

# What fecal analyses reveal about *Manniophyton fulvum* consumption in LuiKotale bonobos (*Pan paniscus*): A medicinal plant revisited

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## Abstract

Observations of animals in the wild can result in the discovery of plants for human medicinal purposes. In this context, our closest relatives, the great apes, are of particular interest. The Euphorbiaceae *Manniophyton fulvum* possesses both phytochemical and biomechanical properties. Its use in the genus *Pan* (*P. troglodytes*; *P. paniscus*) is thought to be based on its mechanical properties promoting the egestion of intestinal parasites, but additional observations from different habitats where the behavior is performed may shed more light on its true purpose. To improve our understanding of what triggers this behavior, we investigated *M. fulvum* consumption in wild bonobos at LuiKotale, Democratic Republic of the Congo between December 2018 and July 2020. Specifically, we tested the hypothesis that *M. fulvum* ingestion is related to gastro-intestinal parasite expulsion. Of 649 focal follows of 37 individuals from two habituated communities, consumption of *M. fulvum* was observed on 111 days ( $N = 507$ ), independent of seasons, environmental factors and the plant's availability. A total of 588 fecal samples were assessed for the presence/absence of gastro-intestinal parasites. We found strongyle eggs in 2.89% of samples and their presence was not associated with the ingestion of *M. fulvum* or environmental conditions. We discuss the importance of seasonality in the life cycle of strongyle species that may influence the pattern of *M. fulvum* consumption observed at LuiKotale. Our data open additional perspectives concerning behavioral parameters such as the existence of a cultural component when comparing ingestion behavior between communities.

## KEYWORDS

bonobo, leaf swallowing, *Manniophyton fulvum*, *Pan paniscus*, self-medication, zoopharmacognosy

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## 1 | INTRODUCTION

Humans (*Homo sapiens*) use medicinal plants (Bailung & Puzari, 2016; Efferth & Greten, 2014; Hardy et al., 2013). Medicinal plant use, however, is not specific to humans. Other animal species also choose organic or inorganic items from their environment that contain pharmacological substances to treat diseases or symptoms (Costa-Neto, 2012; Huffman, 2003; Lozano, 1998). Rodriguez and Wrangham (1993) proposed the term “zoopharmacognosy” to describe the process by which wild animals select and use specific plants with medicinal properties for the treatment and prevention of disease. Zoopharmacognosy in animals describes the use of plants and other items ingested or applied topically in a manner that differs from a species' typical use of these items. The term is often associated with, but not limited to, antiparasitic behaviors (Costa-Neto, 2012). Great apes (orangutans: *Pongo* spp.; gorillas: *Gorilla* spp.; bonobos: *Pan paniscus*; and chimpanzees: *P. troglodytes*) are particularly useful as models for investigating the evolution of medicinal plant use in humans due to their phylogenetic relatedness. Indeed, the *Homo-Pan* lineage only diverged 5 to 7 million years ago (Prüfer et al., 2012), thus making *Pan* species our closest relatives. Since chimpanzees and bonobos use habitats that likely resemble those of early hominins (Sommer & Ross, 2011), inferences about early hominin medicinal plant use can be drawn from studying the overlap in that of indigenous people and great ape species (Cousins & Huffman, 2002; Fruth et al., 2011; Obbo et al., 2013).

One example of ape medicinal plant use that has received considerable attention is the swallowing and subsequent excretion of intact leaves by chimpanzees (e.g., Huffman et al., 1996; McLennan & Huffman, 2012; Wrangham, 1995). Since these leaves are excreted intact, the purpose of performing this behavior must extend beyond the uptake of nutrients or secondary compounds (Messner & Wrangham, 1996). In 1996, Huffman and colleagues ended speculations concerning the mode of action of those leaves, reducing them to their only common denominator: the presence of a rough surface bristled with trichomes. Huffman and Caton (2001) explained the antiparasitic role of this property with the leaves' bristly surface increasing intestinal tract motility when swallowed without chewing, particularly when ingested on an empty stomach. By that, gut passage time was said to be reduced to 6 h, mechanically inducing larvae expulsion by increased peristalsis and “flushing” of the bowel.

African great apes are commonly infected with several species of gastro-intestinal parasites. The leaf swallowing behavior has been suggested to control existing infections by the nematode *Oesophagostomum stephanostomum* and the cestode *Bertiella studeri* (Huffman et al., 2009; McLennan and Huffman, 2012; Wrangham, 1995). *Oesophagostomum* spp. (Chabertiidae) infection occurs by ingesting food on the ground that is contaminated with L3 larvae which is more typical at the onset of the rainy season when climatic conditions favor larval infectivity (Polderman & Blotkamp, 1995). In the wild, great apes can become infected with *Oesophagostomum* sp. larvae and subsequently develop nodules in their gastrointestinal tract without necessarily presenting severe clinical signs (Krief et al., 2008). However, infection may cause oesophagostomiasis, which can cause increased

weight loss, intermittent diarrhea and/or hepatic complications, leading to fatal outcomes in some cases (e.g., in captive conspecifics: Krief et al., 2008; Rousselot & Pellissier, 1952). In the case of *B. studeri*, infection occurs when larvae-carrying oribatid mites (order Oribatida) are ingested and the larvae subsequently migrate to the intestines and develop into adult (Huffman et al., 2009; Metzger, 2015). Pathogenicity in nonhuman primates is comparatively low, with possible symptoms including abdominal pain, diarrhea, loss of appetite, and fatigue (Metzger, 2015). In chimpanzees, the seasonality of *B. studeri* proglottid egestion varies between sites (Huffman et al., 2009). Thus far, there is no published record of *B. studeri* infections in wild bonobos.

*Manniophyton fulvum* (Euphorbiaceae) is a straggling shrub that is widely distributed across Africa's tropical evergreen forests. The surface of its large leaves and young stems is bristled with trichomes, resulting in a rough, sandpaper-like texture that irritates the skin upon contact (Fruth, in press). Across tropical Africa, people use its leaves, sap, roots, bark, and stems inter alia as medicine (Bellomaria & Kacou, 1995; Burkill, 1994; Fruth et al., 2014). Plant parts contain antioxidant, antidiarrheal, antibiotic, antiviral, and/or anti-inflammatory properties (e.g., Mbeunkeu et al., 2018; Musuyu Muganza et al., 2012; Nia et al., 2005) depending on their associated secondary compounds (e.g., alkaloids, saponins, and terpenes: Agbaire et al., 2013). The Nkundo indigenous people in the Democratic Republic of the Congo (DRC) use *M. fulvum* for treating wounds, gastro-intestinal spasms, diarrhea, malaria-induced fever, and eye infections (Musuyu Muganza et al., 2012).

*M. fulvum* is ingested by chimpanzees (Bertin et al., 2018; Huffman & Wrangham, 1994) and bonobos (Dupain et al., 2002; Fruth et al., 2014) without it being part of their regular diet. Both species ingest young leaves, and bonobos in LuiKotale and Kokolopori also ingest the stem-bark of the plant (Fruth et al., 2014; Surbeck, pers. comm.). *M. fulvum* ingestion has been observed at all bonobo research sites active to date: Lomako (Dupain et al., 2002; Fruth, 2013; Henry, 1998), Wamba (Kano & Mulavwa, 1984), LuiKotale (Fruth et al., 2014), Kokolopori (Surbeck, pers. comm.), and Manzano (Narat, 2014). Bonobos typically pick an entire leaf with their hand or front teeth. Depending on its size, they bite off pieces or take one or two additional leaves, folding them slowly and meticulously into an intricate leaf package that they swallow without chewing. Leaf packages are excreted undigested when defecating. The number of leaves ingested per bout ranges from one to a dozen, which significantly distinguishes *M. fulvum* ingestion from habitual leaf ingestion (Fruth et al., 2014). For bonobos at LuiKotale, Fruth and colleagues (2014) described the ingestion of 15–30 cm long strips peeled from young *M. fulvum* rough and hairy shoots. Here, bonobos twist stem strips within their mouth to form balls that they swallow without chewing.

Analyzing fecal samples can provide insight into the frequency and seasonality of *M. fulvum* ingestion and is the only noninvasive approach to diagnose an infection by gastro-intestinal parasites. In 1.5% ( $N = 131$ ), 3.4% ( $N = 1094$ ) and 6.4% ( $N = 110$ ) of the fecal samples analyzed in Lomako (Dupain et al., 2002), LuiKotale (Fruth et al., 2014) and Manzano (Narat, 2014), respectively, researchers found undigested *M. fulvum* leaf-packages. At LuiKotale, stem strip remains had not been found before the onset of this study. Although

site-specific variation is expected, these differences may stem from methodological differences. For example, the first two studies mainly analyzed fecal samples collected beneath night nests, which likely led to an underestimation of the consumption rate. Indeed, hispid leaves swallowed in the early morning are expected to be shed within 6.5 h due to accelerated gut passage and are therefore less likely to occur in samples collected beneath night nests (Huffman & Caton, 2001). Methodology may have also unduly influenced inferences about the seasonality of *M. fulvum* consumption. At Lomako, none of the 33 fecal samples collected during the dry season contained *M. fulvum* remains and only 2 of 98 samples collected during the rainy season did (Dupain et al., 2002). Nevertheless, since 50% of a separate set of fecal samples ( $N = 86$ ) collected at the onset of the rainy season contained *Oesophagostomum* sp. eggs, this study inferred *M. fulvum* ingestion was associated with the rainy season. However, Fruth et al. (2014) found that *M. fulvum* ingestion by bonobos in LuiKotale was more common when ambient temperatures were low, which is a characteristic of the dry season. In Manzano, *M. fulvum* remains were found in feces collected both in the rainy and dry seasons and when the parasitic load was high (Narat, 2014).

Based on previous findings (Fruth et al., 2014) testing Huffman's criteria (1997, 2016) to assess self-medication of LuiKotale bonobos' *M. fulvum* ingestion, we continued investigations to better understand its trigger(s). We focused on the health condition of an individual, before and after ingestion of *M. fulvum*. We also investigated if the consumption of *M. fulvum* affected gut transit time and the consistency of feces.

With regard to *M. fulvum*, we predicted that the frequency of consumption was (1.1) higher during the rainy season; (1.2) higher following periods of heavy rainfall and/or low temperatures and/or when individuals spent more time feeding terrestrially; and (1.3) independent of the plant's availability. Regarding the prevalence of gastro-intestinal parasites in LuiKotale bonobos, we predicted that the frequency with which we found strongyle eggs and/or worms in fecal samples would be greater (2.1) after the onset of the rainy season, and (2.2) when bonobos fed terrestrially; also, (2.3) the egestion of *B. stuederi* proglottids and eggs would not follow a clear seasonal pattern. In line with the above predictions, we expected (3.1) *M. fulvum* ingestion to be positively associated with the egestion of strongyle worms and/or *B. stuederi* proglottids while (3.2) the absence of *M. fulvum* ingestion to be positively associated with the presence of strongyle eggs in feces.

## 2 | MATERIALS AND METHODS

### 2.1 | Ethics statement

All research activities complied with protocols approved by the Institut Congolais pour la Conservation de la Nature (ICCN) and adhered to the legal requirements of the Democratic Republic of the Congo (DRC). The research project was approved by the ethics committee of Liverpool John Moores University (LJMU). This study also complied with the American Society of Primatologists Principles for the Treatment of Non-Human Primates and Code for Best Practices in Field Research.

### 2.2 | Study site and species

Data were collected at the LuiKotale field site (2°45'36"S; 20°22'43"E), located west of Salonga National Park block South, DRC (Hohmann & Fruth, 2003). The site consists of pristine evergreen lowland rainforest and is extremely remote. Two communities of wild, habituated bonobos (Bompusa West:  $N = 22$  adults; Bompusa East:  $N = 15$  adults; July 2020) were followed between December 2018 and July 2020. All individuals were identified (Fruth & Hohmann, 2018).

### 2.3 | Climatologic data

We used LuiKotale long-term data covering 11 years from January 2010 to December 2020. Daily cumulative rainfall was measured using a rain gauge ( $\text{mm}/\text{m}^2$ ) open to the sky, and minimum and maximum temperatures using a Bresser 5 in 1 Weather station deployed in the forest. Following Newton-Fisher (1999), we delineated seasons during the study period using a Walter-Lieth climate diagram (Walter & Lieth, 1967). This diagram characterizes monthly moisture conditions based on the relationship between cumulative monthly rainfall and mean monthly temperature. Months are classified as "wet" when monthly rainfall is  $>100$  mm; "transient" when monthly rainfall is  $\leq 100$  mm; and "dry" when the rainfall figures below the mean temperature line. Whenever possible ( $N = 472$  days), forest temperature was directly used. When forest temperature was not available ( $N = 147$  days), we used a general linear model (GLM) to predict forest temperature from available camp temperature. We characterized the extent of seasonality during the study period using the Perhumidity Index (PI), which measures the annual "continuity of wetness" (Walsh, 1996). PI scores were applied as follows: very wet month ( $\geq 200$  mm) = +2; wet month (100–199 mm) = +1; dry month (50–199 mm) = -1; drought month ( $< 50$  mm) = -2; first dry month after a wet month = -0.5; and first drought month following a wet month = -1.5.

### 2.4 | Behavioral observations

We recorded data ad libitum using focal animal sampling (Altmann, 1974), complemented by health data visually assessed for each individual in sight within the party for a minimum of 15 min, recording symptoms related to typical gastro-intestinal parasitic infections (e.g., diarrhea; fatigue; slow locomotion; Krief et al., 2008; Metzger, 2015). Individuals were followed from nest to nest ( $N = 383$  follows  $> 8$  h) and during shifts ( $N = 266$  shifts 4–8 h; Bompusa West:  $N = 311$ ; Bompusa East:  $N = 338$ ;  $N = 9145$  observation hours including several observers). Independent of the ad-libitum focal sampling, we recorded all occurrences of *M. fulvum* consumption, specifying individual, time of day, part and age of plant, procedure, and duration of ingestion. Following Fruth et al. (2014) we defined an ingestion bout as one or more ingestion events uninterrupted by other activities (e.g., resting, grooming, ingestion of other food). Using behavioral data collected during 5-min activity scans of mature ( $> 10$  years) individuals ( $N = 617$  observation days), we calculated the time each

community fed terrestrially as the percentage of fruit-feeding scans. All behavioral data were collected using CyberTracker 3 (Stevenson et al., 2011) on a Samsung Xcover 5 smartphone.

## 2.5 | *Manniophyton fulvum* availability

We assessed the availability of *M. fulvum* up to three times a day ( $N = 841$ ): at the nest site, at the first stop of the day (e.g., feeding patch) and at a randomly generated stop point during the day. For each stop, we scored the visibility of *M. fulvum* to party members by scanning the surrounding area for 2 min. When plants were visible, we recorded their location (terrestrial, arboreal), visible plant parts (stem, leaf), and stage of maturity (young, old).

## 2.6 | Fecal sampling

Fecal samples were collected systematically from focal individuals and opportunistically from other party members. For each individual, we collected 1–5 fecal samples per day to control for intermittent egestion of parasites (Gool et al., 2003). Feces were collected immediately after defecation using latex gloves and stored in a sealed bag until they could be processed at camp. Time between collection and processing averaged 6.51 h ( $SD \pm 3.06$  h). We did not collect feces contaminated with urine or shed near water (Garcia et al., 2018). For all fecal samples, we described consistency and content, and searched for macroscopically visible worms or proglottids. Packages of folded undigested leaves were unfolded and taxonomically identified. For parasitological investigation, we retrieved 2–5 g of the fecal matrix from the center of the stool to avoid contamination from soil micro-organisms (Krauth et al., 2012). Using a 15 ml tube, the matrix was mixed with a 10% formaldehyde solution, allowing preservation for later investigation.

## 2.7 | Parasitological analyses

We investigated formol samples ( $N = 588$ ) for the presence of gastrointestinal parasites. Of these, 298 samples were analyzed in camp, while 290 were analyzed in the laboratory. Time between collection and analysis was 163 days on average (range 0–517 days,  $SD \pm 155$

days). All samples were analyzed following the same protocol with minor adaptations depending on available resources (e.g., manual/electric centrifuge, analytical scale). We performed flotations with a modified Sheather' sugar solution following protocol described in Modrý et al. (2015). Eggs were identified using morphological features such as shape and size (Garcia et al., 2018; Hasegawa et al., 1983, 2009; Modrý et al., 2015). To allow for control and later identification, specimen photos were taken using a graticule scale. With the exception of *O. stephanostomum*, morphological features are not considered being useful for identification of *Oesophagostomum* spp. (Chabertiidae) and *Necator* spp. (Ancylostomatidae) (Blotkamp et al., 1993, Narat, pers. comm.). Thus, we hereafter refer to them as strongyles (Pit et al., 1999; Polderman & Blotkamp, 1995; Strait et al., 2012).

## 2.8 | Statistical analyses

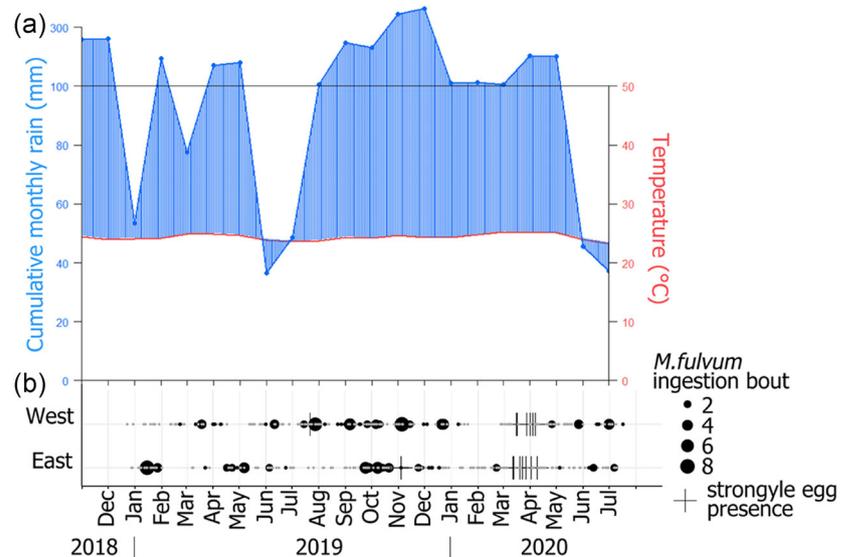
We used  $\chi^2$  tests to compare the occurrence of *M. fulvum* consumption events between (1) sexes, (2) communities, (3) plant parts, and (4) availability. We also used  $\chi^2$  tests to test for differences in (5) fecal consistency; and (6) health status before and after ingestion of *M. fulvum*. We ran a Spearman rank correlation to test for the independence of *M. fulvum* availability in both communities and between months.

To determine if *M. fulvum* consumption was seasonal, we used a logistic regression to assess whether the occurrence ( $Y/N$ ) of *M. fulvum* consumption per observation day ( $N = 507$ ) depended on the season (wet, transient or dry as defined by a Walter–Lieth climate diagram *sensu* Newton-Fisher, 1999) [M1]. We then used a Bernoulli generalized additive mixed model (GAMM) to investigate whether the probability of observing *M. fulvum* consumption during a shift ( $N = 526$ ) depended on (i) cumulative rain; (ii) mean minimum temperature, and (iii) the percentage of terrestrial feeding time [M2]. All values for fixed co-variables were calculated over a period of 14 days before the date of observation to account for the time between potential contamination with L3 infective *Oesophagostomum* larvae and the final molt from L4 stage to adult (Anderson, 2000). We predicted the hatching of the L4 larvae from the nodules embedded in the mucosa causing bleeding and intestinal discomfort (Stewart & Gasbarre, 1989). For this model, we fit community as a random intercept to account for the hierarchical sampling design, and terrestrial feeding tie as a random slope to account for community-level

	Infant (0–5 years) ( $N = 14$ )	Juvenile (>5–10 years) ( $N = 14$ )	Adolescent (>10–15 years) ( $N = 13$ )	Adult (>15 years) ( $N = 37$ )	Total
Female ( $N = 51$ )	11	23	7	118	159
Male ( $N = 27$ )	2	5	6	34	47
Total	13	28	13	152	206

**TABLE 1** *Manniophyton fulvum* ingestion at LuiKotale by sex and age class; December 2018–July 2020

**FIGURE 1** Seasonality, *Manniophyton fulvum* ingestion and strongyle egg presence in bonobo (*Pan paniscus*) feces collected between December 2018 and July 2020 at LuiKotale, DRC: (A) Walter–Lieth climate diagram with cumulative monthly rainfall in mm/m<sup>2</sup> (blue) and smoothed monthly mean temperatures in °C (red). (B) Occurrence of *M. fulvum* ingestion per day (black dots with size representing number of bouts) and presence of strongyle eggs in feces (cross) observed in the Bompusa West (top) and Bompusa East (bottom) communities



differences in the influence of time spent feeding terrestrially on the parasitic infection risk.

To determine whether the presence of strongyle eggs in fecal samples depended on climatic conditions, we used a second Bernoulli GAMM whereby fecal egg presence (Y/N) was fit as the response; cumulative rain and mean minimum temperature as fixed covariates; and individual ID inside community as a nested random effect [M3]. For this model, we averaged values of fixed covariates over the month before sample collection to account for the time between infection by strongyle larvae and production of eggs by adult females (Anderson, 2000). Finally, we used a Bernoulli generalized linear mixed model (GLMM) to assess whether there was an association between the presence of parasite eggs in fecal samples and *M. fulvum* consumption [M4]. Since *M. fulvum* consumption was expected to significantly accelerate gut passage time (Huffman & Caton, 2001), we limited the observation window for *M. fulvum* consumption to the same day the fecal sample was collected and included focal individuals ( $N = 375$ ) only. All models were fit using the packages “brms” (Bürkner, 2017, 2018) and “rstan” (Stan Development Team, 2020) in R version 4.0.5 (R Core Team, 2021). Before modelling, all continuous variables were Z-transformed. After running each model, we checked for autocorrelation in the residuals, and used trace plots to check for chain convergence. We evaluated model performance using posterior predictive checks and checked prior sensitivity by re-running the model with alternate priors. Models are summarized in Table S1.

## 3 | RESULTS

### 3.1 | Behavioral observations

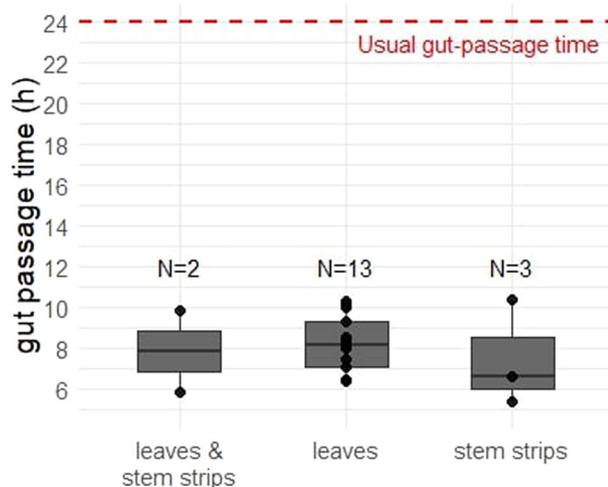
Overall, *M. fulvum* consumption was observed in 60 individuals of different age and sex (Table 1). Considering mature individuals only, *M. fulvum* consumption occurred on 111 out of 507 days (Figure 1b), with one or more individuals ingesting part of the plant's leaf and/or

stem bark. Consumption often occurred early in the morning (median time = 06:26 h, range: 05:31–15:32,  $N = 166$  observations) on an empty stomach. In most cases (92.31%,  $N = 104$ ), individuals that ingested *M. fulvum* did not show symptoms of infection on the day of consumption, nor did they isolate themselves from the group. In 89.7% of cases ( $N = 68$ ) focal individuals did not show any symptoms before or after ingestion. In the remaining 10.3% of observations, we observed individuals with asthenia ( $N = 3$ ) combined with an obvious lack of appetite in one case, diarrhea ( $N = 2$ ), or a combination of both symptoms ( $N = 2$ ). For these seven individuals, we noted an improvement of visually detectable symptoms within a few days (0–6 days) after ingestion of *M. fulvum* (normal feces consistency, normal feeding rate, or energy level).

Individuals from both communities consumed leaves and/or stem strips throughout the study period (median = 2 bouts/individual, range: 1–12). Adult females consumed *M. fulvum* more often than adult males (median = 3 bouts/female [range: 1–12]; median = 2 bouts/male [range: 1–7]). *M. fulvum* consumption occurred independent from the plant's availability (Bompusa West:  $\chi^2 = 1.74$ ,  $p > 0.05$ ; Bompusa East:  $\chi^2 = 0.92$ ,  $p > 0.05$ ).

### 3.2 | Seasonality

LuiKotale climate (2010–2020) was equatorial with abundant annual rainfall (1884 mm/m<sup>2</sup>,  $SD \pm 225$  mm,  $N = 11$ ). Temperatures ranged from 16°C to 39°C, with night temperatures being colder in the small dry season. The Perhumidity Index ranged between 0.5 in 2012 (seasonality) and 13.5 in 2010 (homogenous wetness) averaging  $6.40 \pm SD 3.77$  ( $N = 10$  years) between 2010 and 2020. During the study period 70% of the months were rainy, 15% transient, and 15% dry (Figure 1a). With the exception of two months (December 2018 and March 2020), *M. fulvum* consumption was observed every month when combining consumption of both communities (Figure 1b). We observed two peak periods of *M. fulvum* consumption, one in March–April 2019 (average = 31.58% of monthly follow days with *M. fulvum* bouts), and one in



**FIGURE 2** LuiKotale bonobo gut-passage time from ingestion to shedding (in h) by *Manniophyton fulvum* plant part. Boxplots show median (horizontal line), interquartile range (box) and range (vertical line). Dots indicate observed cases. Dashed line represents the 24 h habitual gut passage time (Beaune et al., 2013)

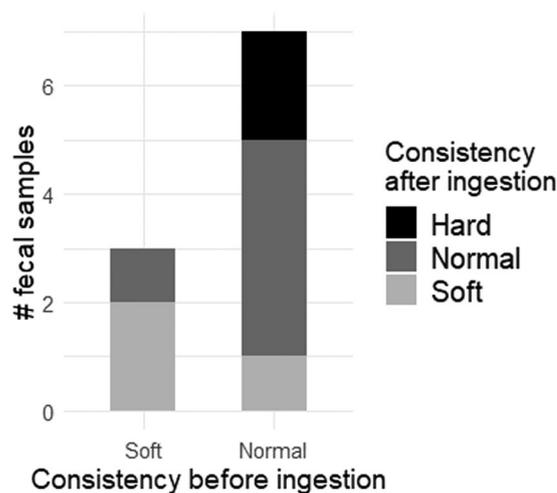
September–December 2019 (average = 29.77%). This was true for both the count of individuals involved and the frequency of consumption bouts. From M1, the posterior distributions for the wet and transition seasons both overlapped zero (Figure S1), meaning there was no significant difference in the likelihood of observing *M. fulvum* consumption between seasons. However, since the probability of the wet season's beta coefficient being positive and nonzero was 0.14, there is an 86% chance that the behavior is less common in the wet season. The occurrence of *M. fulvum* consumption was not predicted by cumulative rain, minimum temperature or time spent feeding terrestrially (Figure S2, Table S2).

### 3.3 | Cultural behavior

*M. fulvum* consumption periods partially overlapped between the two communities, with three and four periods for the Bompusa West and Bompusa East community, respectively (Figure 1b). The *M. fulvum* intake frequency in mature individuals was slightly higher, but not significantly, in the Bompusa East compared to the Bompusa West community (0.013 vs. 0.008 bouts/follow day/individual, Wilcoxon sign ranked test:  $W = 331$ ,  $p > 0.05$ ). However, the frequency of plant part consumption (leaf or stem bark) differed significantly between communities, with Bompusa West choosing stem strips over leaves and Bompusa East choosing leaves over stem strips ( $\chi^2 = 16.4$ ,  $p < 0.001$ ). This difference was independent of the plant's availability (leaves:  $r_s = 87.27$ ,  $p > 0.05$ ; stem strips:  $r_s = 89.25$ ,  $p > 0.05$ ).

### 3.4 | Fecal examination

We collected and described 969 fecal samples from 32 individuals (median = 21.5 samples/individual, range: 1–49). We found *M. fulvum*



**FIGURE 3** LuiKotale bonobo fecal sample consistency (soft; normal; hard) within 48 h before and between 6 and 48 h after ingestion of *Manniophyton fulvum*

remains in 5.3% of samples. No leaves from species other than *M. fulvum* were found intact and folded, even though other species presenting similar characteristics were available. We also found remains of stem strips ( $N = 7$ ) still identifiable by their typical rough surface and dimensions easily distinguishable from fibers of terrestrial herbaceous vegetation. *M. fulvum* egestion occurred on average 8.04 h after ingestion ( $N = 18$ ,  $SD \pm 1.39$  h; leaves: 8.14 h ( $SD \pm 1.27$  h); stem strips: 7.29 h ( $SD \pm 1.52$  h); both: 7.53 h ( $SD \pm 2.46$  h); Figure 2). When focusing on fecal samples ( $N = 355$ ) collected from beneath night nests only (19/05–25/07/2019), 2.25% contained *M. fulvum*. Twenty-five ingestions were directly observed during that time ( $N = 483$  observation hours). When focusing on 28 direct observations of *M. fulvum* ingestion during focal follows, inspecting  $N = 33$  fecal samples collected thereafter (5.30–11.00 h after ingestion), *M. fulvum* remains were present in 42.4% of these samples.

We did not observe the egestion of adult worms or cestode proglottids in any of the fecal samples ( $N = 969$ ) during the study period. Presence or absence of *M. fulvum* remains in fecal samples were unrelated to consistency ( $\chi^2 = 3.67$ ,  $p > 0.05$ ), as was consumption of a specific *M. fulvum* part to the change of fecal consistency when compared with the absence of consumption ( $\chi^2 = 3.84$ ,  $p > 0.05$ ). Based on the comparison between samples collected within 48 h before *M. fulvum* ingestion and after 6 to 24 h after ingestion ( $N = 10$ ), we observed feces to be harder in consistency after ingestion than before (Figure 3), though the difference was not significant ( $\chi^2 = 3.02$ ,  $p > 0.05$ ).

### 3.5 | Parasitological analyses

The flotation experiments revealed the presence of several gastrointestinal parasite species, but here we focused solely on strongyle species (i.e., *Oesophagostomum* spp. and hookworms). Based on shape and dimensions (Hasegawa et al., 1983; Metzger, 2015) we identified strongyle eggs in 2.89% ( $N = 588$ ) of samples from 12 individuals.

Specific morphological features and dimensions of the eggs found in four of these samples resembled those of *O. stephanostomum* (75–80  $\mu\text{m}$  length; 43–48  $\mu\text{m}$  width) (Hasegawa et al., 1983). We only found them in feces from female individuals (Bompusa East:  $N = 1$ ; Bompusa West:  $N = 3$ ) collected between March and April 2020 (Figure 1b). Based on egg dimensions and morphology, other samples contained unidentified strongyles ( $N = 10$ ) and hookworms ( $N = 2$ ). The presence of strongyle eggs in feces was not predicted by rainfall, minimum temperature or the percentage of terrestrial feeding time (Figure S3, Table S2). Finally, feces collected the day of *M. fulvum* ingestion were less likely to contain strongyle eggs (probability of sample containing no egg when collected after consumption of *M. fulvum* = 0.96; Figure S4, Table S2). Between the first (17/03/2020) and the last (09/04/2020) positive sample of *O. stephanostomum* eggs presence, one sample contained hookworms and seven samples contained unidentified strongyles. The remainder ( $N = 29$ ) did not contain additional strongyles. In April 2020, we observed a significant increase in the records of diarrhea in many party members from both communities ( $\chi^2 = 123.62$ ,  $p = 2.2e - 16$ ).

## 4 | DISCUSSION

Here, we set out to understand potential triggers for *M. fulvum* consumption by LuiKotale bonobos, testing the hypothesis that the plant's ingestion is related to (re)-infection by and egestion of strongyle eggs or *B. studeri* proglottids. Between December 2018 and July 2020, we investigated 969 fecal samples at the macroscopic level, 588 of which were also analyzed at the microscopic level.

### 4.1 | Behavioral observations

We observed more *M. fulvum* ingestion occurrences by LuiKotale bonobos than in previous studies (Lomako: Dupain et al., 2002; LuiKotale: Fruth et al., 2014). Considering individuals across both communities, we witnessed leaf swallowing and/or stem stripping of *M. fulvum* almost every month. Independent of the timing of fecal sample collection (i.e., during day or from night nests), we observed *M. fulvum* ingestion more often than we found remains in fecal samples. It is likely we missed fecal samples containing whole leaves and stem strip remains, even following *M. fulvum* consumption. Some leaves may remain in the digestive tract for some time before being egested and were therefore not present in the inspected feces within the expected time window. Alternatively, leaves and stem strips may have been digested when not well folded or too small.

Observations were biased to focal versus nonfocal individuals with all 18 focal individuals ingesting *M. fulvum* at least once and more regularly than nonfocal individuals, emphasizing again how cryptic this behavior is. In addition, these 18 individuals were adults, representing 23.7% of the studied population (48.6% of all adults), meaning we cannot make age-specific inferences as neither infants nor juveniles were focal individuals.

### 4.2 | Seasonality

In contrast to our prediction and expectations based on previous studies in chimpanzees and bonobos (Dupain et al., 2002; Wrangham & Nishida, 1983), the frequency of *M. fulvum* consumption was neither seasonal nor predicted by rainfall, temperature or terrestriality. However, the likelihood of observing *M. fulvum* ingestion during the wet season relative to the dry season was seemingly lower, which aligns with previous results from the LuiKotale research site (Fruth et al., 2014). In the past, leaf swallowing of entire leaves by African great apes has been associated with parasite expulsion (Fowler et al., 2007; Huffman & Caton, 2001; Huffman et al., 1996; Wrangham, 1995). Because re-infection with strongyles likely occurs 1–2 months after the onset of the rainy season, we expected to observe more leaf swallowing and stem-stripping at this time. At LuiKotale, however, bonobos consumed *M. fulvum* throughout the year, independent of wet, transient, and dry months (Figure 1b). When looking at the communities separately, we observed periodic consumption by single individuals interspersed with short periods during which *M. fulvum* was ingested by a larger proportion of the party.

Similar to Budongo (Huffman et al., 2009; Newton-Fisher, 1999), the seasonality at LuiKotale showed extended humid and short dry periods. At Budongo, adults of *Oesophagostomum* spp. were not retrieved from chimpanzees' fecal samples, nor was egestion of eggs associated with rain, or leaf swallowing correlated with monthly rainfall (Huffman et al., 2009). In contrast, at Bulindi, which is located close to Budongo and hence has a similar climate, *Oesophagostomum* eggs were found at higher frequencies in wet compared to transient or dry months, with their presence being associated with low temperatures. However, leaf swallowing here had no clear seasonality. Seasonality may play a critical role in the development and life cycle of parasite species because changing seasonal climate conditions alter the host-parasite dynamic. Strongyles in particular are known to be typically seasonal in some areas of their distribution. At LuiKotale, Budongo and Bulindi dry seasons are short, meaning the soil environment may never be particularly hostile for *Oesophagostomum* eggs to mature into the infective larval stage. Accordingly, 1/bonobos at LuiKotale may not suffer a high burden of re-infection at any particular time during the year (e.g., after the onset of the rainy season) because larvae remain infective over longer periods. Thus, bonobos 2/cope with re-infection all year around by regularly swallowing *M. fulvum* thereby maintaining low levels of infection.

### 4.3 | Cultural behavior

As shown previously by Huffman and colleagues (2010) in captive chimpanzees, naïve zoo-housed chimpanzees started using two different techniques of leaf swallowing to insert and fold the rough leaves into their mouth. These techniques, which have also been observed in the wild, were propagated spontaneously within the respective social groups (see also Tennie et al., 2008). Swallowing without chewing is an acquired behavior as shown by Huffman and

Hirata (2004). Youngsters learn it from their mother or other members of the community. In the present study, we observed immature individuals ingesting *M. fulvum* alongside adults and other group members, and it was not uncommon to observe a juvenile peering at another individual processing and ingesting leaves or stem strips of *M. fulvum*. Bonobos at LuiKotale and Kokolopori ingest leaves and stem bark of *M. fulvum* while bonobos at Lomako and Manzano were only reported to ingest the leaves. This suggests stem-stripping is a cultural behavior. However, even between the two communities in LuiKotale, we documented differences in the plant part chosen, with Bompusa East choosing leaves over stem strips and Bompusa West choosing stem strips over leaves, independent from plant part availability. This may be due to individual preferences or need if different plant parts function differently, though our data did not allow us to define these. We observed individuals combining ingestion of leaves and stem strips in the same bout, which leads us to speculate whether the two parts act synergistically. In some cases, we observed individuals seeking leaves while ignoring abundant stems and vice-versa. To better understand the function of leaves and stem strips and whether techniques for ingestion are cultural, future studies should target immigrant females.

#### 4.4 | Fecal examination

During the study period we did not find adult nematodes or cestode proglottids in fecal samples, independent of the presence of *M. fulvum*. Nevertheless, we cannot exclude bonobos being infected with these parasite species. Unlike at other sites (Huffman et al., 2009; Huffman, pers. comm.), leaf swallowing by LuiKotale bonobos does not appear to be associated with the expulsion of *B. studeri*. Therefore, we consider it very unlikely any of the individuals sampled during the study period were heavily infected with this species. We found leaf packages in 5.3% of collected fecal samples compared to 1.5% at Lomako (Dupain et al., 2002) and 3.5% at LuiKotale in a previous study (Fruth et al., 2014). The fact our sample collection was more systematic and focused on samples collected during all-day follows rather than nest sites may explain this difference. However, we found less *M. fulvum* remains in fecal samples than were found in Manzano (6.4%) (Narat, 2014), despite a larger sample size. Given the discrepancy between the proportion of *M. fulvum* found in feces collected beneath night nests (2.25%) and those during focal follows (42.4%), it is evident that studies relying on fecal collection independent of direct observations considerably underestimate the frequency of this behavior.

Swallowing intact leaves without chewing is generally rare, although variation exists between chimpanzee populations. For study sites in Kibale National Park, Uganda, Wrangham (1995) reported that 2.5% of feces at Kanyawara ( $N = 1,696$ ), and 0.4% at Ngogo ( $N = 1198$ ) contained intact leaves. In Gashaka, Nigeria, Fowler and colleagues (2007) found intact leaves in 3.7% of samples ( $N = 299$ ); and Huffman and colleagues (1996) found intact leaves in 2.4% ( $N = 245$ ) of samples collected in Mahale, Tanzania. In all the above-

mentioned studies, the swallowing of rough-surfaced leaves was associated with parasite infection and/or expulsion. In Bulindi, Uganda, this behavior was more frequent, with McLennan and colleagues (2017) finding intact leaf remains in 11.8% ( $N = 406$ ) of fecal samples. Bulindi is a very disturbed, fragmented area where the risk of parasite transmission between chimpanzees and villagers is high (McLennan et al., 2017); thus, the frequency of *M. fulvum* ingestion may be related to the risk of (re)-infection.

#### 4.5 | Parasitological analyses

*O. stephanostomum* is the most predominant species in African great apes (Hasegawa et al., 1983; Huffman et al., 1996, 2009; Makouloutou et al., 2014). Following previous observations in wild chimpanzees with moderate *O. stephanostomum* infections (Krief et al., 2008), bonobos at LuiKotale did not show obvious symptoms when egesting strongyle eggs, suggesting they do not suffer heavy infection by *O. stephanostomum* or unidentified strongyles.

We retrieved strongyle eggs from 17 of 588 samples analyzed using a flotation technique common in veterinary diagnostics for retrieving helminth eggs (Broussard, 2003; Pouillevet et al., 2017; Vlčková et al., 2018). It is sensitive for detecting strongyle eggs, but has some drawbacks, including the potential to distort the eggs (making identification complicated) and not being adaptable for other egg types (Ballweber et al., 2014, Salvador et al., 2014). It also does not allow identification of larvae making taxonomic identification speculative in our study. In addition, the limitations of field conditions or sample storage may have reduced the chances to retrieve parasite eggs using this method.

While some specialists claim that *Oesophagostomum* and hookworm eggs cannot be distinguished based on egg visualization only (Blotkamp et al., 1993; Narat, pers. comm.), others distinguish between *O. stephanostomum* and other strongyle species microscopically (Hasegawa, pers. comm.). Combining our method with alternative ones such as coproculture or isolation of larval stages would have been pertinent and is encouraged for future studies. However, accurate identification of parasite species can only happen when molecular analyses are used. Lacking genetic identification, we cannot confirm which strongyle genus and species occur at LuiKotale and what their abundance is compared to other sites. Moreover, as there is no published record of parasitological analyses of humans inhabiting the study region, we cannot exclude the presence of helminth species, and if present, whether or not they are shared between humans and bonobos.

At Lomako, researchers found 16.1% and 50.6% of fecal samples ( $N = 87$ ) contained undetermined strongyles and *Oesophagostomum* sp. respectively (Dupain et al., 2009). At Wamba these rates were 21% and 17.9% (Hasegawa et al., 1983) respectively, while at Manzano 44% of samples had undetermined strongyles (Narat et al., 2015). Researchers used different methods of fecal analyses (i.e., sedimentation in Lomako and Wamba; direct smears in Manzano) which might partly explain these differences. In Ugandan

chimpanzees, McLennan and colleagues (2017) found 58% and 66% of all samples ( $N = 38$ ) containing *Oesophagostomum* sp. and hookworm respectively using coproculture. One of the main differences between sites where chimpanzees and bonobos have been studied so far and the LuiKotale study site is the remoteness of the latter. In LuiKotale the nearest village is located approximately 20 km beeline, with a large river in between; thus, the only humans in regular contact with the bonobos are researchers that adhere to a strict hygienic protocol. In this context, cross-species parasite transmission is limited, which could partly explain the very low rate of parasite infections observed in bonobo feces.

Another limitation that we need to consider when analyzing our results is the phenomenon of intermittent shedding (e.g., Khurana & Sethi, 2017; McLennan et al., 2018; Van Gool et al., 2003). Sometimes, parasites are not shed in fecal samples despite the host being infected. Indeed, when analyzing our samples, we found that consecutive fecal samples collected on the same day from the same individual did not have consistent parasite diversity. To account for intermittent parasite and parasite egg egestion and, thereby, obtain a more accurate assessment of an individual's infection status, we recommend increasing the frequency of fecal sample collection and performing molecular analyses in conjunction with classical observational methods.

#### 4.6 | Potential role of *M. fulvum* leaf and stem strip swallowing

So far, the swallowing of unchewed leaves observed in bonobos is known for two species only, *M. fulvum* and *Cola* spp. (Dupain et al., 2002). In the case of *M. fulvum* at least, it is unlikely the plant is consumed for nutritional purposes considering the mode of ingestion (i.e., leaf folding and swallowing without chewing) by bonobos (Messner & Wrangham, 1996) and the absence of taste or odor from any plant part to human taste. We confirmed that *M. fulvum* increases gut motility, with boluses being expelled three times faster than usual without significant modification of fecal consistency and regardless of the plant part consumed. We observed a slight antidiarrheal effect, contradicting what we expected from the increased gut motility; however, this antidiarrheal effect was not evident in all samples and not supported statistically, possibly due to the small sample size.

As found in previous studies, our observations confirmed *M. fulvum* consumption occurs primarily in the early morning and often as the first ingestion of the day. In most cases, bonobos ingesting *M. fulvum* did not present any symptoms of infection on the day of consumption. On only seven occasions, we recorded symptoms such as diarrhea and/or asthenia that were absent after ingestion of *M. fulvum*. In these cases, bonobos showed moderate sociality but remained in the group. Although we cannot prove that the symptoms disappeared as a result of *M. fulvum* ingestion, we also never observed a deterioration in health following ingestion.

Our data showed no association between the consumption or egestion of *M. fulvum* and the egestion of strongyle eggs or adults. As mentioned earlier, independent of leaf presence in feces, we never observed adult worms in feces, which suggests severe parasite infections are rare at LuiKotale.

Interestingly, the frequency of *M. fulvum* ingestion was lower during a period when we found strongyle eggs in feces (between March and May 2020), suggesting a negative association between *M. fulvum* ingestion and the presence of parasite eggs in feces. If *M. fulvum* ingestion helps dislodge larvae from the intestinal wall or expulse adults present in the lumen, we would expect to see a positive association (i.e., worms jointly detected with intact leaves). However, if parasitic load (i.e., estimated by egg count) was very low across individuals and time, this may explain why we did not jointly detect worms and intact leaves in fecal samples. As observed in a previous study on chimpanzees, infection intensity can be very high in one community and low in another one when the prevalence of the given parasite species is comparable between the two communities (Huffman et al., 2009). It is likely that higher infection intensities trigger more leaf swallowing, supporting the need to incorporate egg counts in future studies.

After experiments with captive chimpanzees, Huffman and Hirata (2004) concluded that leaf swallowing is most likely not an innate response to parasite infection as symptom-free individuals performed the behavior. This suggests alternative triggers for *M. fulvum* swallowing by LuiKotale bonobos, such as bloating, intestinal discomfort and spasms observed in chimpanzees (McLennan & Huffman, 2012; McLennan et al., 2017). We speculate that the wound induced by the burst of L4 *Oesophagostomum* larvae from their nodules causes intestinal pain and discomfort that triggers *M. fulvum* swallowing. If true, this suggests the association between the egestion of *Oesophagostomum* worms and leaf swallowing being a byproduct of the self-medicative behavior. It would also support our observation that we detected strongyle eggs in feces more frequently in the absence of *M. fulvum* consumption, as larvae had time to develop into adult reproductive stages.

## 5 | CONCLUSION

Our results provide new insights into the leaf swallowing behavior. We showed that across 20 months of bonobo observation at LuiKotale, leaf swallowing of *M. fulvum* was not seasonal or predicted by environmental variables or associated with the egestion of *Oesophagostomum* worms or proglottids of *B. studeri*. However, we cannot exclude gastro-intestinal infections caused by other parasite species triggering *M. fulvum* ingestion. We showed that leaf swallowing and stem-stripping of *M. fulvum* occurred more often than previously reported. When *M. fulvum* was not ingested, diarrhea and the presence of strongyle eggs in feces were more frequent. Thus, our results support the hypothesis that ingesting *M. fulvum* flushes out larvae before they develop into adults, thereby reducing the likelihood of pathologic nodules and related severe symptoms. We

cannot exclude that *M. fulvum* acts differently from other bristly-leaved species possessing specific characteristics and mechanical and chemical properties. It is possible that its ingestion is triggered by symptoms we cannot detect with the noninvasive methods used here. In future, a more comprehensive study on the health condition of single individuals, including a thorough molecular screening of their intestinal parasite and microbial diversity and abundance, may provide a better understanding of this behavior. Independent of the search for triggers, the evidence that *M. fulvum* ingestion is a cultural trait is a fascinating aspect that warrants further exploration. Studies investigating different bonobo populations may shed light on new components of this potential cultural aspect.

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## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request and will be made available in MPI data repository upon acceptance

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