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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**

4

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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 (Schmitt 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 *Aroramycetes radiatus* (Lloyd) Castellano, Verbeken &
127 Walley, 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 infrequency 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 SEQUENCHER 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 parameters

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 $\geq 75\%$ and $PP \geq 0.95$.

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 $2 \times 4 \mu\text{m}$ 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 $\mu\text{m} \times 8.5$ –10 μm (mean 15 \times 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* Lloyd but has
239 also been placed in *Dendrogaster* Buchholtz and *Gymnoglossum* Masee (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* Masee). 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 $10.8 \times 6.9 \mu\text{m}$ in *A. radiatus* versus $15 \times 9.5 \mu\text{m}$
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 $400 \mu\text{m}$ thick, whereas *H. bonobo* has a 2-layered
266 peridium typically around $100 \mu\text{m}$ 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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355 **LITERATURE CITED**

356 Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool.
357 *Journal of Molecular Biology* 215:403–410.

358

359 Beever RE, Lebel T. 2014. Truffles of New Zealand: a discussion of bird dispersal
360 characteristics of fruit bodies. *Auckland Botanical Society Journal* 69:170–178.

361

362 Beenken L, Sainge MN, Kocyan A. 2016. *Lactarius megalopterus*, a new angiocarpous species
363 from a tropical rainforest in Central Africa, shows adaptations to endozoochorous spore
364 dispersal. *Mycological Progress* 15:1–10.

365

- 366 Bermejo M, Illera G, Pí JS. 1994. Animals and mushrooms consumed by bonobos (*Pan*
367 *paniscus*): new records from Lilungu (Ikela), Zaire. *International Journal of Primatology* 15:879–
368 898.
- 369
- 370 Buyck B, Hosaka K, Masi S, Hofstetter V. 2016. Molecular analyses of first collections of
371 *Elaphomyces* Nees (Elaphomycetaceae, Eurotiales, Ascomycota) from Africa and Madagascar
372 indicate that the current concept of *Elaphomyces* is polyphyletic. *Cryptogamie Mycologie* 37:3–
373 14.
- 374
- 375 Cázares E, Trappe JM. 1994. Spore dispersal of ectomycorrhizal fungi on a glacier forefront by
376 mammal mycophagy. *Mycologia* 86:507–510.
- 377
- 378 Castellano MA, Elliott TF, Truong C, Séné O, Dentinger B, Henkel TW. 2016a. *Kombocles*
379 *bakaiana* gen. sp. nov. (Boletaceae), a new sequestrate fungus from Cameroon. *IMA fungus*
380 7:239–245.
- 381
- 382 Castellano MA, Dentinger B, Séné O, Elliott TF, Truong C, Henkel TW. 2016b. New species of
383 *Elaphomyces* (Elaphomycetaceae, Eurotiales, Ascomycota) from tropical rainforests of
384 Cameroon and Guyana. *IMA fungus* 7:59–73.
- 385
- 386 Castellano MA, Verbeken A, Walley R, Thoen D. 2000. Some new and interesting sequestrate
387 Basidiomycota from African woodlands. *Karstenia* 40:11–21.
- 388

- 389 Claridge AW, May TW. 1994. Mycophagy among Australian mammals. *Austral Ecology*
390 19:251–275.
391
- 392 Colgan W, Claridge AW. 2002. Mycorrhizal effectiveness of *Rhizopogon* spores recovered from
393 faecal pellets of small forest-dwelling mammals. *Mycological Research* 106:314–320.
394
- 395 Dissing H, Lange M. 1962. Gasteromycetes of Congo. *Bulletin de le Jardin Botanique de l’Etat*,
396 32:325–416.
397
- 398 Dring DM, Pegler DN. 1978. New and noteworthy gasteroid relatives of the Agaricales from
399 tropical Africa. *Kew Bulletin* 32:563–569.
400
- 401 Eberhardt U, Verbeken A. 2004. Sequestrate *Lactarius* species from tropical Africa: *L.*
402 *angiocarpus* sp. nov. and *L. dolichocaulis* comb. nov. *Mycological research* 108:1042–1052.
403
- 404 Elliott TF, Bower DS, Vernes K. 2019a. Reptilian mycophagy: A global review of mutually
405 beneficial associations between reptiles and macrofungi. *Mycosphere* 10:776–797.
406
- 407 Elliott TF, Jusino MA, Trappe JM, Lepp H, Ballard G, Bruhl JJ, Vernes K. 2019b. A global
408 review of the ecological significance of symbiotic associations between birds and fungi. *Fungal*
409 *Diversity* 98:161–194.
410

411 Elliott TF, Truong C, Séné O, Henkel TW. 2019c. Animal-fungal Interactions 3: First report of
412 mycophagy by the African Brush-tailed Porcupine *Atherurus africanus* Gray, 1842 (Mammalia:
413 Rodentia: Hystricidae). *Journal of Threatened Taxa* 11:13415–13418.

414

415 Elliott TF, Elliott K. 2019. Vertebrate consumption and dispersal of the Nothofagaceae
416 associated ascomycete *Cyttaria*. *Austral Ecology* 44:747–751.

417

418 Elliott TF, Vernes K. 2019. Superb lyrebird mycophagy, truffles, and soil disturbance. *Ibis*
419 161:198–204.

420

421 Elliott TF, Marshall PA. 2016. Animal-Fungal interactions 1: Notes on bowerbird's use of fungi.
422 *Australian Zoologist* 38:59–61.

423

424 Fogel R, Trappe JM. 1978. Fungus consumption (mycophagy) by small mammals. *Northwest*
425 *Science*. 52:1–31.

426

427 Fossey D. 2000. *Gorillas in the Mist*. Houghton Mifflin Harcourt. Boston, New York. 326 p.

428

429 Georgiev AV, Emery Thompson M, Lokasola AL, Wrangham RW. 2011. Seed predation by
430 bonobos (*Pan paniscus*) at Kokolopori, Democratic Republic of the Congo. *Primates* 52:309–
431 314.

432

- 433 Georgiev AV, Lokasola AL, Nkanga L, Lokondja A, Nsala J, Likenge J, Ilanga-Bomanga A,
434 Likenge JP. 2010. New observations of the terrestrial holoparasite *Chlamydothyrium aphyllum*
435 Mildbr. and its consumption by bonobos at Kokolopori, Democratic Republic of Congo. African
436 Journal of Ecology. 48:849–852.
- 437
- 438 Hanson AM, Hodge KT, Porter LM. 2003. Mycophagy among primates. Mycologist 17:6–10.
- 439
- 440 Hosaka K, Castellano MA, Spatafora JW. 2008. Biogeography of Hysterangiales
441 (Phallomycetidae, Basidiomycota). Mycological Research. 112:448–62.
- 442
- 443 Hashimoto C, Tashiro Y, Kimura D., Enomoto, T., Ingmanson, E.J., Idani, G.I. and Furuichi, T.,
444 1998. Habitat use and ranging of wild bonobos (*Pan paniscus*) at Wamba. International Journal
445 of Primatology 19:1045–1060.
- 446
- 447 Hussain G, Al-Ruqaie IM. 1999. Occurrence, chemical composition, and nutritional value of
448 truffles: an overview. Pakistan Journal of Biological Sciences 2:510–514.
- 449
- 450 Isbell LA, Young TP. 2007. Interspecific and temporal variation of ant species within *Acacia*
451 *drepanolobium* ant domatia, a staple food of patas monkeys (*Erythrocebus patas*) in Laikipia,
452 Kenya. American Journal of Primatology 69:1387-1398
- 453
- 454 Kalač P. 2009. Chemical composition and nutritional value of European species of wild growing
455 mushrooms: A review. Food chemistry 113:9–16.

- 456
- 457 Kano T. 1983. An ecological study of the pygmy chimpanzees (*Pan paniscus*) of Yalosidi,
458 Republic of Zaire. *International Journal of Primatology* 4:1–31.
- 459
- 460 Kano T, Mulavwa M. 1984. Feeding ecology of the pygmy chimpanzees (*Pan paniscus*) of
461 Wamba. In: Susman RL, ed.. *The Pigmy Chimpanzees*. Plenum Press, New York. pp. 233–274.
- 462
- 463 Kimura D, Masuda H, Yamaguchi, R. 2015. Change in land use among the Bongando in the
464 Democratic Republic of the Congo. *African Study Monographs (Suppl.)* 51: 5–35.
- 465
- 466 Maser C, Claridge AW, Trappe JM. 2008. *Trees, truffles, and beasts: how forests function*.
467 Rutgers University Press, Piscataway, New Jersey, USA: Rutgers University Press.
- 468
- 469 Mills MGL. 1978. Foraging behaviour of the brown hyaena (*Hyaena brunnea* Thunberg, 1820)
470 in the southern Kalahari. *Ethology* 48:113–141.
- 471
- 472 Mulavwa M, Furuichi T, Yangozene K, Yamba-Yamba M, Motema-Salo B, Idani GI, Ihobe H,
473 Hashimoto C, Tashiro Y, Mwanza N. 2008. Seasonal changes in fruit production and party size
474 of bonobos at Wamba. In: Furuichi T, Thompson J, eds. *In: The Bonobos*. Springer, New York,
475 pp. 121–134.
- 476

- 477 Nuske SJ, Vernes K, May TW, Claridge AW, Congdon BC, Krockenberger A, Abell SE. 2017.
478 Redundancy among mammalian fungal dispersers and the importance of declining specialists.
479 *Fungal Ecology* 27:1–13.
480
- 481 Orihara T, Smith ME. 2017. Unique phylogenetic position of the African truffle-like fungus,
482 *Octaviania ivoryana* (Boletaceae, Boletales), and the proposal of a new genus, *Afrocastellanoa*.
483 *Mycologia* 109:323–332.
484
- 485 Ronquist F, Teslenko M, Mark P Van Der, Ayres DL, Darling A, Höhna S, Larget B, Liu L,
486 Suchard MA, Huelsenbeck JP. 2012. Mrbayes 3.2: Efficient bayesian phylogenetic inference and
487 model choice across a large model space. *Systematic Biology* 61:539–542.
488
- 489 Schmit JP, Mueller GM. 2007. An estimate of the lower limit of global fungal diversity.
490 *Biodiversity and Conservation* 16:99–111.
491
- 492 Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of
493 large phylogenies. *Bioinformatics*, 30:1312–1313.
494
- 495 Stamatakis, A. 2015. Using RAxML to infer phylogenies. *Current protocols in bioinformatics*
496 51: 6–14.
497

- 498 Stephens RB, Trowbridge AM, Ouimette AP, Knighton WB, Hobbie EA, Stoy PC, Rowe RJ.
499 2019. Signaling from below: rodents select for deeper fruiting truffles with stronger volatile
500 emissions. *Ecology*, e02964.
- 501
- 502 Takemoto, H. 2017. Acquisition of terrestrial life by human ancestors influenced by forest
503 microclimate. *Scientific Reports* 7:1–8.
- 504
- 505 Taylor FW, Thamage DM, Baker N, Roth-Bejerano N, Kagan-Zur V. 1995. Notes on the
506 Kalahari desert truffle, *Terfezia pfeilii*. *Mycological Research* 99:874–878.
- 507
- 508 Trappe JM, Claridge AW, Kagan-Zur V. 2014. Chapter 13. Ecology and distribution of desert
509 truffles in the Kalahari of southern Africa. In: Kagan-Zur V, Roth-Bejerano N, Sitrit Y, Morte A,
510 eds. *Desert truffles—phylogeny, physiology, distribution and domestication*. Springer Berlin
511 Heidelberg. pp. 193–202.
- 512
- 513 Trappe JM, Claridge AW, Arora D, Smit WA. 2008. Desert truffles of the African Kalahari:
514 ecology, ethnomycology, and taxonomy. *Economic Botany* 62:521–529.
- 515
- 516 Trappe JM, Molina R, Luoma DL, Cázares E, Pilz D, Smith JE, Castellano MA, Miller SL,
517 Trappe MJ. 2009. Diversity, ecology, and conservation of truffle fungi in forests of the Pacific
518 Northwest. USDA General Technical Report, Pacific Northwest Research Station. 196 p.
- 519

520 Wallis IR, Claridge AW, Trappe JM. 2012. Nitrogen content, amino acid composition and
521 digestibility of fungi from a nutritional perspective in animal mycophagy. *Fungal Biology*
522 116:590–602.

523

524 Zeller SM, Dodge CW. 1929. *Hysterangium* in North America. *Annals of the Missouri Botanical*
525 *Garden* 16:83–128.

526

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 *Aroramycetes 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 $\geq 75\%$ / posterior probabilities ≥ 0.95 .
547