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RESEARCH ARTICLE

New Evidence for Self-Medication in Bonobos: *Manniophyton fulvum* Leaf- and Stemstrip-Swallowing From LuiKotale, Salonga National Park, DR Congo

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The swallowing of entire leaves by apes across Africa without chewing has been observed for over 40 plant species. Here we add evidence for (a) a new site, LuiKotale where leaf-swallowing of *Manniophyton fulvum* (Euphorbiaceae) is observed in bonobos, (b) a so far unreported ingestion of unchewed stemstrips of *M. fulvum*, we name stemstrip-swallowing; and (c) a test of some of the requirements put forward by Huffman for the assessment of plants ingested for medical purpose. As ecological correlates we analyzed *M. fulvum* phenological data and examined 1,094 dung piles collected between 2002 and 2009. By that we assessed availability and choice of leaves. In addition, we provide the first full description of the behavior related to this plant species' use by chimpanzees or bonobos using 56 bouts of *M. fulvum* ingestion observed between October 2007 and February 2010. With these data we tested and met 4 of the 6 requirements given by Huffman, supporting ingestion of this species as self-medication. Despite species' year-round availability and abundance, *M. fulvum* was ingested only at specific times, in very small amounts, and by a small proportion of individuals per party. In the absence of our own parasitological data, we used *M. fulvum* swallowing as evidence for parasite infestation, and seasonality as a proxy for stressors underlying seasonal fluctuation and impacting immune responses. Using these indirect factors available, we investigated conditions for a parasite to develop to its infective stage as well as conditions for the host to cope with infections. Both rain and temperature were good predictors for *M. fulvum* ingestion. We discuss the use of *M. fulvum* with respect to its hispidity and subsequent purging properties and provide insight into its ethnomedicinal uses by humans, stimulating speculations about potentially additional pharmacological effects. Am. J. Primatol. 76:146–158, 2014.

Key words: ***Pan paniscus*; *M. fulvum*; self-medication; leaf-swallowing; stemstrip-swallowing; seasonality**

INTRODUCTION

Self-Medication in Great Apes

The first reports of great apes using plant parts for apparently non-nutritional purposes date back more than 30 years, when chimpanzees at Gombe were observed swallowing whole leaves in an atypical way [Wrangham, 1975, 1977]. Thereafter, similar feeding habits were reported in a number of other sites, for example, Mahale: [Takasaki & Hunt, 1987; Wrangham & Nishida, 1983]; Kibale: [Huffman & Wrangham, 1994]; Bossou: [Matsuzawa & Yamakoshi, 1996; Sugiyama & Koman, 1992]; and Taï: [Boesch, 1995]. In subsequent decades, this behavior has been observed across Africa involving all chimpanzee subspecies (*Pan troglodytes schweinfurthii*, *P.t. troglodytes*, *P.t. verus*, *P.t. vellerosus*) [Fowler et al., 2007; Huffman, 1997, 2001, 2010; Krief et al., 2006; Pruetz & Johnson-Fulton, 2003], bonobo (*Pan paniscus*) [Dupain et al., 2002; Fruth, 2011], and eastern lowland gorillas (*Gorilla gorilla graueri*) [Yamagiwa pers. comm.] cited in [Huffman & Wrangham, 1994].

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To date, leading studies investigating the function of leaf-swallowing explain the choice of plants utilized by their physical rather than chemical properties [Huffman & Caton, 2001; Huffman et al., 1996; Messner & Wrangham, 1996]. In fact, all species with the observed mode of ingestion, that is, swallowed whole and unchewed, share the characteristic property of having trichomes causing bristly, hispid surfaces [Huffman, 1997]. The inducing of vigorous movements in the intestinal tract leading to a reduced transit time of six hours facilitating the expulsion of *Oesophagostomum stephanostomum* was documented at Mahale [Huffman & Caton, 2001]. Today the swallowing of entire leaves by apes across Africa without chewing has been observed for >40 species [McLennan & Huffman, 2012]. Despite this overwhelming evidence for rough surfaces as criterion of choice, there is also the possibility for the potential role of pharmacologically active compounds that may act in addition to the above cited physical ones in some of these species. Huffman et al. [1996, 1997] and Krief et al. [2008] suggest that leaf-swallowing is of crucial importance in controlling ape intestinal parasites. Huffman et al. [1996] provide evidence from Mahale showing data on sickness and even death in one individual. Krief et al. [2008] report severe clinical lesions caused by *O. stephanostomum* in sanctuary raised apes; and Terio et al. [2011] showed in a study covering 6 years from Gombe, intestinal *Oesophagostomum* spp. infestations for 73% of 11 individuals investigated.

A particular role in host–parasite interactions is attributed to seasonality, as it can influence host–parasite interactions both directly as well as indirectly (for a review see [Altizer et al., 2006]). As seasonality is different across the range of *Pan*-distribution and in addition shows inter-annual variability within sites, assessment of seasonal patterns is crucial for the understanding of two aspects: (1) the conditions for the parasite to develop and become infectious and (2) the conditions for the host to be susceptible to parasite infection.

1. *Conditions for the parasite*: Viability and infectivity of parasite eggs or larvae are known to correlate positively with humidity and warmth [Stepek et al., 2006; Van Voorhis & Weller, 2005]. For species such as *Oesophagostomum* spp. this is well reflected in studies of chimpanzees living in seasonal habitats such as Mahale [Huffman et al., 1990, 1997] and Gashaka [Fowler et al., 2007]; and indicated for bonobos living at Iyema–Lomako [Dupain et al., 2002]. In these studies the considerable increase of infections coincided with the onset of the rainy season, the period needed by, for example, *Oesophagostomum* spp. to undergo development of shedded eggs needing humidity to develop and become infectious stage larvae in the outside environment [Polderman & Blotkamp, 1995]. The reverse effect was found in western lowland gorillas with higher infection rates during the dry than during the wet season [Masi et al., 2012]. In habitats with a more or less equitable distribution of rain throughout the year such as Budongo however, where shedding and reinfection may occur at any time, no such association was found [Huffman et al., 2009]. In any case, leaf-swallowing has been reported to correlate with intestinal parasite infestations, may they be seasonal with respect to rainfall as shown for *Oesophagostomum* at Mahale, Gombe, Gashaka, Fongoli, and Iyema or independent of rainfall as shown for *Bertiella* at Kibale and Budongo. Thus, even if the species of the related parasite is unknown, as in our case, leaf-swallowing may shed light on the state of an individual's intestinal parasite infection.
2. *Conditions for the host*: The hosts' susceptibility to parasites depends on its competence for immune defense against them. Vertebrates are known to possess an immune system being partly innate and partly adaptive, allocating more of their energetic resources to the adaptive one the more long-lived they are [Previtali et al., 2012]. Due to the protected status of endangered species, particularly great apes, data on the immune function of these animals in the wild are limited given that only noninvasive methods can be employed. However, from a comprehensive set of data available for mammals, stress is known to influence immune-responses and by that the individuals' ability to cope with pathogens and parasites [Preston et al., 2009; Webster Marketon & Glaser, 2008]. Stress is known to derive from various variables underlying seasonal fluctuation such as food availability [Wich et al., 2004], food quality [Conklin-Brittain et al., 1998], distribution of and access to mating partners [Hill et al., 2000], pregnancy and lactation [Foerster et al., 2012], rank acquisition and maintenance [Hothorn & Hornik, 2013], and sleep quality [Walker et al., 2008], all potentially impacting immune activity (for a review see [Martin et al., 2008]). In the absence of reliable measurements for any of these potential stressors in our study, we include both rainfall and temperature as proxies for the underlying stress-causing variables.

We focus on *Manniophyton fulvum* (Euphorbiaceae), a species that has been observed being swallowed by chimpanzees at Taï [Boesch, 1995] and Ndoki [Huffman, 1997], and bonobos at Wamba [Huffman, 1997] and Lomako [Dupain et al., 2002; Fruth, 2011]. Unfortunately, investigations of the health status at the time of *M. fulvum* ingestion are lacking from any of these sites. However, infections of *Oesophagostomum* spp. and other intestinal parasites were described for bonobos from Wamba and Lomako [Dupain et al., 2000; Hasegawa et al., 1983].

Here we investigate the use of *M. fulvum* by bonobos of one habituated community at LuiKotale, offering a detailed ethological and ecological description of (a) leaf-swallowing, as well as of (b) stemstrip-swallowing, a so far unreported ingestion of unchewed stemstrips, using both direct and indirect data available. We use four of six characteristics provided by Huffman [1996, 1997] as hypotheses to test the likelihood of *M. fulvum* as a means of self-medication: (1) Infrequent intake of plant species which are not a regular part of the diet; (2) habit of ingestion that provides no nutritional benefit; (3) restriction of plant use to seasons or other periods associated with high risk of parasitic infection; and (4) no interest in or different utilization of the plant by conspecifics (presumably healthy).

In the absence of parasitological data, we use the behavior as sign for intestinal parasite infection as shown by many other studies. We discuss the use of *M. fulvum* leaf- and stemstrip-swallowing with respect to the species' morphological and phytochemical properties.

METHODS

Study Site

Observations were collected during an on-going field study of the Bompusa community of bonobos in LuiKotale, in the Democratic Republic of Congo. The LuiKotale Bonobo Project (LKBP) was initiated in 2002. The field site is located close to the western border of Salonga National Park (South block), 25 km away from the nearest village and stretches from the river Lokoro towards the south [Hohmann & Fruth, 2003]. The climate is equatorial with abundant rainfall (2,132 mm/year ± SD = 546), a small dry season in February and a larger one between May and August. Mean monthly temperatures range between 18 and 29°C with a minimum of 15.7°C and a maximum of 37.3°C (2003–2009). All members of the Bompusa community have been identified by physical features and were habituated to human presence. Long-term follows were possible by the time of the present observations. As this study has not been designed to investigate *M. fulvum* ingestion, but all occurrences of *M. fulvum* swallowing collected let to the desire for a broader investigation, were made use of data accumulated between 2002 and 2009 for the long-term data record for the purpose of other studies. Therefore, study periods vary and are specified according to their use.

Plant Species Investigated

M. fulvum Muell. Arg (Euphorbiaceae) is a monospecific genus occurring in tropical Africa [Jiofack Tafokou, 2010]. It grows as a shrub or woody climber in primary and secondary forests. Leaves are covered by astral hair being responsible for a rough, sandpaper like lower surface and a somewhat smoother upper surface. Mature leaves are dark green, immature leaves light green to yellowish. The stembark is rather soft and when shoots are young they are covered by hair as above, becoming more ligneous as the shoot ages.

Behavioral Observations

Between October 2007 and June 2009, habituated members of the Bompusa community were followed on 276 days investigated by the senior author (AF) and/or two additional field assistants paying attention to *M. fulvum* consumption. For the 211 days between December 2007 and June 2009 observation hours were recorded, 33% were nest to nest follows. All bonobos were individually identified. During this period, the community consisted of 34–39 individuals, including five adult and four adolescent males, and depending on the year, 11 or 12 adults and one up to five adolescent females. Age estimates were based on physical features such as body-, testes-size, dentition, and genital swellings. We categorized individuals in the following way: "immatures," consisting of infants (1–4 years) and juveniles (5–8 years), and "matures," consisting of adolescents (9–13 years) and adults ($\geq$14 years). Party size and composition was recorded every hour.

Whenever occurrence of *M. fulvum* consumption was observed details of ingestion were recorded. All individuals in visual range were controlled for their item of ingestion. Whenever possible, ingestion was videotaped and analyzed post-hoc. In order to assess the specific ingestion procedure and to allow comparison of *M. fulvum* ingestion with other species, we distinguished between ingestion events and ingestion bouts as follows:

An ingestion *event* is the incorporation of selected plant material from putting hand on the given item (leaf, stem), its manipulation to its ingestion (swallowing).

An ingestion *bout* is a sequence of *M. fulvum* ingestion events that are uninterrupted by either ingestion of items other than *M. fulvum* or behavior other than feeding.

For comparison concerning the mode of ingestion, and time of consumption, we used a data-set focusing on food intake (fruit and leaves) obtained from 20 mature individuals (11 females, 9 males) for 61 days between February and June 2009. Each individual was observed for at least 5 min assessing intake rates by counting bites per minute. We considered a bite when lips or teeth enclosed items within the mouth, independent of whether or not items were part of one leaf (as in *Manniophyton* sp.) or of several folioles or leaves (as in *Dialium* spp.). For species comparison of time of consumption we considered for each observation day the first intake per individual.

Fecal Samples

Between 2002 and 2005, 694 dung piles (=100%) were recovered from beneath night nests (97%), feeding spots (1.1%) and during travel (1.9%) to determine dietary parameters. These were taken when considered being the complete fecal deposit of one adult individual of the given defecation event. When multiple nests were close together or spatially distributed, making it impossible to identify the feces from any given nest, samples were not taken. In addition, feces ($N = 400$) were collected consistently between September 2008 and June 2009 from all night nests for at least one nest group per week, as well as independent from the routine sampling the morning after *M. fulvum* consumption was observed. Fecal samples were visually inspected for traces of *M. fulvum* leaves [Fowler et al., 2007] (Fig. 1).

In contrast to leaves, stems of *M. fulvum* swallowed in this way were not detected in the feces. Thus, it was not possible to identify this technique from visual inspection of feces.

Ecological Data

Ecological data collection was conducted in the frame of the Project "The *Cuvette Centrale* as reservoir of medicinal plants," a long-term study of the floral diversity of the Congo Basin with a focus on medicinal plants [Fruth, 2011].

Arboreal availability of *M. fulvum* was measured counting its stems associated with trees (≥10 cm DBH), when stems had developed leaves and reached the canopy layer. A total of 1,000 trees (≥10 cm DBH) were recorded along 8 km of line transects (stripwidth 1 or 2 m, respectively). Transects were established in 2002 to representatively sample three major habitat types: Heterogeneous primary forest on (1) *terra firme* soil, and (2) periodically inundated soil; as well as (3) homogenous primary forest on slopes dominated by *Gilbertiodendron dewevrei*. Overall sample surface was 1 ha.

Terrestrial availability was measured counting presence of *M. fulvum* stems in four randomly chosen plots of 10 m × 10 m setup within each of the three major habitat types mentioned above.

Availability of leaves was recorded for 185 individuals of *M. fulvum* monitored twice per month between April 2002 and March 2005 in the frame of phenological investigations along the above mentioned transects. In addition, between June 2002 and March 2005 for each of these individuals absence/presence of young leaves was noted and if possible the proportion of young leaves visually estimated.

Seasonality

We used data on temperature (°C) and rainfall (mm) to assess seasonal patterns. Temperature was measured from a thermometer being open to the sky. Rain was measured using a rain gauge (mm/m^2) open to the sky.

1. *Conditions for parasite egg/larval development*: In a first assessment we used rainfall data collected between March 2002 and December 2009 to investigate seasonality. For comparability to previous studies we classified monthly cumulative rainfall according to Newton-Fisher [1999] with months being "wet" if rainfall exceeded 100 mm, "transient" if it was between 99 and 51 mm and dry if it was equal or below 50 mm. For inter-site comparability we gave the Perhumidity Index (PI), which measures the degree of continuity of wetness of the annual rainfall, summing monthly scores per year as suggested by Walsh [1996] as follows: Very wet month (>200 mm) = (+) 2; wet

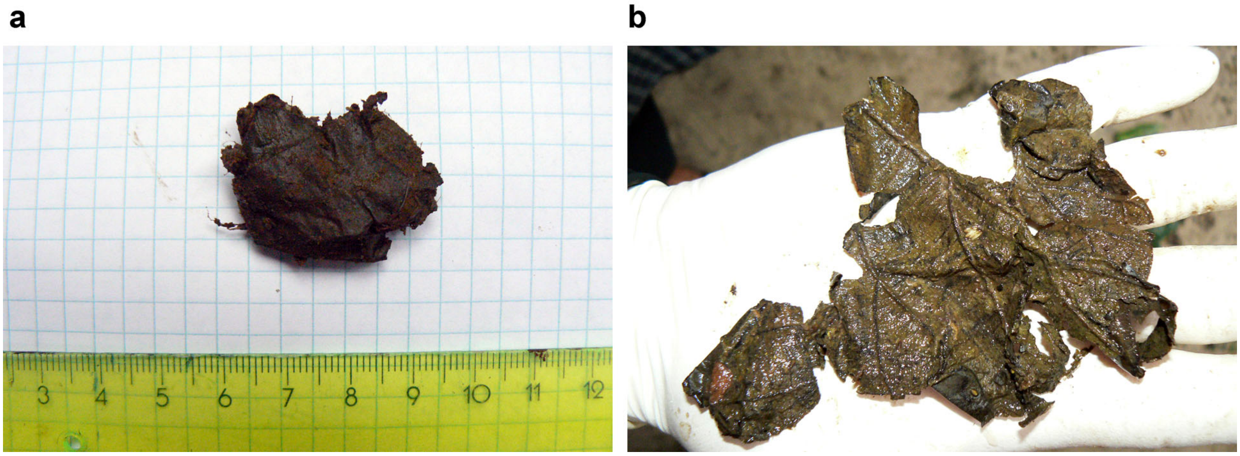

Fig. 1. *M. fulvum* (**a**) package of leaves still folded as recovered from feces; (**b**) package unfolded manually.

month (100–199 mm) = (+) 1; dry month (50–99 mm) = (−) 1; drought month (<50 mm) = (−) 2; the first dry month following a wet month = (−) 0.5 and the first drought month following a wet month = (−) 1.5. To assess seasonality of leaf-swallowing we investigated fresh dung (see above) collected during 50 months of this period.

As the diurnal distribution of rain is considered to be of ecological relevance with night rain being more likely to reach the forest floor due to lower temperatures and reduced evaporation [Walsh, 1996], we used both monthly cumulative rainfall (mm; 24 hr), and monthly cumulative rainfall at night (12 hr; 18:00–5:59 hr) and monthly minimum temperature (°C) (thermometer open to sky) as predictor variables.

2. *Conditions for the host*: We considered seasonality as either a direct or indirect predictor of potential stress variables such as food availability, -quality, or sleep quality and by that as proxy leading to parasite susceptibility. We considered *M. fulvum* ingestion as a response, reflecting parasite infestation of the host with subsequent potential for self-medication.

Statistical Analysis

Terrestrial versus arboreal ingestion

To test whether or not leaf-swallowing bouts took place on the ground with the same probability as stemstrip-swallowing bouts we used a permutation test [Adams & Anthony, 1996] for which we randomized locations within subjects (1,000 permutations into which the original data were included as one permutation) and used chi-square as a test statistic.

Seasonality of ingestion

In order to assess seasonal influences on *M. fulvum* consumption and to allow comparison with previous publications, we first used a traditional approach: We correlated monthly rainfall and temperature with the proportion of all *M. fulvum* swallowing occurrences derived from fecal sample inspection collected between May 2002 and June 2009. Secondly we analyzed a subset of 211 observation days from December 2007 to June 2009 using a Generalized Linear Mixed Model, GLMM [Baayen, 2008], allowing control for individuals. Since the response was binary (*M. fulvum* consumption by an individual on a given day) we fitted the model with binomial error structure and logit link function. We also included temperature and rainfall and their interaction as well as an autocorrelation term (see below) as fixed effects and individual identity as a random effect. To establish the significance of the full model we used a likelihood ratio test [Dobson, 2002], comparing its deviance with that of the null model comprising only the intercept, the autocorrelation term and the offset term (see below). To test the significance of the interaction between *M. fulvum* consumption and seasonality we compared its deviance with that of a corresponding reduced model not comprising this interaction but all other terms being present in the full model.

The response was likely to be temporarily autocorrelated beyond what was explained by the predictors in the model, leading to non-independent residuals thus violating a crucial assumption of linear models. We thus explicitly incorporated a term accounting for such potential temporal autocorrelation into the model. We derived this autocorrelation term by a method essentially identical to that described by Fürtbauer et al. [2011] with the exception that we did not account for individual.

The GLMMs were run in R (version 2.14.1) [R Development Core Team, 2011] using the function *lmer* of the R package lme4 [Bates et al., 2011]. To account for varying observation times per subject and day, we included it (log-transformed) as an offset term into all models. Prior to analysis both predictor variables (rain and temperature) were z-transformed to a mean of 0 and a SD of 1.

For comparison of feeding bouts, time of day, and events per bout we used non parametric tests (exact Wilcoxon matched-pairs signed-ranks test) run in R (version 2.14.1) using the package exactRankTests [Hothorn & Hornik, 2013]. Other nonparametric tests were run in SPSS 20.0.

This research adhered to the American Society of Primatologists principles for the ethical treatment of primates. The methods used to collect observational data in the field are in compliance with animal care regulations and with the requirements and guidelines of the *Institut Congolaise pour la Conservation de la Nature* (ICCN), and adhered to the legal requirements of the host country, the Democratic Republic of Congo.

RESULTS

Of 276 days between October 2007 and June 2009, *M. fulvum* ingestion was observed on 17 days, 14 of which allowed detailed records. Together with one additional day (Feb 27th 2010) we had 15 days with 56 bouts of *M. fulvum* ingestion that provided behavioral details. Both mature and immature individuals of both sexes ingested *M. fulvum*. Of the 5 mature males of the community 2 were observed to do so, of 12 mature females 9 were observed ingesting *M. fulvum*. Eight of these females had dependent offspring, of which four (three males, one female) were observed ingesting *M. fulvum*. Ingestion was observed to take place on the ground (63%) as well as arboreally (37%). We distinguished between two distinct techniques, leaf-swallowing

(LS; $n = 20$ bouts) and stemstrip-swallowing (SS; $n = 35$ bouts), only for one bout were both techniques applied. Excluding the latter, bouts ($n = 55$) consisted of 1.65 events on average (range 1–11), and lasted 33 sec on average (range 6–95).

Leaf-Swallowing Behavior

In a typical leaf-swallowing episode, a young leaf is selected and either manually detached or moved toward the mouth still attached to the stem. A piece of leaf is bitten or ripped off and held in the mouth, flat on the tongue. Usually this is repeated with one or more pieces bitten and laid on top of the first. The leaf segment or package of segments is folded back into the mouth with the tongue, usually keeping the leaves' lower side outer-most. Contact with the lips is avoided. The segment or package of segments folded is swallowed without chewing. The process is repeated until the leaf is finished. The procedure may be repeated.

Leaf-swallowing bouts ($n = 20$) consisted of 2.45 events on average (range 1–11). For 19 of these bouts where the place of ingestion was recorded, 14 were arboreal, and 5 were terrestrial. Eighteen of the 20 bouts refer to adult individuals, two to immature individuals, one juvenile and one infant male. Adult leaf-swallowing bouts lasted on average 129 sec (range 93–180) with events lasting 45 sec on average (range 10–95), while the only entirely observed bout by an infant male of the age of three lasted 137 sec with 11 events lasting 12 sec on average (range 6–20). For 18 leaf-swallowing bouts the number of ingested leaves was observed. It was 25, resulting in an average ingestion of 1.39 leaves per bout.

Stemstrip-Swallowing Behavior

In a typical stem-stripping episode a stem is selected (touched) and its tip broken with the hand or mouth. The break of the stem is brought to the mouth using the hand. An edge is created with the teeth, (at which point latex may be licked from the stem). The soft bark is peeled down with teeth and lips until it either detaches itself or it is broken manually. The detached bark is then rolled up into the mouth forming a ball with the tongue and swallowed. If the process is finished, the stem is discarded, or else the procedure is repeated.

Stemstrip-swallowing bouts ($n = 34$) consisted of 1.18 events on average (range 1–3). For 30 of these bouts where the place of ingestion was recorded, 24 were terrestrial, 4 arboreal, and 2 started in a tree and were finished on the ground. Twenty-five of the 34 bouts refer to adult individuals, 9 to immature individuals, 2 juveniles (6 and 7 years), and 2 infant (3 and 4 years) males and 1 infant female (4 years). Only one stemstrip-swallowing bout was measured entirely. It lasted 120 sec. Events of adult individuals lasted 58 sec on average (range 23–95), while no bout was fully observed, two measured events lasted 15 and 25 sec, respectively.

For 17 stemstrip-swallowing bouts the number of ingested strips was observed. It was 22, resulting in an average ingestion of 1.29 strips per bout.

Comparing both techniques for adults only, there was a tendency for more events per bout for leaf-swallowing (2.06 vs. 1.24; exact Wilcoxon matched-pairs signed-ranks test: $T^+ = 33$, $N = 8$, $P = 0.031$). Leaf-swallowing bouts more often took place in trees, whereas stemstrip-swallowing bouts more often took place on the ground (permutation test: $\chi^2 = 9.72$, $P = 0.01$).

As individuals generally used both techniques interchangeably and the cases of stemstrip-swallowing were only directly witnessed and not reflected in dung-analysis, we combined both techniques as *M. fulvum* ingestion, if not specified otherwise.

Criterion 1: The Plant Part Is Not Eaten Regularly; Despite Wide Spread Availability

Species' availability

Arboreal. Of 1,000 trees (dbh $\geq$ 10 cm) recorded along 8 km of standardized transects across all habitat types, 18.5% of all trees were associated with at least one stem of *M. fulvum* reaching the canopy layer. Considering frequency of the 78 liana species associated with the trees investigated here, *M. fulvum* was the third frequent of all species. Although trees with *M. fulvum* were found more often than expected in periodically inundated forest than in heterogeneous *terra firme* and homogenous slope forests ($\chi^2 = 31.291$, df $= 2$, $P \leq 0.001$), it was present across all habitat types.

Terrestrial. Considering the lower stratum, *M. fulvum* was present in 6 out of 12 plots. While the species was present in both *terra firme* and periodically inundated heterogeneous primary forest, it was absent in the plots set up in slope forest dominated by *G. dewevrei*. Apart from underrepresented slope forests (<7% of all habitats), data from both arboreal and terrestrial availability investigations demonstrate the ubiquity of *M. fulvum*.

Leaves' availability

Of the 185 *M. fulvum* vines monitored between April 2002 and March 2005, 13 happened to be without leaves for very short periods of time (mean = 2.5 months, SD = 3.01). Calculating overall leaf availability for the 185 individual vines across these 36 months, leaves were available all year round with 99.5% of all individuals contributing. Looking at young leaves only, data were available from 33 months. For each of these months young leaves were available (average 27%; range 5–82%), independent of year and season.

Thus availability is not only provided by presence and spread, but also by the suitable phenological stage of the particular item consumed.

Regularity of ingestion

M. fulvum ingestion was directly witnessed on 17 out of 276 observation days (6.28%). When separating consumption of leaves from that of stems, *M. fulvum* leaves were swallowed on 3.98%, while stemstrip-swallowing was seen on 3.62% of these days. When investigating 694 dung piles obtained from 198 days between May 2002 and October 2005 for evidence of *M. fulvum* ingestion, 3.46% of these contained unchewed leaves, increasing evidence for ingestion to 9.09% of days. Similarly, investigation of 400 dung piles obtained from 121 days collected between September 2008 and June 2009 revealed 4.0% containing unchewed leaves, providing evidence for ingestion for 9.92% of days.

Looking at *M. fulvum* leaf consumption in comparison to the consumption of other leaves with respect to overall frequency and time of day, we looked at 61 days of observation when intake of food was observed systematically and intake rates were measured when possible. Leaves were consumed on 36 of these 61 days, involving 18 individuals (10 females, 8 males). Leaves of species of the following six genera were consumed: *Dialium*, *Grewia*, *Ficus*, *Haumania*, *Cynometra*, and *Nymphaea*, *Dialium* spp. leaves were consumed most often (73.8%, $n = 61$), followed by *Haumania* (27.9%) and *Ficus* (4.9%). The other genera (*Cynometra*, *Grewia*, *Nymphaea*) were represented only on 1.6% of these days.

Time of day of ingestion

Figure 2 shows the time of consumption for *M. fulvum* compared to the other plant food species consumed. The difference in the onset of time is significant (Wilcoxon-test: $T^{+} = 28, N = 7, P = 0.016$). Of 14 observed episodes of *M. fulvum* ingestion, 86% were early in the morning (before 7:00 am), while the onset of consumption of leaves of other species was spread across the day.

In sum, criterion 1 is met with *M. fulvum* as compared to other leaves ingested, being consumed rarely and only at specific times of the day, despite widespread availability in the study site.

Criterion 2: The Method of Ingestion Prevents Nutritional Benefit

The method of folding leaves with the tongue in the mouth before swallowing differed from that of other plants where leaves were chewed before swallowing. *M. fulvum* leaves appeared whole and apparently undigested in dung, while other leaves usually appeared as fibrous greenish fecal matrix. Figure 3 shows ingestion rates of other leaves compared to *M. fulvum*. With an average of 26.6 bites/min (range 5–50), average ingestion rate for leaves other than *M. fulvum* was significantly higher than that for *M. fulvum* with 5.7 bites/min (range 1–24) (MW *U*-test: standardized test statistic = 5,715, $N_{\text{Manniophyton}} = 22$, $N_{\text{other}} = 35$, $P < 0.001$). This is even more remarkable as in some species (e.g., *Dialium* spp.) we speak of several leaves per bite, while in *M. fulvum* we speak of several bites per leaf (average = 4; range: 1–7; $N = 26$).

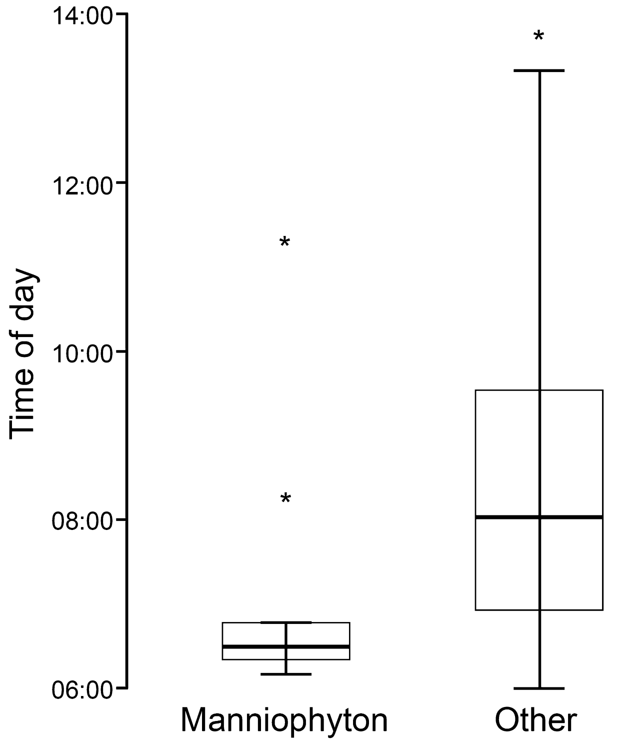

Fig. 2. Leaf consumption of *M. fulvum* and other species in relation to time of day. Horizontal bar in box indicates median, length of box corresponds to interquartile range. Bars outside boxes indicate percentiles (97.5 above; 2.5 below). * indicates outliers.

This is also true for stemstrip-swallowing. In sum, rare occurrence, minor number of leaves, slowness, and method of *M. fulvum* ingestion, were remarkably different from that of other leaves or bark.

In sum, criterion 2 is met with nutritional benefit of *M. fulvum* ingestion being considered as unlikely.

Criterion 3: Restriction of Plant Use to Seasons Associated With High Risk of Parasitic Infection

M. fulvum consumption, rain, and temperature

Rainfall was recorded for 88 months between March 2002 and December 2009. Average annual rainfall was 2,132 mm ± SD 546 ($n = 7$ years). Of these months a total of 78% had more than 100 mm/months and were considered as wet, 17% were

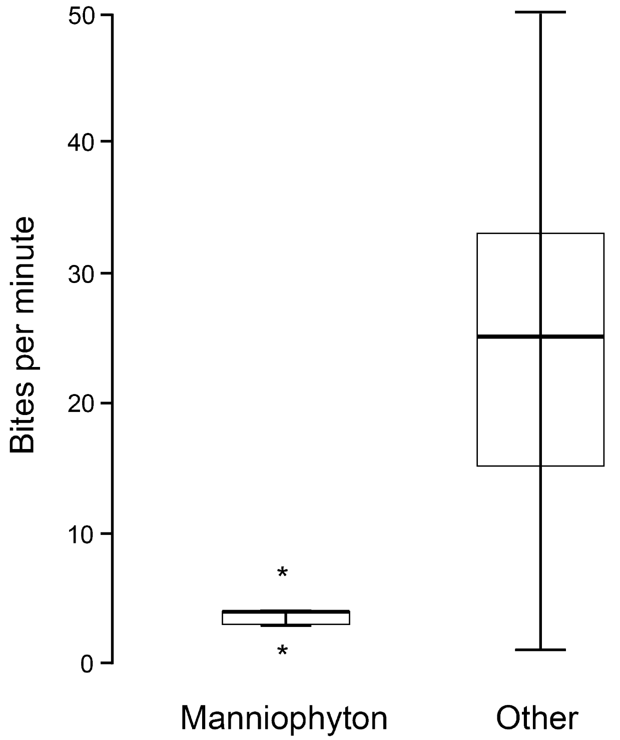

Fig. 3. Bites per minute for *M. fulvum* and other species leaves. Horizontal bar in box indicates median, length of box corresponds to interquartile range. Bars outside boxes indicate percentiles (97.5 above; 2.5 below). * indicates outliers

intermediate with rainfall ranging between 51 and 100 mm/months, and only 5% were dry with ≤50 mm rainfall/month. The Perhumidity Index ranged between 8.5 in 2004 and 17.0 in 2003 averaging 13.86 ± SD 3.01 ($N = 7$ years) between 2003 and 2009. Monthly averages reveal a very short somewhat dryer period usually in February as well as a more extended somewhat dryer season between May and August. Figure 4 shows the monthly distribution of cumulative rainfall, temperature, and monthly occurrence of *M. fulvum* ingestion in relation to either direct or indirect observation periods.

1. *The conditions for the parasite to develop and become infectious*: We monitored the excretion of unchewed *M. fulvum* leaves in the feces across season to investigate the possible link to parasite infection. Between May 2002 and June 2009 we inspected 694 dung collected across 50 months, 10 of which were dry and 40 wet. We found 40 dungs with unchewed *M. fulvum* leaves, 31 in wet and 9 in dry months. There was no significant difference in the seasonal occurrence of the number of dung found to contain unchewed *M. fulvum* leaves (Binominal test; $P = 0.444$).

Correlation of monthly cumulative rainfall with the monthly proportion of dung containing unchewed *M. fulvum* leaves was not significant (Spearman rank correlation: $\rho = -0.210$; $P = 0.592$; $N = 50$), neither was that of monthly cumulative night-rain or of the number of rain-days per month and *M. fulvum* leaves in the dung.

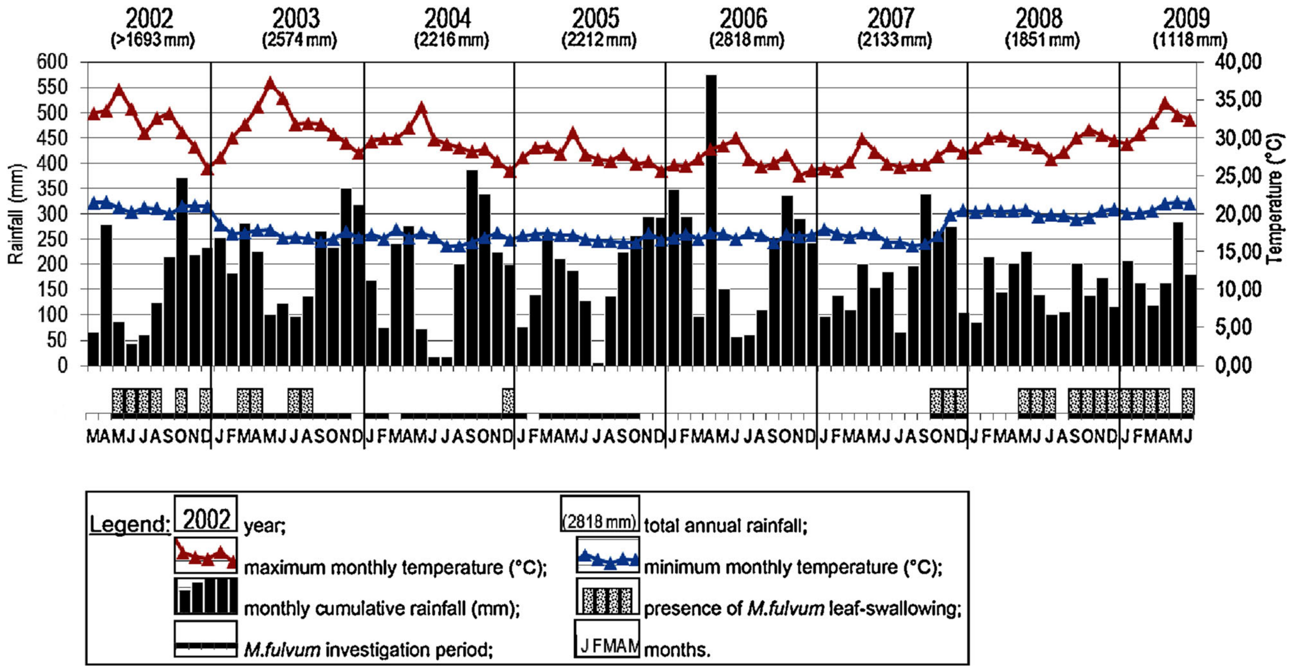

Fig. 4. Monthly distribution of rainfall and temperature and the occurrence of *M. fulvum* leaf-swallowing.

However, when we considered monthly average temperature and the proportion of dung with unchewed *M. fulvum* leaves, a significant relation became apparent with respect to both minimum temperature (Spearman rank correlation: $\rho = 0.521$; $P < 0.001$), and maximum temperature (Spearman rank correlation: $\rho = 0.327$; $P = 0.020$).

2. *The conditions for the host to suffer from increased parasite pressure*: Between December 2007 and June 2009 a total of 41 individuals were observed in changing number and composition across 211 observation days, resulting in 4,052 individual observation days (with or without *M. fulvum* consumption). Thus, the 400 macroscopically inspected dung piles were controlled for individuals and time of observation when investigating monthly cumulative rainfall and average minimum temperature. We found a clear impact of temperature on the probability of *M. fulvum* consumption comparing the full and the null model (likelihood ratio test with autocorrelation included: $\chi^2 = 37.383$, df $= 3$, $P < 0.001$). Since the interaction between rainfall and temperature was not significant (likelihood ratio test: $\chi^2 = 0.723$, df $= 1$, $P = 0.395$; estimate 0.433, SE $\pm$ 0.501) it was removed and the model was run again.

This final model revealed a highly significant effect of temperature on *M. fulvum* consumption with *M. fulvum* consumption being the higher the lower the temperature (-2.82 ± 0.47, $z = -5.987$; $P < 0.001$; Fig. 5).

Only when considering monthly cumulative night rain, did an effect became apparent with *M. fulvum* ingestion being higher at times of reduced than at times of plenty of night rain (-0.95 ± 0.22, $z = -4.285$; $P < 0.001$), although exceptions show that bouts of *M. fulvum* ingestion occurred also during months of abundant rainfall.

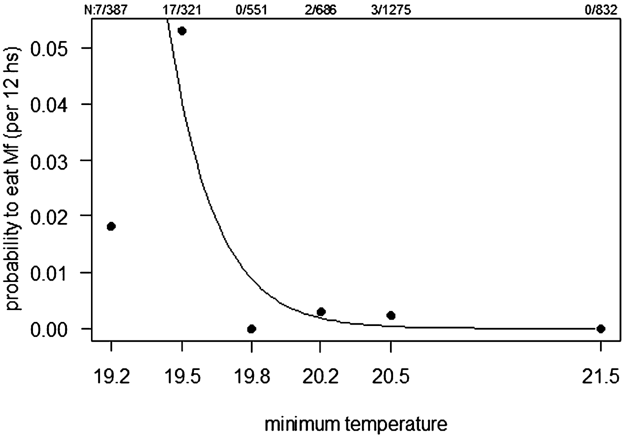

Fig. 5. Probability to eat *M. fulvum* in relation to minimum temperature (°C). Probability represents events/observation time (per 12 hr). Numbers on top of the figure indicate number of samples (observation days with *M. fulvum* consumption) and total number of samples (observation days with and without *M. fulvum* consumption) per binned value of temperature.

In sum, criterion 3 was partially met with *M. fulvum* ingestions being related to one seasonal factor (temperature), although there was no effect of monthly cumulative rainfall on *M. fulvum* ingestion.

Criterion 4: No Interest in or Different Utilization of the Plant by (Presumably Healthy) Conspecifics

Data concerning presence of all individual bonobos in the given party as well as evidence of both absence and presence of consumption of *M. fulvum* among these individuals was available for 13 out of 23 days when *M. fulvum* swallowing was observed. Parties ranged from 9 to 17 mature individuals. On average, 14% of all mature individuals in a given party ingested *M. fulvum* (range 0–26%); the rest did not show any interest in consumption of this plant. On average, 24% (range 0–57%) of mature females, and 10% (range 0–33%) of mature males present, swallowed *M. fulvum*. Thus, adult and sub-adult females were more likely to ingest than males in these age categories (related-samples Wilcoxon signed rank test; standardized test statistic $= -2{,}701$; $P = 0.007$; $N = 13$). Table I shows party size and composition as well as the number of individuals swallowing *M. fulvum* for days when *M. fulvum* ingestion was observed.

In sum, criterion 4 is met with conspecifics of the same party showing no interest in the plant.

DISCUSSION

We presented new evidence for self-medication in bonobos; (a) a new site, LuiKotale where leaf-swallowing is observed, and (b) a so far unreported mode of ingestion of unchewed stemstrips of *M. fulvum* (stemstrip-swallowing). We examined this evidence with regards to a sub-set of the criteria put forward by Huffman [1997], testing our data on four of them for the assessment of whether or not *M. fulvum* may be ingested for medical purposes.

The question of whether or not leaf consumption of a particular species can be considered a regular part of the diet (*criterion 1*) is quite hypothetical. Regularity could be defined as any "rhythm" of consumption related to our Gregorian calendar (frequency per day, week, month, year), or to phenological patterns (season, availability) specific to the site. In this respect, *M. fulvum* ingestion may be witnessed for more observation days than a species undergoing higher seasonality such as *Nymphaea*, but the fact that we talk of more or less one single leaf per individual ingestion day in contrast to intensive harvests for the other species mentioned, allows considering it as a particularly rare ingestion event.

TABLE I. Size and Composition of Parties ($N = 13$) When *M. fulvum* (Mf) Was Ingested

	Mature females			Mature males			Immatures		
Date	In party	Consuming Mf	%	In party	Consuming Mf	%	In party	Consuming Mf	%
13.09.2008	5	1	20	4	1	25	6	1	17
24.09.2008	10	2	20	6	1	17	8	1	13
25.09.2008	12	1	8	8	1	13	6	0	0
30.09.2008	11	0	0	6	0	0	10	0	0
02.10.2008	12	1	8	8	1	13	10	0	0
14.10.2008	10	2	20	7	1	14	10	0	0
20.10.2008	7	4	57	3	0	0	7	3	43
22.10.2008	12	5	42	7	0	0	9	2	22
01.11.2008	9	1	11	8	0	0	7	0	0
21.11.2008	12	1	8	4	0	0	7	0	0
07.12.2008	10	1	10	3	0	0	9	0	0
16.12.2008	9	0	0	6	0	0	9	1	11
23.02.2009	7	2	29	3	0	0	6	0	0
Average	9,69	1,62	18	5,62	0,38	6	8,00	0,62	8
SD	2,250	1,446		1,981	0,506		1,581	0,961	

One row represents one party observed at given "date" reflecting day.month.year. Parties are composed by "mature females," "mature males," and "immatures." Columns "in party" represent all individuals of the respective class present in the given party; columns "consuming Mf" represent all individuals of the respective class actually ingesting Mf in the given party; and columns % (percentage) indicate proportion of *M. fulvum* consumers per age/sex-class (individuals of given sex/age class consuming Mf divided by individuals of this class in party).

Nevertheless, we addressed the question with respect to species availability both in the canopy as well as in the lower strata, being available throughout the range and year round. Neither seasonality nor availability of *M. fulvum* has been investigated in such detail in earlier studies mentioning self-medication, however the discrepancy between consumption and availability we found here is in line with what has been found for another plant suggested to be medicinal, for example, *Vernonia amygdalina* [Huffman & Seifu, 1989; Huffman et al., 1993]. The extreme rarity of ingestion we found here has been mentioned in earlier descriptions for *M. fulvum* leaf consumption for both *Pan* species [Boesch, 1995; Dupain et al., 2002; Fruth, 2011; Kuroda, 1997]. It was also reflected by dung inspection with only 3.4% of dung containing unchewed leaves. Compared to the frequency found in bonobos at Lomako, where Dupain et al. [2002] reported the presence of *M. fulvum* for 1.5% of the dung investigated, the value is high. However our figure falls into the range of what has been reported from chimpanzee sites. These values range from a proportion of 1.5% (Kibale, [Wrangham, 1995]) over 2.1% (Kahuzi, [Basabose, 2002]), 2.8% and 3.1% (Kibale and Gombe, [Wrangham, 1995]) to 10.4% of dung containing unchewed leaves (Bulindi, [McLennan & Huffman, 2012]). In this most recent study, the elevated proportion has been proposed to be due to increased proximity and interactions between chimpanzees and humans and by that increased stress levels, parasite contact and subsequent infections. Particularly infections with *Oesophagostomum* spp. and *Bertiella* spp. were seen as being responsible for the higher frequency of leaf-swallowing [McLennan & Huffman, 2012]. Despite high plausibility of these arguments, an additional cause for the discrepancy in the proportion of unchewed leaves found in dung across sites is likely due to the sampling protocols. While some studies inspected dung only after ingestion of the respective plant during the day [Huffman et al., 2009], others predominantly investigated dung collected from night nests [Dupain et al., 2002; Fowler et al., 2007]. As hispid leaves swallowed (usually in the early morning) were shown to appear in the dung approximately 6 hr after ingestion, protocols collecting dung samples during the day time are expected to reveal higher proportions of unchewed leaves than samples retrieved from morning dung of the subsequent day. This impact should be kept in mind when comparing unchewed leaf proportions across sites. The fact that unchewed, though by digestive liquids visibly macerated, leaves can be found in dung collected beneath night nests at least 16 hr after ingestion, leads to speculations concerning additional effects on top of the purgative ones (see below).

The most obvious to show that nutritional benefit (*criterion 2*) may be of no relevance is the small number of leaves ingested, being expelled whole and undigested [Wrangham & Nishida, 1983].

Investigating seasonality (*criterion 3*), LuiKotale precipitation showed a considerable variability from year to year. A clear distinction of dry- from wet-seasons was not always possible. Average monthly rainfall and Perhumidity Index clearly identify LuiKotale as a typical tropical wet locality with conditions in (1) for the parasite to develop and become infectious resembling those of other less seasonal habitats such as Kibale or Budongo [Huffman et al., 2009]. Monthly cumulative *night* rain proved to be the stronger predictor for the occurrence of *M. fulvum* swallowing than was overall monthly cumulative rainfall (*day* and *night*), indicating that indeed consideration of the diurnal distribution of rain may be of ecological relevance [Walsh, 1996]. Thus if eggs need constant humidity of the forest floor to survive and develop to infectious stages, night rain is more important than day rain. Interestingly, the influence of rain that became apparent when investigating monthly cumulative night rainfall was inverse to what we had expected originally. The higher the rainfall, the lower was the probability of *M. fulvum* consumption. What remains constant across all analyses is the strikingly clear effect of low temperatures on *M. fulvum* ingestion. Given that we have not yet identified a particular intestinal parasite associated with *M. fulvum* leaf- or stemstrip-swallowing, we are unable to further consider the possible effect of these patterns on parasite development.

Ecological factors such as food-availability, as shown by studies linking food constraints to stress and/or parasite susceptibility [Foerster et al., 2012; Masi et al., 2012] may provide more likely explanations focusing on the conditions in (2) for the host to increase parasite susceptibility. Masi et al. [2012] have shown that gorillas have higher parasite egg counts per gram (EPG) during the dry season when fruit availability is low. Other season-related stressors impacting the host's immune-responses are food quality, mating, pregnancy, lactation, and sleep quality. If it was reduced sleep quality, we had expected a strong impact of the combination of both rain and low temperature, which was not the case. Although we can be sure that the potential stressor triggering parasite induced discomfort stimulating the need for self-medication is temperature dependent, unfortunately we do not have the data for its identification.

Investigating representation of the behavior (*criterion 4*), we found individuals ingesting *M. fulvum* being the minority of party members under observation in a group. As females were more likely to swallow *M. fulvum* than were males, further analyses might reveal whether or not this behavior is more likely among certain classes of females within this category, such as pregnant or lactating females, providing evidence for the link between female reproductive costs, immunity, and health.

How can we interpret these results in context of the two hypotheses brought forward explaining the effect taking place on either the (1) physical or the (2) pharmacological level?

The bristly surfaces of leaves observed to be unchewed are considered as the overarching pattern for the expulsion of worms [Huffman, 1997; Huffman & Caton, 2001; Huffman et al., 1996; Page et al., 1997]. *M. fulvum* physical properties of the leaves conform to that of the other 40 plus plant species used by primates, and that of other mammals and birds [Huffman, 1997]. The purging effect caused by these morphological features is common understanding. The role of phytochemistry in the context of leaf-swallowing has been directly refuted for *Aspilia* spp. [Page et al., 1997] and appears insignificant given the overwhelming evidence of worms expelled by the variety of species across sites. Although we lack data to show a phytochemical effect of *M. fulvum* swallowing in bonobos, the above mentioned visibly macerated leaves appearing about 16 hr after ingestion (Fig. 1b), the ethnomedicinal use as well as the species' particularly interesting chemical properties, stimulate consideration of potentially additional effects for future rigorous testing.

M. fulvum use by human populations has been reported across Africa. Leaves are used most frequently, followed by fresh stems and roots. Most applications are by oral ingestion of decoctions or fresh sap. In DRC, applications include a wide range of physical ailments from open wounds and visible bacterial infections to respiratory, gastro-intestinal and sexually transmitted diseases [Bouquet, 1969; Fruth et al., 2010, 2011]. Applications of either leaves or stem in the context of dysentery, stomach troubles, or parasites have also been reported for Sierra Leone [MacFoy & Sama, 1983], Ghana [Abbiw, 1990], and Côte d'Ivoire [Bellomaria & Kacou, 1995]. Of particular interest are ethnomedicinal reports of fresh applications to stop bleeding, help the process of wound healing or scar formation (cicatrization), or treat hemorrhoids and dysentery by applying macerated leaves by clyster or as a suppository [Bellomaria & Kacou, 1995; Bouquet, 1969].

These applications have found support by the discovery of the species' relevant phytochemical substances such as tannins, glycosides, terpenes, saponins, and flavonoids. Their medicinal properties include antiinflammatory [Nia et al., 2005], antioxidant, antidiarrheal [Ezeigbo et al., 2010], antibacterial [Uduak & Kola, 2010], and antiprotozoal agents [Muganza et al., 2012].

Particularly flavonoids, with their antioxidant and antiinflammatory properties found in *M. fulvum* are interesting candidates for speculation: intestinal worms such as strongyles are known to seriously damage the intestinal walls and cause loss of blood and protein. If leaf- or stemstrip-swallowing has evolved as a reward to physiological relief, it is tempting to consider these potentially curing effects. Despite the overwhelming evidence for rough surfaces as criterion of choice of leaves swallowed unchewed, the secondary compounds of *M fulvum* merit systematic testing in future to assess their potentially additional curative role in great apes.

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