Hysterangium bonobo: A newly described truffle species that is eaten by bonobos in the Democratic Republic of Congo

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1   **Short title:** Bonobo mycophagy

2   *Hysterangium bonobo*: **a newly described truffle species that is eaten by bonobos in the**

3   **Democratic Republic of Congo**

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24   **ABSTRACT**

25   Many animals have been shown to eat fungi, and most truffle-like fungi depend on animals for

26   spore dispersal via mycophagy. Although these interactions are widespread, they are

27   understudied in many habitats. In this study, we show that bonobos (*Pan paniscus*) forage and

28   feed on an undescribed truffle species in the rainforests of the Democratic Republic of Congo.

29   Based on morphological and molecular assessment of collections, we show that the species eaten

30   by bonobos is a previously undescribed taxon described here as *Hysterangium bonobo.* This

31   species is known in the local Bantu language (Bongando) as simbokilo and is used for baiting

32   traps to catch several species of small mammals. Our findings highlight the need for further

33   research into mycophagy and systematics of sequestrate fungi in Africa.

34   **KEY WORDS:** African fungi, Hysterangiales, Phallomycetidae, primate mycophagy, truffle

35   taxonomy, 1 new taxon

36   **INTRODUCTION**

37   Fungi play a significant role in the diets and nutrition of diverse vertebrates. Many fungi,

38   particularly truffle-like taxa, have evolved close associations with animals that help to disperse

39   spores via mycophagy (Fogel and Trappe 1978; Elliott et al. 2019a, b). These animal-fungus

40   associations are frequently overlooked, but they are an important part of functional ecosystems

41   and imperative for the dispersal of fungi through these systems. There is a growing interest in

42   better understanding the ecological significance of these associations. Mycophagy is nutritionally

43   important for animals and simultaneously aids dispersal of mycorrhizal fungi (Cázares and

44   Trappe 1994; Hussain and Al-Ruqaie 1999; Colgan and Claridge 2002; Kalač 2009; Wallis et al.

45   2012). These interconnected associations have been studied in various parts of the world but

46   have been mostly overlooked in Africa.

47   The specialization of these associations varies depending on the fungal taxa and types of

48   animals that consume and disperse them. Most studies of vertebrate mycophagy have focused on

49   small mammals in regions outside of Africa (Fogel and Trappe 1978; Claridge and May 1994).

50   In the Middle East and arid North Africa, some bird species are known to feed on truffle-like

51   fungi and may play important roles in the health of desert ecosystems (Elliott et al. 2019b), but in

52   Sub-Saharan Africa only a few vertebrate species have been reported as mycophagous. There are

53   a handful of reports of African primates eating fungi (Kano and Mulavwa 1984; Hanson et al.

54   2003 and references therein; Isbell and Young 2007; Georgiev et al. 2010; Georgiev et al. 2011;

55   Buyck et al. 2016). Among African vertebrates other than primates, four mammal species (Elliott

56   et al. 2019c) and eleven terrestrial turtle species (Elliott et al. 2019a) have been reported to eat

57   fungi. Buyck et al. (2016) noted that animals in the Central African Republic, including different

58   species of duikers, primates, and wild pigs, all consumed *Elaphomyces*. However, this study did

59   not provide the genera and species of the African mycophagous mammals that were observed.

60   As has been shown in other regions, this paucity of mycophagy reports is most likely due

61   to insufficient sampling or the use of methods that do not detect fungal spores. The low number

62   of reports does not necessarily mean that Africa has a low diversity of mycophagous animals

63   (Elliott and Vernes 2019; Elliott et al. 2019b). For many vertebrates it can be difficult to

64   determine whether or not fungi are regularly ingested solely by observing their feeding behavior.

65   Microscopic and/or DNA analysis of feces are useful techniques to determine the presence and

66   diversity of fungi in the diet. It is clear that these methods need to be more thoroughly utilized to

67   study the diets of vertebrates in Africa.

68   Schmitt and Mueller (2007) documented 2,250 described species of macrofungi from

69   Africa (Schmit and Mueller 2007). Mammals on other continents have been shown to eat a wide

70   variety of morphologically diverse fungi (Fogel and Trappe 1978; Claridge and May 1994;

71   Nuske et al. 2017). Fungi that produce sequestrate (enclosed) and/or hypogeous (below ground)

72   fruiting bodies are presumably more dependent on animal vectors than non-sequestrate species

73   because sequestrate and/or hypogeous taxa typically lack the ability to forcibly discharge spores

74   (Trappe et al. 2009). There are more than a dozen species of sequestrate fungi reported from

75   Sub-Saharan Africa (Dissing and Lange 1962; Dring and Pegler 1978; Castellano et al. 2000;

76   Eberhardt and Verbeken 2004; Beenken et al. 2016; Castellano et al. 2016a, b; Orihara and

77   Smith 2017). Several species have also been reported from the arid Kalahari desert and the

78   surrounding region of southern Africa (Taylor et al. 1995; Trappe et al. 2008; Trappe et al.

79   2014). Many of these taxa have been recently described and occur in habitats where primates are

80   known to forage, but no studies have carefully examined the mycophagy of sequestrate fungi by

81   primates or many other African vertebrates. Here we provide new observations of bonobo

82   mycophagy and show that the fungus consumed by these apes is a previously undescribed

83   species of truffle named here as *Hysterangium bonobo* sp. nov..

84   **MATERIALS AND METHODS**

85   *Animal observations*. — The feeding ecology of the Hali-Hali bonobos (Georgiev et al. 2011), a

86   wild unprovisioned community, was studied at the Nsondo Camp (0°12'N, 22°51'E),

87   Kokolopori Bonobo Reserve, Province Equateur, Democratic Republic of Congo from Oct 2006

88   through Jul 2007 with experienced field assistants familiar with the bonobos and the ecology of

89   the forest (Georgiev et al. 2010; Georgiev et al. 2011). The study site is located approx. 30 km

90   east of a long-term bonobo study site at Wamba and shared many of its ecological features

91   (Hashimoto et al. 1998; Kano and Mulavwa 1984). Three main habitat types are present: 1) dry

92   primary forest with portions dominated by trees in Fabaceae subfam. Detarioideae, including

93   ectomycorrhizal (ECM) species of *Gilbertiodendron* and *Brachystegia*, 2) seasonally inundated,

94   riparian swamp forest where *Guibourtia demeusei* (Detarioideae) and ECM *Uapaca* spp.

95   (Phyllanthaceae) are common, and 3) secondary disturbed forest heavily influenced by slash-

96   and-burn agriculture expanding from nearby villages. The seasonality of precipitation at

97   Kokolopori is similar to Wamba, which typically has up to 2900 mm of rainfall per year during

98   one light rainy season from Mar – May and a heavier rainy season from Sep – Nov (Hashimoto

99   et al. 1998; Mulavwa et al. 2008).

100   The Hali-Hali bonobos have been habituated since 2000 by local conservation NGO Vie

101   Sauvage with support from the Bonobo Conservation Initiative (BCI). By Oct 2006, the apes

102   were sufficiently accustomed to human presence to allow behavioral observations on a regular

103   basis. Although the apes allowed detailed monitoring when feeding in the canopy, ground

104   observations were less frequent because they did not always tolerate close human proximity

105   during ground foraging. Observational conditions on the forest floor were also limited to 15–20

106   m or less by dense understory vegetation. The bonobo truffle foraging data presented here are

107   thus considered a conservative estimate of truffle consumption.

108   The diet of the bonobos was scored at 15-min intervals by recording food species and

109   plant parts (if any) eaten by the majority of individuals in view on the sampling point. A 'running

110   food list' was also recoded on a daily basis to note all foods the bonobos were seen to ingest,

111   whether they happened on the 15-min sample point or not (Georgiev et al. 2010; Georgiev et al.

112   2011). We presented data on truffle consumption via a simple dietary score to document the

113   frequency of truffle-eating over the study period, calculated as the monthly proportion of days on

114   which at least one episode of truffle-eating was seen from the total number of days on which

115   bonobos were observed and feeding data were recorded. In some cases it was possible to directly

116   observe truffle consumption by bonobos. At other times, truffle feeding was inferred because the

117   bonobos were feeding on the ground and then observers moved into the area after the bonobos

118   began to move away and were able to observe digging and discarded pieces of truffle

119   basidiomata. In Aug 2007 we were able to directly view bonobos consuming basidiomata and

120   then to collect fresh specimens when they were done feeding (see below).

121   *Morphological studies*. — Four basidiomata were collected from the exact location where

122   bonobos were observed to be feeding on truffles. Specimens were preserved in 99% ethanol. The

123   collection has been accessioned at the Fungal Herbarium of the Florida Museum of Natural

124   History as FLAS-F-64335. Field collected truffles from the Kokolopori bonobo site (FIG. 1)

125   were also directly compared with herbarium specimens from the Oregon State University

126   Mycological Collection (OSC) of *Aroramyces radiatus* (Lloyd) Castellano, Verbeken &

127   Walleyn, one of the only related truffle species known from tropical Africa (see below).

128   Descriptions of macromorphological characters were based on fresh material and photos.

129   Colors were described in general terms. Microscopic characters were examined based on hand

130   sectioned dried tissues rehydrated in Melzer's reagent, 3% KOH, and water. Photomicrographs

131   were taken in water. Basidiospore measurements are based on 20 randomly selected

132   basidiospores. Given the remote nature of the region, logistical difficulties of returning to the

133   site, and the infrequence of hypogeous fungal collections in the region, we were unfortunately

134   forced to base the description of this new species on a single collection.

135   *Molecular methods and phylogenetic analyses*. — Clean fungal tissues were taken from inside

136   the dried specimens that had been previously preserved in ethanol. DNA was extracted using a

137   modified CTAB method (Gardes and Bruns 1993). Amplification of the nuclear rDNA ITS1-

138   5.8S-ITS2 (ITS) region was performed using forward primer ITS1F and reverse primer ITS4

139   (White et al. 1990) and the Phusion Hot Start Flex DNA Polymerase standard protocol (New

140   England BioLabs Inc., Ipswich, Massachusetts). Amplification of a portion of nuc 28S rDNA

141   (28S) was performed using the same protocol with forward primer LROR and reverse primer

142   LR3 (Hopple and Vilgalys 1994). PCR products were visualized on 1.5% agarose gels stained

143   with SYBR Green I (Molecular Probes, Eugene, Oregon). Amplicons were cleaned with EXO

144   (Exonuclease I) and SAP (shrimp alkaline phosphatase) enzymes (Werle et al. 1994) and

145   sequenced by GENEWIZ (South Plainfield, New Jersey). Sequences were then edited with

146   SEQUENCER 5.0.1 (Gene Codes Inc., Ann Arbor, Michigan). The ITS and 28S sequences

147   from our *Hysterangium* collection were compared with those in the NCBI database using the

148   BLASTn tool (Altschul et al. 1990).

149   For phylogenetic analysis, the 28S sequences of FLAS-F-64335 were placed in an

150   alignment of 28S and mitochondrial ATP synthase membrane subunit 6 (*ATP6*) DNA sequences

151   of Hysterangiales and allied fungi in Phallomycetidae previously generated by Hosaka et al.

152   (2006, 2008). We were unable to obtain *ATP6* sequences from FLAS-F-64335. Sequences were

153   downloaded directly from GenBank and aligned in MESQUITE 3.2 (Maddison and Maddison

154   2018) with the aid of MUSCLE 3.8.31 (Edgar 2004). Independent analyses of 28S and *ATP6*

155   showed no conflicting phylogenetic signal (data not shown), so the two loci were combined into

156   a single concatenated analysis. The alignment was edited manually to exclude gaps and

157   ambiguously aligned regions.

158   The concatenated alignment was analyzed with maximum likelihood (ML) and Bayesian

159   Inference (BI) as performed in the Cyberinfrastructure for Phylogenetic Research Science

160   Gateway (CIPRES) 3.1 (Miller et al. 2010). ML was run via RAXML 8.2.10 (Stamatakis 2014)

161   with 1000 bootstrap iterations and a GTRGAMMA model under the default paramaters

162   (Stamatakis 2015). BI was performed in MRBAYES 3.2.7a (Ronquist et al. 2012) using the

163   GTR+I+G model following Hosaka et al. (2008). BI analysis was run on two separate chains

164   using a chain length of one million generations, sampling frequency of 1000, and discarding the

165   first 25% of the samples as the burn-in. The multilocus ML tree was visualized and rooted in

166   FIGTREE 1.4.3 (Rambaut 2016) and Bayesian posterior probability (PP) values were added in

167   ADOBE ILLUSTRATOR CS5.1 (San Jose, California). Nodes were considered strongly

168   supported if ML bootstrap values were ≥75% and PP≥0.95.

169   **RESULTS**

170   *Truffle consumption by bonobos*. — Between Nov 2006 and Jul 2007 bonobos were observed

171   on 155 days (range: 12–26 days per month). Truffles were eaten on 38 observation days in 7 out

172   of the 9 study months on a mean of 4.2 days per month (range: 0–10 days), or for a mean of

173   23.1% of monthly observation days (range: 0.0–52.9% of monthly observation days). Truffles

174   were, however, a minor element of the diet in terms of their overall contribution to feeding

175   observations (less than 3% of all 15-min, group-level feeding scans for all months).

176   *BLASTn and phylogenetic analysis*. — BLASTn analysis of both the ITS and 28S sequences

177   generated from FLAS-F-64335 showed clear affinities with other species of ECM Hysterangiales

178   and related Phallomycetidae. The ITS from FLAS-F-64335 had the highest homology with

179   uncultured Hysterangiales sequences from ECM roots (e.g., KT461360 from an unknown

180   miombo woodland tree from the DRC; KM402914 from *Pseudotsuga menziesii* from British

181   Colombia) as well as specimens of *Hysterangium* (e.g., DQ974810) and *Ramaria* (e.g.,

182   FJ627035). However, the highest hits were only 83–84% similar to the ITS sequence from

183   FLAS-F-64335. The 28S also exhibited obvious affinities with ECM Hysterangiales, including

184   92–93% similarity to a wide range of Phallomycetidae such as species of *Hysterangium*,

185   *Ramaria*, and *Austrogautieria* (e.g., AF336259, JQ408235, KP191776), as well as ECM root tips

186   of Hysterangiales (e.g., JX316465 from a root of *Nothofagus pumilio* from Argentina).

187   Our alignment included 770 nucleotides of aligned 28S sequences and 691 nucleotides of

188   aligned *ATP6* sequences from 171 taxa. Of these, 76 nucleotides were excluded from the 28S

189   portion of the alignment and 31 nucleotides were excluded from the *ATP6* alignment. The ML

190   phylogeny (FIG. 2) depicts FLAS-F-64335 nested among ECM *Hysterangium* and resolved in a

191   clade separate from *Aroramyces radiatus*, the only other described ECM Hysterangiales from

192   tropical Africa. The analyses also revealed that *Aroramyces* is a strongly supported monophyletic

193   group nested within *Hysterangium,* making the latter paraphyletic. This result was previously

194   found by Hosaka et al. (2006, 2008).

195   **TAXONOMY**

196   ***Hysterangium bonobo*** M.E. Sm. & T.F. Elliott, sp. nov.   FIG. 2

197   MycoBank MB834363

198   *Typification*: DEMOCRATIC REPUBLIC OF THE CONGO (DRC). TSHUAPA

199   PROVINCE: Djolu Territory, Kokolopori Bonobo Reserve, in mixed rainforest with

200   ectomycorrhizal trees in the genera *Uapaca* (Phyllanthaceae), *Brachystegia* (Fabaceae), and

201   *Gilbertiodendron* (Fabaceae), Aug 2007, *A. Georgiev MES-127* (**holotype** FLAS-F-64335).

202   GenBank: ITS+28S = MT111903.

203   *Etymology*: *bonobo*, in reference to the common name of the primate *Pan paniscus*,

204   which digs and eats this fungus.

205   *Description*: Basidiomata hypogeous to partially emergent, up to 50 mm broad, more or

206   less globose to irregularly globose. Peridium up to 2 mm thick, light to dull brown, apparently

207   bruising brown when damaged or handled, sometimes cracked, smooth, not easily separable

208   from the gleba, with a somewhat hairy appearance in patches. Gleba dark brown, solid, with

209   narrow meandering hollow veins and small open pockets that are not gel-filled, radiating from

210   indistinct off-white columella less than 1 mm wide at the base but becoming indistinct as it

211   radiates upward through the center of the gleba.

212   Peridium 70–150 μm thick, comprised of two layers; outer layer 10–65 μm thick,

213   composed of interwoven hyphae 3–5 μm wide, hyphae in the outer layer notably darker in color

214   than the inner layer and prominently encrusted with irregular warts 2 × 4 μm or larger, peridial

215   cystidia not observed, clamp connections rare or absent, debris sometimes adhering to the outer

216   layer. Inner peridial layer up to 130 μm thick, composed of thin-walled, tightly packed, highly

217   interwoven hyphae 1–3(–5) μm wide. Gleba trama of tightly interwoven and unorganized,

218   hyaline, gelatinized hyphae 0.5–1.5 μm wide, forming a layer mostly 20–40(–55) μm broad.

219   Columella composed of hyaline interwoven hyphae mostly 1–2 μm wide, up to 100 μm thick at

220   the base but rapidly decreasing in width as it radiates out into the gleba tissues.

221   Basidiospores 14–16 μm × 8.5–10 μm (mean 15 × 9.5 μm), basidiospore walls 1–2 (–3)

222   μm thick, $Q = 1.40–1.68$, mean $Q = 1.58$, brown in mass, spore wall thickness often irregular but

223   in most spores the walls notably thicker toward the apical end of the spores near the attachment

224   to the sterigmata, ornamentation of very short and somewhat indistinct spines or warts (less than

225   0.5 μm tall), apical attachments notable and sometimes with a piece of the sterigma broken off

226   from the basidium and still attached to the spore at maturity, faint oil droplets apparent in some

227   spores, spores somewhat dextrinoid in Melzer's reagent. Basidia mostly 2-sterigmate, difficult to

228   view, deflated and irregularly shaped, apparently collapsing after spore dehiscence; sterigmata

229   irregular in shape, visibly running through the spore wall to attach to the spore apex, 2–5 μm

230   long and 1–2 μm wide.

231   *Notes*: One species of *Hysterangium* and one species of *Aroramyces* (Hysterangiales)

232   have been previously described from Sub-Saharan Africa, *H. niger* Lloyd and *A. radiatus*.

233   *Hysterangium niger* was originally described from South Africa but is morphologically divergent

234   from all other species of *Hysterangium.* It was transferred by Zeller and Dodge (1929) to

235   *Rhizopogon*. We suspect that it is a *Rhizopogon* species introduced to South Africa with pine

236   seedlings, but the type has not been recently studied. However, based on microscopic and

237   macroscopic morphology it is clearly not conspecific with *H. bonobo.*

238   *Aroramyces radiatus* was originally described as *Hymenogaster radiatus* Loyd but has

239   also been placed in *Dendrogaster* Buchholtz and *Gymnoglossum* Massee (Castellano et al. 2000).

240   The genus *Dendrogaster*, however, is currently considered a synonym of *Hymenogaster*

241   (Castellano et al. 2000), and the identity of *Gymnoglossum* remains unverified by sequence data

242   (viz, there are no publicly available sequences in GenBank from the Australian type species

243   *Gymnoglossum stipitatum* Massee). Accordingly, Castellano et al. (2000) established the genus

244   *Aroramyces* to accommodate two species, *Aroramyces gelatinosporus* (J.W. Cribb) Castellano

245   and *A. radiatus*. The genus was morphologically characterized by brown ornamented spores and

246   brownish gleba, the presence of a columella, a multi-layered peridium, and gelatinized tissues.

247   Castellano et al. (2000) reported *A. radiatus* with spores that are strongly truncate at the base of

248   the sterigmata attachment and covered by a wrinkled utricle that conceals the spore ornaments

249   when viewed with SEM. Although Castellano et al. (2000) considered *A. radiatus* in the

250   Cortinariaceae, subsequent phylogenetic studies indicate that *A. radiatus* is a member of

251   Hysterangiales, and that *Aroramyces* is nested within *Hysterangium* (Hosaka et al. 2008).

252   Accordingly, *Hysterangium* is currently a paraphyletic genus and additional taxonomic revisions

253   are needed but are beyond the scope of this study (Hosaka et al. 2008).

254   The type of *H. bonobo* (FLAS-F-64335) from a bonobo foraging site at the Kokolopori

255 Bonobo Reserve is superficially similar to the descriptions of *A. radiatus* in Castellano et al.

256 (2000) and also our direct observations of specimens cited by Castellano et al. (2000) from

257 Zimbabwe. Both species are from tropical Africa and also have a brown gleba, broadly ellipsoid

258 spores and irregular basidia that collapse at maturity. However, the spores of *A. radiatus* are

259 much smaller than those of *H. bonobo* (mean of 10.8×6.9 μm in *A. radiatus* versus 15×9.5 μm

260 in *H. bonobo*), and the spore ornaments of *A. radiatus* are much larger and more notable than in

261 *H. bonobo*. Furthermore, the spore walls are notably thicker near the attachment to the sterigmata

262 in *H. bonobo*, whereas in *A. radiatus* the spores usually taper the opposite direction and are most

263 narrow near the attachment to the sterigmata. The two species also differ in the peridium

264 morphology. Although both species have encrusted hyphae on the outer peridial layer, *A.*

265 *radiatus* has a 3-layered peridium up to 400 μm thick, whereas *H. bonobo* has a 2-layered

266 peridium typically around 100 μm thick. Phylogenetic analysis also clearly separates these taxa

267 (FIG. 2).

268   We do not know which ECM trees are the symbiotic hosts for *H. bonobo*. However,

269 *Hysterangium* spp. are known to be obligate ECM fungi (Hosaka et al. 2008) and *H. bonobo* was

270 found in forests with several confirmed ECM host plants, including species of *Uapaca*,

271 *Brachystegia*, and *Gilbertiodendron*. Species of *Uapaca* and *Gilbertiodendron* are also known to

272 form ECM associations with several other African truffles (Castellano et al. 2016a, b; Orihara

273 and Smith 2017). Bermejo et al. (1994) also noted the presence of *Uapaca* and *Gilbertiodendron*

274 species at sites where they reported bonobos successfully foraging for unidentified truffles.

275 **DISCUSSION**

276   Many mammals rely on aromas released by mature hypogeous fungi to detect where they

277   are fruiting (Mills 1978; Maser et al. 2008; Stephens et al. 2020). Birds and animals with more

278   limited olfactory abilities may rely on visual cues, including mimicking the color or shape of

279   fruits (Beever and Lebel 2014; Elliott and Marshall 2016; Elliott et al. 2019b; Elliott and Elliott

280   2019; Elliott and Vernes 2019). Some of the truffles described from Africa are brightly colored,

281   possibly indicating that their dispersers may rely on their visibility rather than odor (Castellano et

282   al. 2000). It is difficult to determine what alerts bonobos to the presence of subterranean fungi,

283   but it is likely a combination of visual and olfactory cues. Bermejo et al. (1994) described a

284   bonobo that used olfactory cues to locate a truffle: "…standing quadrupedally, digs up the earth,

285   first with one hand, then with the other, in search of subterranean truffles. She puts her face

286   closer to the hole that she has dug and looks closely. Then she carefully puts one hand into the

287   hole and withdraws it immediately, putting her fingers to her nose to detect the scent of truffles."

288   Similarly, our observations of the Hali-Hali bonobos foraging on the ground are consistent with

289   the idea that bonobos rely on olfactory cues to detect hypogeous fungi.

290   The Hali-Hali bonobos consumed truffles on a regular basis (23% of all sampling days),

291   indicating that truffles are a component—but not a staple—in the diet of this community of

292   bonobos. *Hysterangium bonobo* was collected directly after we observed bonobos feeding on

293   truffles, therefore leading to the conclusion that bonobos consume *H. bonobo* as a food source.

294   Although we expect that bonobos may consume other truffle species in the region, further studies

295   are needed to confirm this hypothesis. Previous studies on bonobo diets in the DRC have also

296   reported that bonobos eat truffles (Yalosidi: Kano 1983; Wamba: Kano & Mulawa 1984;

297   Lilungu: Bermejo et al. 1994), but truffles have always been considered a minor component in

298   their diet. The most detailed report on bonobo truffle-eating comes from the Ikela study site,

299   where the apes consumed truffles on 18 days over a 605-day-long study, totaling 686 hrs and 47

300   min of direct observations (Bermejo et al. 1994). We saw bonobos consume truffles on 38 days

301   (from a total of 155 observation days), so it is likely that the Ikela bonobos eat fewer truffles than

302   the bonobos at Kokolopori. The identity of the truffles from previous studies have remained

303   unknown, although Kano (1983) suggested that the puffball *Langermannia fenzlii* (Reichardt)

304   Kreisel might be a food source for bonobos. However, we can find no evidence or specimens to

305   support this hypothesis. Despite being consumed infrequently and in low quantities, the

306   nutritional value of fungi can still constitute an important part of an animal's diet (Wallis et al.

307   2012).

308   Because the truffles at Ikela (Bermejo et al. 1994) were never collected or identified we

309   cannot determine whether the bonobos observed in that study consumed *H. bonobo, A. radiatus*,

310   or other truffle species. It is also possible that the truffle species at the Ikela site were less

311   appealing to bonobos or that the Ikela study occurred during seasons or years with less fungal

312   fruiting than our studies at Kokolopori. Near Kokolopori the local villagers use the Bongando

313   word "simbokilo" to refer to truffles (Takemoto 2017). There is some evidence of wider

314   consumption of simbokilo by other mammals because the truffles are used to bait traps during

315   forest hunting expeditions (Kimura et al. 2015). Simbokilo is specifically useful for trapping

316   *Cricetomys emini* (Emin's pouched rat) but has also been documented by local people to attract

317   *Atherurus africanus* (African brush-tailed porcupine), at least three species of duiker

318   (*Cephalophus monticola*, *C. callipygus*, *C. nigrifrons*), and several species of squirrels (A.

319   Lokasolac, pers. obs.). The word "simbokilo" is derived from 'simba' (don't go away) and

320   'bokilo' (brother-in-law) and derived from a longer phrase "do not allow your brother-in-law to

321   go away because there will be plenty of food coming from traps using simbokilo as a bait." This

322 etymology of simbokilo is indicative of the regular use of this truffle by local people (A.

323 Lokasolac, pers. obs.). However, as far as we know, our collection of *H. bonobo* is the only

324 collection of simbokilo that has been examined microscopically or molecularly. It therefore

325 remains unclear whether this word refers to *H. bonobo* specifically or to a suite of truffles. More

326 direct observations and collections are needed to determine whether simbokilo is one truffle

327 species or several truffle species.

328   There have been scattered reports of mycophagy among primates in Africa and other

329 parts of the world (see Hanson et al. 2003). Most studies are based on visual observations of

330 feeding, but the fungal taxa are rarely or never identified. Most reports of mycophagy give vague

331 descriptions of the macroscopic morphology of the fungi that provide little assistance to

332 taxonomists, e.g. "bracket fungi consumed by gorillas" (Fossey 1983). We urge zoologists

333 working with animal diets in the future to collect, photograph, and preserve voucher specimens

334 of the fungal taxa eaten in order to allow for more in-depth taxonomic studies by fungal

335 biologists.

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527   **FIGURE LEGENDS and FOOTNOTES**

528   **Figure 1**. Morphological features of the holotype collection of *Hysterangium bonobo* collected

529   in a bonobo foraging site after bonobos had recently been feeding. A. Fresh fruiting body of *H.*

530   *bonobo* in hand, note the slight brownish discoloration from handling. B. Broken *H. bonobo*

531   fruiting body revealing brown gleba and columella at the center. C. Peridial hyphae with brown

532   encrusted warts. D. Overview of the gleba showing locules of hymenial tissue lined with basidia

533   and basidiospores interleaved by tramal plates composed of densely interwoven sterile hyphae.

534   Interwoven peridial hyphae on the inner surface of the peridium is visible in the upper left-hand

535   corner of the image. E. Thick-walled basidiospores that are still attached to narrow basidia are

536   visible at the far left and right of the image. Note that the sterigma appear to grow through the

537   thick cell walls. The basidiospore in the center of the image shows the fine ornamentation that is

538   present on mature basidiospores at high magnification. Bars: A–B = 2 cm; C, E = 10 μm, D = 30

539   μm.

540   **Figure 2**. ML phylogenetic tree of Hysterangiales and other fungi in the Phallomycetidae based

541   on analysis of 28S and *ATP6* showing phylogenetic placement of *Hysterangium bonobo* sp. nov.

542   within the genus *Hysterangium*. Note that *H. bonobo* is resolved in a clade that is

543   phylogenetically distant from *Aroramyces radiatus*, the only other described species of

544   Hysterangiales from tropical Africa. Several taxa in Agaricales, Thelephorales, and Boletales

545   served as outgroups. Support values are shown above the nodes using the following format: ML

546   bootstrap values ≥75% / posterior probabilities ≥0.95.

547