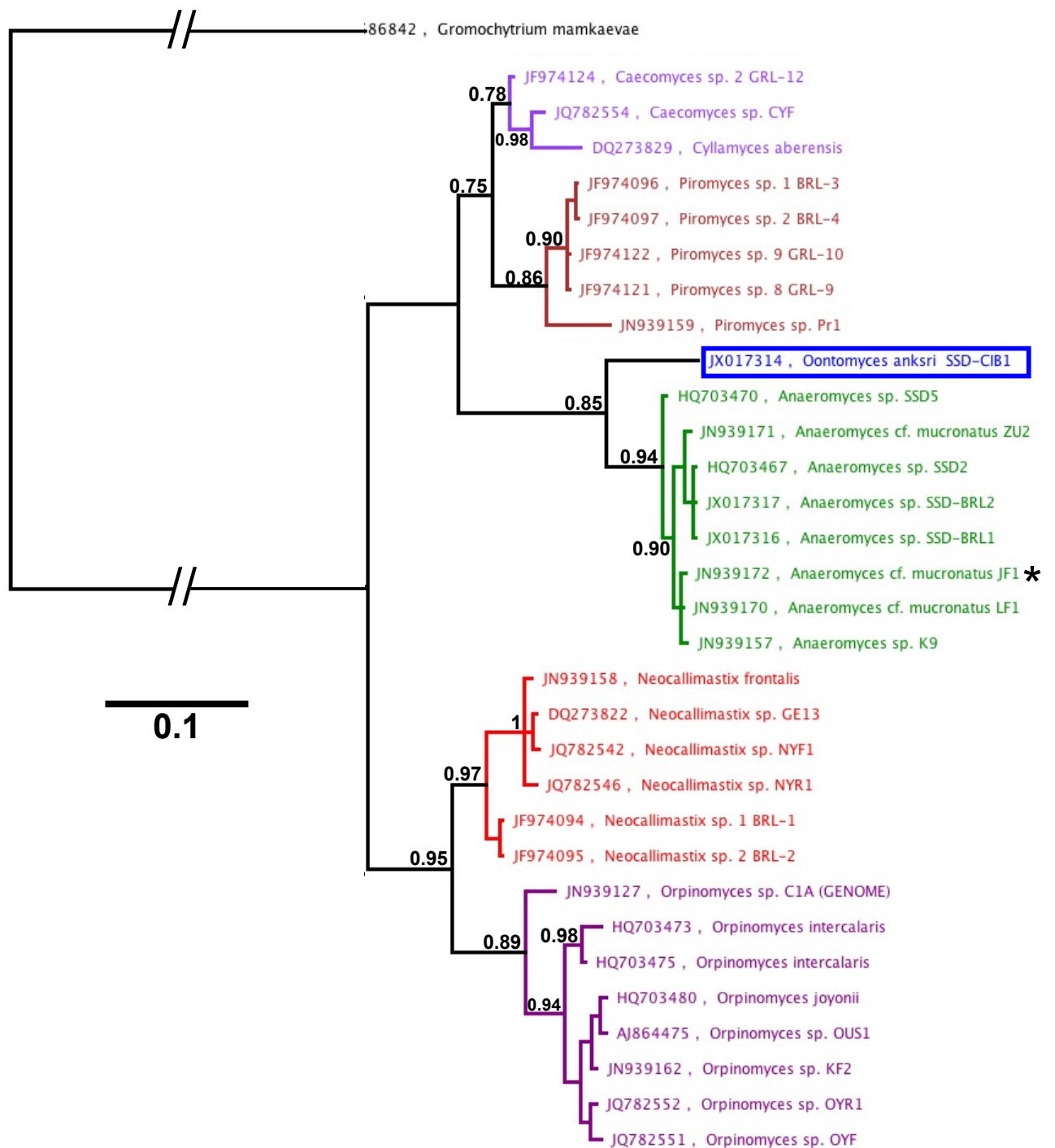


Fig. 2. Bayesian backbone analysis of LSU sequences (750 bp alignment of D1/D2 variable regions) of Neocallimastigomycota rooted with *Gromochytrium mamkaevae* (Chytridiomycota, order Gromochytriales). Bayesian posterior probabilities ≥ 0.75 are shown above the branches. The different genera of Neocallimastigomycota are shown in different coloured font with the *Oontomyces* clade in blue (and boxed). The reference sequence for *Anaeromyces* spp. is indicated by *. Scalebar indicates number of substitutions per site.

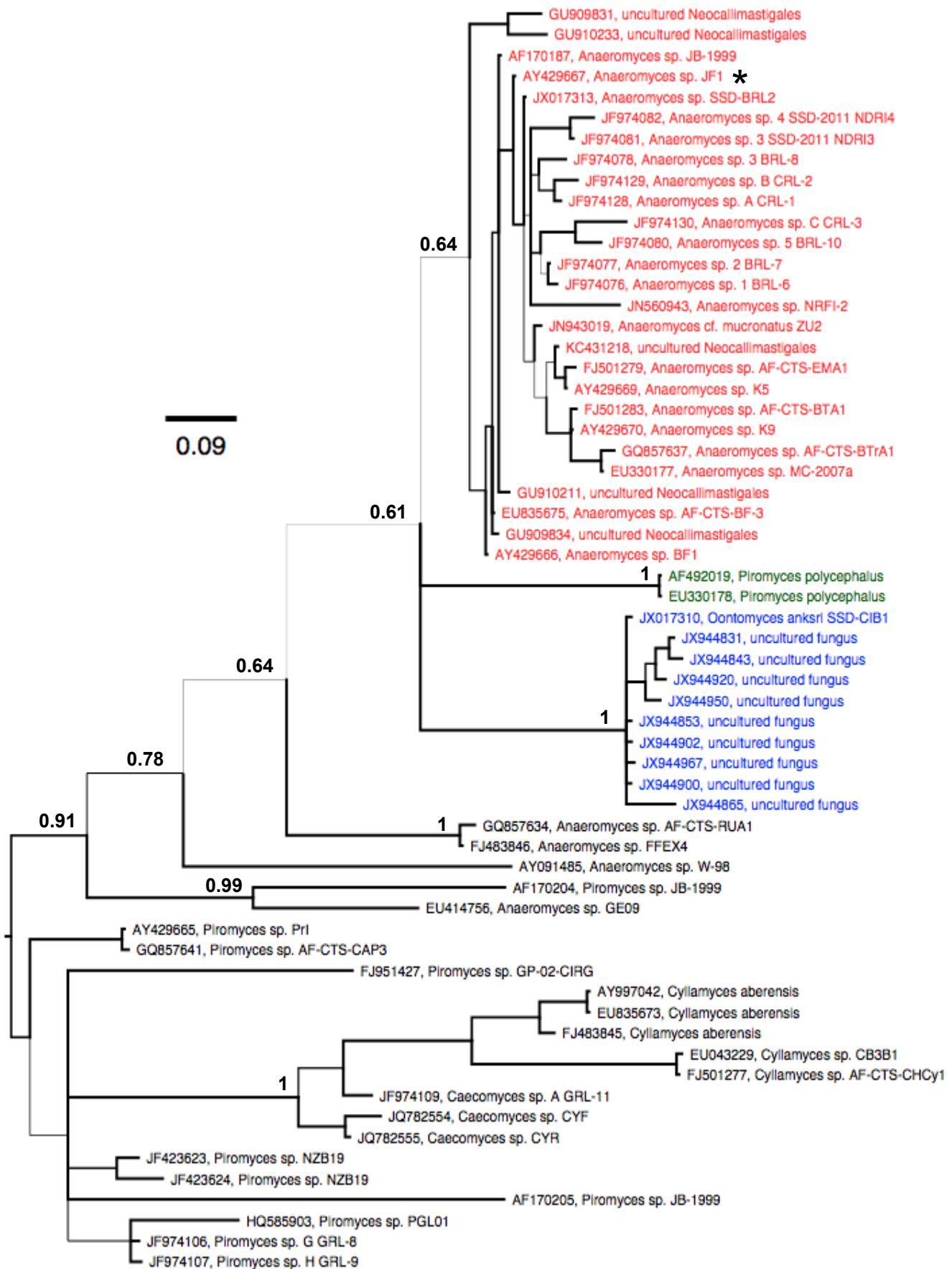
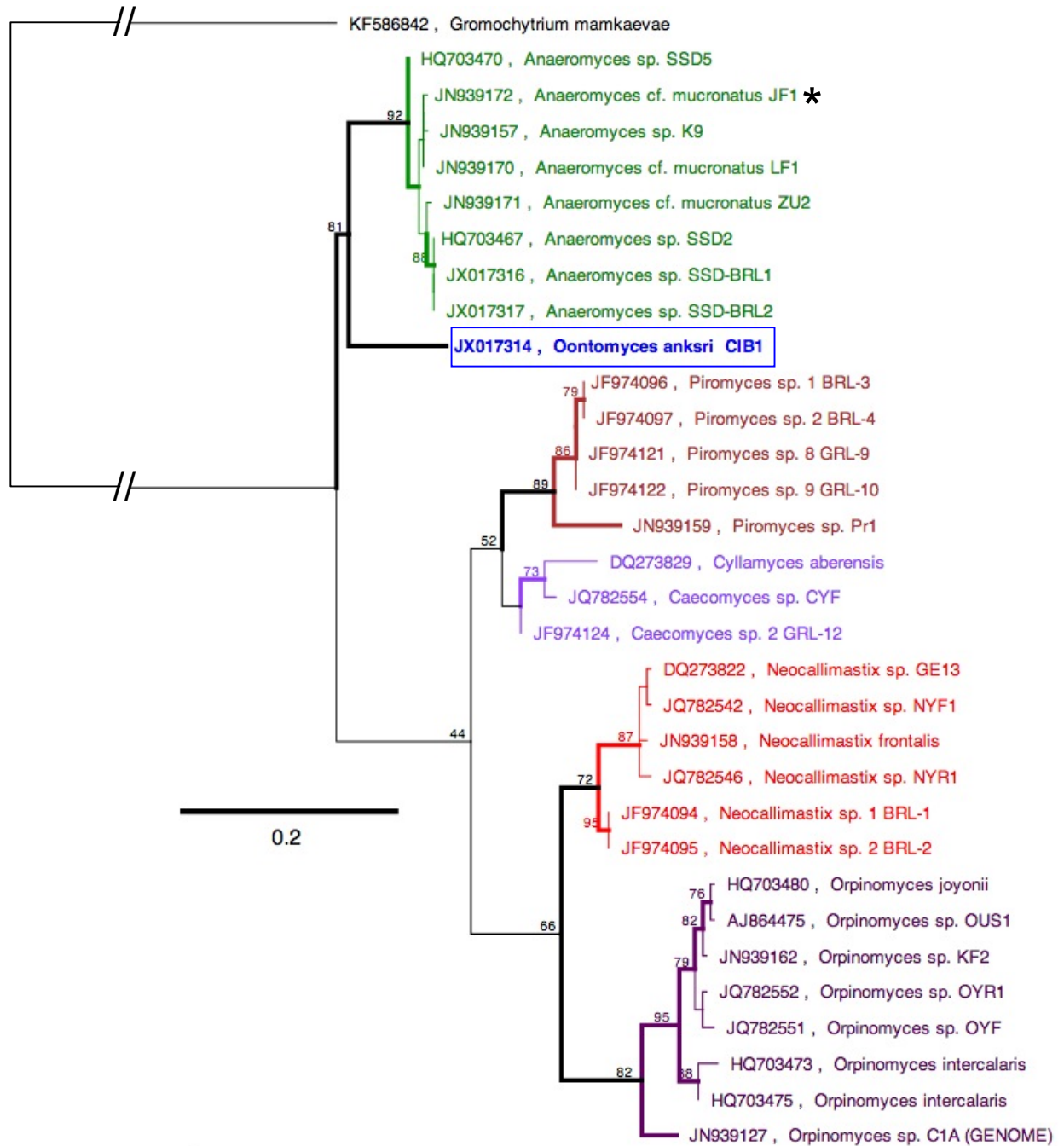
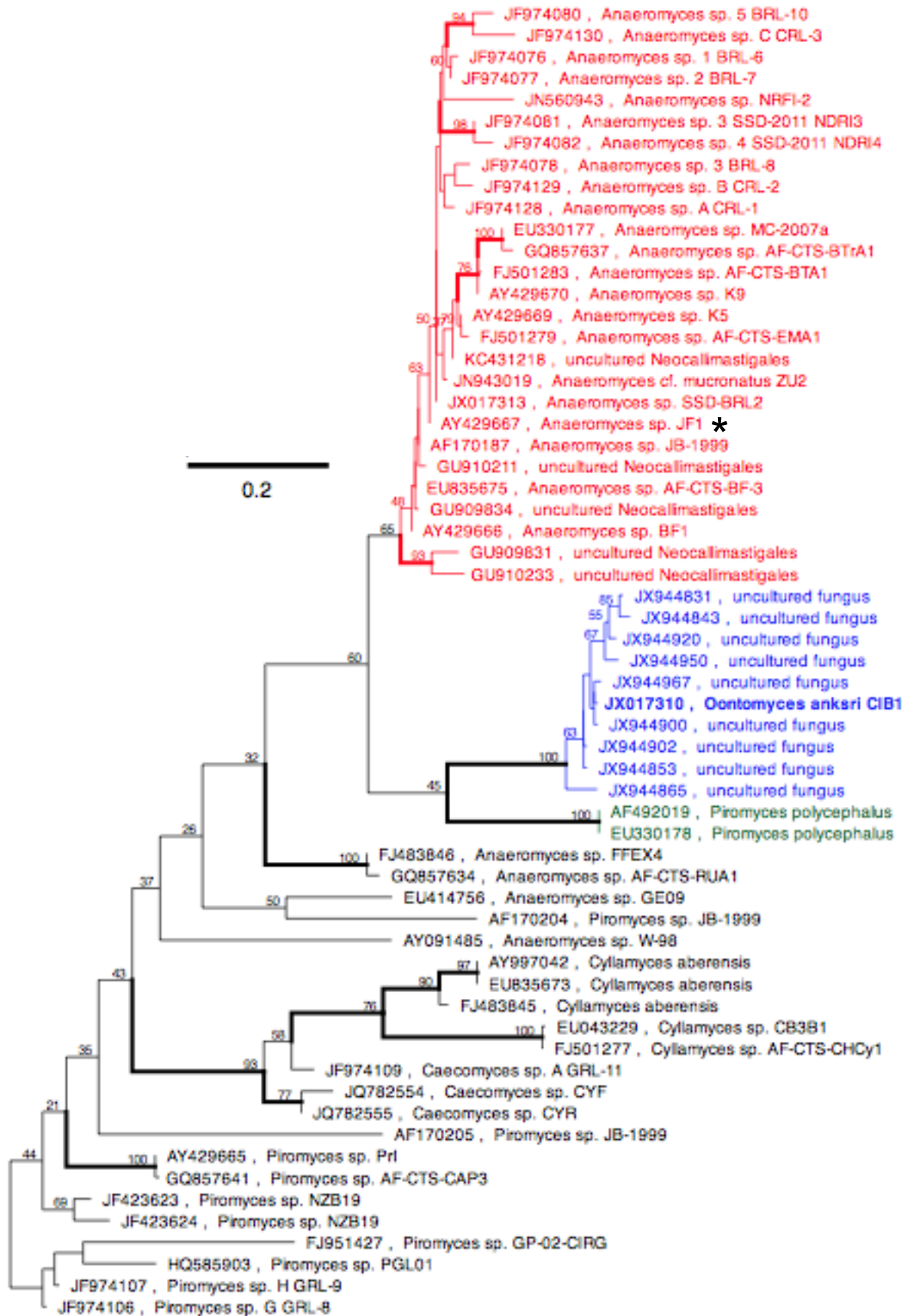


Fig. 3. Bayesian posterior probability analysis of ITS1 sequences of Neocallimastigomycota (290 bp alignment), including the genera with uniflagellate zoospores. The *Oontomyces anksri* clade is shown in blue font (*Anaeromyces* clade in red and the *P. polycephalus* clade in green). Line thickness is proportional to Bayesian posterior probabilities (thin lines = <0.7; thick lines >0.9) and PP probabilities are shown at salient nodes. * indicates the reference sequences for the genus *Anaeromyces*. Scalebar indicates substitutions per site and the tree is midpoint rooted.

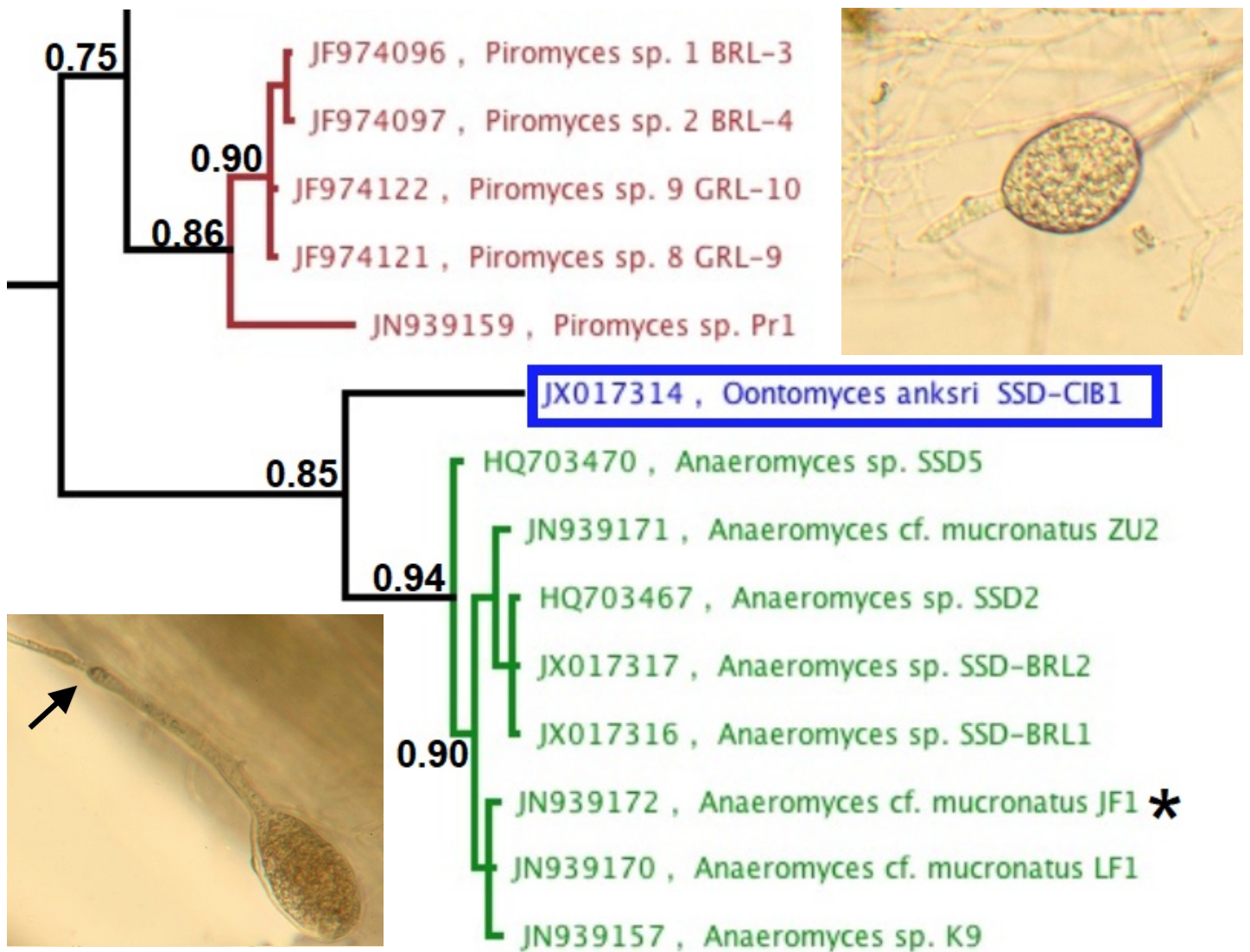


Suppdata1. Maximum Likelihood tree of LSU sequences (750 bp alignment of D1/D2 variable regions; GTR substitution model) of Neocallimastigomycota rooted with *Gromochytrium mamkaevae* (Chytridiomycota, order Gromochytriales). Salient bootstrap values (as %; 1000 bootstrap replicates) are shown at nodes. Branches with >70% bootstrap support are drawn with thick lines. * indicates the reference sequence for the genus *Anaeromyces*. Scalebar indicates substitutions per site. The different genera of Neocallimastigomycota are shown in different coloured font with the *Oontomyces* clade in blue (and boxed). The reference sequence for *Anaeromyces* spp. is indicated by *. Scalebar indicates number of substitutions per site.



Suppdata 2. Maximum Likelihood tree (GTR substitution model) of ITS1 sequences of Neocallimastigomycota (290 bp alignment), including the genera with uniflagellate zoospores. The *Oontomyces anksri* clade is shown in blue font (*Anaeromyces* clade in red and the *P. polycephalus* clade in green. Salient bootstrap values (as %; 1000 bootstrap replicates) are shown at nodes. Branches with >70% bootstrap support are drawn with thick lines. * indicates the reference sequences for the genus *Anaeromyces*. Scalebar indicates substitutions per site.

Graphical abstract



Research Highlights

- Two Neocallimastigomycota cultures were obtained from camel forestomach
- Cultures were monocentric and formed uniflagellate zoospores.
- ITS and LSU sequence analysis placed these in a distinct clade close to *Anaeromyces*
- Environmental sequences also from camel also fell into this clade
- This new fungus is formally named *Oontomyces anksri* gen. nov., sp. nov.