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BUCCAL ENAMEL MICROWEAR VARIABILITY IN CERCOPITHECOIDEA PRIMATES AS A REFLECTION OF DIETARY HABITS IN FORESTED AND OPEN SAVANNA ENVIRONMENTS

ABSTRACT: Dental microwear analysis has proved to be a good indicator of diet and dietary related behaviour in modern humans, fossil hominids and primates. The composition of the diet and the presence of dust and other abrasive particles, are related to microwear rates on the buccal enamel surfaces of molar teeth. Plant food materials such as leaves or stems include phitoliths in larger quantities than fruits or meat. These particles may scratch the enamel surface of teeth during mastication producing a microwear pattern that may be indicative of food choice and food preferences within primate species. In this study we present a dental microwear analysis of extant Cercopithecoidea primates, based on the analysis of more than 200 dental casts obtained from the osteological collection of the National Museum of Kenya (NMK). Specific, sub-specific and also ecological differences are shown to underlie the buccal microwear variability observed within the studied sample.

KEYWORDS: Non-occlusal dental microwear – Microstriation – Primates – Cercopithecoidea

INTRODUCTION

A lot of evidence supports the hypothesis that dental microwear features on the occlusal surface of teeth are determined by dietary and ecological conditions of the *Cercopithecoidea* (Teaford 1994, Ungar 1998, Ungar, Teaford 1996). Hard particles, such as phytolyths present in leaves, shoots or medullas, as well as dust deposited on food items before ingestion, can produce microscopic striations on the enamel surfaces during chewing. Although a close relationship between diet composition and ecological conditions is accepted for living primates for the occlusal surface of teeth, there is little knowledge on how the composition of the diet or the ecology affect to the buccal microwear pattern in primates. Little research on buccal microwear patterns of living primates has been done so far, and buccal microwear may serve as a model for diet/microwear interactions in past human populations based on the lateral surfaces of teeth instead of on the occlusal ones, much more affected by general macroscopic tooth wear. The present study focuses on the interpretation of the differences in the microwear patterns on the buccal surfaces of teeth, looking at differences between several *Cercopithecoidea* species, and how such differences relate to their habitat, ecology, or dietary preferences.

MATERIAL AND METHODS

The sample includes 214 *Cercopithecoidea* specimens for which a dental replica was obtained. All the specimens are curated at the osteological collection of the National Museum of Kenya (NMK), Nairobi. Details on the original sample studied are shown in Galbany *et al.* (2004a). All the specimens analysed are adult individuals of several species (*Table 1*), with both sexes represented, that were

TABLE 1. Total number of analysed moulds (TAM) of *Cercopithecoid* primates from the NMK osteological collection, and number of specimens with well-preserved enamel surfaces (N). All the moulds were made from adult wild-caught specimens. Less than one third of dental casts obtained from the collection offered good information on dental microwear, 30.4% on the average. Last column shows the percentage of good-condition teeth by group considered.

Cercopithecoidea species	TAM	N	%	Group %
Papio anubis	64	27	42.2	42.2
Cercopithecus nictitans mitis	37	6	16.2	
Cercopithecus aethiops	41	14	34.1	31.1
Cercopithecus aethiops pygerythrus	21	10	47.6	
Cercopithecus neglectus	7	3	42.8	
Colobus abyssinicus	34	3	8.8	11.4
Colobus angolensis	10	2	20.0	
TOTAL	214	65	30.4	

wild-caught in nature for different purposes and are now stored in the NMK osteological collection. None of the specimens studied were ever in captivity or fed by humans, so they inhabited their original distribution areas. Definitive species attribution of each specimen was done by original notes taken from the osteological collection (Galbany *et al.* 2004a) and from Kingdom (2001), correcting possible taxonomic inconsistencies. The studied species were: *Papio anubis*, *Cercopithecus nictitans mitis* (or *Cercopithecus albogularis* or *Cercopithecus mitis*), *Cercopithecus aethiops*, *Cercopithecus aethiops pygerytrhus*, *Cercopithecus neglectus*, *Colobus abyssinicus* (or *Colobus guereza*) and *Colobus angolensis*. These species show a clear ecological diversification and an extensive geographical distribution (Fleagle 1999, Kingdom 2001)

Dental casts of buccal surfaces of teeth were obtained from the original primate teeth in the osteological collection of the National Museum of Kenya (NMK) in Nairobi, using the surface activated *President microSystem™ Regular body* polyvinylsiloxane (*Coltène*®). Only one tooth was casted per specimen, normally the lower-left, second molar (LM_2) in order to standardize the analysis methods. Resin replicas where obtained from the casts using epoxy resin Epo-Tek #301. Dental molds where mounted on a stub for SEM image digitalization of the enamel surfaces. SEM images where obtained at 100× magnification using the Scanning Electron Microscopes Hitachi-2300 and Cambridge Stereoscan-120 at the *Serveis Cientificotècnics* (SCT) of the University of Barcelona. The images where taken following standard SEM procedures and were then processed with *Adobe Photoshop 5.0* for image enhancement (Pérez-Pérez *et al.* 1999). Microstriations where counted semi-automatically with *SigmaScan Pro* 5.0, measuring the total number of striations, their lengths, in micrometers (μm), and their orientation (Pérez-Pérez *et al.* 1999, Galbany *et al.* 2004b)

All the striations were classified into an orientation category, in relation to their anatomical position with regard to the cemento-enamel junction of the tooth. Thus, striations were classified as horizontal (H), vertical (V), mesiodistal (MD) or distomesial (DM). Finally, every analyzed tooth was characterized by the density of microstriations (N), their average length in micrometers (X) and the standard deviation of their length (S). Considering at the same time all the orientation categories and original variables measures, a total of 15 normally distributed variables, characteristic of each individual, could be derived. The statistical analyses were done with SPSS 11.0. For a more detailed description of these procedures refer to Pérez-Pérez *et al.* (1999).

RESULTS

Only 65 (30.4%) out of the 214 original teeth studied showed fully well-preserved buccal enamel surfaces, with microwear features clearly visible. There was a great amount of teeth in which microwear features could not be observed, despite all the samples belonged to properly curated, modern primate populations. There are several causes that do not allow for a good preservation of enamel surfaces in modern primates. The main one is the presence of patina on the buccal surface of teeth, a mineral cover on the enamel that impedes the observation of the enamel surfaces. Patinas are very common on buccal surfaces, but not so on occlusal ones (*Figure 1*). The presence of patina, a clear handicap that dramatically reduces the sample to be studied, affected all the species studied. The genus mostly affected by this mineral cover was *Colobus*, where only 11.4% of all the specimens showed unaffected, well preserved enamel surfaces (*Table 1*). This could be caused by the mainly leaf-eating habits of this species, which would drive to the presence of this mineral layer in order to protect the enamel surfaces from lateral abrasion. In contrast, *Papio anubis*, shows the highest percentage of well-preserved enamel surfaces (42.2%), with lack of patinas covering it.

There is a clear difference in the density of microstriations among the different genera studied, as shown for variable NT (total number of microstriation). *Colobus* is the genus that shows the lowest density of features on the enamel surfaces, while *Cercopithecus*, in a broad sense, shows the

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FIGURE 1. Buccal surface of molar teeth showing patina layers at different magnification levels. The pattern shown on the images is very frequent in leaf-eating monkeys (i.e. *Colobus*).
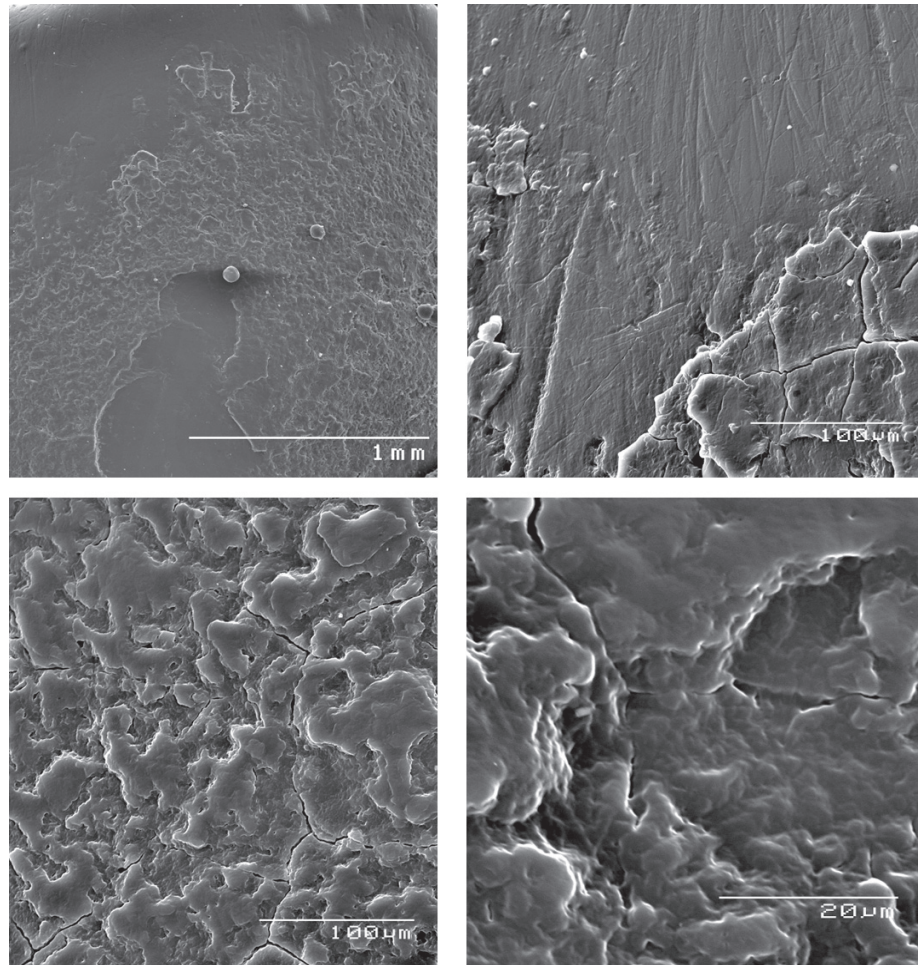

TABLE 2. One-factor ANOVA of the 15 microwear variables considered. Separate analyses were made at the species level (7 groups) and the genus level (3 groups). Almost all variables show significant differences at a 5 % significance level. Only NMD, XDM and SDM show no significant differences for both analyses considered.

Variable	Species		Genus	
	F	p-level	F	p-level
NH	2.808	0.018	6.755	0.002
XH	3.419	0.006	6.310	0.003
SH	2.609	0.026	4.813	0.011
NV	2.724	0.021	4.680	0.013
XV	3.240	0.008	7.326	0.001
SV	4.877	0.000	9.445	0.000
NMD	0.959	0.461	2.662	0.078
XMD	4.517	0.001	9.571	0.000
SMD	3.688	0.004	7.190	0.002
NDM	4.346	0.001	13.564	0.000
XDM	2.038	0.075	2.088	0.133
SDM	1.009	0.429	0.068	0.934
NT	6.833	0.000	17.941	0.000
XT	5.772	0.000	15.570	0.000
ST	4.988	0.000	13.063	0.000

TABLE 3. Bonferroni *post-hoc* test showing the differences between pairs of groups considered at genus level. (Ce: *Cercopithecus*, Co: *Colobus* and Pa: *Papio*). * P=0.05 and ** P=0.01 significance level.

Variables	Ce vs.Pa	Ce vs.Co	Co vs. Pa
NH	**		
XH	*		*
SH			*
NV		*	**
XV		*	**
SV		**	**
NMD			
XMD	**		**
SMD	**		*
NDM	**	**	
XDM			
SDM			
NT	**	**	**
XT	**		**
ST	**	*	**

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FIGURE 2. Box-plot showing total number of microstriations (NT) of the *Cercopithecoidea* species analyzed. Panu (*Papio anubis*), Cmit (Cercopithecus nictitans mitis), Caet (*Cercopithecus aethiops*), Cneg (*Cercopithecus neglectus*), Cpyg (*Cercopithecus aethiops pygerythrus*), Coaby (*Colobus abyssinicus*) and Coang (*Colobus angolensis*).
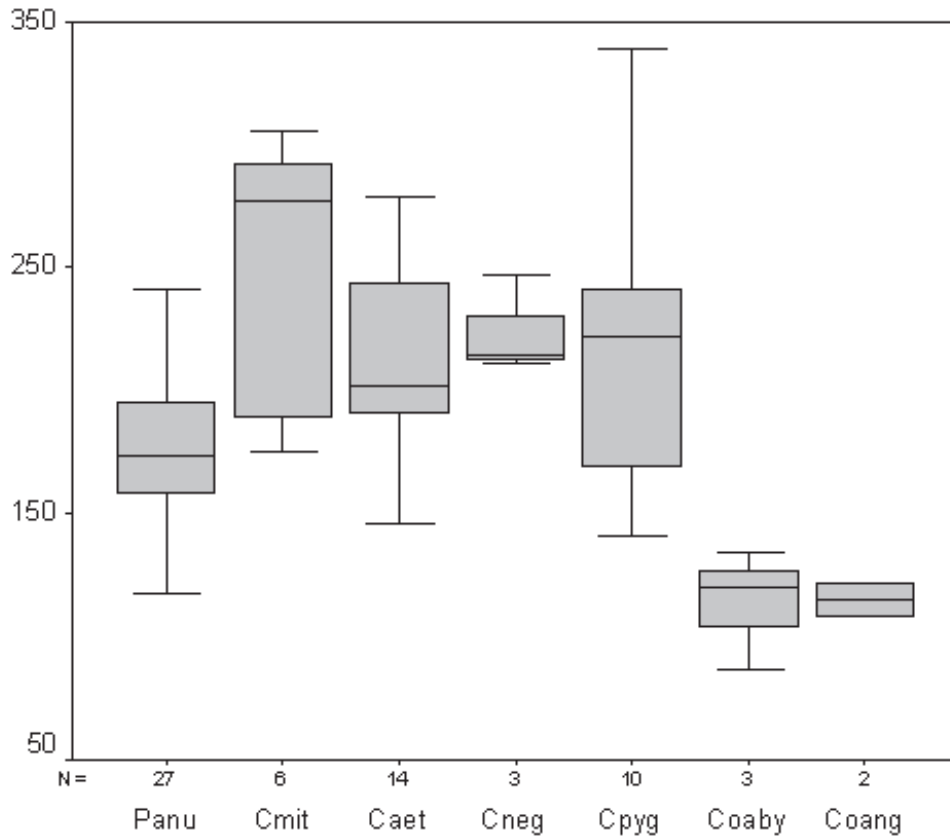

FIGURE 3. Box-plot showing average length of microstriations (XT) in micrometers (?m) in the *Cercopithecoidea* species analyzed. Panu (*Papio anubis*), Cmit (Cercopithecus nictitans mitis), Caet (*Cercopithecus aethiops*), Cneg (*Cercopithecus neglectus*), Cpyg (*Cercopithecus aethiops pygerythrus*), Coaby (*Colobus abyssinicus*) and Coang (*Colobus angolensis*).
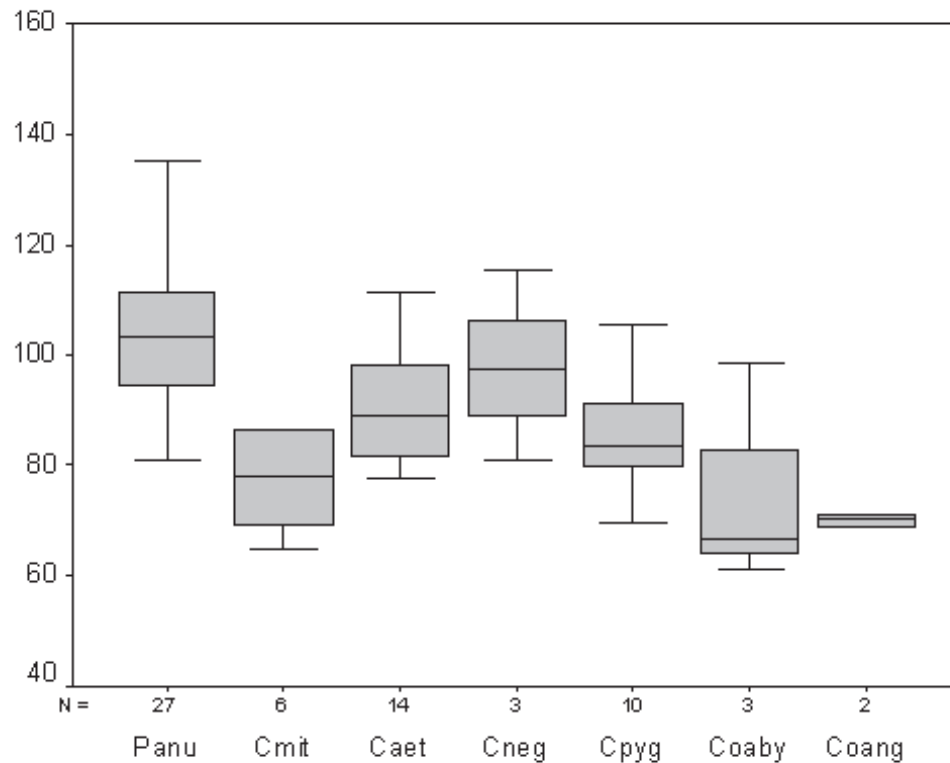
FIGURE 4. Plot of the first two components of the Discriminant Analysis for the *Cercopithecoidea* species studied. The first two functions derived explained 73.0% of total variance, with function 1 explaining 41.2% and function 2 explaining 31.8%. Panu (*Papio anubis*), Cmit (Cercopithecus nictitans mitis), Caet (*Cercopithecus aethiops*), Cneg (*Cercopithecus neglectus*), Cpyg (*Cercopithecus aethiops pygerythrus*), Coaby (*Colobus abyssinicus*) and Coang (*Colobus angolensis*).
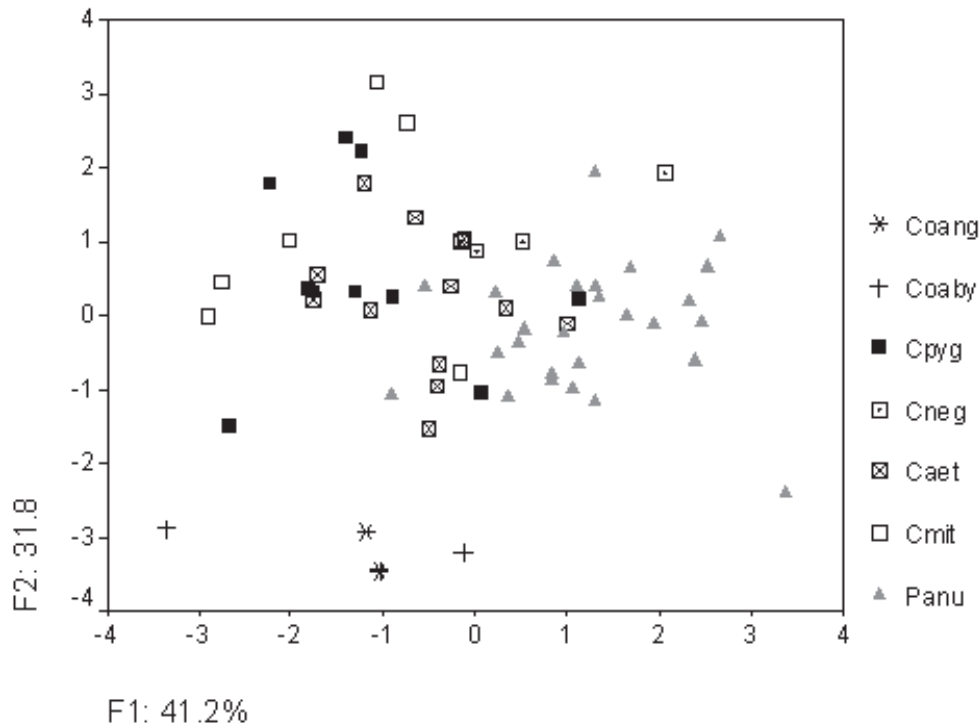
highest NT value. *Papio anubis* is situated between both groups (*Figure 2*). Differences in the lengths of microstriations are also present between the different genera studied. *Papio anubis* shows the longest striations, while *Colobus* shows the shortest ones. One-factor ANOVA analyses show that almost all the analyzed variables significantly differ among the different species studied, as well as when the analyzed species are grouped into genera (*Table 2*). The main significant differences between pairs of genera can be seen with a Bonferroni *post-hoc* test (*Table 3*). In order to analyze the importance of the 15 variables considered, a multivariate Discriminant Analysis was done. The first two derived discriminant factors explain 73.0% of total variability (*Figure 4*). In the first function (explaining 41.2% of total variance) the highest correlations were obtained with variables XT (r=0.60), XDM (r=0.53), ST (r=0.51), SMD (r=0.45), XV (r=0.37) and NH (r=–0.32), while in the second function (31.8% of total variance) the highest correlations were with NT (r=0.72), NDM (r=0.45), NV (r=0.44) and NMD (r=0.27). All the analyzed species show significant between-groups differences. *Colobus* specimens are well grouped and tend to separate from the rest of species for the second function (*Figure 4*). *Cercopithecus* and *Papio* show a gradient for the first component of the discriminant analysis, being *Papio anubis* the species placed on positive values, and *Cercopithecus* that on the left-hand side, where the negative values are. The Discriminant Analysis is not only showing differences in the microwear patterns among the seven species considered, but also between genera. If a step-wise discriminant analysis is done, only variables NT and XT are included. NT strongly correlates with the second function (r=0.96), separating *Colobus* from the rest of species, and XT highly correlates with the first function (r=0.80), showing a clear distinction between *Papio* and the other *Cercopithecus spp.* groups. The descriptive box-plots of NT and XT (*Figures 2* and *3*) show clear between-species and between-genera differences. In general, *Colobus* shows lower densities of microstriations, as well as the shortest ones. *Cercopithecus* has the highest number of microstriations, with an intermediate mean length, and *Papio anubis* has the highest values for the length of the microstriations, but has a moderate mean number of striations. In addition, most of the analysed variables show differences between both species and genera (*Tables 2* and *3*). These results are in accordance to similar studies made for the cercopithecines, both for non-occlusal dental surfaces (Ungar, Teaford 1996), and occlusal ones (Teaford 1994, Ungar 1998).

DISCUSSION

Microwear patterns are clearly related to diet (Teaford 1994, Ungar 1998). Several studies have shown that *Colobus*, that has a mainly folivorous diet (Fleagle 1999, Crissey, Pribyl 1997), has very low wear rates (Teaford, Walker 1984, Teaford 1994, Ungar, Teaford 1996). On the

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other hand, widely frugivorous diets are related to high wear rates and a high number of wear incidences (Teaford 1994, Ungar, Teaford 1996). This could be the situation of *Cercopithecus spp*. because fruit is the most important resource in guenons' diet (Beeson *et al.* 1996, Fleagle 1999). In *Cercopithecus mitis* fruit represents 75.5% of total ingestion (Rudran 1978), although generally guenons also eat other items, including foliar material, flowers or animals (Rudran 1978, Beeson, Lea 1994, Beeson *et al.* 1996). *Papio spp*. shows high microwear incidences in buccal surfaces (Ungar, Teaford 1996), and Daegling and Grine (1999) indicate that *Papio ursinus* shows one of the highest values of microwear on occlusal facets, in relation to the mean pit size and percentage, although no *Cercopithecus spp*. species were compared by them.

In the present study, similar values on the buccal microwear patterns between *Papio* and *Cercopithecus* have been found, clearly differing from *Colobus spp*. This may be explained in part by differences in diet composition between the two groups, as well as by differences in the incidences of terrestrial feeding events. As Ungar and Teaford (1996) suggested, the more terrestrial and/or frugivorous cercopithecines show high incidences of microwear on the buccal surfaces, whereas the more arboreal and folivorous colobines show fewer incidences. Moreover, the discriminant analyses made show a clear difference between *Colobus* and the rest of *Cercopithecoidea* for the second function, which strongly correlates with the total number of microwear features, whereas *Cercopithecus* and *Papio* can be discriminated by the first function, highly related to the mean length of the microstriations.

CONCLUSIONS

It is clear that there are some limiting factors to the study of dental microwear of buccal surfaces of non-human primates due to the presence of patina layers, very frequent in some genus as *Colobus*. The *Cercopithecoidea* analyzed in the present study show clear microwear differences on the buccal surface, coinciding with other studies (Teaford 1994, Ungar 1998, Ungar, Teaford 1996). Leaf-eating monkeys, such as *Colobus spp.*, show a low rate of microwear, as well as a very low number, and short microstriations. Guenons, *Cercopithecus spp.* show the highest wear rates, with a high number of microstriations, and longer on average than in *Colobus*. Finally, *Papio anubis* is placed between both groups, but has longer microstriations than *Cercopithecus*. All these results point to the existence of a clear relationship between dental microwear on buccal surfaces, diet composition, and ecological conditions in *Cercopithecoidea*. Leaf-eating monkeys (i.e. *Colobus*), feeding far from the ground, show low rates of dental microwear, despite leaves frequently include large amounts of phytoliths, probably due to a low dust accumulation on the top of trees, but a high presence of patina layers on buccal surfaces, that could be important to prevent excessive enamel abrasion. Guenons, mainly frugivorous, are unexpectedly associated with high wear rates, similar to *Papio anubis*, probably because of their terrestrial foraging, where more dust accumulation can cause longer striations.

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