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Mycologia

DOI:
10.1080/00275514.2020.1790234

Published: 01/11/2020

Peer reviewed version

Short title: Bonobo mycophagy

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

Todd F. Elliott[a], Alexander V. Georgiev[b], Albert Lotana Lokasola[c] Matthew E. Smith[d*]

[a]Ecosystem Management, University of New England, Armidale, New South Wales 2351, Australia; [b] School of Natural Sciences, Bangor University, Bangor, UK; [c]Vie Sauvage, Kokolopori Bonobo Reserve, Province Equateur, Democratic Republic of Congo; [d]Department of Plant Pathology, University of Florida, Gainesville, Florida 32611, USA

Corresponding author email: [*]trufflesmith@ufl.edu

ABSTRACT

Many animals have been shown to eat fungi, and most truffle-like fungi depend on animals for spore dispersal via mycophagy. Although these interactions are widespread, they are understudied in many habitats. In this study, we show that bonobos (*Pan paniscus*) forage and feed on an undescribed truffle species in the rainforests of the Democratic Republic of Congo. Based on morphological and molecular assessment of collections, we show that the species eaten by bonobos is a previously undescribed taxon described here as *Hysterangium bonobo.* This species is known in the local Bantu language (Bongando) as simbokilo and is used for baiting traps to catch several species of small mammals. Our findings highlight the need for further research into mycophagy and systematics of sequestrate fungi in Africa.

KEY WORDS: African fungi, Hysterangiales, Phallomycetidae, primate mycophagy, truffle taxonomy, 1 new taxon

INTRODUCTION

Fungi play a significant role in the diets and nutrition of diverse vertebrates. Many fungi, particularly truffle-like taxa, have evolved close associations with animals that help to disperse spores via mycophagy (Fogel and Trappe 1978; Elliott et al. 2019a, b). These animal-fungus associations are frequently overlooked, but they are an important part of functional ecosystems and imperative for the dispersal of fungi through these systems. There is a growing interest in better understanding the ecological significance of these associations. Mycophagy is nutritionally important for animals and simultaneously aids dispersal of mycorrhizal fungi (Cázares and Trappe 1994; Hussain and Al-Ruqaie 1999; Colgan and Claridge 2002; Kalač 2009; Wallis et al. 2012). These interconnected associations have been studied in various parts of the world but have been mostly overlooked in Africa.

The specialization of these associations varies depending on the fungal taxa and types of animals that consume and disperse them. Most studies of vertebrate mycophagy have focused on small mammals in regions outside of Africa (Fogel and Trappe 1978; Claridge and May 1994). In the Middle East and arid North Africa, some bird species are known to feed on truffle-like fungi and may play important roles in the health of desert ecosystems (Elliott et al. 2019b), but in Sub-Saharan Africa only a few vertebrate species have been reported as mycophagous. There are a handful of reports of African primates eating fungi (Kano and Mulavwa 1984; Hanson et al. 2003 and references therein; Isbell and Young 2007; Georgiev et al. 2010; Georgiev et al. 2011; Buyck et al. 2016). Among African vertebrates other than primates, four mammal species (Elliott et al. 2019c) and eleven terrestrial turtle species (Elliott et al. 2019a) have been reported to eat fungi. Buyck et al. (2016) noted that animals in the Central African Republic, including different species of duikers, primates, and wild pigs, all consumed *Elaphomyces*. However, this study did not provide the genera and species of the African mycophagous mammals that were observed.

As has been shown in other regions, this paucity of mycophagy reports is most likely due to insufficient sampling or the use of methods that do not detect fungal spores. The low number of reports does not necessarily mean that Africa has a low diversity of mycophagous animals (Elliott and Vernes 2019; Elliott et al. 2019b). For many vertebrates it can be difficult to determine whether or not fungi are regularly ingested solely by observing their feeding behavior. Microscopic and/or DNA analysis of feces are useful techniques to determine the presence and diversity of fungi in the diet. It is clear that these methods need to be more thoroughly utilized to study the diets of vertebrates in Africa.

Schmitt and Mueller (2007) documented 2,250 described species of macrofungi from Africa (Schmit and Mueller 2007). Mammals on other continents have been shown to eat a wide

variety of morphologically diverse fungi (Fogel and Trappe 1978; Claridge and May 1994; Nuske et al. 2017). Fungi that produce sequestrate (enclosed) and/or hypogeous (below ground) fruiting bodies are presumably more dependent on animal vectors than non-sequestrate species because sequestrate and/or hypogeous taxa typically lack the ability to forcibly discharge spores (Trappe et al. 2009). There are more than a dozen species of sequestrate fungi reported from Sub-Saharan Africa (Dissing and Lange 1962; Dring and Pegler 1978; Castellano et al. 2000; Eberhardt and Verbeken 2004; Beenken et al. 2016; Castellano et al. 2016a, b; Orihara and Smith 2017). Several species have also been reported from the arid Kalahari desert and the surrounding region of southern Africa (Taylor et al. 1995; Trappe et al. 2008; Trappe et al. 2014). Many of these taxa have been recently described and occur in habitats where primates are known to forage, but no studies have carefully examined the mycophagy of sequestrate fungi by primates or many other African vertebrates. Here we provide new observations of bonobo mycophagy and show that the fungus consumed by these apes is a previously undescribed species of truffle named here as *Hysterangium bonobo* sp. nov..

MATERIALS AND METHODS

Animal observations. **—** The feeding ecology of the Hali-Hali bonobos (Georgiev et al. 2011), a wild unprovisioned community, was studied at the Nsondo Camp (0°12’N, 22°51’E), Kokolopori Bonobo Reserve, Province Equateur, Democratic Republic of Congo from Oct 2006 through Jul 2007 with experienced field assistants familiar with the bonobos and the ecology of the forest (Georgiev et al. 2010; Georgiev et al. 2011). The study site is located approx. 30 km east of a long-term bonobo study site at Wamba and shared many of its ecological features (Hashimoto et al. 1998; Kano and Mulavwa 1984). Three main habitat types are present: 1) dry primary forest with portions dominated by trees in Fabaceae subfam. Detarioideae, including

ectomycorrhizal (ECM) species of *Gilbertiodendron* and *Brachystegia*, 2) seasonally inundated, riparian swamp forest where *Guibourtia demeusei* (Detarioideae) and ECM *Uapaca* spp. (Phyllanthaceae) are common, and 3) secondary disturbed forest heavily influenced by slash-and-burn agriculture expanding from nearby villages. The seasonality of precipitation at Kokolopori is similar to Wamba, which typically has up to 2900 mm of rainfall per year during one light rainy season from Mar – May and a heavier rainy season from Sep – Nov (Hashimoto et al. 1998; Mulavwa et al. 2008).

The Hali-Hali bonobos have been habituated since 2000 by local conservation NGO Vie Sauvage with support from the Bonobo Conservation Initiative (BCI). By Oct 2006, the apes were sufficiently accustomed to human presence to allow behavioral observations on a regular basis. Although the apes allowed detailed monitoring when feeding in the canopy, ground observations were less frequent because they did not always tolerate close human proximity during ground foraging. Observational conditions on the forest floor were also limited to 15–20 m or less by dense understory vegetation. The bonobo truffle foraging data presented here are thus considered a conservative estimate of truffle consumption.

The diet of the bonobos was scored at 15-min intervals by recording food species and plant parts (if any) eaten by the majority of individuals in view on the sampling point. A ‘running food list’ was also recoded on a daily basis to note all foods the bonobos were seen to ingest, whether they happened on the 15-min sample point or not (Georgiev et al. 2010; Georgiev et al. 2011). We presented data on truffle consumption via a simple dietary score to document the frequency of truffle-eating over the study period, calculated as the monthly proportion of days on which at least one episode of truffle-eating was seen from the total number of days on which bonobos were observed and feeding data were recorded. In some cases it was possible to directly

observe truffle consumption by bonobos. At other times, truffle feeding was inferred because the bonobos were feeding on the ground and then observers moved into the area after the bonobos began to move away and were able to observe digging and discarded pieces of truffle basidiomata. In Aug 2007 we were able to directly view bonobos consuming basidiomata and then to collect fresh specimens when they were done feeding (see below).

Morphological studies. **—** Four basidiomata were collected from the exact location where bonobos were observed to be feeding on truffles. Specimens were preserved in 99% ethanol. The collection has been accessioned at the Fungal Herbarium of the Florida Museum of Natural History as FLAS-F-64335. Field collected truffles from the Kokolopori bonobo site (FIG. 1) were also directly compared with herbarium specimens from the Oregon State University Mycological Collection (OSC) of *Aroramyces radiatus* (Lloyd) Castellano, Verbeken & Walleyn, one of the only related truffle species known from tropical Africa (see below).

Descriptions of macromorphological characters were based on fresh material and photos. Colors were described in general terms. Microscopic characters were examined based on hand sectioned dried tissues rehydrated in Melzer's reagent, 3% KOH, and water. Photomicrographs were taken in water. Basidiospore measurements are based on 20 randomly selected basidiospores. Given the remote nature of the region, logistical difficulties of returning to the site, and the infrequence of hypogeous fungal collections in the region, we were unfortunately forced to base the description of this new species on a single collection.

Molecular methods and phylogenetic analyses. **—** Clean fungal tissues were taken from inside the dried specimens that had been previously preserved in ethanol. DNA was extracted using a modified CTAB method (Gardes and Bruns 1993). Amplification of the nuclear rDNA ITS1-5.8S-ITS2 (ITS) region was performed using forward primer ITS1F and reverse primer ITS4

(White et al. 1990) and the Phusion Hot Start Flex DNA Polymerase standard protocol (New England BioLabs Inc., Ipswich, Massachusetts). Amplification of a portion of nuc 28S rDNA (28S) was performed using the same protocol with forward primer LROR and reverse primer LR3 (Hopple and Vilgalys 1994). PCR products were visualized on 1.5% agarose gels stained with SYBR Green I (Molecular Probes, Eugene, Oregon). Amplicons were cleaned with EXO (Exonuclease I) and SAP (shrimp alkaline phosphatase) enzymes (Werle et al. 1994) and sequenced by GENEWIZ (South Plainfield, New Jersey). Sequences were then edited with SEQUENCHER 5.0.1 (Gene Codes Inc., Ann Arbor, Michigan). The ITS and 28S sequences from our *Hysterangium* collection were compared with those in the NCBI database using the BLASTn tool (Altschul et al. 1990).

For phylogenetic analysis, the 28S sequences of FLAS-F-64335 were placed in an alignment of 28S and mitochondrial ATP synthase membrane subunit 6 (*ATP6*) DNA sequences of Hysterangiales and allied fungi in Phallomycetidae previously generated by Hosaka et al. (2006, 2008). We were unable to obtain *ATP6* sequences from FLAS-F-64335. Sequences were downloaded directly from GenBank and aligned in MESQUITE 3.2 (Maddison and Maddison 2018) with the aid of MUSCLE 3.8.31 (Edgar 2004). Independent analyses of 28S and *ATP6* showed no conflicting phylogenetic signal (data not shown), so the two loci were combined into a single concatenated analysis. The alignment was edited manually to exclude gaps and ambiguously aligned regions.

The concatenated alignment was analyzed with maximum likelihood (ML) and Bayesian Inference (BI) as performed in the Cyberinfrastructure for Phylogenetic Research Science Gateway (CIPRES) 3.1 (Miller et al. 2010). ML was run via RAXML 8.2.10 (Stamatakis 2014) with 1000 bootstrap iterations and a GTRGAMMA model under the default paramaters

(Stamatakis 2015). BI was performed in MRBAYES 3.2.7a (Ronquist et al. 2012) using the GTR+I+G model following Hosaka et al. (2008). BI analysis was run on two separate chains using a chain length of one million generations, sampling frequency of 1000, and discarding the first 25% of the samples as the burn-in. The multilocus ML tree was visualized and rooted in FIGTREE 1.4.3 (Rambaut 2016) and Bayesian posterior probability (PP) values were added in ADOBE ILLUSTRATOR CS5.1 (San Jose, California). Nodes were considered strongly supported if ML bootstrap values were ≥75% and PP≥0.95.

RESULTS

Truffle consumption by bonobos. **—** Between Nov 2006 and Jul 2007 bonobos were observed on 155 days (range: 12–26 days per month). Truffles were eaten on 38 observation days in 7 out of the 9 study months on a mean of 4.2 days per month (range: 0–10 days), or for a mean of 23.1% of monthly observation days (range: 0.0–52.9% of monthly observation days). Truffles were, however, a minor element of the diet in terms of their overall contribution to feeding observations (less than 3% of all 15-min, group-level feeding scans for all months).

BLASTn and phylogenetic analysis. **—** BLASTn analysis of both the ITS and 28S sequences generated from FLAS-F-64335 showed clear affinities with other species of ECM Hysterangiales and related Phallomycetidae. The ITS from FLAS-F-64335 had the highest homology with uncultured Hysterangiales sequences from ECM roots (e.g., KT461360 from an unknown miombo woodland tree from the DRC; KM402914 from *Pseudotsuga menziesii* from British Colombia) as well as specimens of *Hysterangium* (e.g., DQ974810) and *Ramaria* (e.g., FJ627035). However, the highest hits were only 83–84% similar to the ITS sequence from FLAS-F-64335. The 28S also exhibited obvious affinities with ECM Hysterangiales, including 92–93% similarity to a wide range of Phallomycetidae such as species of *Hysterangium*,

Ramaria, and *Austrogautieria* (e.g., AF336259, JQ408235, KP191776), as well as ECM root tips of Hysterangiales (e.g., JX316465 from a root of *Nothofagus pumilio* from Argentina).

Our alignment included 770 nucleotides of aligned 28S sequences and 691 nucleotides of aligned *ATP6* sequences from 171 taxa. Of these, 76 nucleotides were excluded from the 28S portion of the alignment and 31 nucleotides were excluded from the *ATP6* alignment. The ML phylogeny (FIG. 2) depicts FLAS-F-64335 nested among ECM *Hysterangium* and resolved in a clade separate from *Aroramyces radiatus*, the only other described ECM Hysterangiales from tropical Africa. The analyses also revealed that *Aroramyces* is a strongly supported monophyletic group nested within *Hysterangium*, making the latter paraphyletic. This result was previously found by Hosaka et al. (2006, 2008).

TAXONOMY

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

MycoBank MB834363

Typification: DEMOCRATIC REPUBLIC OF THE CONGO (DRC). TSHUAPA PROVINCE: Djolu Territory, Kokolopori Bonobo Reserve, in mixed rainforest with ectomycorrhizal trees in the genera *Uapaca* (Phyllanthaceae), *Brachystegia* (Fabaceae), and *Gilbertiodendron* (Fabaceae), Aug 2007, *A. Georgiev MES-127* (**holotype** FLAS-F-64335). GenBank: ITS+28S = MT111903.

Etymology: *bonobo*, in reference to the common name of the primate *Pan paniscus*, which digs and eats this fungus.

Description: Basidiomata hypogeous to partially emergent, up to 50 mm broad, more or less globose to irregularly globose. Peridium up to 2 mm thick, light to dull brown, apparently bruising brown when damaged or handled, sometimes cracked, smooth, not easily separable

from the gleba, with a somewhat hairy appearance in patches. Gleba dark brown, solid, with narrow meandering hollow veins and small open pockets that are not gel-filled, radiating from indistinct off-white columella less than 1 mm wide at the base but becoming indistinct as it radiates upward through the center of the gleba.

Peridium 70–150 μm thick, comprised of two layers; outer layer 10–65 μm thick, composed of interwoven hyphae 3–5 μm wide, hyphae in the outer layer notably darker in color than the inner layer and prominently encrusted with irregular warts 2 × 4 μm or larger, peridial cystidia not observed, clamp connections rare or absent, debris sometimes adhering to the outer layer. Inner peridial layer up to 130 μm thick, composed of thin-walled, tightly packed, highly interwoven hyphae 1–3(–5) μm wide. Gleba trama of tightly interwoven and unorganized, hyaline, gelatinized hyphae 0.5–1.5 μm wide, forming a layer mostly 20–40(–55) μm broad. Columella composed of hyaline interwoven hyphae mostly 1–2 μm wide, up to 100 μm thick at the base but rapidly decreasing in width as it radiates out into the gleba tissues.

Basidiospores 14–16 μm × 8.5–10 μm (mean 15 × 9.5 μm), basidiospore walls 1–2 (–3) μm thick, Q = 1.40–1.68, mean Q = 1.58, brown in mass, spore wall thickness often irregular but in most spores the walls notably thicker toward the apical end of the spores near the attachment to the sterigmata, ornamentation of very short and somewhat indistinct spines or warts (less than 0.5 μm tall), apical attachments notable and sometimes with a piece of the sterigma broken off from the basidium and still attached to the spore at maturity, faint oil droplets apparent in some spores, spores somewhat dextrinoid in Melzer's reagent. Basidia mostly 2-sterigmate, difficult to view, deflated and irregularly shaped, apparently collapsing after spore dehiscence; sterigmata irregular in shape, visibly running through the spore wall to attach to the spore apex, 2–5 μm long and 1–2 μm wide.

Notes: One species of *Hysterangium* and one species of *Aroramyces* (Hysterangiales) have been previously described from Sub-Saharan Africa, *H. niger* Lloyd and *A. radiatus*. *Hysterangium niger* was originally described from South Africa but is morphologically divergent from all other species of *Hysterangium.* It was transferred by Zeller and Dodge (1929) to *Rhizopogon*. We suspect that it is a *Rhizopogon* species introduced to South Africa with pine seedlings, but the type has not been recently studied. However, based on microscopic and macroscopic morphology it is clearly not conspecific with *H. bonobo.*

Aroramyces radiatus was originally described as *Hymenogaster radiatus* Loyd but has also been placed in *Dendrogaster* Buchholtz and *Gymnoglossum* Massee (Castellano et al. 2000). The genus *Dendrogaster*, however, is currently considered a synonym of *Hymenogaster* (Castellano et al. 2000), and the identity of *Gymnoglossum* remains unverified by sequence data (viz, there are no publicly available sequences in GenBank from the Australian type species *Gymnoglossum stipitatum* Massee). Accordingly, Castellano et al. (2000) established the genus *Aroramyces* to accommodate two species, *Aroramyces gelatinosporus* (J.W. Cribb) Castellano and *A. radiatus*. The genus was morphologically characterized by brown ornamented spores and brownish gleba, the presence of a columella, a multi-layered peridium, and gelatinized tissues. Castellano et al. (2000) reported *A. radiatus* with spores that are strongly truncate at the base of the sterigmata attachment and covered by a wrinkled utricle that conceals the spore ornaments when viewed with SEM. Although Castellano et al. (2000) considered *A. radiatus* in the Cortinariaceae, subsequent phylogenetic studies indicate that *A. radiatus* is a member of Hysterangiales, and that *Aroramyces* is nested within *Hysterangium* (Hosaka et al. 2008). Accordingly, *Hysterangium* is currently a paraphyletic genus and additional taxonomic revisions are needed but are beyond the scope of this study (Hosaka et al. 2008).

The type of *H. bonobo* (FLAS-F-64335) from a bonobo foraging site at the Kokolopori Bonobo Reserve is superficially similar to the descriptions of *A. radiatus* in Castellano et al. (2000) and also our direct observations of specimens cited by Castellano et al. (2000) from Zimbabwe. Both species are from tropical Africa and also have a brown gleba, broadly ellipsoid spores and irregular basidia that collapse at maturity. However, the spores of *A. radiatus* are much smaller than those of *H. bonobo* (mean of 10.8 × 6.9 μm in *A. radiatus* versus 15 × 9.5 μm in *H. bonobo*), and the spore ornaments of *A. radiatus* are much larger and more notable than in *H. bonobo*. Furthermore, the spore walls are notably thicker near the attachment to the sterigmata in *H. bonobo*, whereas in *A. radiatus* the spores usually taper the opposite direction and are most narrow near the attachment to the sterigmata. The two species also differ in the peridium morphology. Although both species have encrusted hyphae on the outer peridial layer, *A. radiatus* has a 3-layered peridium up to 400 μm thick, whereas *H. bonobo* has a 2-layered peridium typically around 100 μm thick. Phylogenetic analysis also clearly separates these taxa (FIG. 2).

We do not know which ECM trees are the symbiotic hosts for *H. bonobo*. However, *Hysterangium* spp. are known to be obligate ECM fungi (Hosaka et al. 2008) and *H. bonobo* was found in forests with several confirmed ECM host plants, including species of *Uapaca*, *Brachystegia*, and *Gilbertiodendron*. Species of *Uapaca* and *Gilbertiodendron* are also known to form ECM associations with several other African truffles (Castellano et al. 2016a, b; Orihara and Smith 2017). Bermejo et al. (1994) also noted the presence of *Uapaca* and *Gilbertiodendron* species at sites where they reported bonobos successfully foraging for unidentified truffles.

DISCUSSION

Many mammals rely on aromas released by mature hypogeous fungi to detect where they are fruiting (Mills 1978; Maser et al. 2008; Stephens et al. 2020). Birds and animals with more limited olfactory abilities may rely on visual cues, including mimicking the color or shape of fruits (Beever and Lebel 2014; Elliott and Marshall 2016; Elliott et al. 2019b; Elliott and Elliott 2019; Elliott and Vernes 2019). Some of the truffles described from Africa are brightly colored, possibly indicating that their dispersers may rely on their visibility rather than odor (Castellano et al. 2000). It is difficult to determine what alerts bonobos to the presence of subterranean fungi, but it is likely a combination of visual and olfactory cues. Bermejo et al. (1994) described a bonobo that used olfactory cues to locate a truffle: "…standing quadrupedally, digs up the earth, first with one hand, then with the other, in search of subterranean truffles. She puts her face closer to the hole that she has dug and looks closely. Then she carefully puts one hand into the hole and withdraws it immediately, putting her fingers to her nose to detect the scent of truffles." Similarly, our observations of the Hali-Hali bonobos foraging on the ground are consistent with the idea that bonobos rely on olfactory cues to detect hypogeous fungi.

The Hali-Hali bonobos consumed truffles on a regular basis (23% of all sampling days), indicating that truffles are a component—but not a staple—in the diet of this community of bonobos. *Hysterangium bonobo* was collected directly after we observed bonobos feeding on truffles, therefore leading to the conclusion that bonobos consume *H. bonobo* as a food source. Although we expect that bonobos may consume other truffle species in the region, further studies are needed to confirm this hypothesis. Previous studies on bonobo diets in the DRC have also reported that bonobos eat truffles (Yalosidi: Kano 1983; Wamba: Kano & Mulawa 1984; Lilungu: Bermejo et al. 1994), but truffles have always been considered a minor component in their diet. The most detailed report on bonobo truffle-eating comes from the Ikela study site,

where the apes consumed truffles on 18 days over a 605-day-long study, totaling 686 hrs and 47 min of direct observations (Bermejo et al. 1994). We saw bonobos consume truffles on 38 days (from a total of 155 observation days), so it is likely that the Ikela bonobos eat fewer truffles than the bonobos at Kokolopori. The identity of the truffles from previous studies have remained unknown, although Kano (1983) suggested that the puffball *Langermannia fenzlii* (Reichardt) Kreisel might be a food source for bonobos. However, we can find no evidence or specimens to support this hypothesis. Despite being consumed infrequently and in low quantities, the nutritional value of fungi can still constitute an important part of an animal's diet (Wallis et al. 2012).

Because the truffles at Ikela (Bermejo et al. 1994) were never collected or identified we cannot determine whether the bonobos observed in that study consumed *H. bonobo*, *A. radiatus*, or other truffle species. It is also possible that the truffle species at the Ikela site were less appealing to bonobos or that the Ikela study occurred during seasons or years with less fungal fruiting than our studies at Kokolopori. Near Kokolopori the local villagers use the Bongando word "simbokilo" to refer to truffles (Takemoto 2017). There is some evidence of wider consumption of simbokilo by other mammals because the truffles are used to bait traps during forest hunting expeditions (Kimura et al. 2015). Simbokilo is specifically useful for trapping *Cricetomys emini* (Emin's pouched rat) but has also been documented by local people to attract *Atherurus africanus* (African brush-tailed porcupine), at least three species of duiker (*Cephalophus monticola*, *C. callipygus*, *C. nigrifrons*), and several species of squirrels (A. Lokasolac, pers. obs.). The word "simbokilo" is derived from 'simba' (don't go away) and 'bokilo' (brother-in-law) and derived from a longer phrase "do not allow your brother-in-law to go away because there will be plenty of food coming from traps using simbokilo as a bait." This

etymology of simbokilo is indicative of the regular use of this truffle by local people (A. Lokasolac, pers. obs.). However, as far as we know, our collection of *H. bonobo* is the only collection of simbokilo that has been examined microscopically or molecularly. It therefore remains unclear whether this word refers to *H. bonobo* specifically or to a suite of truffles. More direct observations and collections are needed to determine whether simbokilo is one truffle species or several truffle species.

There have been scattered reports of mycophagy among primates in Africa and other parts of the world (see Hanson et al. 2003). Most studies are based on visual observations of feeding, but the fungal taxa are rarely or never identified. Most reports of mycophagy give vague descriptions of the macroscopic morphology of the fungi that provide little assistance to taxonomists, e.g. “bracket fungi consumed by gorillas” (Fossey 1983). We urge zoologists working with animal diets in the future to collect, photograph, and preserve voucher specimens of the fungal taxa eaten in order to allow for more in-depth taxonomic studies by fungal biologists.

ACKNOWLEDGMENTS

We thank Sally Coxe and Michael Hurley from the Bonobo Conservation Initiative (BCI) for access to Kokolopori and for field support. Permission for the study was facilitated by the BCI, Vie Sauvage, and Mwanza Ndunda at the Centre Recherche de Ecologie et Forestrie at Mbandaka. BCI staff in Kinshasa and Mbandka, the late Veronique Lokasola, and her colleagues at Vie Sauvage provided assistance in the DRC. Leonard Nkanga, Antoine Lokondja, Jean Nsala, Jacques Likenge, Antoine Ilanga-Bomanga, and Jean-Pierre Likenge assisted in the field. Richard Wrangham provided encouragement and advice on AG’s bonobo research. Jim Trappe and Michael Castellano for provided specimens of *A. radiatus* from OSC. We thank Rosanne

Healy, Donald Pfister and Nattapol Kraisitudomsook for laboratory support. Funding was provided by Harvard University and the Arthur L. Greene Fund to AG. This study complied with the requirements of the Institutional Animal Care and Use Committee (IACUC) at Harvard University, as well as with legislation of the Democratic Republic of the Congo. MES received funding from USDA NIFA (FLA-PLP-005289), the Farlow Herbarium at Harvard and IFAS at the University of Florida. TFE received an International Postgraduate Research Scholarship from The School of Environmental and Rural Science at the University of New England. Kelsey Myers Elliott provided helpful insights and editorial comments. Konstanze Bensch and Jim Trappe provided clarity on etymology. The authors thank Terry Henkel and Brandon Matheny for editorial suggestions.

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FIGURE LEGENDS and FOOTNOTES

Figure 1. Morphological features of the holotype collection of *Hysterangium bonobo* collected in a bonobo foraging site after bonobos had recently been feeding. A. Fresh fruiting body of *H. bonobo* in hand, note the slight brownish discoloration from handling. B. Broken *H. bonobo* fruiting body revealing brown gleba and columella at the center. C. Peridial hyphae with brown encrusted warts. D. Overview of the gleba showing locules of hymenial tissue lined with basidia and basidiospores interleaved by tramal plates composed of densely interwoven sterile hyphae. Interwoven peridial hyphae on the inner surface of the peridium is visible in the upper left-hand corner of the image. E. Thick-walled basidiospores that are still attached to narrow basidia are visible at the far left and right of the image. Note that the sterigma appear to grow through the thick cell walls. The basidiospore in the center of the image shows the fine ornamentation that is present on mature basidiospores at high magnification. Bars: A–B = 2 cm; C, E = 10 μm, D = 30 μm.

Figure 2. ML phylogenetic tree of Hysterangiales and other fungi in the Phallomycetidae based on analysis of 28S and *ATP6* showing phylogenetic placement of *Hysterangium bonobo* sp. nov. within the genus *Hysterangium*. Note that *H. bonobo* is resolved in a clade that is

phylogenetically distant from *Aroramyces radiatus*, the only other described species of Hysterangiales from tropical Africa. Several taxa in Agaricales, Thelephorales, and Boletales served as outgroups. Support values are shown above the nodes using the following format: ML bootstrap values ≥75% / posterior probabilities ≥0.95.