

1 **Supplementary Information for: “Dental calculus evidence of Tai Forest Chimpanzee plant**
2 **consumption and life history transitions”**

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21 **Supplementary Texts**

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23 1. Study population

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25 The chimpanzee calculus samples derive from the Tai Chimpanzee osteology collection of 77
26 chimpanzees curated at the Max Planck Institute for Evolutionary Anthropology (MPI-EVA) in
27 Leipzig, Germany. The remains were collected with as many details as possible on sex, age and
28 cause of death. All Tai Forest material and data collected complied with the requirements and
29 guidelines of the Ministère de l'Enseignement Supérieure et de la Recherche Scientifique of
30 Côte d'Ivoire, and adhered to its legal requirements. When possible we sampled chimpanzees
31 who had known life histories, and ideally with comprehensive dietary records. Much of the
32 observational data relates to chimpanzees that are not part of this osteology collection. Dietary
33 records vary from thousands of observations over a decade to a limited number over the
34 course of a single day. After death, these individuals were interred for defleshing and then later
35 exhumed. Some of the skeletal material was cleaned using strong disinfectants before storage
36 to minimize the risk of disease transmission.

37
38 It has been noted that chimpanzees produce less salivary α -amylase than humans, especially
39 humans from agricultural societies that consume high levels of starch¹. Thus starch entering the
40 chimpanzee mouth may be less readily hydrolysed than in human groups, which may make it
41 more likely for starches to enter and preserve in chimpanzee dental calculus than in human

42 dental calculus. However if this patterns occurs in our samples it is unclear and it cannot
43 testable with our data.

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45 2. Collection of calculus samples

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47 Occasionally, chimpanzee calculus showed substantial flecks of dark material that did not
48 resemble calculus and appeared to be sediment contamination. Chimpanzee samples where
49 sediment contamination was suspected were omitted. All chimpanzee remains sampled are
50 curated at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany. Samples
51 from two chimpanzees (Vanessa and 13438) were omitted from analysis because their age at
52 death was not recorded, though we did count microremains recovered from these individuals in
53 the Supplementary Data 3. A sample from a further chimpanzee (Loukoum) was omitted due to
54 surface adherents on the calculus. The calculus we chose for the final complete analysis came
55 from molars of 24 individuals (12 male and 12 female) ranging in age from between 12 and 552
56 months (1 and 46 years) old (Table 1; Supplementary Table 3).

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58 3. *Tai Forest plant reference collection*

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60 A microremain reference collection with 119 plant species was built using the most frequently
61 consumed chimpanzee plant foods in the Tai forest (Supplementary Table 7). Tai chimpanzees
62 consume a particularly diverse range of foods. We collected plant parts that were documented
63 as a specific component of the diet (fruits, seeds, piths, leaves, stems, bark, flowers, and roots.)
64 We also include fungal fruiting bodies known to be consumed. Effort was made to include other
65 rainforest edible plants not recorded as chimpanzee foods. Although our reference collection is
66 not exhaustive, it incorporates the most important plants foods of the Tai chimps, achieving
67 coverage of 89 % of the total dietary observations. Plants collected in the Tai Forest were
68 immediately preserved onsite either by freezing or by drying in 15 or 50 ml centrifuge tubes
69 with silica gel (Roth - T858.1 and P077.1, Karlsruhe, Germany). Additionally, we collected some
70 plant material from the University of Leipzig Botanical Garden (marked as fresh in Supp. Table
71 7) and analysed this material fresh for starch or dried for phytoliths. We did not make a
72 reference collection for unsilicified plant microremains as these microremains are unlikely to be
73 undiagnostic.

74

75 Starch was analysed by directly mounting finely sliced dry plant material on slides with
76 approximately 10 µl of distilled water and 10 µl of a 25 % glycerol solution. Starches were
77 observed at 200-640 x magnification using a Zeiss Axioscope. Phytoliths were isolated from
78 plant material by dissolving weighed dried plant material in ≥65 % nitric acid with a heating

79 block to expedite the reaction. Small quantities of potassium chlorate were added to encourage
80 the process.

81

82 In most chimpanzee foods we observed either very few starch grains or none at all, suggesting
83 quantities too negligible to be detected or a complete lack of starch in the plant
84 (Supplementary Table 2). Plants that produced negligible numbers of starches were not
85 analysed for the identification model, because they did not have enough starch grains to build a
86 reference set of 50 starches. We found phytoliths were common in many species, but many
87 morphotypes are poorly studied in morphometric studies and cannot be easily described using
88 the variables we chose for our model (e.g. hair cells, epidermal, cylindroids, plates and tracheid
89 phytoliths). These morphotypes were found in a number of genera in the reference collection
90 plant but only in low numbers.

91

92 Plants that had few phytoliths were not included. Furthermore, if microremains were found in
93 parts of a plant that chimpanzees do not eat, the plants were not included (e.g. starch from
94 *Beilschmedia mannii* seed). Thirteen starch- and seven phytolith-producing plants were
95 selected for developing identification criteria. We chose to measure or quantify several
96 variables on 50 microremains per species (Supplementary Data 1 and 2), focusing on variables
97 that past studies have shown to be effective in distinguishing among starches and phytoliths^{2,3}.
98 Our variables include max length, max width, area, shape, surface regularity, the number of
99 echinate spines, length of longest cross axis, type, number and length of cracks, number of
100 facets and lamellae (Supplementary Table 8). If abundant starches or phytoliths were
101 recovered, their abundance was analysed in order to assess the expected starch and phytolith
102 contribution to dental calculus (Table 1). Starch content was established by combining previous
103 nutritional content studies^{4,5}. For species where this data was not available we assessed starch
104 content per gram dried plant material colourimetrically using an Amyloglucosidase / α -amylase
105 method with a Megazyme Total Assay Kit (AA/AMG 11/01, AOAC Method 996.11, AACC
106 Method 76.13, ICC Standard Method No. 168). Phytolith content was estimated by calculating
107 the total weight of sample left after nitric acid digestion.

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109 *4. Identification of microremains by classification*

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111 Statistical approaches are increasingly used for the study and classification of microremains^{2,6-9}.
112 A variety of approaches have been implemented in past studies such as image analysis¹⁰, linear
113 discrimination³, and factor regression analysis by principal components². We used random
114 forest-based classification because it is robust, non-parametric and easily accommodates both
115 large number of variables and categorical data. Using this approach, we can easily see the most
116 important variables that drive the differences among the microremain types. The most

117 important variables in our phytolith model include length and the number of spines
118 (Supplementary Table 8). In the starch random forest model area and length were the most
119 important variables (Supplementary Table 9).

120

121 5. Model design and formulae

122

123 We predicted that number of microremains should increase with age, and might vary by sex.
124 We tested this using a negative binomial regression, with microremain count as the response,
125 and age and sex as predictors, weighting each observation by the weight of the calculus sample
126 (see detailed methods below). We ran separate tests for phytoliths, unsilicified remains and
127 starches.

128 The models described in R terminology are as follows:

129

130 Microremain type count ~ chimpanzee age + chimpanzee sex, weights=calculus sample weight

131

132 Expressed as a mathematical formula, this analysis is written as follows:

$$y_i = \text{Negbin}(\mu_i, k)$$

$$133 \log(\mu_j) = \beta_0 + X_j\beta_j + \varepsilon$$

134 where $\beta_0 = 0$

$$\log(\mu_j) = \beta_0 + \sum_{j=1}^p [\beta_{11j}\text{chimp_age}_j + \beta_{12j}\text{chimpanzee sex}_j] + \varepsilon_j$$

135 where $\beta_0 = 0$

136

137 We predicted that more frequently consumed plants should be highly represented in the
138 chimpanzee calculus. To test this, we used an observational random effect Poisson model
139 (Supplementary Text 5). The count of microremains (starches or phytoliths) belonging to a
140 particular genus was our response variable, and the fixed predictors were: (a) minutes spent
141 consuming each genus, and (b) chimpanzee age in months. Sex was included as a control
142 predictor, and both calculus sample weight and successful identification rate of each genus
143 were included as weights. We accounted for the variation in production of microremains in
144 different genera by using microremains content as an offset. We used counts of each genus
145 predicted to be present with the total minutes spent consuming each genus. The chimpanzee
146 individual was included as a random slope term, while year of death, tooth and food type were
147 treated as random intercept terms

148

149 The models described in R terminology are as follows:

150

151 The observational feeding records model. Key: obs_id=observation id, plant_id= Plant genus,
152 death_year = year that chimpanzee died, mr_content=Prevalence of starch in each plant
153 species, wt= Milligrams in each sample, class_rate=Rate of successful identification in this
154 species.

155 Count of each plant species~ mins+ age+ sex+ (1|obs_id)+ (1|plant_id)+ (1|tooth)+
156 (1|chimp_name)+ (1|death_year)+ (0+mins|chimp_name)+ (0+mins|tooth)+ (0+
157 mins|death_year)+ (0+age|plant_id)+ (0+age|tooth)+ offset(log(mr_content)),
158 weight=class_rate+ calculus samples weight

159

160 In mathematical notation, the models are written as follows:

$$\begin{aligned} \log_e(\lambda) = & -n\lambda + \log_e(\lambda) \sum_{j=1}^p [\beta_{11j}\text{mins}_j + \beta_{12j}\text{age}_j + \beta_{13j}\text{sex}_j) + \beta_{21j} + u_{11j})\text{tooth}_j \\ & + (\beta_{22j} + u_{12j})\text{death_year}_j + (\beta_{23j} + u_{13j})\text{plant_id}_j + (\beta_{24j} + u_{14j})\text{age}_j \\ & - \sum_{j=1}^p \ln[\beta_{11j}\text{mins}_j + \beta_{12j}\text{age}_j + \beta_{13j}\text{sex}_j) + \beta_{21j} + u_{11j})\text{tooth}_j \\ & + (\beta_{22j} + u_{12j})\text{death_year}_j + (\beta_{23j} + u_{13j})\text{plant_id}_j + (\beta_{24j} + u_{14j})\text{age}_j]! \\ & + u_{01} + u_{02} + u_{03} + u_{04} + u_{05} + \varepsilon_j \end{aligned}$$

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164 **References**

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Supplementary Tables

Supplementary Table 1: Details of the random forest model used to predict genus of origin for the phytolith-producing taxa. Identification rate = rate of successful identification per genus.

Number of variables tried at each split (mtry)						15
Tune length						3
Tree number						500
Out of bag estimate of error rate						25.75%
Confusion matrix						
	<i>Aframomum</i>	<i>Ancistrophyllum</i>	<i>Elaeis</i>	<i>Eremospatha</i>	<i>Sarcophrynium</i>	<i>Identification rate</i>
<i>Aframomum</i>	39	3	1	5	2	0.78
<i>Ancistrophyllum</i>	3	32	3	12	0	0.64
<i>Elaeis</i>	2	3	40	5	0	0.8
<i>Eremospatha</i>	5	11	1	33	0	0.66
<i>Sarcophrynium</i>	2	0	1	0	47	0.94

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Supplementary Table 2: Details of the random forest model used to predict genus of origin for the starch-producing taxa. Identification rate=rate of successful identification per genus.

Number of variables tried at each split (mtry)														14
Tune length														3
Tree number														500
Out of bag estimate of error rate														32.77%
Confusion matrix														
	<i>Aframomum</i>	<i>Calpocalyx</i>	<i>Cola</i>	<i>Coula</i>	<i>Eremospatha</i>	<i>Gilbertiodendron</i>	<i>Napoleona</i>	<i>Panda</i>	<i>Piper</i>	<i>Sacoglottis</i>	<i>Sarcophrynium</i>	<i>Treulia</i>	<i>Xylica</i>	<i>Identification rate</i>
<i>Aframomum</i>	45	1	0	0	0	0	0	0	2	0	0	1	1	0.9
<i>Calpocalyx</i>	0	40	0	0	7	0	0	2	0	0	0	0	1	0.8
<i>Cola</i>	0	0	26	0	0	3	0	2	0	5	0	11	3	0.52
<i>Coula</i>	0	0	0	44	3	0	0	0	0	2	0	0	1	0.88
<i>Eremospatha</i>	0	10	0	0	31	0	0	7	0	0	0	0	2	0.62
<i>Gilbertiodendron</i>	0	0	4	0	0	38	1	0	0	7	0	0	0	0.76
<i>Napoleona</i>	0	2	0	0	1	1	18	7	0	2	0	8	11	0.36
<i>Panda</i>	0	3	1	0	6	0	11	11	0	0	0	6	12	0.22
<i>Piper</i>	2	0	0	0	0	0	0	0	47	1	0	0	0	0.94

<i>Sacoglottis</i>	0	0	0	0	0	6	0	0	0	43	0	0	1	0.86
<i>Sarcophrynium</i>	0	0	0	0	0	2	0	0	1	0	47	0	0	0.94
<i>Treculia</i>	0	0	7	0	0	2	4	6	0	1	0	26	4	0.52
<i>Xylia</i>	0	3	0	0	6	0	7	7	0	3	0	3	21	0.42

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Supplementary Table 3: Table of total recovered plant microremains, both in the full sample and per milligram of calculus with cause of death of the sampled chimpanzees, condition of their dental calculus and skeleton treatment a) Buried for unknown duration, cleaned and dried (1984-1994, 1996-2004 b) Necropsy, burial for 1 year, possible boiling and dried (1994-1996) and c) Necropsy, burial for 1 year, disinfection with chlorine, 10% formalin and dried (2004-onwards).

Name	Phy	Phy/mg	Starch	Starch/mg	Unsilicified/mg	Unsilicified remains	Cause of death	Calculus condition	Skeleton treatment
Ophelia	0	0	1	40	0	0	Pneumonia	White	C
Leonardo	0	0	0	0	0	0	Starvation	White/grey	A
Bambou	0	0	0	0	1	7.41	Tree fall	White	A
Piment	0	0	0	0	0	0	Ebola	White	B
Oreste	40	74.63	4	7.46	1	1.87	Pneumonia	Grey	C
Hector	24	34.83	2	2.9	6	8.71	Anthrax	Orange	A
Noah	47	52.51	2	2.23	32	35.75	Unknown	Brownish	A
Lefkas	19	31.93	11	18.49	13	21.85	Pneumonia	White	A
Tina	29	21.21	8	5.85	6	4.39	Leopard	Brownish	A
Dorry	159	214.29	5	6.74	4	5.39	Unknown	White	A
Zerlina	147	167.43	0	0	9	10.25	Ebola?	Moderate	B
Clyde	27	23.87	4	3.54	3	2.65	Poacher	White	A
Agathe	94	15.47	13	2.14	22	3.62	Ebola?	Brown/creamy	A
Bijou	87	17.26	10	1.98	22	4.36	Unknown disease	Brownish	A
Leo	126	116.13	5	4.61	9	8.29	Unknown	Brownish	A
Castor	65	9.31	25	3.58	6	0.86	Pneumonia	White	A
Fanny	109	27.84	54	13.79	11	2.81	Ebola?	White brown	B
Kendo	233	235.59	0	0	25	25.28	Ebola?	Grey	B
Venus	96	59.26	16	9.88	2	1.23	Unknown	Brownish	C
Goma	98	7.42	181	13.7	17	1.29	Anthrax	White	A
Rubra	120	17.78	10	1.48	30	4.44	Anthrax?	Mixed/white	C
Ondine	26	17	0	0	10	6.54	Ebola?	Brown/green	A
Mkubwa	11	33.95	0	0	1	3.09	Unknown	Whitish green	A
Brutus	161	49.6	5	1.54	25	7.7	Unknown	Brownish	a

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Supplementary Table 4: Summary counts of identified genera in Tai Chimpanzee calculus samples.

Name	Phytolith		Starch	
	Genera count	% of total genera	Genera count	% of total genera
Ophelia	0	0	0	0
Leonardo	0	0	0	0
Bambou	0	0	0	0
Piment	0	0	0	0
Oreste	5	100	2	15.38
Hector	3	60	2	15.38
Noah	5	100	0	0.00
Lefkas	2	40	4	30.77
Tina	3	60	2	15.38
Dorry	4	80	3	23.08
Zerlina	4	80	0	0
Clyde	3	60	3	23.08
Agathe	4	80	4	30.77
Bijou	5	100	5	38.46
Leo	4	80	2	15.38
Castor	5	100	3	23.08
Fanny	4	80	10	76.92
Kendo	5	100	0	0.00
Venus	4	80	5	38.46
Goma	5	100	9	69.23
Rubra	5	100	5	38.46
Ondine	3	60	0	0.00
Mkubwa	2	40	0	0.00
Brutus	5	100	3	23.08

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Supplementary Table 5: Summary of coefficients of our statistical models.

Model	Term	Estimate	Std. Err.	Z value	P
Tests of effect of age and sex on microremain numbers					
Phytolith Negative binomial	Intercept	3.969	0.160	24.790	1.1398e-135
	Age	0.002	0.0005	3.833	1.2616e-04
	Sex	-0.027	0.157	-0.170	8.6469e-01
Starch Negative binomial	Intercept	3.009	0.426	7.052	1.7575e-12
	Age	0.003	0.001	2.661	7.7805e-03
	Sex	-2.569	0.437	-5.873	4.2665e-09
Unsilicified remains	Intercept	2.210	0.202	10.904	1.0978e-27

Negative binomial	Age	0.001	0.0006	3.093	1.9775e-03
	Sex	-0.048	0.199	-0.245	8.0594e-01
Tests of effect of consumption frequency on microremain numbers					
Phytolith poisson model	Intercept	-0.231	0.876	-0.263	0.791
	z.min	1.707	0.680	2.509	0.0120
	z.age	3.612	2.075	1.740	0.081
	sex	-0.801	0.934	-0.858	0.390
Starch logistic regression model	Intercept	-14.2189228	0.8709593	-6.325589	6.4911e-60
	z.min	0.5912703	0.5056228	1.169390	2.4224e-01
	z.age	0.4893117	0.4425319	1.105709	2.6885e-01
	sex	-1.2664400	0.9964118	-1.271001	2.0372e-01

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Supplementary Table 6: Complete inventory of plants and fungus analysed in reference collection. x=no microremain found. o=microremains found and used for identification model. 1=found but not used in classification model due to their complex morphology, 2=found but not included as they are very rare, 3=found but only in parts that are not eaten. Prep=preparation. d=dried, fn=frozen and fh=fresh.

		Leaf	Fruit pulp	Seed	Stem	Pith	Shell	Flower	USO	Bark	Leaf	Fruit pulp	Seed	Stem	Pith	Shell	Flower	USO	Bark	Prep
Plants																				
Genus	Species	Starch										Phytoliths								
<i>Aframomum</i>	<i>exscapum</i> (Sims) Hepper	x		x																d
<i>Aframomum</i>	<i>cereum</i> (Hook.f.) K.Schum.										x		x							d
<i>Afzelia</i>	<i>bella</i> Harms										1									d
<i>Agaricus</i>	<i>bispourus</i> (J.E.Lange) Emil J. Imbach				x															d
<i>Anchomanes</i>	<i>difformis</i> (Bl.) Engl.													x						fn
<i>Antiaris</i>	<i>toxicaria</i> subsp. <i>welwitschii</i> (Engl.) C.C.Berg		x	2																d
<i>Auricularia</i>	<i>auricula-judae</i> . (Bull.) J.Schröt.				x									x						d
<i>Beilschmiedia</i>	<i>mannii</i> (Meisn.) Benth. & Hook.f.			2																d
<i>Bombax</i>	<i>buonopozense</i> P.Beauv.			x																d
<i>Bombax</i>	<i>ceiba</i> L.	x									2									fh
<i>Calpocalyx</i>	<i>Sp.</i>		o																	d
<i>Calpocalyx</i>	<i>aubrevillei</i> Pellegr.	x									x									d
<i>Canarium</i>	<i>schweinfurtii</i> Engl.		x	x																fn
<i>Castanola</i>	<i>paradoxa</i> (Gilg)											x	x							d

235 Supplementary Table 8: Variable importance in phytolith random forest.

Variable	Importance
Length	100.00
Spine number	75.301
Spine ang	74.109
LW Ratio	43.996
Spine length	42.854
Area	29.581
Width	22.056
Irregul	10.236
Spherical	6.667
Angularpoint	6.575
Polygon	4.590
Ovoid	1.663
Prolate	1.620
Triangular	1.447
Elongate	0.440
Quadrangular	0.228
Facets	0.184
Conjoined	0.106
Prolate concave-convex	0.043
Polygon concave	0.042

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238 Supplementary Table 9: Variable importance in starch random forest.

Variable	Importance
Area	100
Length	75.8434
Width	67.5876
Dist	61.1718
Facets	60.4963
LW Ratio	56.2298
Type	55.9587
Lam	35.8372
Spherical	31.833
Prolate	8.2554
Ovoid	7.157
Polygon	5.8693
Hemispherical	4.9279
Oblate conovoid	4.6926
Striaelen	2.4395
Elongate ovoid	2.4011
Striae no	2.1051
Triangular	1.8956
Quadrangular	0.9141
Pyriform	0.4986

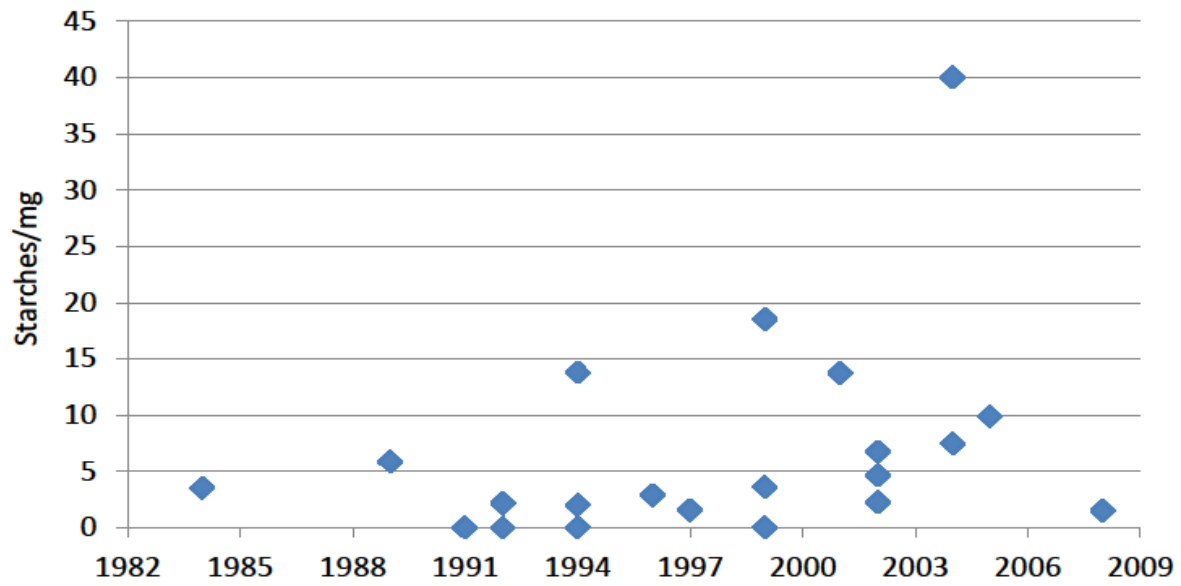
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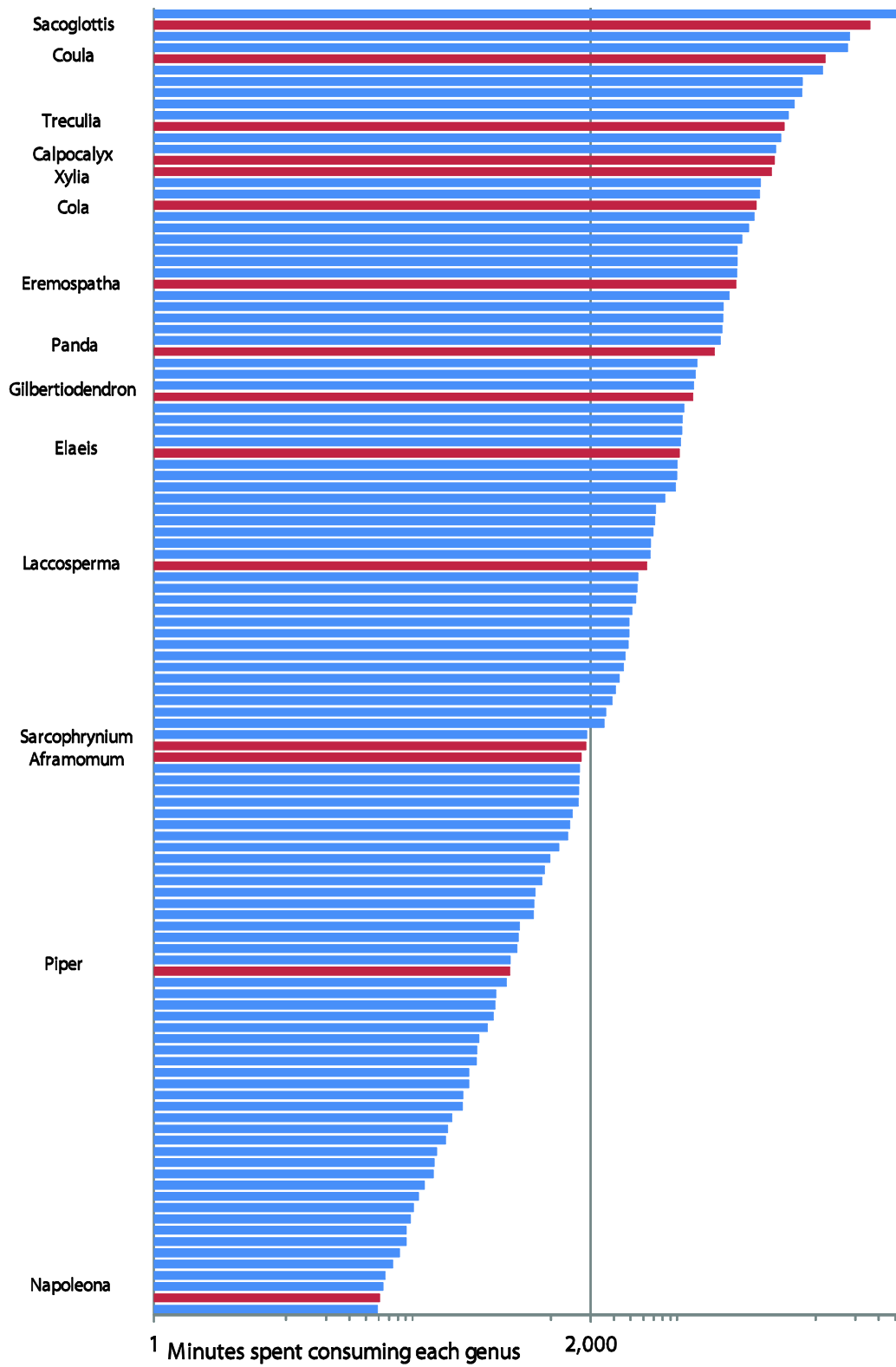
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242 **Supplementary Figures**

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245 Supplementary Figure 1: Scatter plot of starches per mg in each chimpanzee calculus samples
246 and year of chimpanzee death. Starches /mg includes the possible starch microremain category.
247 Treatment of the skeletal remains and year of chimpanzee death does not predict variation of
248 starches per mg.
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Supplementary Figure 2: Bar chart of chimpanzee plant foods, ranked by the number of minutes each was consumed. Plants in random forest model are in red and those that are not

254 are in blue. Chart omits foods eaten for <40 minutes. Our sample includes plants that are
255 frequently consumed (e.g. *Sacoglottis* and *Coula*) as well as those less often eaten (e.g. *Piper*
256 and *Napoleona*).

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