


ARTICLE

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

Alba Hendier^{1,2}  | Cyrille Chatelain³ | Pierre-Emmanuel Du Pasquier¹ |
 Monique Paris^{4,5} | Karim Ouattara^{2,6} | Inza Koné² | Daniel Croll¹ |
 Klaus Zuberbühler^{1,2,7}

¹Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland

²Centre Suisse de Recherches Scientifiques en Côte d'Ivoire (CSRS), Abidjan, Ivory Coast

³Conservatoire et Jardin Botaniques de la ville de Genève, Geneva, Switzerland

⁴Institute for Breeding Rare and Endangered African Mammals (IBREAM), Edinburgh, UK

⁵Mammal Research Institute, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria, South Africa

⁶Université Félix Houphouët-Boigny, Abidjan, Ivory Coast

⁷School of Psychology and Neuroscience, University of St Andrews, St Andrews, UK

Correspondence

Alba Hendier, Institute of Biology, University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland.
 Email: alba.hendier@gmail.com

Funding information

This research was funded by 'Fond des donations' of the University of Neuchâtel and the 'Willy Müller Award' of the Centre Suisse de Recherches Scientifiques en Côte d'Ivoire

Abstract

Diet determination of endangered species is an essential element in defining successful conservation strategies and optimising captive breeding programmes. In this study, we developed a new diet identification system, derived from standard faecal analysis, to determine the diet of an elusive and endangered herbivore, the pygmy hippopotamus (*Choeropsis liberiensis*). We collected faecal samples from 10 free-ranging individuals covering a combined home range area of about 50 km² in Taï National Park, Côte d'Ivoire. In subsequent laboratory analyses, we extracted a large number of leaf epidermis fragments from spatially separated faecal samples and compared them with a reference plant database. Using Multiple Correspondence Analysis (MCA) of epidermis fragments combined with direct visual inspection, we identified the most frequently consumed plant species, which revealed that pygmy hippopotami qualified as intermediate feeders. Their diet was based on at least seven species of monocotyledonae, dicotyledonae and fern groups, with a preference for a small number of other plant species. We evaluate the merit of our method and discuss our findings for developing effective conservation and captive breeding strategies in an endangered species with a wild population of less than 2500 adult individuals.

KEYWORDS

Africa, conservation, faecal analysis, foraging, Multiple Correspondence Analysis

Résumé

La détermination de l'alimentation des espèces en voie de disparition est essentielle afin de définir des stratégies de conservation efficaces et d'optimiser les programmes de reproduction en captivité. Dans cette étude, nous avons mis au point un nouveau système d'identification de l'alimentation dérivé d'une analyse des selles standard, afin de déterminer le régime alimentaire de l'hippopotame pygmée, un herbivore insaisissable en voie de disparition (*Choeropsis liberiensis*). Nous avons prélevé des échantillons de selles auprès de 10 individus en liberté au sein d'une superficie totale d'environ 50 km² dans le parc national de Taï, en Côte d'Ivoire. Lors des analyses en laboratoire qui ont suivi, nous avons extrait un grand nombre de fragments d'épiderme foliaire dans les échantillons fécaux séparés dans l'espace et avons comparé ces derniers à

une base de données de plantes de référence. En utilisant une méthode d'analyse des correspondances multiples (ACM) des fragments d'épiderme, combinée à une inspection visuelle directe, nous avons identifié les espèces végétales les plus fréquemment consommées, ce qui a révélé que l'alimentation des hippopotames pygmées peut être considérée comme mixte. Leur régime alimentaire était basé sur au moins sept espèces de monocotylédones, dicotylédones et de fougères, avec une préférence pour un petit nombre d'autres espèces végétales. Nous évaluons le bien-fondé de notre méthode et discutons de nos constatations afin d'élaborer des stratégies efficaces de conservation et de reproduction en captivité pour les espèces en voie de disparition dont la population sauvage compte moins de 2500 individus adultes.

1 | INTRODUCTION

The pygmy hippopotamus, *Choeropsis liberiensis* (Morton, 1849), named hereafter as pygmy hippo, is an endemic species to West Africa (Côte d'Ivoire, Guinea, Liberia and Sierra Leone; Prothero & Foss, 2007). It originally occurred as two subspecies, *C. l. liberiensis* and *C. l. heslopi* (Corbet, 1969), but the Nigerian subspecies

C. l. heslopi has gone extinct in the 1940s (Robinson, 2013), while the remaining West African subspecies is classified by the IUCN as Endangered (Ransom et al., 2015) due to habitat loss and poaching for bushmeat (Lewison & Oliver, 2008). The population is still declining due to political instability in the region, sustained lack of law enforcement and absence of coordinated conservation efforts (Conway, 2013; Mallon et al., 2011). The current population size of

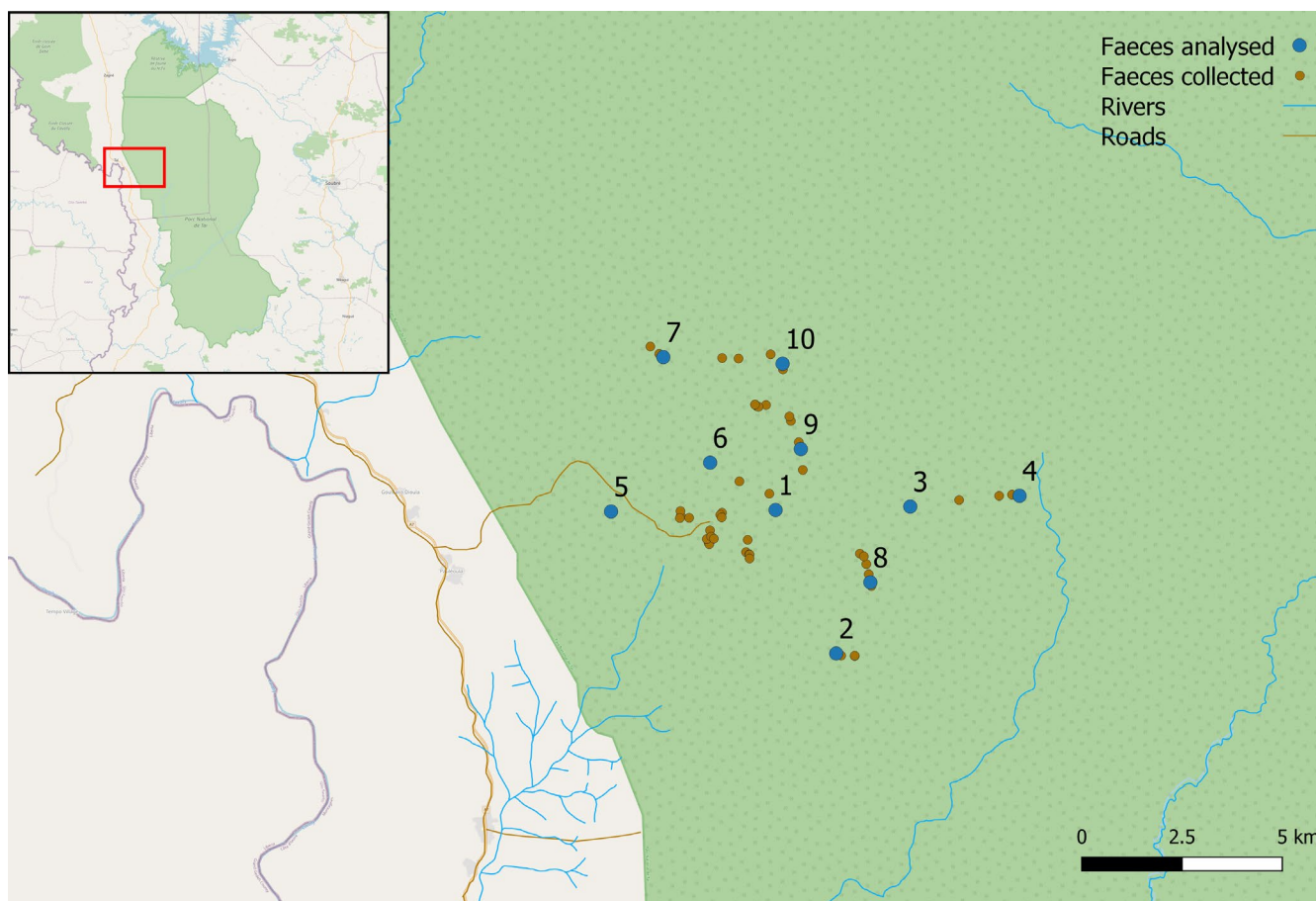


FIGURE 1 Taï National Park (TNP). On the top left is the map of the TNP. The red rectangle represents our research area. On the right, zoom into our research area. The brown circles represent the faeces collected during the fieldwork and the blue ones represent the ten faeces used for this study

TABLE 1 Plant species collected in the TNP

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 ^a
Chrysobalanaceae	Parinari	excelsa	sp46	abun ^a
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 ^a
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	Taumathococcus	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	Campylospermum	calomelanos	sp39	abun
Olacaceae	Coula	eduils	sp37	mark
Olacaceae	Strombosia	glaucescens	sp54	mark

(Continues)

TABLE 1 (Continued)

Family	Genus	Species	Identity	Type
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 ^a
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

Note: The first column represents the Family, the second one the genera, the third one the species and the fourth one the number we gave to simplify the identification. The last column represents the different reasons why these plants were collected. We noted 'ref' for reference plants; plants already suggested by other authors to be eaten by pygmy hippos. 'abun' for plants that seemed abundant in our research area and 'mark' for plants on which we found a hippo's territorial marking.

^aRepresents the four species deleted from the analysis because the two sides of the leaf's epidermis removed were not workable.

pygmy hippos is estimated to be less than 2500 adult individuals, the majority of which are believed to reside in Taï National Park in Côte d'Ivoire (Ransom et al., 2015; Roth et al., 2004).

Although there are a good number of studies on pygmy hippos (Bogui et al., 2016; Conway, 2013; Flacke & Decher, 2019; Garthey, 2013; Hillers et al., 2017; Roth et al., 2004), much of the available information is from captive animals (Flacke et al., 2015, 2016). There is little information from the wild, which is mainly due to the species' cryptic behaviour and difficulties accessing their natural habitat. In captivity, individuals suffer from persistent health problems, such as polycystic kidney disease and dental skin and foot problems, which may originate from inadequate diet (Flacke et al., 2017; von Houwald et al., 2007). Pygmy hippos appear to forage mainly at night over a period of about 6 hours (Eltringham, 1999; Mallon et al., 2011; Robinson, 1981). They are thought to consume a wide variety of ferns, roots, grasses, stems, leaves of young trees and crops (Robinson, 1970, Eltringham, 1999; Bülow, 1987; Hentschel, 1990; Robinson et al., 2017), but we are not aware of any systematic data on diet preference and composition in free-ranging animals.

The purpose of this study was to address this issue with a new methodology, based on progress in plant identification techniques: microscopic analysis of plant fragments of leaf epidermis in faeces (Cuartas & Garcia-Gonzalez, 1996). Identification via microscopy is different from traditional Linnaean classification, which is based on the plant reproductive system. However, recent botanical studies have shown that the microscopically determined foliar anatomy can produce reliable information for species identification, particularly at the family and group level (Metcalf and Chalk, 1950, 1957; Shah

et al., 2018a, b; Ullah et al., 2018a, b). The foliar anatomy and epidermis type are determined from five features (four microscopic, one macroscopic) through Multiple Correspondence Analysis (MCA) complemented by a visual analysis (by pictures) of the targeted epidermis.

We took advantage of this recent development by combining standard faecal analysis with the newly established plant identification system in order to determine the diet of free-ranging pygmy hippos in Taï National Park. Our first goal was to describe the species' diet composition; our second goal was to develop an identification key for tropical plant species based on microscopic features, which could be used for other studies and contribute to welfare decisions in captivity and conservation efforts in the wild.

2 | MATERIALS AND METHODS

2.1 | Study site

The study was conducted in Taï National Park (TNP), Côte d'Ivoire, from July to November 2017, in an area of about 50 km² (Figure 1), surrounding the research station of the 'Centre de Recherche en Ecologie' (CRE). Since 1982, the TNP is a UNESCO World Heritage site that covers an area of 536,000 ha, the largest protected tropical forest of West Africa (Lauginie, 2007; OIPR, 2018; UNESCO World Heritage, 2018). The vegetation of the park is rich with 1365 documented plant species (Scoupe, 2011). Regular censuses of the flora have been carried throughout the park both in the Northern

and Eastern parts (Scoupe (2011) as well as in the South (Adou Yao et al., 2000) and the Southwest (Adjanooun & Guillaumet (1963); Adou Yao et al. (2005); Aké Assi & Pfeffer (1975); Aké Assi (1984); Menziès (2000)), which has resulted in an extensive plant database (Sattler, 2000).

2.2 | Plant reference library

The standard method for obtaining diet information from elusive wild animals is by microscopic analysis of plant epidermis cells recovered from faeces with the help of a reference catalogue (Butet, 1985, 1987; Chapuis, 1980; Chatelain et al., 2000; Crocker, 1959; Cuartas & Garcia-Gonzalez, 1996; Rech, 2011; Storr, 1961). Our selection criteria for including plant species for a reference library were as follows: (1) high abundance, less than 1 m high and encountered in pygmy hippo habitat at the edges of transects or along rivers ('abun', Table 1), (2) covered by pygmy hippo territorial markings ('mark'; Table 1), (3) mentioned in literature as eaten by pygmy hippos in the park ('ref'; Table 1; Bülow, 1987; Hentschel, 1990). A voucher of $n = 60$ plant species (Table 1) was collected and deposited at the herbarium of the 'Centre Suisse de Recherches Scientifiques' (www.csr.ch) for subsequent validation by the assistant curator of the herbarium, Saturnin Dougoune, following botanical nomenclature from African Plant Database (African Plant Database, 2018).

The microscopic food items for the reference library were prepared using two methods to enhance reliability: the 'nail-polish method' (Hilu and Randall, 1984; Miller & Ashby, 1968) and Rech's (2011) protocol (see below). The nail-polish method consists of applying a thin layer of commercial, transparent nail-polish on the sample leaves. Once dry, the nail-polish layer is then removed and placed on a slide in a drop of water. For both methods, semi-permanent slides were created with the two leaf sides. All the slides were photographed at 40 \times , 100 \times and 200 \times with an inverted microscope.

2.3 | Faecal sample library

Pygmy hippo faeces are easy to locate in the natural habitat. Similar to common hippos (*Hippopotamus amphibius* Linnaeus, 1758), pygmy hippo faeces can be classified as either territorial or as litter droppings (Robinson et al., 2017), depending on their consistency and shape (soft/shapeless or solid, respectively). We collected both types, following the sampling method for common hippos (Michez, 2006; Scotcher et al., 1978). In total, $n = 70$ faeces were collected. Whenever we had evidence for pygmy hippo presence (footprint or droppings), we determined the exact location using a GPS device, which resulted in a total of $n = 330$ location points. To obtain independent data points, only samples that were at least 2 km apart were selected (home range estimates; females = 0.5 km²; males = 1.5 km²; Bülow, 1987; Hentschel, 1990) (Figure 1). The final sample size consisted of faecal material from $n = 10$ different locations, that is, 10 different individuals.

An extract of 2 g from each faecal sample was used for a macroscopic classification into four categories: leaves, roots/stems, seeds and unidentifiable material following Michez (2006). Then, we randomly selected $n = 48$ leaf fragments per sample, which were placed in two 24-well cell culture clusters (i.e., 48 wells) and photographed under a binocular magnifying glass at 7.5 \times , 25 \times , and 60 \times . Some samples having more leaves than others, $n = 48$, allowed us to have a representative subsample and to keep the same number of leaf fragments per faeces. After photographing, fragments were soaked in ethanol and sodium hypochlorite until they were transparent following the Rech's protocol (2011) for animal faeces studies. Finally, the discoloured fragments were placed between a slide and a lamella in a drop of glycerine. A layer of commercial nail-polish was added to better preserve the samples for at least two months. In total, $n = 480$ fragments with 2880 pictures (1440 microscopic and 1440 macroscopic) were taken with inverted microscope.

2.4 | Data analysis

Based on different morphological features used in diet studies (Butet, 1985, 1987; Chapuis, 1980; Kok and van der Schijff, 1973; Metcalfe and Chalk 1950, 1957; Rech, 2011; Stoddard, 1965), we selected five categorical features (Table 2) as parameters for both the plant reference library and the faecal library. Morphological features for the plant reference library concerned the epidermis cells of 56 referent plants along these five features. As the two sides of the leaves can be difficult to identify in faeces, we measured each side of the leaf in this library. In total, $n = 112$ reference epidermis samples were described. From the $n = 480$ faeces fragments (2880 images) prepared from the $n = 10$ faecal samples, a selection of 11-16 fragments per faecal sample was chosen, following careful inspection of all images, which resulted in a final sample size of $n = 130$ fragments. Finally, we subjected the results to statistical analysis.

2.5 | Statistical analyses

Multivariate analyses (Benzécri, 1973) were used in order to (1) assess the diagnostic value of the five categorical features (i.e., 'variables') in order to identify the taxonomic groups from the plant reference library (separately for both leaf sides, i.e., 'individual'; $n = 112$), and (2) compare the fragments found in the hippo faecal sample library (i.e., 'additional individual') with our plant reference library. A first MCA with the reference library ($n = 112$) allowed us to explore the structure in our data set and try to detect a possible taxonomic clustering (i.e., plant classes, families, genus or species) (Crawley, 2007). In parallel, an agglomerative Hierarchical Clustering analysis (HC) was conducted based on the MCA coordinates of all axes using the Ward method (Ward, 1963). In a second MCA, the faeces library ($n = 130$) was added as 'additional individuals'. Thus, these individuals were not taken into account for calculating the MCA axes. This allowed us to consider the spatial position of our faeces fragments (additional

TABLE 2 List of 15 variables, which describe our reference library with the code used in our data frame

Macroscopic criteria	
Leaf vein shape ^a	macro_veins (3): <i>pinnate_leaf, reticulate_leaf, parrallel_leaf</i>
Microscopic criteria	
Epidermal cells	
Width	width_epid_cells (2): <i>ML_25_ep, More_25_ep</i>
Length ^a	length_epid_cells (3): <i>small_ep, medium_ep, large_ep</i>
Layout ^a	layout_epid_cells (2): <i>aligned, non_aligned</i>
Cell shape	shape_epid_cells (3): <i>alongated, pentagonal, winding</i>
Wall shape ^a	shape_wall_cells (5): <i>straight_wall, angular_wall, wavy_wall, slightly_wavy_wall, round_wall</i>
Silica	silica (3): <i>absence_silica, concave_parallel, concave_perpendicular</i>
Scale	scale (3): <i>absence_scale, flat_thiny, flat_thick</i>
Trichome	
Trichome cellularity	trichome (3): <i>absence_trichome, uni, multi</i>
Insertion	insertion_trichome (3): <i>absence_insertion, flower, other_insertion</i>
Stomata	
Quantity ^a	quantity_stomata (4): <i>absence_quantity, large, medium, low</i>
Direction	direction_stomata (3): <i>absence_direction, different, same</i>
Width	width_stomata (3): <i>absence_width, ML_25_stom, More_25_stom</i>
Length	length_stomata (3): <i>absence_length, ML_25_stomata, More_25_stomata</i>
Type	stomata_type (8): <i>absence_type, actinocytic, anomocytic, anisocytic, diacytic, gramineous, paracytic, tetracytic</i>

Note: In italic are the categories and in brackets is the number of categories used for each variable.

^aRepresents the five most relevant variable finally selected.

individuals) in relation to our plant reference library (individuals). In order to interpret the results, we calculated a Hierarchical Clustering (HC) dendrogram, based on the coordinates of the individuals (plant reference library) on all the MCA axes including the additional individuals (faeces reference library). The HC tree allowed us to focus on the similarities and differences between the food item references and the faeces fragments. From this HC dendrogram, we continued the analysis by looking carefully at pictures that were targeted as similar, and we approved or disapproved the species targeted by the MCA analysis.

All statistical analyses were performed with the R software (R Development Core Team, 2018), using the package 'ade4' with the 'dudi.acm' function (Dray and Dufour, 2007). The interface 'explor'

(from the explor package) was used to observe the results of MCA and edit the different graphs (Barnier, 2020). For the HC analysis, we used the 'hclust' function.

3 | RESULTS

3.1 | Plant reference library

We carried out a Multiple Correspondence Analysis (MCA) to establish the plant reference library for future identification of fragments in faeces. The MCA ($n = 112$ individuals; 5 variables) had a total inertia of 2.4. The first two axes (F1 and F2) explained 35.2%, and the first five axes 65.7% of the total variance. We observed a light horseshoe shape (Guttman effect) on the active individual's (reference plants; blue) projection in F1xF2 axes (Figure 2, Appendix 1 for the projection on F1xF3 axes). This effect was due to two variables that contributed in similar ways, most likely the macroscopic variable 'macro veins' and the 'microscopic layout'. Despite this effect, three main groups were observed through the categories of the variables (Appendix 2), which corresponded mostly to the three major taxonomic groups: monocots, dicots and ferns. There was no other structure apart from the distinction between these three groups. Indeed, the MCA did not allow us to gather taxonomic ranks (species) inside the families and even less at the generic level.

3.2 | Faecal samples

In a second step, the faeces fragments were added as supplementary elements to the previous MCA analysis (named as supplementary in Figure 2, in red). This allowed us to see whether there were similar species between our reference plants and our faeces fragments. We noticed that the reference library and the faeces library shared similar positions on the spatial projection (Figure 2). In the HC tree, the clustering of the plant groups (monocots, dicots and ferns) was represented along the two main branches as well as some plant families: Rapataceae, Marantaceae, Pteridaceae and Rubiaceae. However, many plant species of the reference plant list shared the same position on the tree (Figure S1). The HC tree helped to target similar epidermis, but visual inspection was still necessary to determine the species in the faeces fragments. After analysing each branch of the tree (Figure S1) with the corresponding pictures, we were able to confirm the presence of seven species of plants from our reference list (Table 3).

Results showed that 8 of 10 faecal samples contained monocots, dicots and fern species. The two remaining samples (2, 6) did not contain any fern (Table 3). For the ferns, *Nephrolepis bisserata* could be identified in 7 of 10 faecal samples (Appendix 3). For the monocots of the Poaceae family, we found evidence for *Cenhoteca lappa-ceae*, and *Streptogyna crinita* (Appendix 4 and 5). For Marantaceae family, they were present in 7 of 10 samples (Appendix 6 and 7;

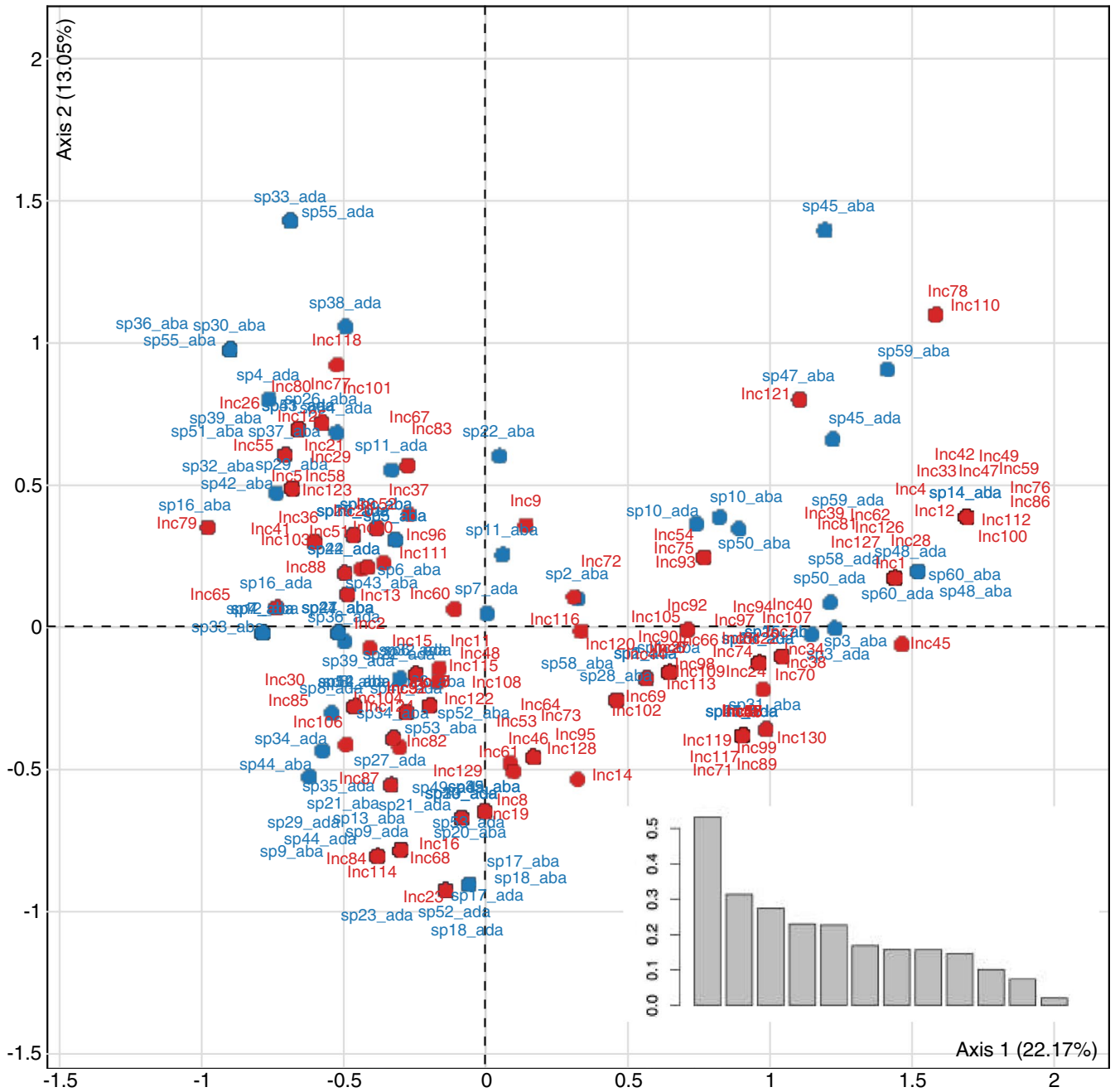


FIGURE 2 Projection of the faeces fragments (as additional individuals) in the food items on MCA F1x2 axes. On blue, are represented the reference library (56 plants species, i.e., 112 leaves sides), and on red are the $n = 130$ plants fragments found in the 10 droppings (named as supplementary individual). On the bottom right is represented the barplot of the MCA. For the projection of the 5 variables with their categories on F1x2 axes, see Appendix 2

often *Marantocloa purpurea*). Finally, in the dicots, we found four times *Herritiera utilis* remnants (Figure 3).

4 | DISCUSSION

We analysed the diet of 10 individual pygmy hippos over a combined range of about 50 km² and found evidence for commonly found plants in the faecal samples. We combined a new statistical analysis of microscopic epidermis fragments with traditional faeces-based

diet determination. This analysis was based on only five categorical variables, thus demonstrating the potential of the method as an interactive research tool for rapid diet identification in combination with established criteria, such as trichomes, silicates or scales. With this method, we found that, firstly, free-ranging pygmy hippos have a varied diet, which includes major plant groups including monocots, dicots and ferns. Secondly, we were able to identify at least seven plant species, although several epidermis fragments remained unidentified (limitations below). Hence, we can confirm that pygmy hippos are to be classified as herbivore generalists, a foraging strategy

TABLE 3 Summary of the visual analysis based on the HC tree in Figure S1

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

Note: The green tick (✓) represents the plant species found in each faeces sample (numbered 1 to 10).

The first column represents the plants groups and families, the second one the plants species identified and finally their presence in the ten faeces.

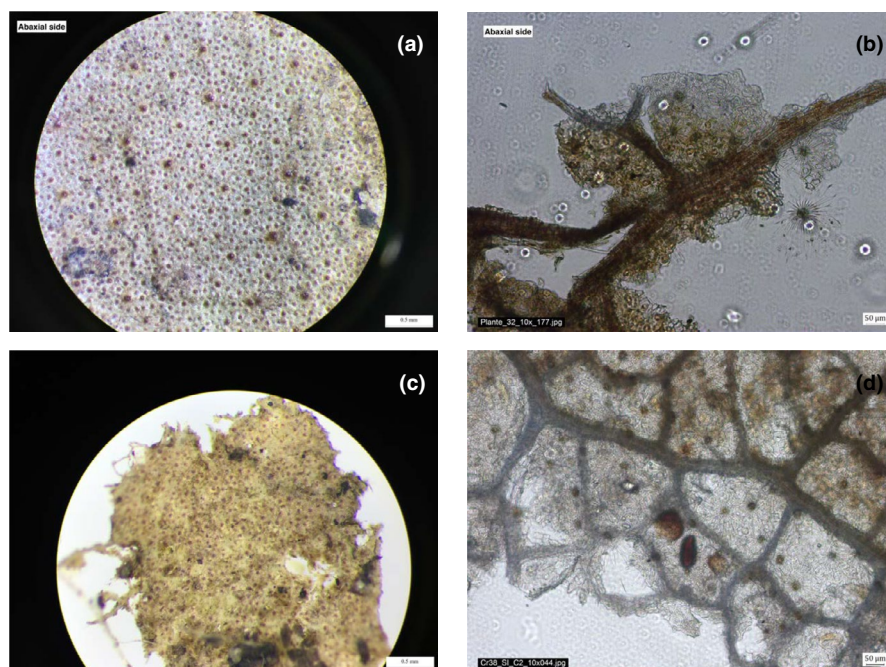


FIGURE 3 Comparison between *Heritiera utilis* (dicot) and a faeces fragment. (a) macroscopic view of *H. utilis*, abaxial side (b) microscopic view of *H. utilis*, abaxial side with a magnification of 100× (c) macroscopic view of a faeces fragment similar to *H. utilis* (d) microscopic view of the same faeces fragment with a magnification of 100

that allows them to avoid over-ingestion of plant toxins (Freeland and Janzen, 1974).

Generalist feeding does not exclude the possibility that herbivorous mammals can develop preferences for certain plant species (Belovsky, 1978), which is supported by our observation of low variability between different droppings. The seven plant species described in the results were frequently found in most faecal samples, particularly the plants from the Poaceae family (grasses). More generally, the data suggest that pygmy hippos in TNP have a preference for *N. biserrata*, *Pteris burtonii*, *Marantaceae* species and *S. crinita*, in line with what has been proposed by Bülow (1987) and Hentschel (1990). However, we could not find any evidence

for the following dicots: *Desmodium ascendens*, *Dissotis rotundifolia*, *Geophila afzelii*, *Geophila hirsuta*, and *Cercestis afzelii*. This may be due to the fact that we focussed on large fragments only (large particles ingested). Dicot species have a thin cell wall (Bodmer, 1990; Shipley, 1999), suggesting that they are better absorbed and therefore may have been overlooked by our method. In captive pygmy hippos, it has been argued that low digestibility of some particles may be due to ineffective mastication (Schwarm et al., 2009), suggesting that by including smaller fragments, we should probably find these species.

Following Bülow (1987) and Hentschel (1990)'s database, we can add two new species that were frequently observed in the

droppings: *Centoteca lappaceae* (grass, found in all samples) and *Herritiera utilis* (tree leaves, found in at least four of the ten samples). This new observation could be explained by the fact that previous studies were based on feeding trials, feeding signs and direct observations in a restricted study area (Hentschel, 1990). When comparing our data to the already published list, we could only confirm about 50% of species, while species classified as favourites were difficult to find or absent in our area (e.g., *Staurogyne paludosa*, *Justicia tenella*, and *Floscopa africana*). This fact further supports our findings that pygmy hippos qualify as herbivorous generalists (Hentschel, 1990; Robinson et al., 2017), such that an individual's diet composition will largely depend on the local flora encountered during foraging. Pygmy hippos occupy very small home ranges in swampy areas confirming the relationship between home range size and nutritional requirement of pygmy hippos (Robinson et al., 2017).

All these observations support that pygmy hippos are non-ruminant generalist intermediate feeders. This is in contrast to mixed feeders that forage on grasses and forbs, which tend to contain higher proportions of cellulose (Demment and Van Soest, 1985) as well as shrubs and tree leaves, which contain higher proportions of lignin (Bodmer, 1990; Van Soest, 1996). Furthermore, intermediate feeders are able to adapt their diets according to the availability of resources and the seasons (Hofmann, 1989). Classifying pygmy hippos as intermediate feeders is also supported by their gregarious and territorial behaviour, dentition (Lang, 1975), and other dietary studies (Bülow, 1987; Hentschel, 1990).

Based on observations in zoos, Flacke (2017) pointed out that pygmy hippos were incorrectly classified as non-ruminant generalist browsers by the Nutritional Advisory Group (Lintzenich & Ward, 1997) and the Pygmy Hippo Husbandry Manual (von Houwald et al., 2007). Our data show that grasses are an integral part of the pygmy hippo diet, but it is unclear whether this is properly taken into account by captive facilities. It has been suggested that captive pygmy hippos receive a food intake that is too energy rich, which can lead to obesity and disease (Flacke et al., 2016; Flacke, 2017; von Houwald et al., 2008). In a recent study, it was found that reducing the number of pellets and providing hay ad libitum, captive pygmy hippos will approach body weights similar to their wild counterparts (Taylor et al., 2013). This is in line with what is predicted for intermediate feeders, which rely on a diet that is rich in slowly digestible plant fibres (Shiple, 1999).

4.1 | Limitations

A first limitation concerns the size of the plant reference library. Less than 4% of the plant species of TNP were part of the library (60 of 1356 documented species by Scoupe (2011)). However, pygmy hippos are unlikely to target most of these plants as food items, but will focus on shrubs and herbaceous plants, which represent only between 10 and 15% of all plant species. Therefore, we assume that

almost one third of these plant species were analysed in this study. We recommend to extend the database produced by this study to increase the knowledge of the diet of pygmy hippos as well as the diet of other species within TNP. It would be important to add faecal samples from other TNP areas and across the seasons, which is likely to produce a fuller picture of the dietary flexibility of pygmy hippos. Besides enlarging the database, it would also be relevant to conduct chemical analyses on the plants consumed, to get a better understanding of the nutritional needs of wild pygmy hippos (Freeland and Janzen, 1974).

A second limitation concerns the choice of variables for the MCA. Although a macroscopic variable would be valuable, it is not possible when operating with small plant fragments. Furthermore, many of the food item references share the same characteristics and, sometimes, it is difficult to distinguish the epidermis of different species without a confirmation by picture. Future studies may also want to add information on the stomata (i.e., the shape and number of cells around the stomata), which are good indicators to determine the family and species level (Metcalf and Chalk, 1957). In this study, this was not possible due to the quality of our reference slides. Rech (2011) recommends analysing only the abaxial side because it is more characteristic of the plant species. Indeed, as there are fewer features visible on the adaxial side, we were limited in the descriptions. The cells looked very similar, and it was difficult to distinguish one from another (i.e., adaxial side of *Dialium aubrevillei* and *Napoleona leonensis*). Unfortunately, the side of the faeces fragments removed was not always an option. Also, increasing the number of food item species would give more comparisons to identify more faeces fragments that remain unidentifiable.

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

Finally, these analyses could be compromised by the fact that male and female home ranges can overlap, suggesting that two different individuals could have contributed to each sampling location (Roth et al., 2004). We also tried an approach by camera traps to identify the plants eaten by pygmy hippos (182 videos taken over

two years by Noémie Capelle from the Max Planck Institute (MPI). However, it was almost impossible to carry plant identification, and recognise different individuals based on the videos. First, because there were not many videoclips that showed eaten plants, and second, because the videos did not always allow to observe the plants correctly. However, the activity level (Rowcliffe et al., 2014) and density (Buckland et al., 2000; O'Connell et al., 2011; Trollet et al., 2014) of pygmy hippos are currently analysed based on the large database of camera trap material.

An alternative approach for individual recognition is to utilise DNA barcode analysis. However, this type of method requires storage of fresh faeces, that is, to collect the samples shortly after defecation in order to store them at -20°C or in ethanol to preserve the gut cells (Blekhman et al., 2016; Reed et al., 1997). These conditions are difficult to meet in the remote field locations, especially when working with cryptic and nocturnal species where it is difficult to collect fresh faeces. Furthermore, appropriate markers need to be developed first to enable discrimination at the individual level (Reed et al., 1997; Valentini et al., 2009).

5 | CONCLUSIONS

We applied a new plant identification system to infer the diet of free-ranging pygmy hippos. Our results confirmed that pygmy hippos are generalist herbivores with a wide range of plant species consumed, including grasses and shrubs, suggesting they should be classified as intermediate feeders. Indeed, we observed similar fragments of monocots (grasses), dicots (shrubs and tree leaves) and ferns in almost all faeces analysed, collected from ten different individuals distributed over a combined home range area of about 50km^2 . Moreover, if these analyses were combined with additional evidence, then pygmy hippos in the Taï area appear to have a food preference for specific species, notably *N. bisserata* (fern), *S. crinita* (monocot), *Marantaceae* species (monocot), *C. lappaceae* (monocot), and *H. utilis* (dicot). The latter two species were not considered part of the pygmy hippo diet before. In addition, *C. lappaceae* (monocot) was found in all samples and confirmed the importance of grasses in the diet of this species. High diversity of plants in the diet and the fact that they should be classified as intermediate feeders (rather than browsers; Flacke, 2017) is important for pygmy hippo welfare and conservation strategies both in the wild and in captivity.

With this study, we also compiled a tropical plant database consisting of 60 species that may serve future faunistic studies in West African forests. Future research may focus on the chemical composition of the preferred food items, which is essential in welfare programmes designed to improve the diet offered in captivity and to combat common health problems. For free-ranging pygmy hippos, the data presented here will help to identify and conserve specific microhabitats that contain plant species essential for the survival of this enigmatic forest mammal.

ACKNOWLEDGEMENTS

We thank the Centre Suisse des Recherches Scientifiques (CSRS) for logistic support. Data collection and analyses have been done in collaboration with the Evolutionary Genetics, the Soil Biodiversity and the Comparative Cognition laboratories of the University of Neuchâtel, with further support by the Conservatoire et Jardin Botaniques of Geneva (CJBG), the Max Planck Institute for Evolutionary Anthropology (MPI-EVAN; Leipzig) and the Institute for Breeding Rare and Endangered African Mammals (IBREAM; Edinburgh). We thank that Office Ivoirien des Parcs et Réserves (OIPR) and the Taï Monkey Project (TMP) for giving us permission to carry out the research. Finally, we would like to thank the curator of Basel Zoo as well as Emilie Chanclud, Vinciane Mossion, Fred Stauffer, Anthelme Gnagbo, Elie Bandama Bogui, Hjalmar Kuehl, Noémie Cappelle, Mark Van Heukelum, Saturnin Dougoune, Donatien Bélé, Mamadou Ouattara, Radu Slobodeanu and Mahmoud Bouzelboudjen for discussions and advice during the different stages of this study.

CONFLICT OF INTEREST

This study was part of an on-going collaboration between the Institute for Breeding Rare and Endangered African Mammals (IBREAM) and the Centre Suisse de Recherches Scientifiques: Côte d'Ivoire's Pygmy Hippo Conservation Project (CSRS - THP).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

ORCID

Alba Hendier  <https://orcid.org/0000-0002-3123-9845>

REFERENCES

- Adjanohoun, E., & Guillaumet, J. L. (1963). *Etude botanique entre Bas-Sassandra et Bas-Cavally (missions militaires entre 1960-1961)* (pp. 80-87). Adiopodoumé: ORSTOM.
- Adou Yao, C. Y. (2000). *Inventaire et étude de la diversité floristique du Sud du Parc National de Taï (Côte d'Ivoire)*. Abidjan: Mémoire de D.E.A.
- Adou Yao, C. Y., Blom, E. C., Dengueadhé, K. T. S., van Rompaey, R. S. A. R., N'Guessan, E. K., Wittebolle, G., & Bongers, F. J. J. M. (2005). *Diversité floristique et Végétation dans le Parc National de Taï*. Abidjan: Tropenbos International (Tropenbos Côte-d'Ivoire séries).
- African Plant Database (APD; version 3.4.0) (2018). *Conservatoire et Jardin botaniques de la Ville de Genève and South African National Biodiversity Institute*. Retrieved from: <http://www.africanplantdat abase.ch>
- Aké Assi, L. (1984). *Flore de la Côte d'Ivoire*. Thèse de Doctorat. Université d'Abidjan.
- Aké Assi, L., & Pfeffer, P. (1975). *Etude d'aménagement touristique du Parc National de Taï. Tome 2: Inventaire de la flore et de la faune*. BDPA.
- Barnier, J. (2020). Exploration interactive de résultats d'ACP/ACM avec explor. Retrieved from https://cran.r-project.org/web/packages/explor/vignettes/introduction_fr.htm
- Belovsky, G. (1978). Diet optimization in a generalist herbivore: The moose. *Theoretical Population Biology*, 14(1), 105-134. [https://doi.org/10.1016/0040-5809\(78\)90007-2](https://doi.org/10.1016/0040-5809(78)90007-2)

- Benzécri, J. P. (1973). *L'analyse des données: L'analyse des correspondances*. Dunod.
- Blekhman, R., Tang, K., Archie, E. A., Barreiro, L. B., Johnson, Z. P., Wilson, M. E., Kohn, J., Yuan, M. L., Gesquiere, L., Grieneisen, L. E., & Tung, J. (2016). Common methods for fecal sample storage in field studies yield consistent signatures of individual identity in microbiome sequencing data. *Scientific reports*, 6, 31519. <https://doi.org/10.1038/srep31519>
- Bodmer, R. E. (1990). Ungulate frugivore and the browser-grazer continuum. *Oikos*, 57, 319–325. <https://doi.org/10.2307/3565960>
- Bogui, E. B., Koffi, D. A., Koné, I., Ouattara, K., Kouakou, Y. C., & Gnagbo, A. (2016). Distribution of pygmy hippopotamus (*Choeropsis liberiensis*) in Taï National Park, Ivory Coast: Influences of natural and anthropogenic factors. *International Journal of Research in Biosciences*, 5(4), 27–35.
- Bradley, B. J., Stiller, M., Doran-Sheehy, D. M., Harris, T., Chapman, C. A., Vigilant, L., & Poinar, H. (2007). Plant DNA sequences from faeces: Potential means for assessing diets of wild primates. *American Journal of Primatology*, 69(6), 699–705. <https://doi.org/10.1002/ajp.20384>
- Buckland, S., Goudie, I., & Borchers, D. (2000). Wildlife population assessment: Past developments and future directions. *Biometrics*, 56(1), 1–12. <https://doi.org/10.1111/j.0006-341X.2000.00001.x>
- Bülow, W. (1987). *Untersuchungen am Zwergflusspferd Choeropsis liberiensis in Azagny National Park, Elfenbeinküste*. Diploma thesis, University of Braunschweig (Germany).
- Butet, A. (1985). Méthode d'étude du régime alimentaire d'un rongeur polyphage (*Apodemus sylvaticus* L., 1758) par l'analyse microscopique des fèces. *Mammalia*, 49, 455–483.
- Butet, A. (1987). L'analyse microscopique des fèces: une technique non perturbante d'étude des régimes alimentaires des mammifères phytophages. *Arvicola*, 4(1), 33–38.
- Chapuis, J. L. (1980). Méthodes d'étude du régime alimentaire du lapin de garenne *Oryctolagus cuniculus* (L.) par l'analyse micrographique des fèces. *Revue Ecologique, Terre et Vie*, 34, 159–198.
- Chatelain, C., Kadjo, B., Koné, I., & Refisch, J. (2000). *Relations Faune - Flore dans le Parc National de Taï: une étude bibliographique*. Tropicbos Côte d'Ivoire.
- Conway, A. (2013). *Conservation of the Pygmy hippopotamus (Choeropsis liberiensis) in Sierra Leone, West Africa*. Dissertation, University of Georgia.
- Corbet, G. B. (1969). The taxonomic status of the pygmy hippopotamus, *Choeropsis liberiensis*, from the Niger Delta. *Journal of Zoology*, 158(3), 387–394. <https://doi.org/10.1111/j.1469-7998.1969.tb02156.x>
- Crawley, M. J. (2007). Chapter 23: Multivariate statistics. *The R Book* (pp. 731–747). John Wiley & Sons, Ltd. <https://doi.org/10.1002/9780470515075.ch23>
- Crocker, B. H. (1959). A method of estimating the botanical composition of the diet of sheep. *New Zealand Journal of Agricultural Research*, 2, 72–85. <https://doi.org/10.1080/00288233.1959.10427125>
- Cuartas, P., & Garcia-Gonzalez, R. (1996). Review of available techniques for determining the diet of large herbivores from their faeces. *Oecologia Montana*, 5, 47–50.
- Demment, M. W., & Van Soest, P. J. (1985). A nutritional explanation for body-size patterns of ruminant and non ruminant herbivores. *The American Naturalist*, 125(5), 641–672.
- Dray, S., & Dufour, A. B. (2007). The ade4 Package: Implementing the duality diagram for ecologists. *Journal of Statistical Software*, 22, 1–20. <https://doi.org/10.18637/jss.v022.i04>
- Eltringham, S. K. (1999). *Les hippopotames*. Academic Press.
- Flacke, G. L., Chambers, B., Martin, G., & Paris, M. (2015). The pygmy Hippopotamus *Choeropsis liberiensis* (Morton, 1849): bringing to light research priorities for the largely forgotten, smaller hippo species. *Der Zoologische Garten*, 84(5–6), 234–265. <https://doi.org/10.1016/j.zoolgart.2015.07.006>
- Flacke, G. L., Tkalčić, S., Steck, B., Warren, K., & Martin, G. B. (2016). A retrospective analysis of mortality in captive pygmy hippopotamus (*Choeropsis liberiensis*) from 1912 to 2014. *Zoo Biology*, 35(6), 556–569. <https://doi.org/10.1002/zoo.21336>
- Flacke, G. L., Tomkins, J., Black, R., & Steck, B. (2017). Demographics of polycystic kidney disease and captive population viability in pygmy hippopotamus (*Choeropsis liberiensis*). *Zoo Biology*, 36(2), 136–151. <https://doi.org/10.1002/zoo.21351>
- Flacke, G. L., (2017). *The pygmy hippopotamus (Choeropsis liberiensis)—an enigmatic oxymoron: how a not-so-small species presents a sizeable conservation challenge*. Dissertation, University of Western Australia. <https://doi.org/10.4225/23/5953425346527>
- Flacke, G. L., & Decher, J. (2019). *Choeropsis liberiensis (Artiodactyla: Hippopotamidae)*. *Mammalian Species*, 51(982), 100–118. <https://doi.org/10.1093/mspecies/sez017>
- Freeland, W., & Janzen, D. (1974). Strategies in herbivory by mammals: The role of plant secondary compounds. *The American Naturalist*, 108, 269–289.
- Garthey, C. J. (2013). *Studying the distribution and abundance of the endangered pygmy hippopotamus (Choeropsis liberiensis) in and around the Gola Rainforest National Park in southeastern Sierra Leone*. PhD Njala University, Sierra Leone.
- Hentschel, K. (1990). *Untersuchung zu Status, Ökologie und Erhaltung des Zwergflusspferdes (Choeropsis liberiensis) in der Elfenbeinküste*. PhD thesis, University of Braunschweig. Braunschweig, Germany.
- Hillers, A., Buchanan, G. M., Garteh, J. C., Tommy, S. M., Fofana, M. L., & Lindsell, J. A. (2017). A mix of community-based conservation and protected forests is needed for the survival of the endangered pygmy hippopotamus *Choeropsis liberiensis*. *Oryx*, 51(2), 230–239. <https://doi.org/10.1017/S003060531600020X>
- Hilu, K. W., & Randall, J. L. (1984). Convenient method for studying grass leaf epidermis. *Taxon*, 33(3), 413–415. <https://doi.org/10.1002/j.1996-8175.1984.tb03896.x>
- Hofmann, R. R. (1989). Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative review of digestive system. *Oecologia*, 78, 443–457. <https://doi.org/10.1007/BF00378733>
- Iwanowicz, D., Vandergast, A., Cornman, R., Adams, C., Kohn, J., Fisher, R., & Brehme, C. (2016). Metabarcoding of fecal samples to determine herbivore diets: A case study of the endangered pacific pocket mouse. *PLoS One*, 11(11), e0165366. <https://doi.org/10.1371/journal.pone.0165366>
- Kok, P. D. F., & van der Schijff, H. P. (1973). A key based on epidermal characteristics for the identification of certain highveld grasses. *Koedoe: African Protected Area Conservation and Science*, 16(1), 27–43. <https://doi.org/10.4102/koedoe.v16i1.883>
- Lang, E. M. (1975). *Das Zwergflusspferd*. Wittenberg Lutherstadt: A.Ziensen Verlag.
- Lauginie, F. (2007). *Conservation de la nature et aires protégées en Côte d'Ivoire*. CEDA/NEI Hachette et Afrique Nature.
- Lewis, R., & Oliver, W. (2008). *Choeropsis liberiensis*. In: IUCN 2011. *IUCN red list of threatened species*. Version 2011.1. Retrieved from www.iucnredlist.org
- Lintzenich, B. A., & Ward, A. M. (1997). *Hay and pellet ratios: considerations in feeding Ungulates*. Nutrition Advisory Group Handbook, Fact sheet 006.
- Mallon, D., Wightman, C., De Ornellas, P., Collen, B., & Ransom, C. (Compilers) (2011). *Conservation strategy for the pygmy Hippopotamus*. IUCN Species Survival Commission.
- Menziès, A. (2000). *Structure et composition floristique de la forêt de la zone Ouest du Parc National de Taï (Côte d'Ivoire)*. Diplôme Université de Genève.
- Metcalfe, C. R., & Chalk, L. (1950). *Anatomy of the Dicotyledons*. Clarendon Press Oxford 1. <https://doi.org/10.1111/j.2042-7158.1950.tb13008.x>

- Metcalfe, C. R., & Chalk, L. (1957). *Anatomy of the Dicotyledones*. Clarendon press, Oxford 2.
- Michez, A. (2006). *Etude de la population d'hippopotames (Hippopotamus amphibius L.) de la rivière Mouena Mouele au Parc National du Loango-Sud (Gabon)*. Travail de fin d'étude, Université de Liège.
- Miller, N. A., & Ashby, W. C. (1968). Studying stomates with polish. *Turtax News*, 46, 322–324.
- Morton, S. G. (1849). *Additional observations on a new species of hippopotamus of Western Africa (Hippopotamus liberiensis)*. *Journal of the Academy of Natural Sciences*, 2nd Series 1:3.
- O'Connell, A. F., Nichols, J. D., & Karanth, K. U. (2011). *Camera traps in animal ecology; methods and analyses*. Springer Verlag.
- OIPR (2018). *Parc National de Taï*. Retrieved from <http://www.oipr.ci/index.php/parcs-reserves/parcs-nationaux/parc-national-de-tai>
- Prothero, D. R., & Foss, S. E. (2007). *The evolution of artiodactyls*. Johns Hopkins University Press.
- Ransom, C., Robinson, P. T., & Collen, B. (2015). *Choeropsis liberiensis*: e. T10032A18567171. <https://dx.doi.org/10.2305/IUCN.UK.2015-2.RLTS.T10032A18567171.en>
- R development core team. (2018). *R: The R project for statistical computing*. Retrieved from <https://cran.r-project.org/>
- Rech, J. (2011). *Microscopie des plantes consommées par les animaux*. Quae eds.
- Reed, J. Z., Tollit, D. J., Thompson, P. M., & Amos, W. (1997). Molecular scatology: the use of molecular genetic analysis to assign species, sex and individual identity to seal faeces. *Molecular ecology*, 6(3), 225–234. <https://doi.org/10.1046/j.1365-294X.1997.00175.x>
- Robinson, P. T. (1970). *The status of the pygmy hippopotamus and other wildlife in West Africa (Unpubl.)*. A thesis submitted to Michigan State University in partial fulfillment of the requirements for the degree of Master of Science, department of Fisheries and Wildlife.
- Robinson, P. T. (1981). The reported use of denning structures by the pygmy hippopotamus (*Choeropsis liberiensis*). *Mammalia*, 45, 506–508.
- Robinson, P. T. (2013). *Choeropsis liberiensis pygmy Hippopotamus. Mammals of Africa. Volume VI: Pigs, Hippopotamuses, Chevrotain, Giraffes, Deer and Bovids*. Bloomsbury Publishing.
- Robinson, P. T., Flacke, G. L., & Hentschel, K. M. (2017). *The pygmy Hippo story: West Africa's enigma of the rainforest*. Oxford University Press.
- Roth, H. H., Hoppe-Dominik, B., Muhlenberg, M., Steinhauer-Burkart, B., & Fischer, F. (2004). Distribution and status of the hippopotamids in the Ivory Coast. *African Zoology*, 39, 211–224.
- Rowcliffe, J., Kays, R., Kranstauber, B., Carbone, C., & Jansen, P. (2014). Quantifying levels of animal activity using camera trap data. *Methods in Ecology and Evolution*, 5(11), 1170–1179. <https://doi.org/10.1111/2041-210X.12278>
- Sattler, D. (2000). *Flore du Parc National de Taï (Côte d'Ivoire)*. Kasperek.
- Schwarm, A., Ortmann, S., Wolf, C., Streich, W., & Clauss, M. (2009). More efficient mastication allows increasing intake without compromising digestibility or necessitating a larger gut: Comparative feeding trials in banteng (*Bos javanicus*) and pygmy hippopotamus (*Hexaprotodon liberiensis*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 152(4), 504–512. <https://doi.org/10.1016/j.cbpa.2008.12.006>
- Scotcher, J. S. B., Stewart, D. R. M., & Breen, C. M. (1978). The diet of the Hippopotamus in Ndumu game reserve, natal, as determined by faecal analysis. *South African Journal of Wildlife research*, 8, 1–11.
- Scoupe, M. (2011). *Composition floristique et diversité de la végétation de la zone Est du Parc National de Taï (Côte d'Ivoire)*. Master thesis, Université de Genève.
- Shah, S., Ahmad, M., Zafar, M., Malik, K., Rashid, N., Ullah, F., Zaman, W., & Ali, M. (2018a). A light and scanning electron microscopic diagnosis of leaf epidermal morphology and its systematic implications in Dryopteridaceae: Investigating 12 Pakistani taxa. *Micron*, 111, 36–49. <https://doi.org/10.1016/j.micron.2018.05.008>
- Shah, S., Ahmad, M., Zafar, M., Razzaq, A., Malik, K., Rashid, N., Ullah, F., Iqbal, M., & Zaman, W. (2018b). Foliar epidermal micromorphology and its taxonomic implications in some selected species of Athyriaceae. *Microscopy Research and Technique*, 81(8), 902–913. <https://doi.org/10.1002/jemt.23055>
- Shiple, L. A. (1999). Grazers and browsers: how digestive morphology affects diet selection. *Grazing Behaviour of Livestock and Wildlife*, 70, 20–27.
- Stoddard, E. M. (1965). *Identifying plants by leaf epidermal characters*. The Connecticut Agricultural Experiment Station.
- Storr, G. M. (1961). Microscopic analysis of faeces, a technique for ascertaining the diet of herbivores mammals. *Australian Journal of Biological Sciences*, 14(1), 157–164. <https://doi.org/10.1071/B19610157>
- Taylor, L. A., Rudd, J., Hummel, J., Clauss, M., & Schwitzer, C. (2013). Weight loss in pygmy hippos (*Choeropsis liberiensis*). *International studbook for the year 2012 - Pygmy Hippopotamus* (pp. 20–25). Basel: Basel zoo.
- Trolliet, F., Vermeulen, C., Huynen, M. C., & Hamburgers, A. (2014). Use of camera traps for wildlife studies: A review. *Biotechnologie, Agronomie, Société et Environnement*, 18(3), 446–454.
- Ullah, F., Zafar, M., Ahmad, M., Shah, S. N., Razzaq, A., Sohail, A., Zaman, W., Çelik, A., Ayaz, A., & Sultana, S. (2018a). A systematic approach to the investigation of foliar epidermal anatomy of subfamily Caryophylloideae (Caryophyllaceae). *Flora*, 246, 61–70. <https://doi.org/10.1016/j.flora.2018.07.006>
- Ullah, F., Zafar, M., Amhad, M., Sultana, S., Ullah, A., Shah, S., Butt, M., & Mir, S. (2018b). Taxonomic implications of foliar epidermal characteristics in subfamily Alsinoideae (Caryophyllaceae). *Flora*, 242, 31–44. <https://doi.org/10.1016/j.flora.2018.02.003>
- UNESCO World Heritage (2018). *Taï National Park*. Retrieved from <https://whc.unesco.org/en/list/195>
- Valentini, A., Pompanon, F., & Taberlet, P. (2009). Trends in ecology & evolution. *Cell Press*, 24(2), 110–117. <https://doi.org/10.1016/j.tree.2008.09.011>
- Van Heukelum, M. (2010). *In search of the illusive pygmy Hippo; Establishment of methods to determine population structure of pygmy Hippos in Tai forest, and assessment of their role in seed dispersal*. Wageningen University, Master thesis.
- Van Soest, P. J. (1996). Allometry and ecology of feeding behaviour and digestive capacity in herbivores: A review. *Zoo biology*, 15(5), 455–479. [https://doi.org/10.1002/\(SICI\)1098-2361\(1996\)15:5<455:AID-ZOO3>3.0.CO;2-A](https://doi.org/10.1002/(SICI)1098-2361(1996)15:5<455:AID-ZOO3>3.0.CO;2-A)
- Von Houwald, F., Mcdonald, A. A., Pagan, O., & Steck, B. (2007). *Husbandry guidelines for the pygmy Hippopotamus (Hexaprotodon liberiensis)*. Basel: Basel zoo.
- Ward, J. H. (1963). Hierarchical grouping to optimize an objective function. *Journal of the American Statistical Association*, 58, 236–244. <https://doi.org/10.1080/01621459.1963.10500845>

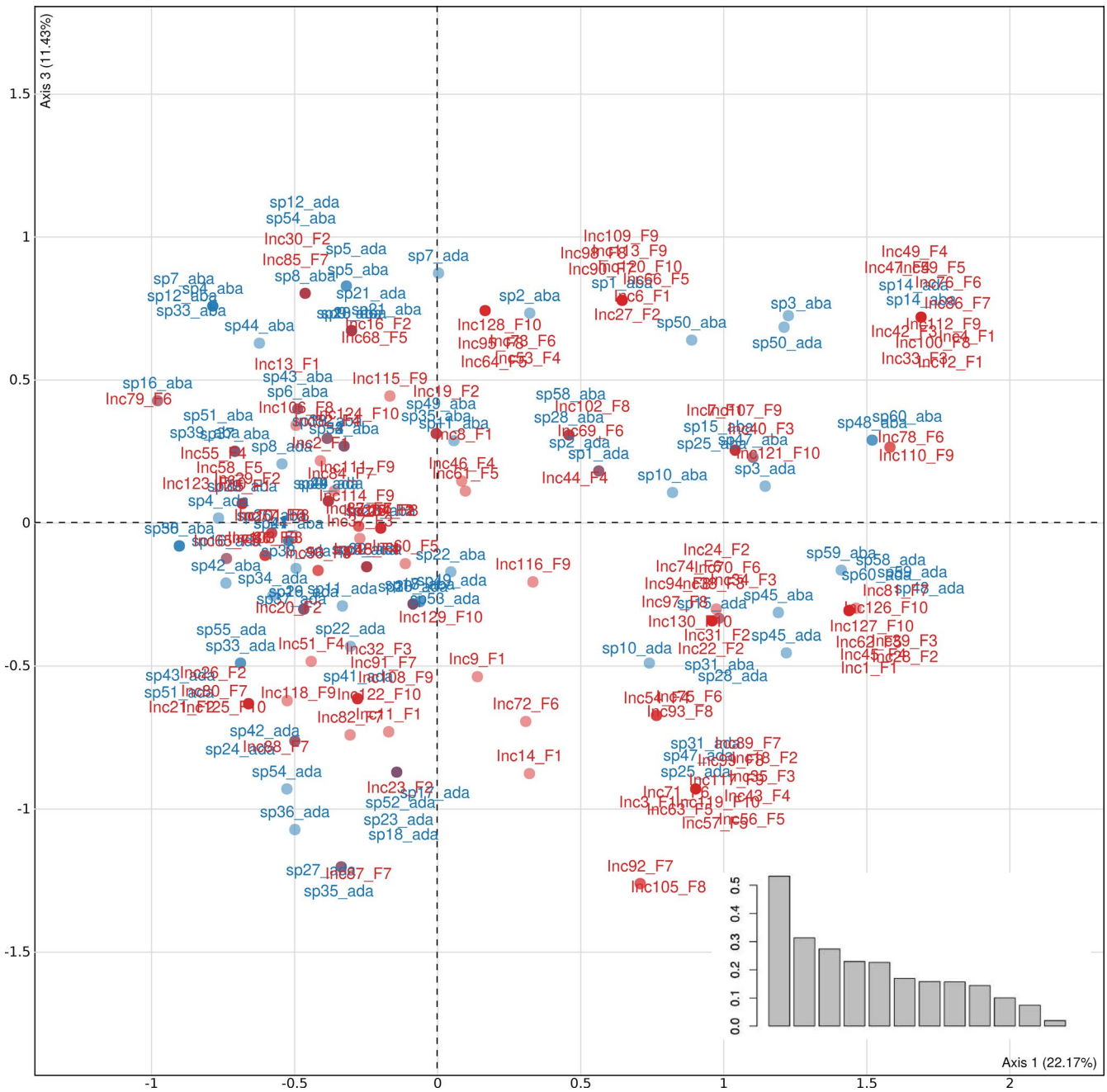
SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Hendier A, Chatelain C, Du Pasquier P-E, et al. A new method to determine the diet of pygmy hippopotamus in Taï National Park, Côte d'Ivoire. *Afr J Ecol*. 2021;00:1–17. <https://doi.org/10.1111/aje.12888>

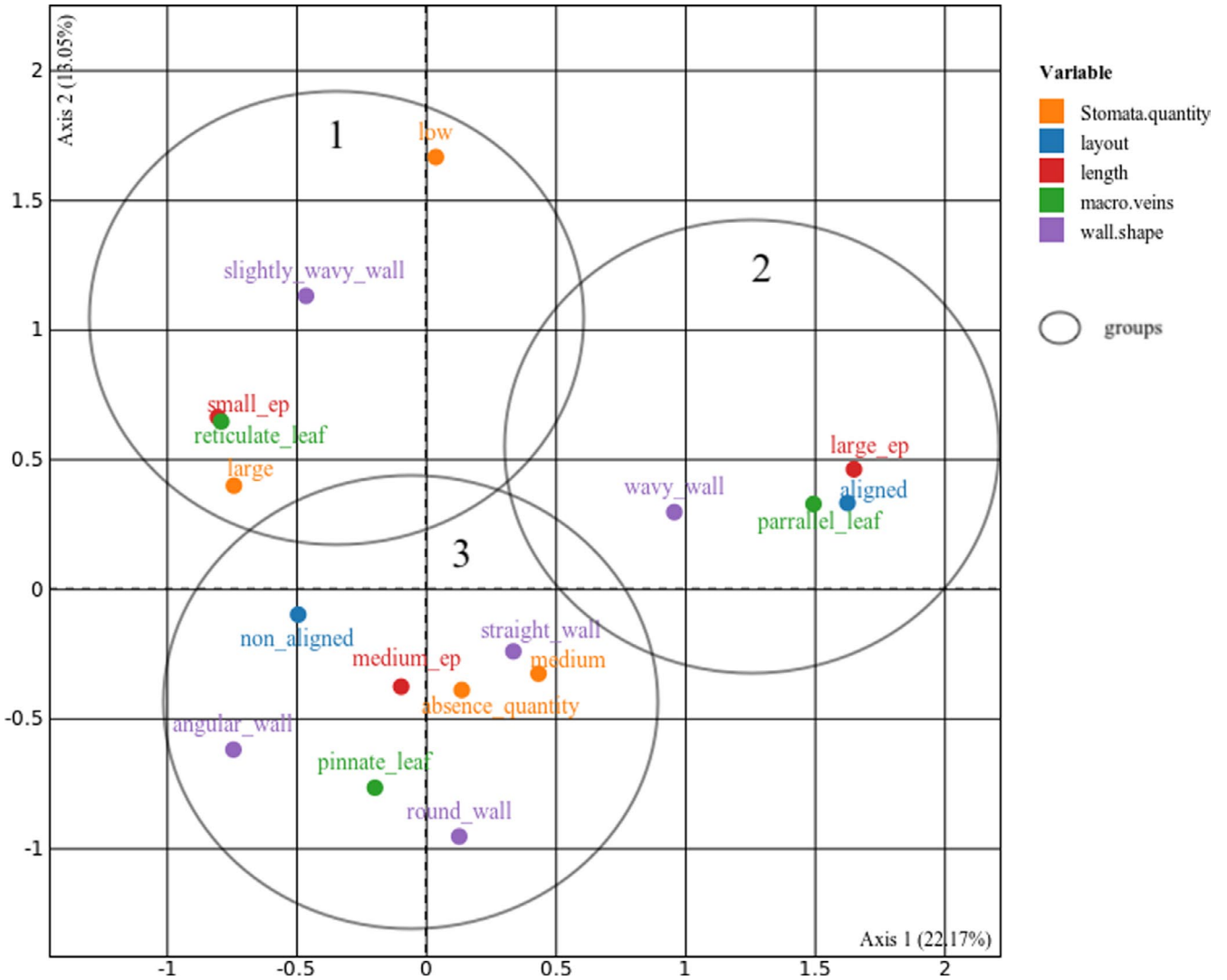
APPENDIX 1

Projection of the faeces fragments (as additional individuals) in the food items on MCA F1×F3 axes. On blue, are represented the reference library (56 plants species, i.e., 112 leaves sides), and on red are the n = 130 plants fragments found in the 10 droppings (named as supplementary individuals). On the bottom right is represented the barplot of the MCA.



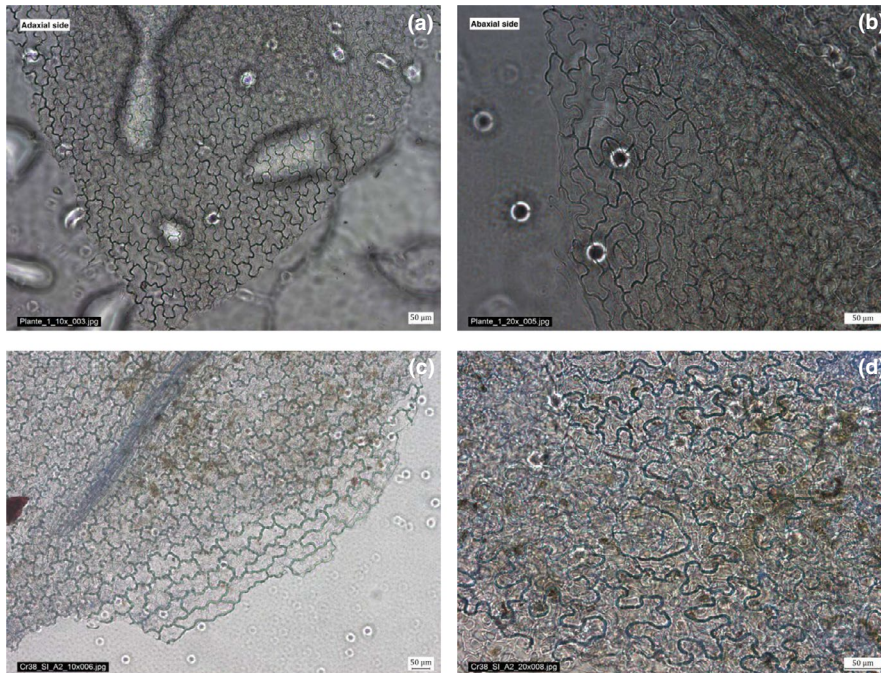
APPENDIX 2

Projection of the reference library's variables on F1×F2 axes. The colours represent each variable with their different categories. We added three circles to highlight three groups of individuals.



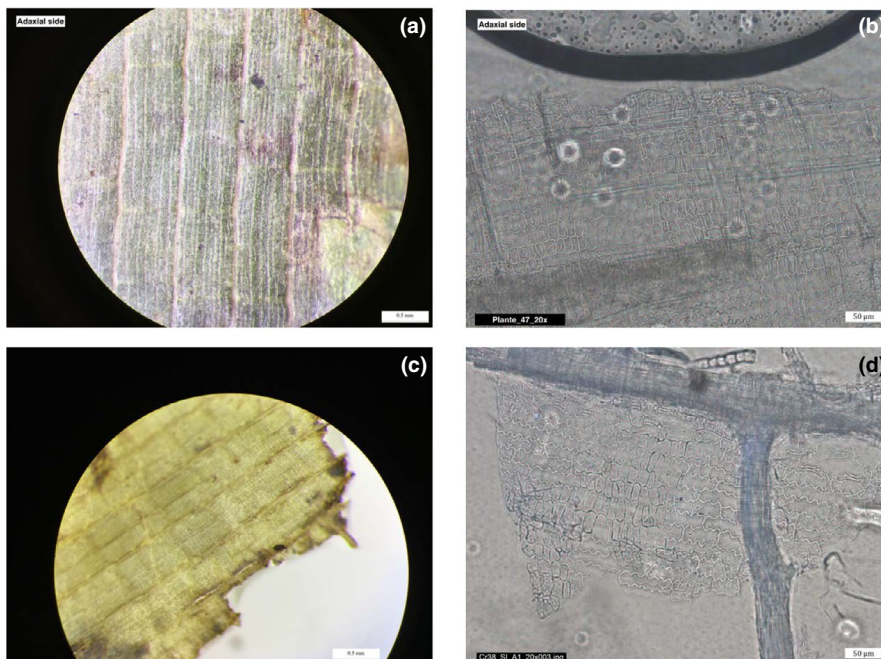
APPENDIX 3

Comparison between *Nephrolepis bisserata* (fern) and a faeces fragment. (a, b) microscopic view of *N. bisserata*. (c, d) microscopic view of a faeces fragment similar to *N. bisserata*. (a, c) adaxial sides, with a magnification of 100 \times . (b, d) abaxial side, with a magnification of 200 \times . (a–d) The slides were prepared with the discoloration method.



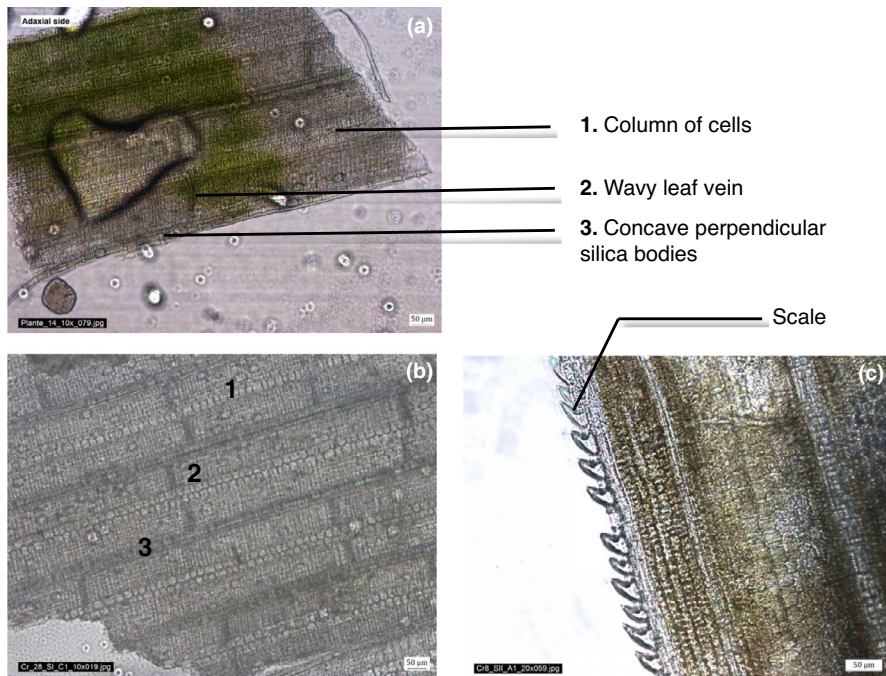
APPENDIX 4

Comparison between *Centhoteca lappaceae* and a faeces fragment. (a, c) macroscopic view of *C. lappaceae*, a, and a faeces fragment in c. (b, d) microscopic view of *C. lappaceae* in b and a faeces fragment in d, with a magnification of 200 \times . The slides were prepared with the discoloration method.



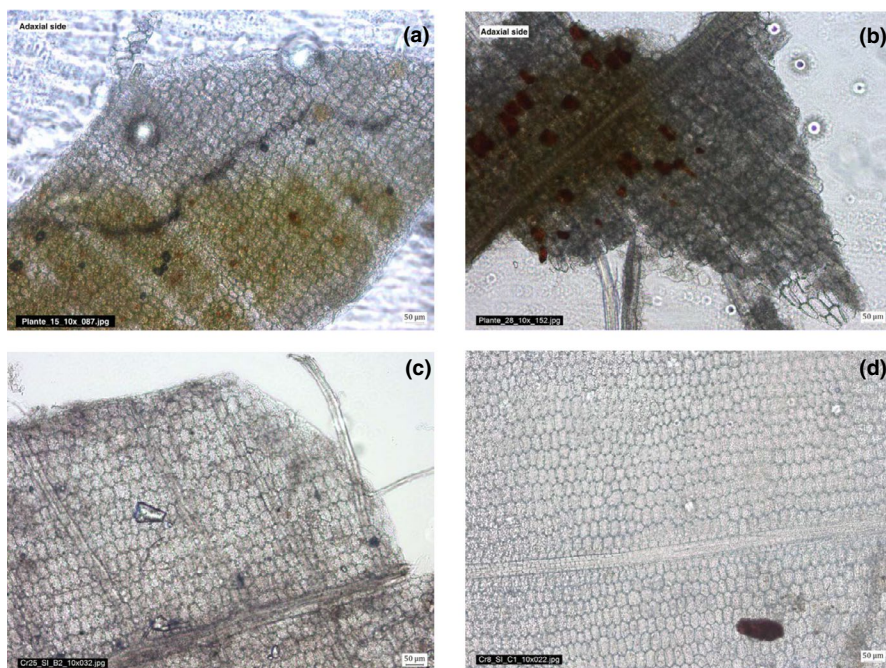
APPENDIX 5

Comparison between *Streptogyna crinita* and a faeces fragment. (a) microscopic view of the adaxial side of *S. crinita* with a magnification of 100×. (b) microscopic view of a faeces fragment with a magnification of 100× (c) microscopic view of a faeces fragment with a magnification of 200×. (a–c) The slides were prepared with the discoloration method.



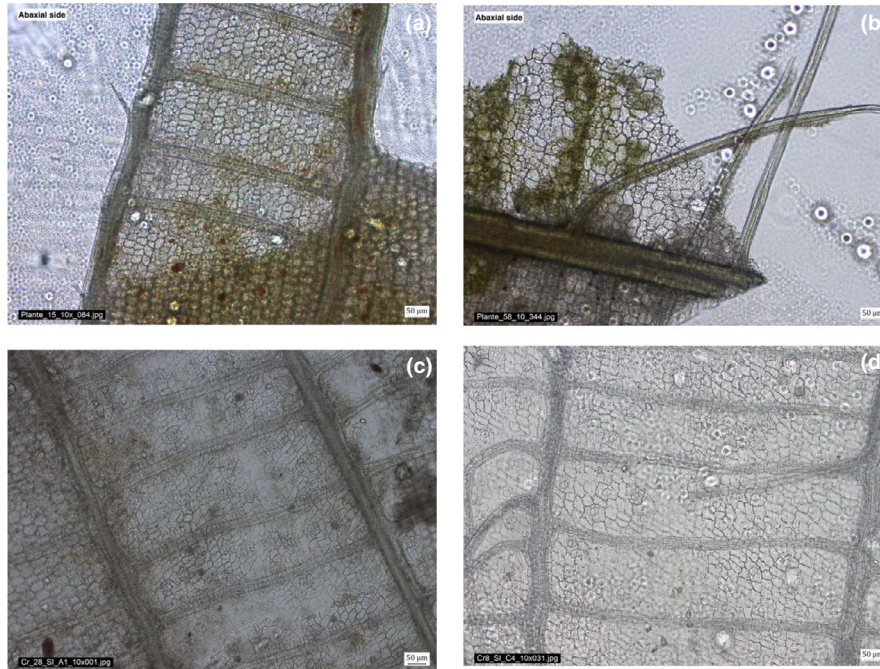
APPENDIX 6

Comparison between *Marantaceae* species and faeces fragments (adaxial sides). (a, b) microscopic view of the adaxial sides of *Marantochloa purpurea*, a, and *Hypselodelphys violaceae*, b. (c, d) microscopic view of two faeces fragment similar to *Martantaceae* species. (a–d) the slides were prepared with the discoloration method and photographed with a magnification of 100×.



APPENDIX 7

Comparison between *Marantochloa purpurea*, *Costus afer* and faeces fragments. (a) microscopic view of the abaxial side of *Marantochloa purpurea*. (b) microscopic view of the abaxial side of *Costus afer*. (c, d) microscopic view of faeces fragments similar to *Marantochloa purpurea* and *Costus afer*. (a–d) The slides were prepared with the discoloration method and photographed with a magnification of 100×.

**APPENDIX 8**

Seed found in faeces. (a) internal view of the seed. (b) external view of the seed. (a–b) the two different views were photographed with a magnification of 25×.

