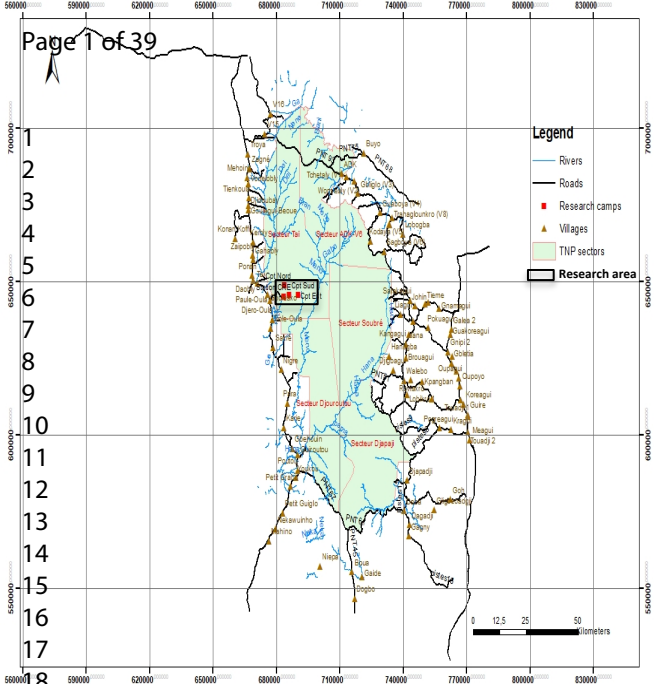
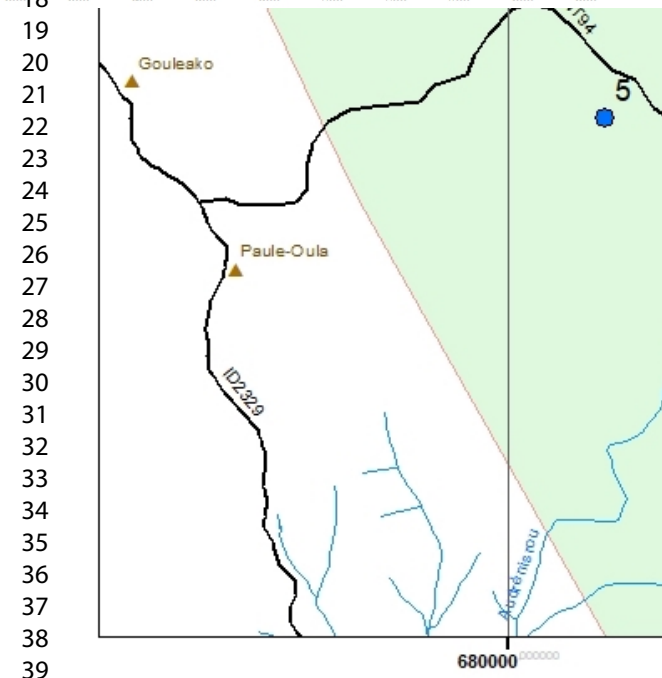
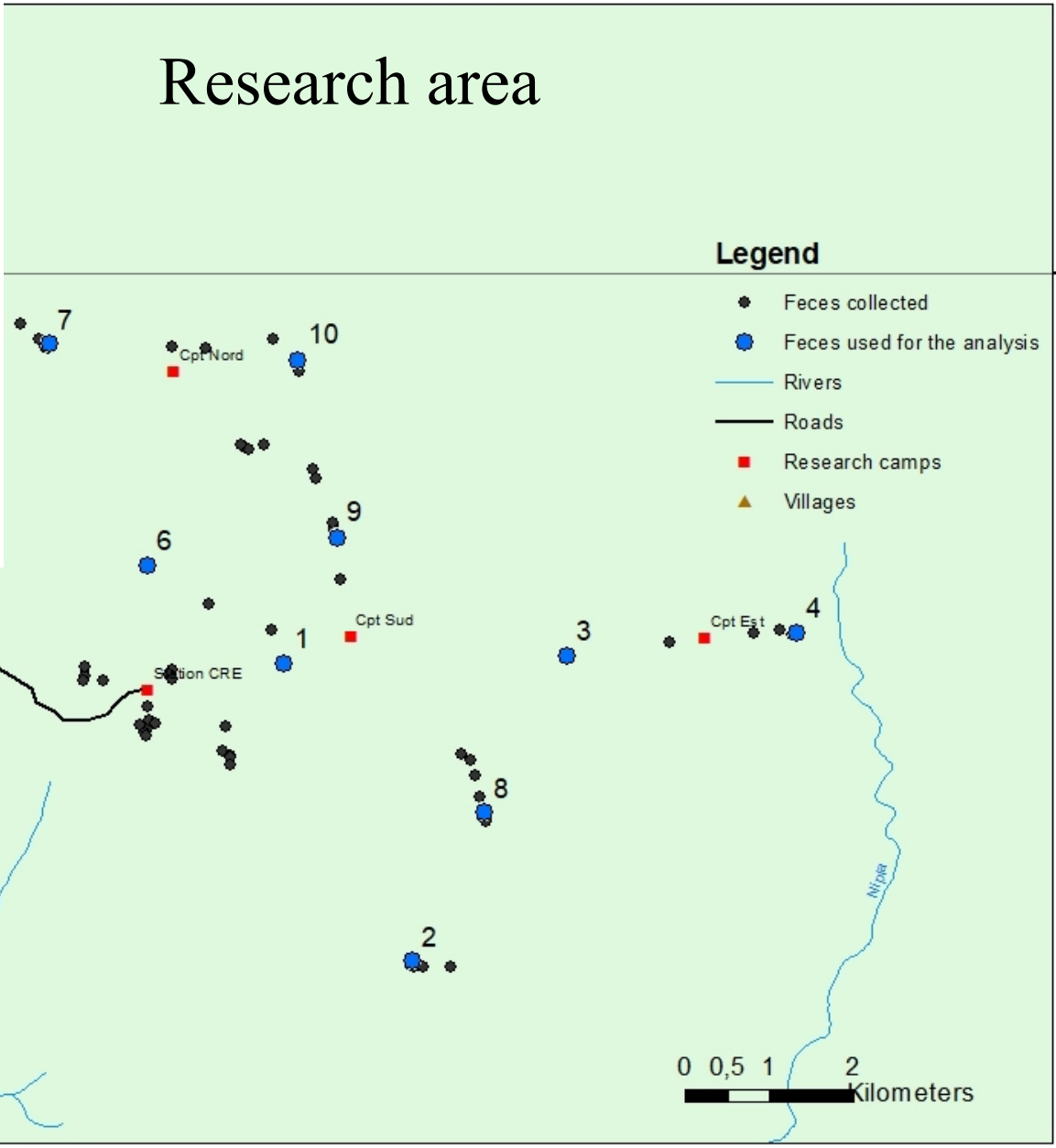


## A new method to determine the diet of pygmy hippopotamus in Taï National Park, Côte d'Ivoire

Journal:	<i>African Journal of Ecology</i>
Manuscript ID	AFJE-21-015
Manuscript Type:	Article
Date Submitted by the Author:	14-Jan-2021
Complete List of Authors:	Hendier, Alba Chatelain, Cyrille Du Pasquier, Pierre-Emmanuel Paris, Monique Ouatarra, Karim Kone, Inza Croll, Daniel Zuberbühler, Klaus
Main Subject Area:	Conservation
Geographical Location:	West Africa
Detailed Subject Area:	diet
Additional Keywords:	Multiple Correspondence Analysis, Faecal analysis, Foraging



# Research area

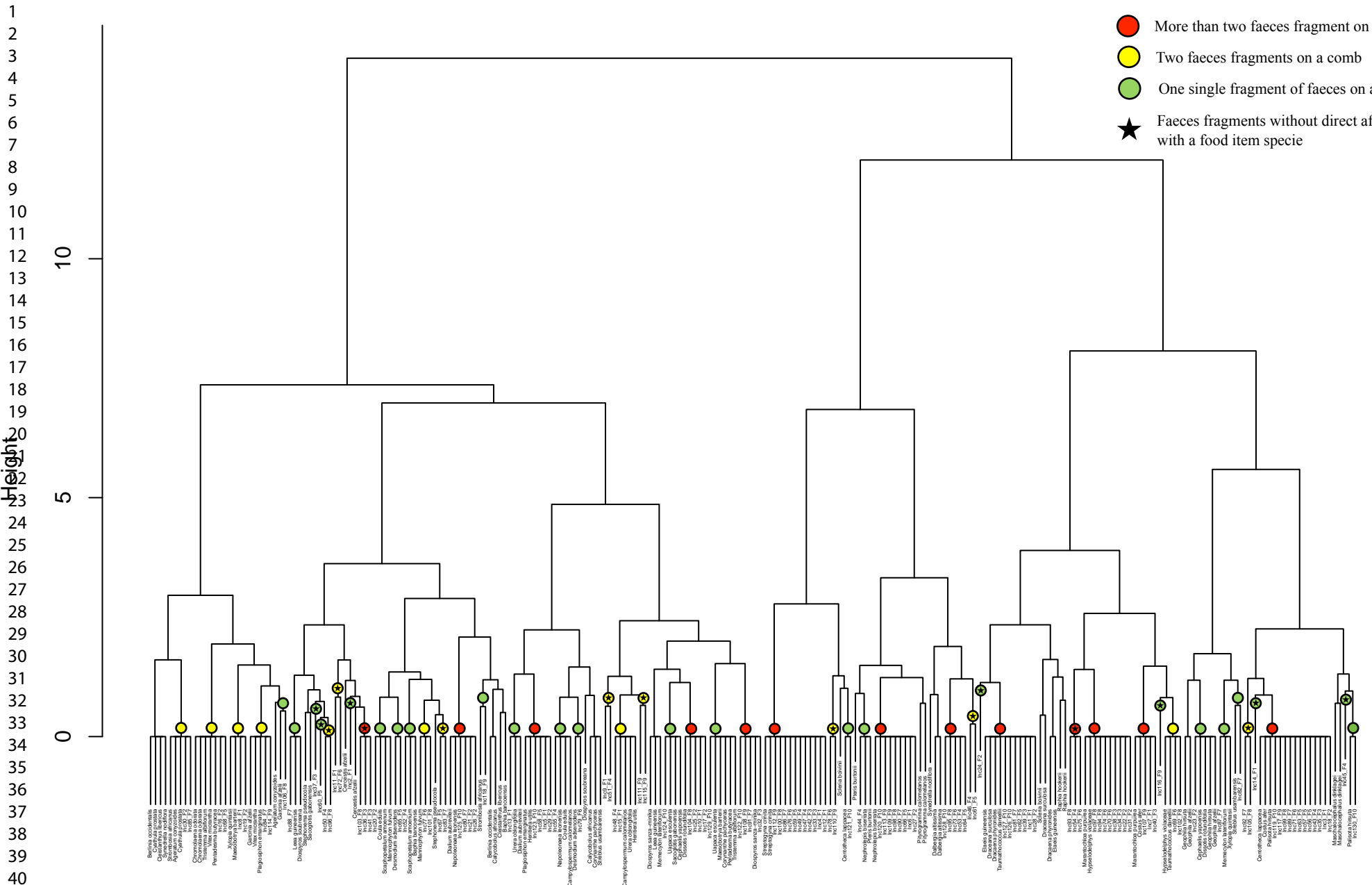




# African Journal of Ecology

## Cluster Dendrogram

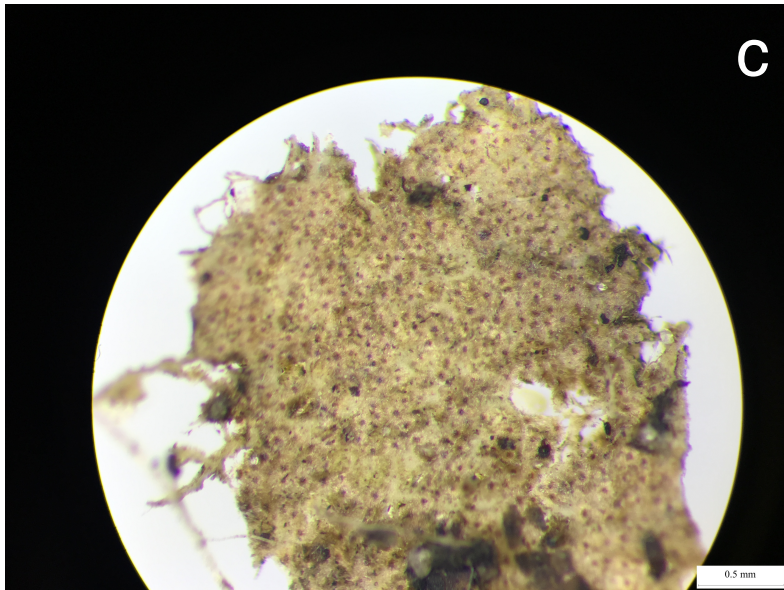
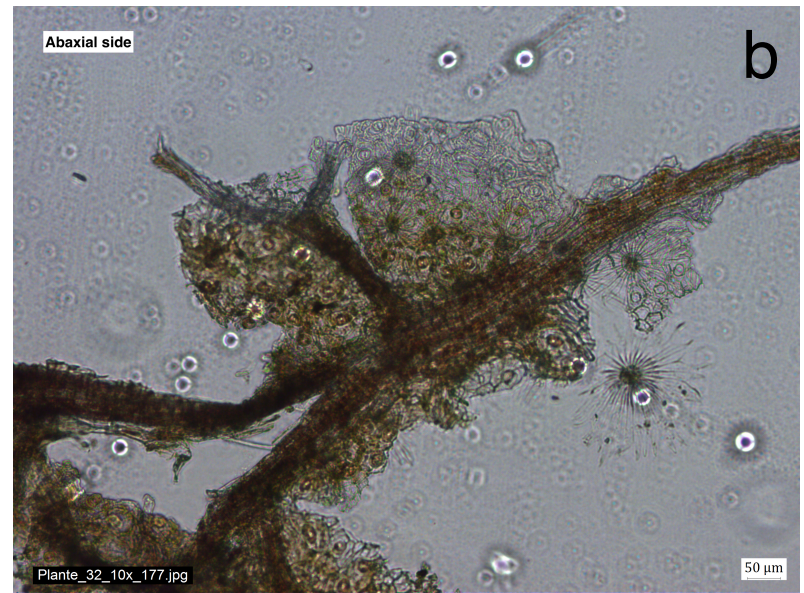
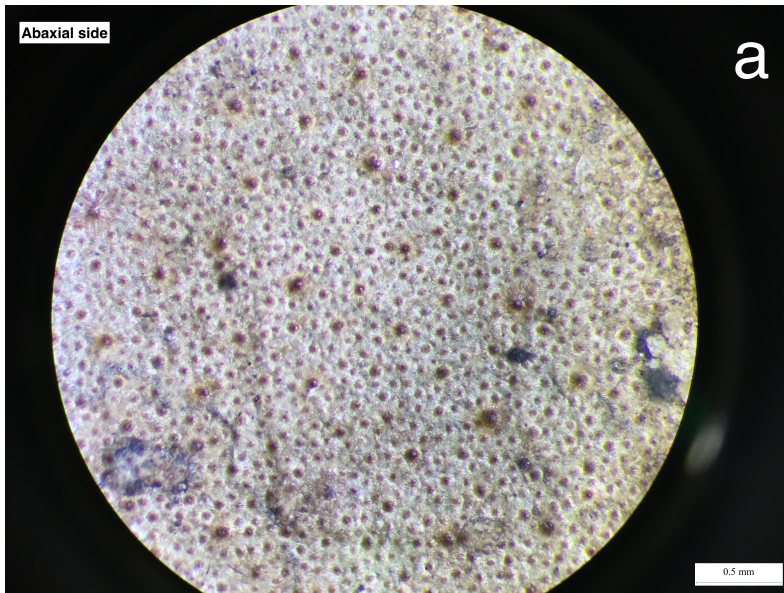
- Legend
- More than two faeces fragment on a comb
  - Two faeces fragments on a comb
  - One single fragment of faeces on a comb
  - ★ Faeces fragments without direct affinity with a food item specie



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4 1 A new method to determine the diet of pygmy hippopotamus in Taï National Park, Côte  
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## 24 **Abstract**

25 Diet determination of endangered species is an essential element in defining successful  
26 conservation strategies and optimizing captive breeding programmes. In this study, we  
27 developed a new diet identification system, derived from standard faecal analysis, to  
28 determine the diet of an elusive and endangered herbivore, the pygmy hippopotamus  
29 (*Choeropsis liberiensis*). We collected faecal samples from 10 free-ranging individuals  
30 covering a combined home range area of about 50 km<sup>2</sup> in Taï National Park, Côte d'Ivoire. In  
31 subsequent laboratory analyses we extracted a large number of leaf epidermis fragments from  
32 spatially separated faecal samples and compared them with a reference plant database. Using  
33 Multiple Correspondence Analysis (MCA) of epidermis fragments combined with direct  
34 visual inspection, we identified the most frequently consumed plant species, which revealed  
35 that pygmy hippopotami qualified as intermediate feeders. Their diet was based on at least  
36 seven species of monocotyledonae, dicotyledonae and fern groups, with a preference for a  
37 small number of other plant species. We evaluate the merit of our method and discuss our  
38 findings for developing effective conservation and captive breeding strategies in an  
39 endangered species with a wild population of less than 2,500 adult individuals.

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45 **Key words:** Conservation, Africa, faecal analysis, foraging, Multiple Correspondence  
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47 **Analysis**  
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## 43 Introduction

44 The pygmy hippopotamus, *Choeropsis liberiensis* (Morton, 1849), named hereafter as pygmy  
45 hippo, is an endemic species to West Africa (Côte d'Ivoire, Guinea, Liberia and Sierra Leone;  
46 Prothero et al., 2007). It originally occurred as two subspecies, *C. l. liberiensis* and *C. l.*  
47 *heslopi* (Corbet, 1969), but the Nigerian subspecies *C.l heslopi* has gone extinct in the 1940s  
48 (Robinson, 2013), while the remaining West African subspecies is classified by the IUCN as  
49 Endangered (Ransom et al., 2015) due to habitat loss and poaching for bushmeat (Lewison &  
50 Oliver, 2008). The population is still declining due to political instability in the region,  
51 sustained lack of law enforcement and absence of coordinated conservation efforts (Mallon et  
52 al., 2011; Conway, 2013). The current population size of pygmy hippos is estimated to be less  
53 than 2,500 adult individuals, the majority of which are believed to reside in Taï National Park  
54 in Côte d'Ivoire (Roth et al., 2004; Ransom et al., 2015).

55 Although there are a good number of studies on pygmy hippos (Roth et al., 2004; Garthey,  
56 2013; Conway, 2013; Bogui, 2016; Hillers et al., 2017; Flacke & Decher, 2019), much of the  
57 available information is from captive animals (Flacke et al., 2015, 2016). There is little  
58 information from the wild, which is mainly due to the species' cryptic behaviour and  
59 difficulties accessing their natural habitat. In captivity, individuals suffer from persistent  
60 health issues, such as polycystic kidney disease and dental skin and foot problems, which may  
61 originate from inadequate diet (Steck, 2008; Flacke et al., 2017). Pygmy hippos appear to  
62 forage mainly at night over a period of about 6 hours (Mallon et al., 2011; Robinson, 1981;  
63 Eltringham, 1999). They are thought to consume a wide variety of ferns, roots, grasses, stems,  
64 leaves of young trees and crops (Robinson, 1970, 1999; Bülow, 1988; Hentschel, 1990;  
65 Robinson et al., 2017) but we are not aware of any systematic data on the diet preference and  
66 composition of free-ranging animals.

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3 68 The purpose of this study is to address this issue with a new methodology, based on recent  
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5 69 progress in plant identification techniques: microscopic analysis of plant fragments of leaf  
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7 70 epidermis in faeces (Cuartas, 1996). Identification via microscopy is different from traditional  
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9 71 Linnaean classification, which is based on the plant reproductive system. However, recent  
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11 72 botanical studies have shown that the microscopically determined foliar anatomy can produce  
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13 73 reliable information for species identification, particularly at the family and group level  
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15 74 (Metcalf and Chalk, 1950, 1957; Shah et al., 2018a, b; Ullah et al., 2018a, b). The foliar  
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17 75 anatomy and epidermis type is determined from five features (four microscopic, one  
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19 76 macroscopic) through Multiple Correspondence Analysis (MCA) complemented by a visual  
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21 77 analysis (by pictures) of the targeted epidermis.

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26 78 We took advantage of this recent development by combining standard faecal analysis with the  
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28 79 newly established plant identification system in order to determine the diet of free ranging  
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30 80 pygmy hippos in Taï National Park. Our first goal was to better describe the species' diet  
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32 81 composition. Our second goal was to develop an identification key for tropical plant species  
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34 82 based on microscopic features, which could be used for other studies and so contribute to both  
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36 83 welfare decisions in captivity and conservation efforts in the wild.

## 84 **Material and Methods**

### 85 Study site

86 The study was conducted in Taï National Park (TNP), Côte d'Ivoire, from July to November  
87 2017, in an area of 49 km<sup>2</sup> (Figure 1), surrounding the research station of the Centre de  
88 Recherche en Ecologie (CRE). The TNP is a UNESCO World Heritage since 1982 and  
89 currently covers an area of 536,000 ha, the largest protected tropical forest of West Africa  
90 (UNESCO World Heritage, 2018; OIPR, 2018; Lauginie, 2007). The vegetation of the park is  
91 rich with 1,365 documented plant species (Scoupe, 2011). Regular censuses of the flora have

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3 92 been carried throughout the park both in the Northern and Eastern parts (Scoupe (2011), in  
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5 93 the South (Adou Yao et al., 2000) and Southwest (Menziès (2000), Adjanohoun &  
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7 94 Guillaumet (1963), Aké Assi & Pfeffer (1975), Aké Assi (1984), Adou Yao et al. (2005)),  
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9 95 which has resulted in an extensive database (Sattler, 2000).  
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### 13 96 Plant reference library

14  
15 97 The standard method for obtaining diet information from elusive wild animals is by  
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17 98 microscopic analysis of plant epidermis cells contained in faeces with the help of a reference  
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19 99 catalogue (Crocker, 1959; Storr, 1961; Chapuis, 1980; Butet, 1985, 1987; Cuartas, 1996;  
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21 100 Chatelain et al., 2000; Rech, 2011). Our selection criteria for including plant species for a  
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23 101 reference library were as follows: (i) high abundance, less than 1m high, and encountered in  
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25 102 pygmy hippo habitat at the edges of transects or along rivers ('abun', Table 1), (ii) covered by  
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27 103 pygmy hippo territorial markings ('mark'; Table 1), (iii) mentioned in literature as eaten by  
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29 104 pygmy hippos in the park ('ref'; Table 1; Bülow, 1987; Hentschel, 1990). A voucher of n=60  
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31 105 species (Table 1) was collected and deposited at the herbarium of the Centre Suisse de  
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33 106 Recherches Scientifiques (www.csrs.ch) in Abidjan for subsequent validation by the assistant  
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35 107 curator of the herbarium, Saturnin Dougoune, following botanical nomenclature from African  
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37 108 Plant Database (APD, 2018).  
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44 109 The microscopic food items for the reference library were prepared using two methods to  
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46 110 enhance reliability: the 'nail-polish method' (Miller et al., 1968; Hilu and Randall, 1984) and  
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48 111 Rech's (2011) protocol (see below). The nail-polish method consists of applying a thin layer  
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50 112 of commercial, transparent nail-polish on the sample leaves. Once dry, the nail-polish layer is  
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52 113 then removed and placed on a slide in a drop of water. For both methods, semi-permanents  
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54 114 slides were created with the two leaf sides. All the slides were photographed at 40x, 100x,  
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56 115 and 200x with inverted microscope.  
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3 116 Faecal sample library  
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6 117 Pygmy hippo faeces are easy to locate in the natural habitat. Similar to the common hippo  
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8 118 (*Hippopotamus amphibius Linnaeus, 1758*), pygmy hippo faeces can be classified as either  
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10 119 territorial or as litter droppings (Robinson et al., 2017), depending on their consistency and  
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12 120 shape (soft/shapeless or solid, respectively). We collected both types, following the sampling  
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14 121 method for common hippos (Scotcher et al., 1978; Michez, 2006). In total, n=70 faeces were  
15  
16 122 collected. Whenever we had evidence for pygmy hippo presence (footprint or droppings), we  
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18 123 determined the exact location using a GPS device, which resulted in a total of n=330 location  
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20 124 points. To obtain independent data points, only samples that were at least 2 km apart were  
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22 125 selected (home range estimates; females = 0.5 km<sup>2</sup>; males = 1.5 km<sup>2</sup>; Bülow, 1988;  
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24 126 Hentschel, 1990) (Figure 1). The final sample size consisted of faecal material from n=10  
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26 127 different locations, i.e. 10 different individuals.  
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31 128 An extract of 2g from each faecal sample was used for a macroscopic classification into four  
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33 129 categories: leaves, roots/stems, seeds and unidentifiable material following Michez (2006).  
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35 130 Then, we randomly selected n=48 leaf fragments per sample, which were placed in two 24-  
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37 131 well cell culture clusters (i.e., 48 wells) and photographed under a binocular magnifying glass  
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39 132 at 7.5x, 25x, 60x. Some samples having more leaves than others, n=48 allowed us to have a  
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41 133 representative subsample and to keep the same number of leaf fragments per faeces. After  
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43 134 photographing, fragments were soaked in ethanol and sodium hypochlorite until they were  
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45 135 transparent following the protocol by Rech (2011) for animal faeces studies. Finally, the  
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47 136 discoloured fragments were placed between a slide and a lamella in a drop of glycerine. A  
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49 137 layer of commercial nail polish was added to better preserve the samples for at least two  
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51 138 months. In total, n=480 fragments with 2,880 pictures (1,440 microscopic and 1,440  
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53 139 macroscopic) were taken with inverted microscope.  
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### 140 Data analysis

141 Based on different morphological features used in diet studies (Butet, 1985, 1987; Metcalfe  
142 and Chalk 1950, 1957; Stoddard, 1965; Kok and van der Schijff, 1973; Chapuis, 1980; Rech,  
143 2011) we selected five categorical (qualitative) features (Table 2) to measure in the plant  
144 reference library and in the faecal library. Morphological features for the plant reference  
145 library concerned the epidermis cells of 56 referent plants along these five features. As the  
146 two sides of the leaves can be difficult to identify in the faeces, we measured each side of the  
147 leaf in this library. In total, n=112 reference epidermis were described. From the n=480 faeces  
148 fragments (2,880 images) prepared from the n=10 faecal samples, a selection of 11-16  
149 fragments per faecal sample was chosen, following careful inspection of all images, which  
150 resulted in a final sample size of n=130 fragments. Finally, we subjected the results to  
151 statistical analysis.

### 152 Statistical analyses

153 Multivariate analyses was used in order to i) assess the diagnostical value of the 5 categorical  
154 features (named as variables herein below) in the identification of taxonomic group in the  
155 plant reference library with their respective leaves' sides (named as individuals; n=112), and  
156 ii) compare the fragments found in the hippo faecal samples library (named as additional  
157 individuals) with our plants reference library. A first MCA with the reference library (n=112)  
158 allowed to explore the structure in our dataset and try to detect a possible taxonomic  
159 clustering (i.e. plants classes, families, genus or species). In parallel, an agglomerative  
160 Hierarchical Clustering analysis (HC) was conducted based on the MCA coordinates of all  
161 axes and using the Ward method. In a second MCA, the faeces library (n=130) was added as  
162 additional individuals. Thus, these individuals were not taken into account for calculating the  
163 MCA axes. This allowed us to consider the spatial position of our faeces fragments



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3 164 (additional individuals) in relation to our plant reference library (individuals). In order to  
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6 165 interpret the results, a Hierarchical clustering (HC) dendrogram based on the coordinates of  
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8 166 the individuals from the plants reference library on all the MCA axes including the additional  
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10 167 individuals coordinates (Faeces library). The HC tree allowed us to focus on the similarities  
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12 168 and differences between the food item references and the faeces fragments. From this HC  
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15 169 dendrogram, we continued the analysis by looking carefully at pictures that were targeted as  
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17 170 similar, and we approved or disapproved the species targeted by the MCA analysis.

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20 171 All statistical analyses were performed with the R software (R Development Core Team,  
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22 172 2018), using the package “ade4” with the “dudiacm” function (Dray and Dufour, 2007). The  
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24 173 interface “explor” (from explore package) was used to observe the results of MCA and edit  
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27 174 the different graphs (<https://CRAN.R-project.org/package=explor>). And, for the HC analysis  
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29 175 we used the “hclust” function.

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## 33 34 35 177 **Results**

### 36 37 38 178 Plants reference library

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41 179 Multiple Correspondence Analysis (MCA) was relevant to establish a plant reference library  
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43 180 to facilitate decision-making when identifying fragments found in the pygmy hippo faeces.  
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46 181 The MCA performed in the reference library (on 112 individuals) and 5 variables had a total  
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48 182 inertia of 2.4. The first two axes (F1 and F2) explained 35.2% of the total variation and 65.7%  
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50 183 was explained by the first 5 axes. A light horseshoe shape (Guttman effect) on Figure 2 is  
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52 184 observed on the active individual's (reference plants; coloured in blue) projection in F1xF2  
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54 185 axes (Appendix 1 for the projection on F1xF3 axes). This effect was due to two variables that  
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56 186 contributed in similar ways, most likely the macroscopic variable macro veins and the  
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59 187 microscopic layout. Despite this effect, three main groups are observed through the categories

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3 188 of the variables (Appendix 2), which corresponded mostly to the three major taxonomic  
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5 189 groups: monocots, dicots and ferns. There was no other structure apart from the distinction  
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8 190 between these 3 groups. Indeed, the MCA did not allow us to gather taxonomic ranks  
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10 191 (species) inside the families and even less at the generic level.  
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### 13 192 Faecal samples

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16 193 In a second step, the faeces fragments were added to the MCA previous analysis (named as  
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18 194 supplementary in Figure 2, in red) as supplementary elements in the MCA. This allowed us to  
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20 195 see if there were similar species between our reference plants and our faeces fragments. We  
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22 196 notice that the reference library and the faeces library shared similar positions on the spatial  
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24 197 projection (Figure 2). In the HC tree, the clustering of the plant groups (monocots, dicots and  
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26 198 ferns) are represented along the two main branches as well as some plants families:  
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28 199 Rapataceae, Marantaceae, Pteridaceae and Rubiaceae. However, many plants species of the  
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30 200 reference plants list shared the same position on the tree (Figure 3). The HC tree helped to  
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32 201 target similar epidermis, but visual inspection was still necessary to determine the faeces  
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34 202 fragments species. After analysing each branch of the tree (in Figure 3) with the  
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36 203 corresponding pictures, we were able to confirm the presence of 7 species of plants from our  
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38 204 reference list (Table 3).  
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44 205 Results showed that 8 of 10 faecal samples contained monocots, dicots and fern species. The  
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46 206 two remaining samples (2, 6) did not contain any fern (Table 3). For the ferns, *Nephrolepis*  
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48 207 *bisserata* could be identified in 7 of 10 faecal samples (Appendix 3). For the monocots of the  
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50 208 Poaceae family, particularly the following species *Cenhoteca lappaceae* and *Streptogyna*  
51  
52 209 *crinita* (Appendix 4 and 5). For Marantaceae family they were present in 7 of 10 samples  
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54 210 (Appendix 6 & 7; often *Marantocloa purpurea*). Finally, in the dicots, we found four times  
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56 211 the species *Herritiera utilis* (Figure 4).  
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## 212 Discussion

213 We combined a newly developed statistical analysis of microscopic epidermis fragments with  
214 traditional faeces-based diet determination. We analysed the diet of 10 individuals over a  
215 combined range of about 50 km<sup>2</sup> and found evidence for commonly found plants in the faecal  
216 samples. Our analysis was based on five categorical variables only, thus demonstrating the  
217 potential of the method as an interactive research tool for rapid diet identification in  
218 combination with established criteria, such as trichomes, silicates or scales. In particular, we  
219 found that, firstly, free-ranging pygmy hippos have a varied diet, which includes major plant  
220 groups including monocots, dicots and ferns. Secondly, we were able to identify at least seven  
221 plant species, although several epidermis fragments remained unidentified (limitations  
222 below). Hence, we can confirm that pygmy hippos are to be classified as herbivore  
223 generalists, a foraging strategy that allows them to avoid over-ingestion of plant toxins  
224 (Freeland and Janzen, 1974).

225 Generalist feeding does not exclude the possibility that herbivorous mammals can develop  
226 preferences for certain plant species (Belovsky, 1978), which is supported by our observation  
227 of low variability between different droppings. The seven plant species described in the  
228 results were frequently found in most faeces samples, particularly the plants from the Poaceae  
229 family (grasses). More generally, the data suggest that pygmy hippos in TNP have a  
230 preference for *Nephrolepis bisserata*, *Pteris burtonii*, *Marantaceae* species and *Streptogyna*  
231 *crinita*, in line with what has been proposed by Bülow (1987) and Hentschel (1990).

232 However, we could not find any evidence for the following dicots: *Desmodium adscendens*,  
233 *Dissotis rotundifolia*, *Geophila afzelii*, *Geophila hirsuta* and *Cercestis afzelii*. This may be  
234 due to the fact that we focussed on large fragments only (large particles ingested). Dicots'  
235 species have a thin cell wall (Bodmer, 1990; Shipley, 1999), suggesting that they are better

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3 236 absorbed by pygmy hippos, and therefore may have been overlooked by our method. In  
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5 237 captive pygmy hippos, it has been argued that low digestibility of some particles may be due  
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8 238 to ineffective mastication (Schwarm et. al, 2009), suggesting that by including smaller  
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10 239 fragments we should probably find these species.

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13 240 Following Bülow (1987) and Hentschel (1990)'s database, we can add two new species that  
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15 241 were frequently observed in the droppings: *Centoteca lappaceae* (grass, found in all samples)  
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17 242 and *Herritiera utilis* (tree leaves, found in at least four of the ten samples). This new  
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20 243 observation could be explained by the fact that previous studies were based on feeding trials,  
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22 244 feeding signs and direct observations in a restricted study area (Hentschel, 1990). When  
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24 245 comparing our data to the already published list, we could only confirm about 50% of species,  
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26 246 while species classified as favourites were difficult to find or absent in our area (e.g.,  
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28 247 *Staurogyne paludosa*, *Justicia tenella* and *Floscopa africana*). This fact further supports our  
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30 248 findings that pygmy hippos qualify as herbivorous generalists (Hentschel, 1990; Robinson et  
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32 249 al., 2017), such that an individual's diet composition will largely depend on the local flora  
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34 250 encountered during foraging. Pygmy hippos occupy very small home ranges in swampy areas  
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36 251 confirming the relationship between home range size and nutritional requirement of pygmy  
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38 252 hippos (Robinson et al., 2017).

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41 253 All these observations support that pygmy hippos are non-ruminant generalist intermediate  
42  
43 254 feeders. This is in contrast to mixed feeders that forage on grasses and forbs, which tend to  
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45 255 contain higher proportions of cellulose (Demment and Van Soest, 1985) as well as shrubs and  
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47 256 tree leaves, which contain higher proportions of lignin (Bodmer, 1990; Van Soest, 1996).  
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50 257 Furthermore, intermediate feeders are able to adapt their diets according to the availability of  
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53 258 resources and the seasons (Hofmann, 1989). Classifying pygmy hippos as intermediate  
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3 259 feeders is also supported by their gregarious and territorial behaviour, dentition (Lang, 1975),  
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5 260 and other dietary studies (Bülow, 1987; Hentschel, 1990).  
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9 261 Based on observations in zoos, Flacke (2017) pointed out that pygmy hippos were incorrectly  
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11 262 classified as non-ruminant generalist browsers by the Nutritional Advisory Group (Lintzenich  
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13 263 & Ward, 1997) and the Pygmy Hippo Husbandry Manual (von Houwald et al., 2007). Our  
14  
15 264 data show that grasses are an integral part of the pygmy hippo diet, but it is unclear whether  
16  
17 265 this is properly taken into account by captive facilities. It has been suggested that captive  
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19 266 pygmy hippos receive a food intake that is too energy rich, which can lead to obesity and  
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21 267 disease (Flacke et al., 2016; Flacke, 2017; Steck, 2008). In a recent study it was found that  
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23 268 reducing the number of pellets and providing hay ad libitum, captive pygmy hippos will  
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25 269 approach body weights similar to their wild counterparts (Taylor, 2013). This is in line with  
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27 270 what is predicted for intermediate feeders, which rely on a diet that is rich in slowly digestible  
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29 271 plant fibers (Shipley, 1999).  
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### 35 272 Limitations

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38 273 A first limitation concerns the size of the plant reference library. Less than 4% of the plant  
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40 274 species of TNP were part of the library (60 of 1,356 documented species by Scoupe (2011)).  
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42 275 However, pygmy hippos are unlikely to target most of these plants as food items, but will  
43  
44 276 focus on shrubs and herbaceous plants, which represent only between 10-15% of all plant  
45  
46 277 species. Therefore, we assume that almost one third of these plant species were analysed in  
47  
48 278 this study. We recommend to extend the database produced by this study to increase the  
49  
50 279 knowledge on pygmy hippo's diet as well as the diet of other species within TNP. It would be  
51  
52 280 important to add faecal samples from other TNP areas and across the, seasons, which is likely  
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54 281 to produce a fuller picture of the dietary flexibility of pygmy hippos. Besides enlarging the  
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56 282 database, it would also be relevant to conduct chemical analysis on the plants consumed, to  
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3 283 get a better understanding of the nutritional needs of wild pygmy hippos (Freeland and  
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5 284 Janzen, 1974).

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9 285 A second limitation concerns the variable choice for the MCA. The macroscopic variable is  
10  
11 286 very helpful, but this application with small fragments is not possible. Furthermore, many of  
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13 287 the food items references share the same characteristics and sometimes it is difficult to  
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15 288 distinguish the epidermis of different species without a confirmation by picture. Future  
16  
17 289 studies may also want to add information on the stomata (i.e. the shape and number of cells  
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19 290 around the stomata), which are good indicators to determine the family and species level  
20  
21 291 (Metcalf and Chalk, 1957). In this study, this was not possible due to the quality of our  
22  
23 292 reference slides. Rech (2011) recommends analysing only the abaxial side because it is more  
24  
25 293 characteristic to the plant's species. Indeed, as there are fewer features visible on the adaxial  
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27 294 side, we were limited in the descriptions. The cells looked very similar and it was difficult to  
28  
29 295 distinguish one from another (i.e. adaxial side of *Dialium aubrevillei* and *Napoleona*  
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31 296 *leonensis*). Unfortunately, the side of the faeces fragments removed was not always an option.  
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33 297 Also, increasing the number of food item species would give more comparisons to identify  
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35 298 more faeces fragments that remain unidentifiable.

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42 299 A third limitation is that this study focused only on large plant fragments. In order to consider  
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44 300 the complete diet of the pygmy hippos, one should look at smaller fragments and also include  
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46 301 fruits and seeds, because fruits and seeds are also part of the pygmy hippo diet. During the  
47  
48 302 sorting of the faeces, we found matching seeds across samples collected during the rainy  
49  
50 303 season (Appendix 8). We were unable to determine the species identity of the seeds. In one  
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52 304 report (van Heukelum, 2010) wooden remains in the faeces were found from the seeds or  
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54 305 fruits they have eaten, suggesting that pygmy hippos consume seeds in their entirety. As the  
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56 306 majority of the seed and fruit were not preserved in their entirety, DNA barcoding analysis  
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3 307 with specific markers would be required for further analyses (Bradley et al., 2007; Iwanowicz  
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5 308 et al., 2016). Furthermore, concerning the methodology, we worked with dry material  
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8 309 (reference plants and droppings) but it may be preferable to boil the material first (see  
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10 310 Metcalfe and Chalk, 1957), allowing the cells to rehydrate and regain their shape. This would  
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12 311 provide a better comparison and would allow us to look at more digested fragments.

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15 312 Finally, these analyses could be compromised by the fact that male and female home ranges  
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17 313 can overlap, suggesting that two different individuals could have contributed to each sampling  
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19 314 location (Roth et al., 2004). We also tried an approach by camera traps to identify the plants  
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21 315 eaten by pygmy hippos (182 videos taken over two years by Noémie Capelle from the Max  
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23 316 Planck Institute (MPI)). However, it was almost impossible to carry plants identification and  
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25 317 recognize different individuals based on the videos. First because there were not many  
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27 318 videoclips that showed eaten plants and second because the videos do not always allow to  
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29 319 observe correctly the plants. However, the activity level (Rowcliffe et al., 2014) and density  
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31 320 (Buckland et al., 2000; O'Connell et al., 2011; Trollet et al., 2014) of pygmy hippos is  
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33 321 currently analysed using this material.

## 34 35 36 37 38 39 40 322 Conclusions

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44 323 We applied a new plant identification system to better understand the diet of free-ranging  
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46 324 pygmy hippos. Our results confirmed that pygmy hippos are generalist herbivores with a wide  
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48 325 range of plant species consumed, including grasses and shrubs, suggesting they should be  
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50 326 classified as intermediate feeders. Indeed, we observed similar fragments of monocots  
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52 327 (grasses), dicots (shrubs, tree leaves) and ferns in almost all faeces analysed, i.e. from ten  
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54 328 different individuals distributed over about 50km<sup>2</sup>. Moreover, if these analyses were  
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56 329 combined with additional evidence, then pygmy hippos in the Taï area appear to have a food  
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58 330 preference for specific species, notably *Nephrolepis bisserata* (fern), *Streptogyna crinita*

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3 331 (monocot), *Marantaceae* species (monocot), *Cenhoteca lappaceae* (monocot) and *Herritiera*  
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5 332 *utilis* (dicot). The latter two species were not considered part of the pygmy hippos' diet until  
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7  
8 333 now. In addition, *Cenhoteca lappaceae* (monocot) was found in all samples and confirmed  
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10 334 the importance of grasses in the diet of pygmy hippos. High diversity of plants in the diet and  
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12 335 the fact that they should be classified as intermediate feeders (rather than browsers; Flacke,  
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14 336 2017) is important for pygmy hippo welfare and conservation strategies both in the wild and  
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17 337 in captivity.

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20 338 With this study we also compiled a tropical plant database concerning 60 species that can  
21  
22 339 serve other faunistic studies in West African forests. Future research may focus on the  
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24 340 chemical composition of the preferred food items which would be essential in welfare  
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26 341 programmes designed to improve the diet offered in captivity and to combat common health  
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28 342 problems. For free-ranging pygmy hippos, the data presented here will help to identify and  
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30 343 conserve specific microhabitats that contain plant species essential for the survival of this  
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32 344 enigmatic forest mammal.

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#### 38 39 40 346 **Acknowledgements**

41  
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44 347 We thank the Centre Suisse des Recherches Scientifiques (CSRS) for logistic support. Data  
45  
46 348 collection and analyses have been done in collaboration with the Evolutionary Genetics, the  
47  
48 349 Soil Biodiversity and the Comparative Cognition laboratories of the University of Neuchâtel,  
49  
50 350 with further support by the Conservatoire et Jardin Botaniques of Geneva (CJBG), the Max  
51  
52 351 Planck Institute for Evolutionary Anthropology (MPI-EVAN; Leipzig) and the Institute for  
53  
54 352 Breeding Rare and Endangered African Mammals (IBREAM; Edinburgh). We thank that  
55  
56 353 Office Ivoirien des Parcs et Réserves (OIPR) and the Tai Monkey Project (TMP) for giving us  
57  
58 354 permission to carry out the research. Finally, we would like to thank the curator of Basel Zoo



1  
2  
3 355 as well as Emilie Chanclud, Vinciane Mossion, Fred Stauffer, Anthelme Gnagbo, Elie  
4  
5 356 Bandama Bogui, Hjalmar Kuehl, Noémie Cappelle, Mark Van Heukelum, Saturnin  
6  
7  
8 357 Dougoune, Donatien Bélé, Radu Slobodeanu and Mahmoud Bouzelboudjen for discussions  
9  
10 358 and advice during the different stages of this study.  
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15  
16 360 Declaration: This study was part of an on-going collaboration between the Institute for  
17  
18 361 Breeding Rare and Endangered African Mammals (IBREAM) and the Centre Suisse de  
19  
20 362 Recherches Scientifiques: Côte d'Ivoire's Pygmy Hippo Conservation Project (CSRS - THP)  
21  
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25  
26 364 Funding: This research was funded by "Fond des donations" of the University of Neuchâtel  
27  
28 365 and the "Willy Müller Award" of the Centre Suisse de Recherches Scientifiques en Côte  
29  
30 366 d'Ivoire.  
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586 **Tables**

Family	Genus	Species	Identity	Type
Agavaceae	Dracaena	phyronides	sp48	abun
Amaranthaceae	Cyathula	prostata	sp12	abun
Annonaceae	Xylopia	quintasii	sp35	mark
Araceae	Cercestis	afzelii	sp11	ref
Arecaceae	Raphia	hookerii	sp50	mark
Arecaceae	Elaeis	guineensis	sp60	abun
Asparagaceae	Draceana	surculosa	sp59	mark
Asteraceae	Synedrella	nodiflora	sp7	abun
Asteraceae	Ageratum	conyzoides	sp8	abun
Asteraceae	Chromolaena	odorata	sp21	abun
Caesalpiniaceae	Plagiosiphon	emarginatus	sp29	mark
Caesalpiniaceae	Berlinia	occidentalis	sp33	mark
Caesalpiniaceae	Dialium	aubrevileii	sp43	mark
Caesalpiniaceae	Gilbertiodendron	preusti	sp57	mark †
Chrysobalanaceae	Parinari	excelsa	sp46	abun †
Clusiaceae	Pentadesma	butyracea	sp13	abun
Clusiaceae	Garcinia	afzelii	sp44	mark
Combretaceae	Strephonema	pseudocola	sp22	abun
Commelinaceae	Palisota	hirsuta	sp31	mark
Convolvulaceae	Calycobolus	africanus	sp55	mark
Cyperaceae	Scleria	boivinii	sp45	abun
Ebenaceae	Diospyros	manii	sp40	mark †
Ebenaceae	Diospyros	sanza-minika	sp41	mark
Ebenaceae	Diospyros	soubreana	sp42	mark
Euphorbiaceae	Cleistanthus	libericus	sp4	abun
Euphorbiaceae	Manniophyton	fulvum	sp26	mark
Euphorbiaceae	Maesobotrya	barterii	sp49	mark
Euphorbiaceae	Uapaca	esculenta	sp53	abun
Fabaceae	Dalbergia	altissima	sp5	abun
Fabaceae	Desmodium	adsencdens	sp16	ref
Fabaceae	Baphia	bancoensis	sp38	mark
Humiriaceae	Sacoglottis	gabonensis	sp34	abun

Lamiaceae	Vitex	micrantha	sp9	abun
Lecythidaceae	Napoleonaea	leonensis	sp51	mark
Marantaceae	Marantochloa	purpurea	sp15	ref and mark
Marantaceae	Hypselodelphys	violaceae	sp28	abun
Marantaceae	Taumatococcus	daniellii	sp58	abun
Melastomataceae	Tristemma	albiflorum	sp20	abun
Melastomataceae	Dissotis	rotundifolia	sp23	ref
Melastomataceae	Memecylon	lateriflorum	sp27	abun
Moraceae	Streblus	usambarensis	sp36	abun
Nephrolepidaceae	Nephrolepis	biserrata	sp1	ref and mark
Ochnaceae	Campylopermum	calomelanos	sp39	abun
Olacaceae	Coula	eduils	sp37	mark
Olacaceae	Strombosia	glaucescens	sp54	mark
Poaceae	Streptogyna	crinita	sp14	ref
Poaceae	Centotheca	lappacea	sp47	mark
Pteridaceae	Pteris	burtonii	sp2	ref and mark
Pteridaceae	Pityrogramma	calomelanos	sp3	abun
Rapataceae	Maschalocephalus	dinklagei	sp10	ref
Rubiaceae	Geophila	hirsuta	sp17	ref and mark
Rubiaceae	Geophila	afzelii	sp18	ref and mark
Rubiaceae	Corynanthe	pachyceras	sp30	abun
Rubiaceae	Cephaelis	yapoensis	sp52	mark
Rubiaceae	Massularia	acuminata	sp56	mark †
Sterculiaceae	Scaphopetalum	amoenum	sp19	abun
Sterculiaceae	Heritiera	utilis	sp32	mark
Urticaceae	Urera	oblongifolia	sp6	abun
Vitaceae	Leea	guineensis	sp24	abun
Zingiberaceae	Costus	afer	sp25	abun

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3 587 Table 1 : Plant species collected in the TNP. The first column represents the Family, the second  
4  
5 588 one the genera, the third one the species and the fourth one the number we gave to simplify the  
6  
7 589 identification. The last column represents the different reasons why these plants were collected.  
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10 590 We noted 'ref' for reference plants; plants already suggested by other authors to be eaten by  
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12 591 pygmy hippos. 'abun' for plants that seemed abundant in our research area and 'mark' for plants  
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14 592 on which we found a hippo's territorial marking. The symbol † represents the four species  
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16 593 deleted from the analysis because the two sides of the leaf's epidermis removed were not  
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18 594 workable.  
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For Review Only

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3 595 **MACROSCOPIC CRITERIA**

4 596 1. **Leaf vein shape †** macro\_veins (3): *pinnate\_leaf, reticulate\_leaf, parrallel\_leaf*

597

7 598 **MICROSCOPIC CRITERIA**

9 599 Epidermal cells

10 600 2. Width width\_epid\_cells (2): *ML\_25\_ep, More\_25\_ep*

11 601 3. **Length †** length\_epid\_cells (3): *small\_ep, medium\_ep, large\_ep*

12 602 4. **Layout †** layout\_epid\_cells (2): *aligned, non\_aligned*

13 603 5. Cell shape shape\_epid\_cells (3): *alongated, pentagonal, winding*

14 604 6. **Wall shape †** shape\_wall\_cells (5): *straight\_wall, angular\_wall, wavy\_wall,*  
15 605 *slightly\_wavy\_wall, round\_wall*

16 606 7. Silica silica (3): *absence\_silica, concave\_parallel, concave\_perpendicular*

17 607 8. Scale scale (3): *absence\_scale, flat\_thiny, flat\_thick*

18 608 Trichome

19 609 9. Trichome cellularity trichome (3): *absence\_trichome, uni, multi*

20 610 10. Insertion insertion\_trichome (3): *absence\_insertion, flower, other\_insertion*

21 611 Stomata

22 612 11. **Quantity †** quantity\_stomata (4): *absence\_quantity, large, medium, low*

23 613 12. Direction direction\_stomata (3): *absence\_direction, different, same*

24 614 13. Width width\_stomata (3): *absence\_width, ML\_25\_stom, More\_25\_stom*

25 615 14. Length length\_stomata (3): *absence\_length, ML\_25\_stomata, More\_25\_stomata*

26 616 15. Type stomata\_type (8): *absence\_type, actinocytic, anomocytic, anisocytic, diacytic,*  
27 617 *gramineous, paracytic, tetracytic*

28 618

29 619 Table 2 : List of 15 variables, which describe our reference library with the code used in our

30 620 data frame. In italic are the categories and in brackets is the number of categories used for

31 621 each variable. The symbol † represents the five most relevant variable finally selected.



Groups/Families	Plants species	Faeces									
		1	2	3	4	5	6	7	8	9	10
<b>FERNS</b>		✓	✓		✓	✓		✓	✓	✓	✓
Nephrolepidaceae	<i>Nephrolepis biserrata</i>	✓	✓		✓	✓		✓	✓	✓	
Pteridaceae	<i>Pteris burtonii</i>				✓						
<b>MONOCOTYLEDONAE</b>		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Marantaceae specie		✓	✓	✓			✓		✓	✓	✓
	<i>Marantocloa purpurea</i>	✓	✓	✓					✓		
	<i>Taumatococcus danielii</i>	✓	✓								
Poaceae species		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	<i>Centotheca lappaceae</i>	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	<i>Streptogyna crinita</i>	✓		✓	✓	✓	✓	✓	✓	✓	✓
<b>DICOTYLEDONAE</b>		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Sterculiaceae	<i>Heritiera utilis</i>	✓	✓			✓					✓

622 Table 3: Summary of the visual analysis based on the HC tree in Fig.3. The first column  
 623 represents the plants groups and families, the second one the plants species identified and finally  
 624 their presence in the ten faeces.

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3 625 **Figure legends**  
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6 626 Figure 1 : Taï National Park (TNP), research area. On the top left is the map of the TNP. The  
7  
8 627 Park is divided in 5 sectors (Taï, ADK-V6, Djouroutou, Soubré and Djapaji) defined by the  
9  
10 628 Office of Parks and Reserves (OIPR). The black rectangle represents our research area. On  
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13 629 the right, zoom in into our research area. The black circles represent the faeces collected  
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15 630 during the fieldwork and the blue ones represent the ten faeces used for this study.  
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18 631 Figure 2: Projection of the faeces fragments (as additional individuals) in the food items on MCA  
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20 632 F1xF2 axes. On blue, are represented the reference library (56 plants species, i.e. 112 leaves  
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22 633 sides), and on red are the N=130 plants fragments found in the 10 droppings (named as  
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24 634 supplementary individual). On the top left is represented the barplot of the MCA. On the  
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27 635 bottom right is represented the projection of the 5 variables with their categorises on the  
28  
29 636 F1xF2 axes.  
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32 637 Figure 3: HC tree for all the individuals (food items and Faeces fragments). On the comb of  
33  
34 638 the tree are written the species names as well as the faeces fragments name (number; from 1  
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36 639 to 130, and faeces number; from 1 to 10). The red circles represent the combs of the tree that  
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38 640 are shared by more than two faeces fragments. The yellow circles represent the combs of the  
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40 641 tree that are shared by two faeces fragments and the green circles represents a single faeces  
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42 642 fragment on a comb. Finally, the stars indicate the faeces fragments that have no direct  
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44 643 affinity with the food items species.  
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49 644 Figure 4: Comparison between *Herritiera utilis* (dicot) and a faeces fragment. (a)  
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51 645 macroscopic view of *Herritiera utilis*, abaxial side (b) microscopic view of *Herritiera utilis*,  
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53 646 abaxial side with a magnification of 100x (c) macroscopic view of a faeces fragment similar  
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55 647 to *Herritiera utilis* (d) microscopic view of the same faeces fragment with a magnification of  
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58 648 100.  
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## 649 Appendices

650 Appendix 1: Projection of the faeces fragments (as additional individuals) in the food items  
651 on MCA F1xF3 axes. On blue, are represented the reference library (56 plants species, i.e.  
652 112 leaves sides), and on red are the n=130 plants fragments found in the 10 droppings  
653 (named as supplementary individuals). On the top left is represented the barplot of the MCA.  
654 On the bottom right is represented the projection of the 5 variables with their categorises on  
655 the F1xF3 axes.

656 Appendix 2: Projection of the reference library's variables on F1xF2 axes. The colours  
657 represent each variable with their different categories. We added three circles to highlight  
658 three groups of individuals.

659 Appendix 3: Comparison between *Nephrolepis bisserata* (fern) and a faeces fragment. (a, b)  
660 microscopic view of *Nephrolepis bisserata*. (c, d) microscopic view of a faece fragment  
661 similar to *Nephrolepis bisserata*. (a, c) adaxial sides, with a magnification of 100x. (b, d)  
662 abaxial side, with a magnification of 200x. (a-d) The slides were prepared with the  
663 discoloration method.

664 Appendix 4: Comparison between *Centhoteca lappaceae* and a faece fragment. (a, c)  
665 macroscopic view of *Centhoteca lappaceae* in a and a faece fragment in c. (b, d) microscopic  
666 view of *Centhoteca lappaceae* in b and a faece fragment in d, with a magnification of 200x.  
667 The slides were prepared with the discoloration method.

668 Appendix 5: Comparison between *Streptogyna crinita* and a faece fragment. (a) microscopic  
669 view of the adaxial side of *Streptogyna crinita* with a magnification of 100x. (b) microscopic  
670 view of a faeces fragment with a magnification of 100x (c) microscopic view of a faece  
671 fragment with a magnification of 200x. (a-c) The slides were prepared with the discoloration  
672 method.

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3 673 Appendix 6: Comparison between *Marantaceae* species and faeces fragments (adaxial sides).

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5 674 (a, b) microscopic view of the adaxial sides of *Marantochloa purpurea*, a, and

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7 675 *Hypselodelphys violaceae*, b. (c, d) microscopic view of two faeces fragment similar to

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9 676 *Marantaceae* species. (a-d) the slides were prepared with the discoloration method and

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11 677 photographed with a magnification of 100x.

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15 678 Appendix 7: Comparison between *Marantochloa purpurea*, *Costus afer* and faeces fragments.

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17 679 (a) microscopic view of the abaxial side of *Marantochloa purpurea*. (b) microscopic view of

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19 680 the abaxial side of *Costus afer*. (c, d) microscopic view of faeces fragments similar to

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21 681 *Marantochloa purpurea* and *Costus afer*. (a-d) The slides were prepared with the

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23 682 discoloration method and photographed with a magnification of 100x.

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27 683 Appendix 8: Seed found in faeces. (a) internal view of the seed. (b) external view of the seed.

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29 684 (a-b) the two different view were photographed with a magnification of 25x.