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Deciphering the mandibular shape variation in a group of Malagasy primates using Fourier outline analysis.

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Abstract

Objectives : Among extant Malagasy primates, the family Lemuridae has previously been recognized as presenting a higher mandibular morphological variation than other families. We conducted a quantitative analysis of mandibular size and shape within the five genera (*Lemur, Eulemur, Hapalemur, Prolemur* and *Varecia*) associated with a set of covariables that could explain this variation.

Materials and Methods : We used Fourier outline analysis on the left hemimandible of 182 specimens covering the Lemuridae family. The influence of the phylogeny but also of seven covariables (genus, diet, sex, sexual behavior, mating system, ecoregion and forest type) on mandibular variation was examined using multivariate statistics and models selection.

Results : Our results indicate that the high level of morphological variation within the family, associated with a phylogenetic effect and differences in diet, is due to a strong distinction between the genera *Prolemur* and *Hapalemur* and the other genera of the family. A second analysis, correcting this strong effect, indicate that mandibular shape variation is influenced not only by the phylogeny and the diet but by a combination of all the covariables.

Discussion : The analysis of morphological variation is a powerful tool with major applications, both for the estimation of biological diversity and for the understanding of the fundamental parameters of a species' ecology. Our work indicates that, if mandibular shape variation is mainly driven by dietary adaptation, other variables describing ecology and habitat should be considered and taken into account for an integrative understanding of species resources and the establishment of conservation measures.

Keywords: Lemuridae, mandible, Fourier analysis, Geometric Morphometrics.

Introduction:

Madagascar is one of the world major hotspot of biodiversity, with about 3.2% of the world's endemic plants and 2.8% of endemic vertebrates (De Wit, 2003; Tattersall, 2006). In mammals, the endemicity is observed in the orders of Chiroptera (eight endemic families), Carnivora (one endemic family), Afrosoricida (one endemic family) and Primates (five endemic families). This endemic biodiversity, as the entire biodiversity of Madagascar, is threatened with extinction, due to the continuous decrease in habitat size and quality (e.g. Campera et al. 2014; Herrera & Dàvalos, 2016) and 90% of Madagascar's species are forest dependent (Allnutt et al., 2008). This is especially the case for the Malagasy primates that combines threats from habitat loss due to agriculture which affects about 90% of the species population, logging and wood harvesting (50%), cattle ranching (25%) and mining (18%) (Harper et al., 2007; Estrada et al., 2017; Vieilledent et al., 2018; Morelli et al., 2020; Eppley et al., 2020) but also threats from poaching, hunting for trade or domestication which affects about 65% species (Estrada et al., 2017; Reuter & Schaefer, 2017; LaFleur et al., 2019). Thus, it has been estimated that 87% of Malagasy primates species are nowadays threatened and that 100% of these species have declining populations (Estrada et al., 2017). Factors threatening primates are dynamic and interact with each other at local, regional, and national scales, leading to a reduction in population sizes and, a probable extinction (Estrada et al., 2017). Previous studies have shown that morphology covaries with ecological parameters in Malagasy primates (e.g. Ravosa, 1990; 1991; 1992; Raveloson et al., 2005; Ross et al., 2012; Meloro et al., 2015; Pitirri & Begun, 2019, 2020; Andrews et al., 2020) but also that the mandible is too variable to be useful in understanding taxonomic relationships (e.g. Schmittbuhl et al, 2007; Humphrey et al., 1999; Pitirri & Begun, 2020; Taylor & Groves, 2003) as well as in understanding diet adaptation (Ravosa, 1990; Raveloson et al., 2005; Meloro, 2015; Ross et al., 2012; Marcé-Nogué, Püschel, & Kaisr, 2017). In addition, morphology (phenotype) also clearly carries a phylogenetic signal (Fleagle et al., 2010; 2016; Masters et al., 2014; Masters & Couette, 2015; St Clair & Boyer, 2016).

Lemuriforms is a monophyletic group that diverged from the lorisiforms, between 50 and 70 Ma on the basis of calibrated molecular phylogenies (Yoder and Yang, 2000; Perelman et al., 2011; Pozzi et al., 2014a; Kistler et al., 2015). Resolving the phylogeny of lemuriforms using molecular data alone has proven difficulties (Yoder and Yang, 2000; Springer et al., 2012). The placement of extinct giant lemurs in the phylogeny was originally based on the morphometric affinities of extinct lemurs to living species (e.g. Jungers et al., 1991; 1997; Herrera & Dàvalos, 2016). Among the lemuriforms, the family Lemuridae is among the most studied Malagasy primates. It is also considered to be a monophyletic group, as evidenced by the many phylogenies published over time, in which the relationships between taxa of this family are well resolved. (e.g. Simons & Rumpler, 1988; Tattersall & Schwartz, 1991; Yoder et al., 1996; Stanger-Hall, 1997; Randria, 1999; Yoder & Irwin, 1999; Wyner, DeSalle & Absher, 2000; Delpero et al., 2001). The Lemuridae is the most taxonomically diverse of the five lemuriform families (Yoder et al., 1996), this specific biodiversity associated with a large variation of morphological characters, of diets, of behaviors and ecology, but also a large variation of distribution over the island.

In primates, the craniofacial complex and teeth have been the subject of several studies (e.g. Fleagle et al., 2010; Perez et al., 2011; Bennett & Goswami 2012; Baab et al., 2014; Dumont, 1997; Sauther et al., 2001; Cuozzo, 2012; Scott, 2012) focusing on morpho-functional adaptation or taxonomy. Concerning the mandible, previous studies have shown that mandibular morphology of primates is influenced by several factors or parameters, including diet, allometric scaling, sexual dimorphism, geographical distribution, behavior, growth and phylogeny (Daegling & Grine, 2006; Daegling & Jungers, 2000; Guy et al, 2008; Humphrey, Dean & Stringer, 1999; Ravosa, 1991; Ross et al., 2012; Schmittbuhl et al., 2007; Meloro et al., 2015; Pitirri & Degun. 2019). However, in Malagasy primates, the mandible has been less studied than the skull, as attested by the few numbers of articles on this subject, among which the ones of Ravosa,1990; Raveloson et al., 2005; Viguier & Tort, 2000; Ross et al., 2012; Meloro, 2015 or even Marcé-Nogué et al., 2017. Most of these studies have focused on one

or another factors and parameters influencing the mandibular morphology (e.g. diet, or sexual dimorphism, or phylogeny, etc.), but rarely on a set of factors. The distinction of folivorous species from the frugivorous ones is one of the most reported result explaining mandibular variation in Lemuridae (e.g. Tattersall & Schwartz, 1974). Other studies have been conducted in order to understand the functional role of each mandibular parts in jaw movements for chewing and bitting actions (see Vinyard et al., 2007 for a review). Differences in diet between species are linked to morphofunctional adaptations of the mandibule muscular forces and strains that could lead to bony differences in size and shape (Ravosa, 1991). The mandibular stress pattern is dependent on the fusion of the symphysis (never present in Lemuridae) and of the bony morphology of the temporomandibular joint (Beecher, 1977; Hylander, 1979; Ravosa, 2000; Terhune et al., 2022). In Lemuridae, the main functional differences between species are related to the ingestion of leafs, and especially highly fibrous or hard items (Hylander et al., 2005). It concerns several species of the genus *Eulemur*, *Varecia variegata*, *Lemur catta* for the consumption of young leaves and *Hapalemur* and *Prolemur* for the consumption of bamboo shoots and mature leaves (Mittermeier et al., 2013). However, these factors covary and it seems important to decipher the covariation of each of these factors, and their interactions with the morphology of the mandible.

The family Lemuridae is composed of species that have very contrasted diet, that inhabit different habitats, present different behaviors. An integrative knowledge of the covariation between shape (here mandibular shape) and other variable describing ecology and behaviour is necessary to establish conservation measures (Estrada et al., 2017). Ours study aims to identify the main factors controlling morphological variation, thus highlighting the links between individuals' morphology, their ecology and their environment. We hypothesized the fact that mandibular variation could be under the influence of a combination of variables rather than a single one or a couple. We established several models that we compared to test this hypothesis. Such information is necessary to make decisions and develop the good strategies in the framework of biodiversity conservation and sustainable remediation.

Materials and methods :

Sample

We studied 182 specimens belonging to 12 living species including *Eulemur fulvus, Eulemur rufus, Eulemur albifrons, Eulemur coronatus, Eulemur macaco, Eulemur mongoz, Eulemur rubriventer, Lemur catta, Varecia variegata, Hapalemur griseus* and *Prolemur simus* of the Lemuridae family (Table 1, Figure 1). These specimens (dry hemi-mandibular bones) come from the osteological collections of the Muséum national d'Histoire naturelle (MNHN) in Paris, France. All the specimens have fully erupted teeth on the mandible and on the maxillary, and are thus considered as adults.

Data collection

Morphological data acquisition consists of representative 2D images from 3D mandible shape bones. Each hemi-mandible was placed on a black background to obtain a contrast between the light-coloured spot representing the object of interest and was illuminated by two lamps (on both sides) with a voltage variator diffusing a homogeneous light (Figure 2).

The focal axis of the camera was placed perpendicular to the plane parallele to the long axis of the hemi-mandible, and then centered on the left lower first molar. We used a Nikon D750 camera equipped with a Nikon 105 mm macro lens. All the focusing of the shots was set and controlled from the computer using the DigiCamControl v 2.1.4.0 software (Duka Istvàn, 2018). Our acquisition protocol is consistent with the one described by Raveloson et al. (2005). All the images of our sample were newly taken (they were not coming from Raveloson et al. study) by one of us (JE.M.). As tooth shape variation was not the aim of our study, and as many mandibles had missing teeth, we kept the parts of the pictures related to the mandible and removed manually the parts related to the teeth, first because our study was not focusing

on teeth and second because several specimens had missing teeth. We thresolded the images to remove teeth using Adobe Photoshop CS6 (version 13.0, Thomas & John (2012)). Then with this same software, we converted the images in grayscale and the images were all reduced to the same size to avoid potential differences in image resolution.

Outlining

We used the Elliptic Fourier Analysis (EFA) method to quantify the mandible shape and size. This method is one of the Geometric Morphometrics methods that can be used for the characterization of biological objects shape and their quantification by continuous quantitative variables. EFA consists of approaching the outline of the mandible by a sum of ordered trigonometric functions (harmonics), each harmonic participating in the reconstruction of the original outline, adding complexity (see for instance Kuhl & Giardina, 1982; Zelditch, 2012; Claude, 2008). Elliptic Fourier is used to quantify closed contours whatever their degree of morphological complexity. With this method, we can keep the fixed position of the mandible and we can quantify all angles. However, with 3D landmarks, it is a point to point (anatomical points). Our study is complementary to the one published by Raveloson et al. in 2005. We used a similar protocol, based on EFA (rather than 3D landmarks) in order to facilitate the comparison of results.

Outline of the hemi-mandibles were produced by the TPS series of software, and especially the tpsDIG2 version 2.31 and tpsUtil (Rohlf, 2015). The outlines are automatically calculated after the definition of a starting point. In our protocol, all the outlines were aligned along the axis of elongation, we defined a reference direction of rotation for outline opening (anticlockwise) and we placed the starting points at the same place on every outline (Figure 3). The power of reconstruction of the original outline is calculated and used to make a decision about the harmonics number to retain. Four coefficients are associated with each harmonic and used for shape characterization. Harmonic coefficients are used in multivariate analysis for shape comparisons (Rohlf & Archie 1984; Crampton, 1995; Renaud & Michaux,

2003). The outline analysis was performed with the Momocs Package v 1.1.0 (Bonhomme et al., 2016) on R v. 3.2.5 (R Core Team, 2021). A quantification of outline size (centroid size) is obtained with the same package, calculated as the magnitude of the semi-major axis of the first fitting ellipse (Claude, 2008).

We estimated the measurement error by measuring twice a subsample of 10 specimens of the same species. We estimated the variance within replicates, the variance between replicates and thus estimated the effect of replication using F statistics. A percentage of measurement error can also be calculated based on the variance values, following the method explained by Bailey and Byrns (1990).

Phylogeny:

We used the phylogeny proposed by Herrera & Dávalos (2016) to estimate the phylogenetic structure of our morphological results and to establish the phylomorphospaces (Figure 4). This phylogeny is one of the most complete analyses for strepsirrhines, and is based on a combination of 421 morphological characters and 5,767 base pairs from two mitochondrial and four nuclear loci. We selected the branches corresponding to the Lemuride family among the majority rule consensus tree. We use two versions of the phylogeny, a first version with the 12 taxa of our sample (Figure 4A) and a second version with a reduced sample on which we removed the species *Prolemur simus* and *Hapalemur griseus* (Figure 4B).

Covariables

We investigated the covariation of mandibular shape with seven variables possibly related to mandibular morphological variation.

We tested the covariation of the groups (here the genera) with the shape of the mandible. The family Lemuridae constitutes five genera of which: *Eulemur, Lemur, Varecia, Hapalemur* and *Prolemur* (Mittermeier et al., 2010).

We tested the covariation of diet with lemurids mandibular shape. Three diet categories were defined with specimens classified as folivore/frugivore for the species of *Eulemur*, frugivore/folivore for *Lemur catta* and *Varecia variegata* and folivore or feeding on hard objects (bamboo shoots, bamboo culm, sugar cane, etc.) for *Hapalemur griseus* and *Prolemur simus*. We based this classification on data summarized in previous studies on primate dietary adaptation (e.g. Wright & Willis, 2012; Winchester et al., 2014; Coiner-Collier et al., 2016; Steffens, 2020; Eronen et al., 2017, St. Clair et al., 2018; Andrews et al., 2020).

We also investigated the influence of sex, sexual behavior and mating system on mandibular shape variation, hypothesizing that mandibular shape would express a sexual dimorphism, would vary depending on dominant and non-dominant specimens and over different mating systems. Indications for specimen sex were collected from collection labels. We used the shape of the mandible to evaluate the extent of sexual dimorphism in Malagasy prosimians.

For sexual behaviour, females are most of the time social dominant (coded “Dominant” for females and “Non-dominant” for males) in Lemuridae (e.g. Mittermeier et al., 2006, 2010; Sauther, 2012). However, in *Prolemur simus*, the males are socially dominant over females (Roullet, 2012). We also hypothesized the covariation of mating system with mandibular shape and tested this hypothesis by coding both mating systems polygynous (also including multimale-multifemale system) and monogamous for the different species composing our sample.

The covariations of mandibular shape with ecoregions and forest types have been tested. Lemurs are distributed in the forested zones remaining around the periphery of Madagascar (Mittermeier, 2010). In order to test these hypotheses, we coded Madagascar's ecoregions and habitat (Figure 5). Madagascar can be divided into several bioclimatic or

phytogeographical domains depending on latitude, altitude, temperature, rainfall, seasonality and vegetation (Battistini, 1996; DuPuy & Moat, 1996). Ecoregions were defined following climatic (Cornet, 1974) and vegetation criteria. Forest types are defined on the basis of their belonging to one of the ecoregions (Vieilledent, 2018; Harper, 2007): the central plateau is temperate, with marked wet and dry seasons, is characterized by mid-altitude montane rainforest and by sclerophyllous forest at the highest altitudes. The moist forest with dense evergreen lowland rainforest is located in the east of the island where *H. griseus*, *P. simus*, *V. variegata*, *E. fulvus*, *E. rubriventer* are distributed and in south-east where *E. collaris*, *H. griseus* and *E. rubriventer* are distributed. *E. collaris* are also distributed in the tropical rainforest if the south-east. The west of the island is characterized by distinct seasons, with deciduous forests. The cliamte becomes drier from the north to the south, where *E. fulvus*, *E. mongoz, E. rufus and E. coronatus* are distributed. The southern domain is hot and semiarid, with subarid thorn scrub and gallery forest where *L. catta* are distributed. The dry forest is present in Mayotte, where *E. fulvus* are distributed (species introduced by humans). Finally, some specimens of our sample come from parks and zoos.

Statistical analyses

We applied a Principal Component Analysis (PCA) on harmonic coefficients in order to compute the morphospace. The inverse transformation, done on the harmonic coefficients extracted from every outline, allowed a reconstruction of mandibular shapes describing the variation expressed by each PCs. Size differences between taxonomic groups are calculated using the ANOVA method and multivariate morphological differences were tested using MANOVAs with the help of the package MASS (Venables & Ripley, 2002).

We tested the structure of the morphospace explained by the phylogenetic hypothesis using a phylogenetic test available on the Geomorph R package (Adams & Otárola-Castillo, 2013; Adams, 2014). Then, we plotted the phylogeny onto the morphospace, producing a phylomorphospace, using the package R v. 3.2.5 (R core Team, 2021). For this analysis we

computed the mean shape for each species (as we have only one observation per group in the phylogeny), computed the morphospace from these mean shapes and projected the observations in the morphospace. We obtained a graphic in which the phylogeny and the morphological variation within each group can be associated.

We then tested the effects of covariables on mandibular shapes using a linear model. We built nine different linear models considering mandibular shape variation in function of:

- Null model
- Phylogeny
- Phylogeny + Diet
- Phylogeny + Sex (covariables sex, sexual behaviours and mating system)
- Phylogeny + Habitat (covariables ecoregion and forest type)
- Phylogeny + Diet + Sex
- Phylogeny + Diet + Habitat
- Phylogeny + Sex+ Habitat
- Phylogeny + Diet + Habitat + Sex

The robustness of each model is estimated by verifying the distribution and homogeneity of variances of the residuals. We also detected the presence of potential outliers. R^2 values of each model is given in tables 2 and 3. We used AICc (corrected Akaike Iteration Criterion) values for model comparison (Burnham & Anderson, 2004). The model with the lower value of AICc was considered as the one that best describes the data structuration. We compared the models with the function aictab of the R package AICmodavg (Mazerolle, 2020). Same models were tested on centroid size.

Result:

Measurement error

The measurement error is lower than 2% and results from the MANOVA indicate that the effect of the replicates is not significant (Pillai = 0.15, p=0.81).

Morphological variation in lemurids

The morphospace composed of the two first PCs, accounting for 55.5 and 16.9% of total variation, clearly separates specimens of the genera *Hapalemur* and *Prolemur* from the specimens of the genera *Eulemur*, *Lemur* and *Varecia* (Figure 6). This distinction occurs mainly along PC1 that describes mandibles with high coronoid processes, with tips arched posteriorly, high vertical ramuses and shallow angles but developed and low angular processes on positive values of the axis (illustrated by *Hapalemur griseus*). Negative values of PC1 (illustrated by *Eulemur rufus*) describe mandibles with pronounced symphyses, bodies with convex ventral edges, moderately angular processes, ramuses with marked grooves between the angular processes and the condyles, marked sigmoid notches and almost vertical coronoid processes. The variation along this first axis is important between genera (as presented previously) but also within some genera (especially in *Hapalemur*, *Prolemur* and *Eulemur*). PC2 describes mandibles with thin bodies, low symphyses and vertical ascending ramus with vertically developed coronoid processes on positive values (illustrated by *Lemur catta*). The mandibles described by the negative values of this second axis (illustrated by *Eulemur fulvus*) have thin bodies, with marked symphysis, moderately developed ascending ramus with marked angles, condyles and coronoid processes directed posteriorly more than vertically. Shape variation along this axis is also important. The amount of variation seems homogeneous between genera and thus only describes differences within genera. The mandibular shape differences described on the two first PCs is also true when considering the entire variation. The results of the MANOVA calculated on all the PCs indicate a significant distinction of mandibular shape between genera (Pillai = 3.57, $p<0.001$). The difference in mandibular shape between genera is accompanied by a difference in mandibular size (ANOVA results: $F = 7.18$, $p<0.01$), with the mandibular centroid size in *Prolemur* being significantly higher than mandibular centroid size in the other genera.

The mandibular shape difference between the genera *Hapalemur*, *Prolemur* and the other genera of the lemuridae family also reflects the phylogenetic signal, as presented by plotting the phylogenetic tree onto the morphospace (Figure 6) but also as established by the results of the phylogenetic test (K statistics = 1.27, p = 0.03). The high difference between *Hapalemur*/*Prolemur* and the other genera of the family is so high that it is not easy to understand the morphological structure of mandibular shape variation in the genera *Eulemur*, *Lemur* and *Varecia*.

In order to investigate this phylogenetic variation, we ran a new analysis on a subsample composed of the specimens of these three genera. Results are presented on Figure 7. The two first PCs account for 49.25 and 23.88% of total variation respectively. Shape variation along PC1 describes, for negative values, mandibles with thin bodies, developed ascending rami, with high and vertical coronoid processes, marked mandibular (sigmoid) notches, low condylar neck but developed condyles, and small angular processes. Positive values of PC1 describe mandibles with thin bodies and small symphyses, low ascending rami with coronoid processes developed posteriorly, shallow condyles and small angular processes (Figure 7). PC2 describes mandibles, for negative values, with relatively low coronoid processes, with tips directed posteriorly, the condyle is marked but without necks, and the angles and the angular processes are low. Positive values of the second PC describe mandibles with marked symphyses, developed ascending rami, with high coronoid processes, protruding condyles and marked necks, and developed angular processes. Specimens of the different taxonomic groups are overlapping on this morphospace but all the specimens of the genera *Varecia* and *Lemur* have positive values on PC1 when specimens of the genus *Eulemur* are more widely distributed along this axis. Within the genus *Eulemur*, specimens of the different species are also overlapping showing a high amount of shape variation in comparison to the variation expressed by the specimens of the two other genera. When projecting the phylogeny onto the morphospace, we can see that the shape variation in this subsample is not structured by the

phylogenetic signal. This is reinforced by the non significant result of the phylogenetic test (K statistics = 0.64, p = 0.08).

Analysis of covariations

covariations with mandibular shape

We calculated the AICc values for the nine tested models. Results are presented on Table 2. Our first analysis was made on the entire sample, but, regarding the high distinction of the specimens of the *Hapalemur* and *Prolemur* genera, we conducted a second analysis, removing these specimens. AICc values are here computed as the sum of the AICc relative to the first, second and third PC axes. We considered the three first PCs as they cumulated more than 90% of total variation in both PCA analyses.

Models on shape of the entire dataset were all significant (except the null model), with high values of R^2. The most likely model being the one with the lowest AICc value, it is noticeable that the two first models (Phylogeny and Phylogeny + Diet) can be selected to describe the interactions between covariables and mandibular shape. These models are similar as the two genera *Hapalemur* and *Prolemur* are also the only ones of the sample having a folivorous diet. Our results also indicate that the phylogenetic signal (here the difference in mandibular shape between genera) associated with the diet signal is predominant and that the other covariables do not appear as relevant in this situation.

Results for mandibular shape variation (Table 3) are very different when considering the reduced sample (i.e. the sample without *Hapalemur* and *Prolemur* specimens). In this case, all the models, except the null model, were significant but the R^2 values were different from one model to another. The most likely model is the one considering the covariables Phylogeny, Diet, Habitat and Sex. If we decipher the different covariables of this model responsible for mandibular shape variation, Diet still plays a significant role ($F=131.7$, $p<0.001$). For Habitat covariables, mandibular shapes are different between Ecoregions ($F=3.617$, $p<0.001$) but they

are not different between forest types. Mandibular shapes are not different between males and females nor between the two types of sexual behaviours expressed in our sample (dominant females or dominant males). However, mandibular shapes are significantly different between the two different types of mating systems (monogamous and polygynous). Then, we also found a difference of mandibular shape between the three genera included in this analysis ($F=18.5$, $p<0.01$).

- **Covariations with mandibular size**

The most likely models to explain mandibular size variation are the one with the phylogeny and diet as covariables, for the full sample (Table 2). In the case of the full sample analysis, the effect of Diet on mandibular size variation is highly significant ($F=12$, $p<0.001$), the effect of the phylogeny is also significant but with lower values ($F=3.4$, $p=0.031$). In the case of the reduced sample, if the best models are the same as with the entire sample, the AICc values are very similar and the R^2 values are low and not significant. In this case, size variation is probably too low to be explained.

Discussion

Our results illustrate the high level of mandibular shape variation in the family Lemuridae. This high variation is mainly driven by the fact that the specimens of the genera *Prolemur* and *Hapalemur* have different mandibular morphologies than the other genera of the family. Such differences are likely explained by a phylogenetic effect combined with an adaptation to folivory. As specimens of the genera *Hapalemur* and *Prolemur* are the only folivorous of our sample, it is not possible, in this case to explain the differences in mandibular shape from the other genera by a phylogenetic effect or by a difference in diet. Specimens of these genera indeed present mandibular shapes with very robust and short mandibular bodies, with deep mandibular symphysis, even deeper and wider than those of the great apes (Taylor, 2003; Ravosa, 1991), and a large muscular insertion on the ascending ramus. This is certainly an

adaptive specialization to their diet, which in *Hapalemur* is based on bamboo (Wright & Willis, 2012; Grassi, 2006). The difference has been located not only in the choice of food variety but also in the choice of food qualities (Tan, 1999; Grassi, 2006). Indeed, *Hapalemur griseus* has been exploiting mainly shoot stems and young bamboo leaves (Rabarivola et al., 2007) while *Prolemur simus* also feeds on hard leaves, also requiring high bite strength (Vinyard et al., 2008).

Among most living prosimians (with the exception of *Daubentonia* and the Indriids), it is inferred that the mandibular corpus is probably not powerfully bent during biting along the anterior dentition since their anterior teeth, which are either absent or are quite gracile and not firmly rooted, are poorly adapted to withstand powerful axial and bending stresses (Kay & Hylander, 1978). Leaf chewing species probably requires a larger average biting force than frugivorous species, and the leaf chewing species spend a greater amount of time chewing food compared to the frugivorous (Hylander, 1979).

In this case, *Prolemur simus* has an even deeper and wider body and mandibular symphysis and a higher ascending ramus than *Hapalemur griseus*. Such developed structures imply, on the coronoid process, larger insertions of the muscle *temporalis* and of the sphenomandibular ligaments (along the lingula of the mandible), responsible for an optimal elevation of the mandible, maximal leverage and accurate contractile strength for hard item consumption (Pick & Howden, 1977). Developed ascending ramus combined with developed angular processes allow large insertions of the muscles *masseter* (deep and superficial), *zygomatico mandibularis* and *medial pterygoid* also responsible for elevation of the mandible during mastication movements. A developed symphysis rather indicates developed insertions for the muscles *digastric*, *stylo-hyoid*, *mylo-hyoid* and *genio-hyoid*, all depressors of the mandible. These large insertions indicate large and powerful muscles with higher leverage and thus higher chewing strength (Pick & Howden, 1977).

In contrast, in the *Eulemur, Lemur* and *Varecia* genera, the mandible reflects a longitudinal development, a long aspect of the articular condyle and a long and shallow mandibular body and symphysis with short ascending rami. These traits are explained by a rather soft diet (frugivorous), composed essentially of leaves, flowers and fruits. The masticatory apparatus, that is to say, the surfaces of muscular insertions on bones and the volume of muscles, is less developed with lower chewing strength (Raveloson, 2007; Ravosa, 1991). The mandibular shape differences between genera within lemurids can also be explained by the manner of food processing employed by the animals. For instance, specimens of the species *Varecia variegata* use the anterior teeth to grasp and move the feeding objects (Raveloson et al., 2005). As a result, much of the stress flow is reported to the anterior part of the jaw (Chivers et al., 1984; Preuschoft & Witzel, 2005), biting forces being higher on the anterior parts of the mandible (at incisors, canine and premolar) rather than on the posterior part (Marcé-Nogué et al., 2017). The chewing movement of the mandible has been reported by previous authors working on chewing movements (Ross et al., 2009). They identified the peculiar length of lemurids mandible (especially in *Eulemur*, *Lemur* and *Varecia* species), comparatively to mandibular mass and body mass, that is out of the range of a general primate model. The authors conclude that the lemurids use their gracile and long mandibles in faster chewing cycles than the primates, reinforcing the idea of singularity for the group.

Such variation within the lemurids and the high difference between folivorous and non folivorous species have already been reported on cranial shape (*e.g.* Andrews et al., 2020 work on palate shape variation) as well as on mandibular shape. For instance, in the work published in 2005 Raveloson et al., highlighted this difference, and separated their analysis in two sub analyses, with and without the folivorous specimens (all classified as *Hapalemur* in their case). The aim of Raveloson et al. 2005's work was to evaluate the method and separate the mandibular morphologies of the genera composing the sample. Our work has different aims but could be seen as complementary to Raveloson's earlier work. Our results support

Raveloson et al. 2005's conclusions of a great mandibular shape variation within the family. This high variation was also suggested by other authors, based on the morphometric analysis of the mandibular shape (Viguier, 2004 ; St Clair et al., 2018, Oh et al., 2019). Here, we also report an high level of variation with the genera. Such variation was also reported by Raveloson et al., 2005 but for *Hapalemur* only. The authors explained this variation by differences in dietary compositions between species. Our results indicate that the high level of variation for each genera composing our sample, not only for *Hapalemur*. The direct correlation between mandibular shape variation and dietary shifts could explain such variation. However, the direct link between mandibular shape variation and diet specialization is questionable. It is quite clear that the folivorous species have a particular mandibular morphology but one could think that, in the case of a selective pressure driven by a morphofunctional adaptation, the expected level of variation should be low (Futuyma & Moreno, 1988). This is not the case for the folivorous species of our sample. The difference in diet could explain the mandibular shape morphology for the folivorous but some other covariables could explain the intra generic shape variation. Some previous studies have mentioned the fact that other covariables than diet could have an effect on mandibular shape variation in strepsirrhines. For instance, in 2018, Fabre et al. have quantified the covariation between mechanical and architectural properties of the mandibular muscles and the mandible shape variation. The level of covariation found by these authors led them to the conclusion that, because it was difficult to identify the biomechanical patterns associated with diet, the mandible could reflect other non-dietary systems that permit it to vary. An assumption of mandibular random shape variation was even formulated. Our results indicate a true covariation of mandibular morphology with diet, but also with other covariables non-dietary covariables.

Another important result is the position of the ring-tailed lemurs in the morphospace. *Lemur catta* is frugivorous/folivorous but its mandibular morphology is highly different from the other frugivorous/folivorous species. *Lemur catta* became frugivorous/folivorous or even opportunistic because of the destruction of its habitat and the insufficiency of food. The species

shifted its diet seconderly and managed to eat seeds and leaves of the tamarind species *Tamarindus indica* (Jolly et al., 2006; Yamashita et al., 2015). Given the hardness of this fruit, the shift in food items is related to the increase in the ascending branch of their mandible by exerting masseter muscular forces during mastication relatively long (Vinyard et al., 2006). The consumption of tamarind leaves also has consequences. As the dietary shift is relatively recent, the adaptation process of the mandible shape is probably still incomplete. Dental pathologies, mandibular wear are observed (Sauther & Cuozzo, 2009) and unconsumed part of leaves are found in fecal matters (Yamashita, 2003). In that case, dental and masticatory morphologies are not fully adapted to processing these foods items and thus a mismatch is observable between diet of *Lemur catta* and mandibular morphology (Yamashita et al., 2012).

In our study based on the reduced sample (without *Hapalemur* and *Prolemur*), the most likely model is the one considering the entire set of covariables with significant effect of mating system and ecoregions when the variables sex, sexual behaviour and type of forest were not covarying with mandibular shape. It is well known that sexual dimorphism in low in strepsirrhines and probably not correlated with body weight, or canine size (Kappeler, 1989, 1990, 1991; Kappeler & Heymann, 1996; Jenkins & Albrecht, 1991; Thorén et al., 2006). It is thus not very surprising that we did not find significant differences in mandibular shape between sexes (even if few studies focused on mandibular shape variation). Sexual behaviour does not covary with mandibular shape. Here again, several authors have previously expressed that the lack of sexual dimorphism was probably related to the dominance of females over males (Richard, 1987; Richard & Nicoll, 1987). Female dominance could diminish the competition between males and, as a consequence, reduce the level of sexual dimorphism (Jenkins & Albrecht, 1991). Our results indicate that the mating system of lemurids has a significant effect on mandible shape variation. Several authors have reported the fact that intrasexual competition is higher in polygynous species. The male-male competition and agonistic behaviors can be observed for female access during mating season as described in *Lemur catta* (Mittermeier et al., 2010). Moreover, the availability of food for each individual in

a group also depends on the number of individuals in the group, and the monogamous mating system provides an advantage over the polygynous one on this point (Kling & Wright, 2018). Intergroup agonistic behaviors are also described for food access in *Eulemur macaco* (Bayard & Simmen, 2005). It is known that the availability of food for each individual in a group depends on the number of individuals in the group and that, on this point, monogamous groups have more advantages than multimale-multifemale groups (Kling and Wright, 2018). Our results suggest that inter individual competition in large groups has an effect on mandibular shape variation, possibly in modifying the musculature. Several authors have shown that in strepsirrhines, canine size is not a sexually dimorphic trait and that canine size is not covarying with the mating system (Kapeller 1990, 1996). Very little is known about the mandibular shape variation and we think that it would be important to focus on this anatomical part.

Our results indicate that mandibular shape does not vary with types of inhabited forests. We expected a significant covariation of these variables mainly because the type of forest is supposed to describe the type of available food items and thus to correspond in a way to the dietary categories. Our results do not show such interaction. Two conclusions can be expressed: the first one is that the possible covariation between the type of forest and the mandibular shape variation is indeed not significant. The second is that the possible covariation between the type of forest and mandibular shape exists but the method to classify the forests is not optimal to categorize the animal's habitats. Previous authors have shown that forest classifications are difficult to homogenize (Humbert, 1954; Faramalala, 1995; Moat, 1996). Then, the Madagascar landscapes are more structured by the biogeographic regions rather than by a classification of the forest types (Ganzhorn et al., 2006; Mittermeier et al., 2014).

Our results indicate a significant covariation of mandibular shape with ecoregions. Covariations of primate morphology with geographical distribution (taking ecoregions as great categories of distribution) are known for many years (Albrecht, 1990; Albrecht & Miller, 1993). These studies mainly focused on cranial size variation. Albrecht et al. (1990) explained the pattern of ecogeographical size difference by the fact that larger animals are found in wetter and more

productive habitats than smaller ones. Moreover, other works reported that western species in the tropical forest or dry forest are smaller compared to eastern species in rainforest (Eppley et al., 2017). The ecogeographical difference is not only visible on size (cranial size in the case of Viguier, 2002), but also on cranial shape variation (in this case). Our results on the mandible support these results considering the entire sample, but not considering the reduced one were size variation was not explained by the covariables.

The natural forest of Madagascar has suffered a great loss of forest cover inhabited by lemurs (Vieilledent et al., 2017; Ganzhorn et al., 2000). Habitat loss, habitat fragmentation due to anthropogenic activities or climate change have a negative impact on primates (Almeida-Rocha et al., 2017). Madagascar is a hotspot of biodiversity but it is also one of the most threatened areas with almost half of its forest cover lost since 1953 (Vieilledent et al., 2017; Kling et al., 2019). Landscape fragmentation mainly causes a decrease in lemur species richness (Ganzhorn, 1998; Ganzhorn et al., 2000). Our results illustrate the fact that multiple factors can act simultaneously on the morphological variation. These biotic and abiotic confounding factors are highly important to identify the true effects of environmental changes and their consequences. Such results are important in the context of conservation and sustainable remediation, and contribute to a good knowledge of biodiversity.

Conclusion

A quantitative estimation of the morphological variation, using 2D geometric morphometric methods have been proposed. Our results illustrate the high level of mandibular variation within the family Lemuridae. We have highlighted a strong phylogenetic effect in this family, with the clear separation of the genera *Hapalemur* and *Prolemur* from the other genera of the family. This result was already stated in previous studies. Our results showed that diet was not the only factor interacting with mandible shape variation, which, rather is influenced by a complex signal with a combination of factors. These findings are important for the understanding of variation and diversity and should be taken into account for instance for conservation plans.

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Data are available upon request to the authors

Authors contribution :

Miarisoa Jeanne Emma: Investigation ; writing-original draft ; writing-review and editing. **Raveloson Herimalala:** writing-review and editing. **Randrianambinina Blanchard:** writing-review and editing, **Sébastien Couette:** Conceptualization ; formal analysis ; writing-original draft; writing-review and editing.

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References:

Adams, D.C. (2014). A generalized K statistic for estimating phylogenetic signal from shape and other high-dimensional multivariate data. *Systematics Biology,* 63, 685–697. DOI: 10.1093/sysbio/syu030

Adams, D.C. & Otárola-Castillo, E. (2013). Geomorph: An R package for the collection and analysis of geometric morphometric shape data. *Methods in Ecology and Evolution,* 4(4), 393-399. https://doi.org/10.1111/2041-210X.12035

Albrecht, G.H. & Miller, J. (1993). Geographic variation in primates. In: Kimbel, W. & Martin & L.B. (Eds.). Species, species concepts and primate evolution. Springer, 123-161. https://doi.org/10.1007/978-1-4899-3745-2_6

Albrecht, G.H., Jenkins, P.D. & Godfrey, L.R. (1990). Ecogeographic size variation among the living and subfossil prosimians of Madagascar. *American Journal of Primatology*, 22(1), 1-50. https://doi.org/10.1002/ajp.1350220102

Allnutt, T.F., Ferrier, S., Manion, G., Powell, G.V.N., Ricketts, T.H. & Fisher, B.L. et al. (2008). A method for quantifying biodiversity loss and its application to a 50-years record of deforestation across Madagascar. *Conservation Letters,* 1, 173 -181. https://doi.org/10.1111/j.1755-263X.2008.00027.x

Almeida-Rocha, J.M.D, Peres, C.A. & Oliveira, L.C. (2017). Primates responses to anthropogenic habitat disturbance: A pantropical meta analysis. *Biological Conservation*, 215, 30-38. https://doi.org/10.1016/j.biocon.2017.08.018

Andrews, C., Masters, J.C., Génin, F., Couette, S. (2020). The evolution of palate shape in the Lepilemur-Cheirogaleidae clade (Primates: Strepsirrhini). *American Journal of Physical Anthropology, 24093*, 1-15. DOI:10.1002/ajpa.24093

Baab, K.L., Perry, J.M.G., Rohlf, J.F. and Jungers, W.L. (2014). Phylogenetic, Ecological, and allometric correlates of cranial shape in malagasyMalagasy lemuriforms. *The Society for the Study of Evolution,* 68(5), 1450–1468.https://doi.org/10.1111/evo.12361

Bailey, R.C. & Byrnes, J. (1990). A new, old method for assessing measurement error in both univariate and multivariate morphometric studies. *Sytematic zoology*, 39(2), 124-130.https://doi.org/10.2307/2992450

Balestri, M., Barresi, M., Campera, M., Serra, V., Ramanamanjato, J.B., Heistermann, M., Donati, G. (2014). Habitat Degradation and Seasonality Affect Physiological Stress Levels of *Eulemur collaris* in Littoral Forest Fragments. *PLoS ONE*, 9(9), e107698.https://doi.org/10.1371/journal.pone.0107698

Battistini, R. (1996). Paléogéographie et variété des milieux naturels à Madagascar et dans les îles voisines : quelques données de base pour l'étude biogéographique de la « région Malgache ». In : Lourenço, W.R. (ed.). *Biogéographie de Madagascar*, Paris : Éditions de l'ORSTOM, 1-17.

Bayard, F. & Simmen, B. (2005). Demography, range use, and behavior in black lemurs (*Eulemur macaco macaco*) at Ampasikely, northwest Madagascar. *American Journal of Primatology*, 67(3), 299-312. https://doi.org/10.1002/ajp.20186

Beecher, R. M. (1977). Function and fusion at the mandibular symphysis. *American Journal of Physical Anthropology*. 47, 325-336.

Bennett, C.V. and Goswami, A. (2012). Morphometric Analysis of Cranial Shape in Fossil and Recent Euprimates. *Anatomy Research International*, 1-7. https://doi.org/10.1155/2012/478903

Bonhomme, V., Picq, S., Gauchel, C. & Claude, J. (2014). Momocs : Outilne Analysis Using R. journal of Statistical Software, 56(13), 1-24. https://doi.org/10.18637/jss.v056.i13

Burnham, K. P., Anderson, D. R. (2004) Multimodel inference: understanding AIC and BIC in model selection. *Sociological Methods and Research* 33, 261–304.

Chivers, D.J., Andrews, P., Preuschoft, H., Bilsborough, A. & Wood, B.A. (1984). Food acquisition and processing in primates: concluding discussion. In: Chivers, D.J., Wood, B.A. & Bilsborough, A. (Eds.). *Food acquisition and processing in Primates*, Boston: Springer, 554-556.https://doi.org/10.1002/ajpa.1330690313

Claude, J. (2008). *Morphometrics with R*. Springer Science & Business Media. 330 p. https://doi.org/10.1007/978-0-387-77789-4

Coiner-Collier, S., Scott, R.S., Chalk-Wilayto, J., Cheyne, S.M., Constantino, P. & Dominy N.J. et al. (2016). Primate dietary ecology in the context of food mechanical properties. *Journal of Human Evolution*, 98: 103–118. https://doi.org/10.1016/j.jhevol.2016.07.005

Cornet, A. (1974). *Essai de cartographie bioclimatique à Madagascar.* Paris : ORSTOM, 28 p.

Crampton, J. S. (1995). Elliptic Fourier shape analysis of fossil bivalves: some practical considerations. *Lethaia*, 28(2), 179-186.https://doi.org/10.1111/j.1502-3931.1995.tb01611.x

Cuozzo, F.P., Sauther, M.L. (2006). Severe wear and tooth loss in wild ring-tailed lemurs (*Lemur catta*): A function of feeding ecology, dental structure, and individual life history. *Journal of Human Evolution*, 51(5), 490–505. https://doi.org/10.1016/j.jhevol.2006.07.001

Dausmann , K.H. and Warnecke, L. 2015. Primate Torpor Expression: Ghost of the Climatic Past. *Physiology*. 31 : 398-408. doi:10.1152/physiol.00050.

Daegling, D.J., Grine, F.E. (2006). Mandibular biomechanics and the paleontological evidence for the evolution of human diet. In P. Ungar (Ed.), Evolution of the human diet: *The known, the unknown, and the unknowable*, 77–105. Oxford University Press.

Daegling, D.J., Jungers, W.L. (2000). Elliptical Fourier analysis of symphyseal shape in great ape mandibles. *Journal of Human Evolution*, 39(1), 107-122. https://doi.org/10.1006/jhev.2000.0402

De Wit, M.J. (2003). Madagascar: Heads It's a Continent, Tails It's an Island. Annual Review of Earth and Planetary Sciences, 3151, 213–248. https://doi.org/10.1146/annurev.earth.31.100901.141337

Du Puy, D.J. & Moat, J. (1996). A refined classification of the primary vegetation of Madagascar based on the underlying geology: using GIS to map its distribution and to assess its conservation status. In: Lourenço, W.R. (ed.). In : Lourenço, W.R. (ed.). *Biogéographie de Madagascar*, Paris : Éditions de l'ORSTOM, 205-218.

Dumont, E.R. (1997). Cranial Shape in Fruit, Nectar, and Exudate Feeders : Implications for Interpreting the Fossil Record. *American Journal of Physical Anthropology*, 102, 187– 202. https://doi.org/10.1002/(SICI)1096-8644(199702)102:2<187::AID-AJPA4>3.0.CO;2-W

Eppley, T.M., Santini, L., Tinsman, J.C., Donati, G. (2020). Do functional traits offset the effects of fragmentation? The case of large-bodied diurnal lemur species. *American Journal of Primatology*, e23104. https://doi.org/10.1002/ajp.23104

Eppley, T.M., Watzek, J., Hall, K., Donati, G. (2017). Climatic, social and reproductive influences on behavioural thermoregulation in a female-dominated lemur. *Animal Behaviour*, 134, 25-34.https://doi.org/10.1016/j.anbehav.2017.10.003

Eronen, J.T., Zohdy, S.E., Tecot, S.R., Wright, P.C., & Jernvall J. (2017). Feeding Ecology and Morphology Make a Bamboo Specialist Vulnerable to Climate Change. *Current Biology*, 27(21), 3384–3389.https://doi.org/10.1016/j.cub.2017.09.050

Estrada, A., Garber, P.A., Rylands, A.B., Roos, C., Fernandez-Duque, E. & Di Fiore, A. et al. (2017). Impending extinction crisis of the world's primates: Why primates matter. *Science Advances*, 3(1), e1600946. https://doi.org/10.1126/sciadv.1600946

Fabre, A-C., Perry, J.M.G., Hartstone-Rose, A., Lowie A., Boens A. & Dumont, M. (2018). Do Muscles Constrain Skull Shape Evolution in Strepsirrhines? *The Anatomical Record*, 301(2), 291-310. https://doi.org/10.1002/ar.23712

Faramalala, M.H. (1995). Formations végétales et domaine forestier national de Madagascar. *Conservation International*, 1.

Fleagle, J.G., Gilbert, C.C. and Baden, A.L. (2010). Primate Cranial Diversity. *American Journal of Physical Anthropology,* 142, 565–578. https://doi.org/10.1002/ajpa.21272

Fleagle, J.G., Gilbert, C.C., Baden, A.L. (2016). Comparing primate crania: The importance of fossils. *American Journal of Physical Anthropology*, 161: 259–275.https://doi.org/10.1002/ajpa.23026

Futuyma, D.J. & Moreno, G. (1988). The evolution of ecological specialization. *Annual review of Ecology and Systematics*, 19(1), 207-233.

Ganzhorn, J.U., Goodman, S.M., Nash, S. & Thalmann, U. (2006). Lemur biogeography. In: Lehman, S.M. & Fleagle, J.G. (Eds.). *Primate biogeography*, Boston: Springer, 229-254. https://doi.org/10.1007/0-387-31710-4_8

Ganzhorn, J.U., Goodman, S.M., Ramanamanjato, J.-B., Ralison, D.J., Rakotondravony, & Rakotosamimanana, B. (2000). Effects of fragmentation and assessing minimum viable populations of lemurs in Madagascar. In: Isolated Vertebrate Communities in the Tropics Proc. 4th. International Symposium, Bonner zoologische Monographien, 46, 365-372.

Glander, K.E., Wright, P.C., Daniels, P.S., Merenlender, A.M. (1992). Morphometrics and testicle size of rainforest lemur species from southeastern Madagascar. *Journal of Human Evolution*, 22(1), 1-17.https://doi.org/10.1016/0047-2484(92)90025-5

Grassi, C. (2006). Variability in habitat, diet, and social structure of *Hapalemur griseus* in Ranomafana National Park, Madagascar. *American Journal of Physical Anthropology*, 131(1), 50-63.https://doi.org/10.1002/ajpa.20423

Groves, C.P., Eaglen, R.H. (1988). Systematics of the Lemuridae (Primates, Strepsirhini). *Journal of Human Evolution*, 17(5), 513-538.https://doi.org/10.1016/0047-2484(88)90040-1

Guy, F., Mackaye, H.T., Likius, A., Vignaud, P., Schmittbuhl, M., Brunet, M. (2008). Symphyseal shape variation in extant and fossil hominoids, and the symphysis of *Australopithecus bahrelghazali*. *Journal of Human Evolution*, 55(1), 37-47. https://doi.org/10.1016/j.jhevol.2007.12.003

Harper, G.J., Steininger, M.K., Tucker, C.J., Juhn, D., Hawkins, F. (2007). Fifty years of deforestation and forest fragmentation in Madagascar. *Environmental Conservation*, 34(4), 25-333.DOI: https://doi.org/10.1017/S0376892907004262

Herrera, J.P. & Dàvalos, L.M. (2016) Phylogeny and Divergence Times of Lemurs Inferred with Recent and Ancient Fossils. *Society of Systematic Biologists*, 65(5), 772-79. DOI: 10.1093/sysbio/syw035

Humbert, H. (1954). Flore de Madagascar et des Comores, 130ème famille: Canellacées. Paris, 46- 52.

Humphrey, L.T., Dean, M.C., Stringer, C.B. (1999). Morphological variation in great apes and modern human mandibles. *Journal of Anatomy*, 195(4):491–513.DOI: https://doi.org/10.1046/j.1469-7580.1999.19540491.x

Hylander, W.L. (1979) The functional significance of primate mandibular form. *Journal of Primatologica*, 160(2), 223-239. https://doi.org/10.1002/jmor.1051600208

Hylander, W.L. (1979). The functional significance of primate mandibular form. *Journal of Morphology.* 160:223–240.

IUCN, 2021. *The IUCN Red List of Threatened Species. Version 2021-3*. https://www.iucnredlist.org.

Jenkins, P.D. & Albrecht, G.H. (1991) Sexual dimorphism and sex ratios in Madagascan prosimians. *American Journal of Primatology*, 24(1), 1-14. https://doi.org/10.1002/ajp.1350240102

Jolly, A., Sussman, R. W., Koyama, N., & Rasamimanana, H. (Eds.). (2006). *Ringtailed lemur biology: Lemur catta in Madagascar*. Springer Science & Business Media.

Jungers, W.L. (1991) Scaling of Postcranial Joint Size in Hominoid Primates. *Human Evolution,* 6(5-6), 391-399. https://doi.org/10.1007/BF02435532

Jungers, W.L., Smith, R.J. (1997). Body mass in comparative primatology. *Journal of Human Evolution*, 32, 523–559. https://doi.org/10.1006/jhev.1996.0122

Kappeler, P. M. (2014). Lemur behavior informs the evolution of social monogamy. *Trends in Ecology & Evolution*, 29(11), 591-593. https://doi.org/10.1016/j.tree.2014.09.005

Kappeler, P.M. (1989) Agonistic and grooming behavior of captive crowned lemurs (*Lemur coronatus*) during the breeding season. *Human Evolution*, 4(2-3), 207-215. https://doi.org/10.1007/BF02435450

Kappeler, P.M. (1990) The evolution of sexual size dimorphism in prosimian primates. *American Journal of Primatology*, 21(3), 201-214. https://doi.org/10.1002/ajp.1350210304

Kappeler, P.M. (1991) Patterns of Sexual Dimorphism in Body Weight among Prosimian Primates. *Folia Primatologica*, 57(3), 132-146. https://doi.org/10.1159/000156575

Kappeler, P.M. & Heymann, E.W. (1996) Nonconvergence in the evolution of primate life history and socio-ecology. *Biological Journal of the Linnean Society*, 59(3), 297-326. https://doi.org/10.1006/bijl.1996.0067

Kay, R.F. & Hylander, W.L. (1978) The dental structure of mammalian folivores with special reference to primates and phalangeroidea (Marsupialia). In: Montgomery, G.G. (Ed.) *Ecology of Arboreal Folivores.* Washington DC: Smithsonian Institution, 173-191.

Kistler, L., Ratan, A., Godfrey, L.R., Crowley, B.E., Hughes, C.E. & Lei, R., et al. (2015). Comparative and population mitogenomic analyses of Madagascar's extinct, giant subfossil lemurs. *Journal of Human Evolution*, 79, 45-54. https://doi.org/10.1016/j.jhevol.2014.06.016

Kling, K.J. & Wright, P.C. (2018). Reference Module in Life Sciences II Mating Systems of Lemurs. *Encyclopedia of Animal Behavior*, 2nd edition (4), 1-7.https://doi.org/10.1016/B978-0-12-809633-8.20693-6

Kuhl, F. P. & Giardina, C.R. (1982) Elliptic Fourier features of a closed contour. *Computer Graphics and images processing*, 18(3), 236-258. https://doi.org/10.1016/0146-664X(82)90034-X

LaFleur, M., Clarke, T.A., Reuter, K.E., Shaefer, M.S., Horst, C. (2019) Illegal Trade of Wild-Captured *Lemur catta* within Madagascar. *Folia Primatologica*, 90, 199-214. https://doi.org/10.1159/000496970

Lawing, A.M. and Polly, P.D. (2010). Geometric morphometrics: recent applications to the study of evolution and development. *Journal of Zoology*, 280(1), 1-7. https://doi.org/10.1111/j.1469-7998.2009.00620.x

Marcé-Nogué, J., Püschel, T.A., Kaiser, T.M. (2017). A biomechanical approach to understand the ecomorphological relationship between primate mandibles and diet. *Scientific Reports*, 7, 8364.https://doi.org/10.1111/j.1469-7998.2009.00620.x

Masters, J.C., Couette, S. (2015). Characterizing cryptic species: A morphometric analysis of craniodental characters in the dwarf galago genus Galagoides. *American Journal of Physical Anthropology*, 158, 288–299. https://doi.org/10.1002/ajpa.22792

Masters, J.C., Génin, F., Silvestro, D., Lister, A. M., DelPero, M. (2014). The red Island and the seven dwarfs: Body size reduction in Cheirogaleidae. *Journal of Biogeography*, 41, 1833-1847.https://doi.org/10.1111/jbi.12327

Mazerolle, M.J. (2020). AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package, version 2.3-1. https://cran.r-project.org/package=AICcmodavg.

Meloro, C., Carlos, C.N., Carotenuto, F., Sponchiado, J., Melo, G.L. & Passaro, F. et al. (2015) Chewing on the trees: Constraints and adaptation in the evolution of the primate mandible. *The Society for the Study of Evolution*, 69(7), 1690-1700. https://doi.org/10.1111/evo.12694

Mittermeier, R.A., Konstant, W.R., Hawkins, F., Louis, E.E., Langrand, O. & Ratsimbazafy, J., et al. (2006). Lemurs of Madagascar : Conservation International Tropical Field Guide Series. Washington, Conservation International.

Mittermeier, R.A., Louis, E.E., Richardson, M., Schwitzer, C., Langrand, O. & Rylands, A.B. et al. (2010) Lemurs of Madagascar. *Third edition. Conservation International, Washington, DC, USA*.

Moat, J. (1996). A refined classification of the primary vegetation of Madagascar based on the underlying geology: Using GIS to map its distribution and to assess its conservation status. *Biogeographic de Madagascar*, 205-218.

Morelli, T.L., Smith, A.B., Mancini, A.N., Balko, E.A., Borgerson, C., & Dolch R., et al. (2020). The fate of Madagascar's rainforest habitat. *Nature Climate Change*, 10(1), 89-96. https://doi.org/10.1038/s41558-019-0647-x.

Oh, H., Knigge R., Hardin, A., Sherwood, R. & Duren, D. et al. (2019). Predicting adult facial type from mandibular landmark data at young ages. *Orthodontics & Craniofacial Research*, 22(S1), 154-162.https://doi.org/10.1111/ocr.12296

Perelman, P., Johnson, W.E., Roos, C., Seuánez, H.N., Horvath, J.E. & Moreira M.A.M. et al. (2011). A Molecular Phylogeny of Living Primates. *PLoS Genetics*, 7(3), e1001342. https://doi.org/10.1371/journal.pgen.1001342

Perez, S.I., Klaczko, J., Rocatti, G. and Dos Reis, S.F. (2011). Patterns of cranial shape diversification during the phylogenetic branching process of New World monkeys (Primates: Platyrrhini). *Journal of Evolutionary Biology* 24, 1826-1835. https://doi.org/10.1111/j.1420-9101.2011.02309.x

Pick, T.P. & Howden, R. (1977). Anatomy, descriptive and surgical. *Gray. A revised American from the Fifteenth English Edition*.

Pitirri, M.K. & Begun, D. (2019). A new method to quantify mandibular corpus shape in extant great apes and its potential application to the hominoid fossil record. *American Journal of Physical Anthropology*, 168(2), 318-328. https://doi.org/10.1002/ajpa.23749

Pitirri, M.K., Vermeulen, E., Komza, K. & Begun, D. (2020). Mandibular shape variation in mainland and insular hylobatids. *American Journal of Primatology,* e23175. https://doi.org/10.1002/ajp.23175

Pozzi, L., Hodgson, J.A., Burrell, A.S., Sterner, K.N., Raaum, R.L., Disotell, T.R. (2014). Primate phylogenetic relationships and divergence dates inferred from complete mitochondrial

genomes. *Molecular Phylogenetics and Evolution*, 75, 165-183. https://doi.org/10.1016/j.ympev.2014.02.023

Preuschoft, H. & Witzel, U. (2005). Functional shape of the skull in vertebrates: Which forces determine skull morphology in lower primates and ancestral synapsids? *The Anatomical Record,* 283A(2), 402-413. https://doi.org/10.1002/ar.a.20176

R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rabarivola, C., Prosper, P., Zaramody, A., Andriaholinirina, A. & Hauwy, M. (2007). Cytogenetics and taxonomy of the genus *Hapalemur*. *Lemur News* 12, 46-49.

Raveloson, H., (2007). Contribution à l'étude de la variabilité morphologique du squelette crânien et à l'étude comparative de la morphologie externe de l'appareil génital chez les Lemuridae (Lenuriformes, Primates). *Mémoire de thèse*, 209 pages.

Raveloson, H., Le Minor, J-M., Rumpler, Y. & Schmittbuhl, M. (2005). Shape of the Lateral Mandibular Outline in Lemuridae: A Quantitative Analysis of Variability Using Elliptical Fourier Analysis. *Folia Primatologica*, 76, 245-261. https://doi.org/10.1159/000088033

Ravosa, M.J. (1990). Functional assessment of subfamily variation in maxillomandibular morphology among Old-World monkeys. *American Journal of Physical Anthropology,* 82(2), 199–212.https://doi.org/10.1002/ajpa.1330820209

Ravosa, M.J. (1991). Structural allometry of the mandibular corpus and symphysis in prosimian primates. *Journal of Human Evolution,* 20, 3-20. https://doi.org/10.1016/0047-2484(91)90042-T

Ravosa, M.J. (1992). Allometry and heterochrony in extant and extinct malagasyMalagasy primates. *Journal of Human Evolution,* 23(2), 197-217. https://doi.org/10.1016/0047-2484(92)90107-K

Ravosa, M.J. (2000). Size and scaling of the mandible in living and extinct apes. *Folia Primatologica* 71:305–322.

Renaud, S., & Michaux, J. R. (2003). Adaptive latitudinal trends in the mandible shape of *Apodemus* wood mice. *Journal of Biogeography*, 30(10), 1617-1628. https://doi.org/10.1046/j.1365-2699.2003.00932.x

Reuter, K.E. & Schaefer, M.S. (2017). Motivations for the ownership of captive lemurs in Madagascar. *Anthrozoös,* 30, 33-46. https://doi.org/10.1080/08927936.2017.1270589

Reuter, K.E., Schaefer, M.S. (2016a). Illegal captive lemurs in Madagascar: comparing the use of online and in-person data collection methods. *American Journal of Primatology*, 79: 1-9. https://doi.org/10.1002/ajp.22541

Richard, A. (1987). MalagasyMalagasy prosimians: female dominance. In: Smuts, B.B, Cheney, D.L., Seyfarth, R.M., Wranghram, R.W. & Struhsaker, T.T. (Eds.): *Primate societies*. Chicago: University of Chicago press, 25-33.

Richard, A. & Nicoll, M. (1987). Female social dominance and basal metabolism in a malagasyMalagasy primate, *Propithecus verreauxi*. *American Journal of Primatology,* 12, 309-314. https://doi.org/10.1002/ajp.1350120308

Rohlf, F. J. (2015). The tps series of software. *Hystrix*, 26(1). https://doi.org/10.4404/hystrix-26.1-11264

Rohlf, F. J., & Archie, J. W. (1984). A comparison of Fourier methods for the description of wing shape in mosquitoes (Diptera: Culicidae). *Systematic Zoology*, 33(3), 302-317. https://doi.org/10.2307/2413076

Ross, C.F, Washington, R.L., Eckhardt, A., Reed, D.A., Vogel, E.R. & Dominy, N.J. et al. (2009). Ecological consequences of scaling of chew cycle duration and daily feeding time in

Primates. *Journal of Human Evolution*, 56(6), 570-585. https://doi.org/10.1016/j.jhevol.2009.02.007

Ross, C.F., Iriarte-Diaz, J., Charles, L.N. (2012). Innovative Approaches to the Relationship Between Diet and Mandibular Morphology in Primates. *International Journal of Primatology* , 33(3), 632–660.https://doi.org/10.1007/s10764-012-9599-y

Roullet, D. (2012). The role of the captive population of greater bamboo lemurs *Prolemur simus* in the conservation of the species. *Lemur News*, 16, 20-25.

Sauther, M.L., Cuozzo, F.P. (2009) Behavioral responses to tooth loss in wild ring-tailed lemurs (*Lemur catta*) at the Beza Mahafaly Special Reserve, Madagascar. *American Journal of Physical Anthropology*, *140*(1), 120-134. https://doi.org/10.1002/ajpa.21045

Sauther, M.L., Cuozzo, F.P. (2012). Understanding Eocene primate palaeobiology using a comprehensive analysis of living primate ecology, biology and behavior. *Palaeobiodiversity and Palaeoenvironments,* 92(4), *573–583.*https://doi.org/10.1007/s12549-012-0089-1

Sauther, M.L., Cuozzo, F.P., Sussman, R.W. (2001). Analysis of Dentition of a Living Wild Population of Ring-Tailed Lemurs (*Lemur catta*) From Beza Mahafaly, Madagascar. *American Journal of Physical Anthropology,* 114, 215–223. https://doi.org/10.1002/1096-8644(200103)114:3<215::AID-AJPA1021>3.0.CO;2-J

Schmittbuhl, M., Rieger, J., Le Minor, J.M., Schaaf, A. & Guy, F. (2007). Variations of the mandibular shape in extant hominoids: Generic, specific, and subspecific quantification using elliptical Fourier analysis in lateral view. *American Journal of Physical Anthropology*, 132(1), 119-131. https://doi.org/10.1371/journal.pone.0049521

Scott, J. E. (2012). Molar size and diet in the Strepsirrhini: Implications for size-adjustment in studies of primate dental adaptation. *Journal of Human Evolution,* 63, 796-804.

Seiler, M., Holderied, M. & Schwitzer, C. (2013). Effects of Habitat Degradation on Sleeping Site Choice and Use in Sahamalaza Sportive Lemurs (*Lepilemur sahamalazensis*). *International Journal of Primatology,* 34(2), 260-280. 10.1007/s10764-013-9658-z

Springer, M.S., Meredith, R.W., Gatesy, J., Emerling, C., Park, J., & Rabosky, D.L. et al. (2012). Macroevolutionary dynamics and historical biogeography of primate diversification inferred from a species supermatrix. *PLoS One,* 7 (11), e49521.

St Clair, E. M., & Boyer, D.M. (2016). Lower molar shape and size in prosimian and platyrrhine primates. *American Journal of Physical Anthropology*, 161, 237-258. https://doi.org/10.1002/ajpa.23021

St Clair, E.M., Reback, N. & Perry, J.M.G. (2018). Craniomandibular Variation in Phalangeriform Marsupials: Functional Comparisons with Primates. *The Anatomical Record*, 301(2), 227-255. https://doi.org/10.1002/ar.23717

Steffens, K.J.E. (2020). Lemur food plants as options for forest restoration in Madagascar. *The journal of Society for Ecological Restoration*, 28(6), 1517–1527. https://doi.org/10.1111/rec.13234

Tan, C.L. (1999). Behavior and Ecology of Gentle Lemurs (Genus *Hapalemur*). *International Journal of Primatology,* 20(4), 547-566. https://doi.org/10.1007/978-0-387-34586-4_17

Tattersall, I. (2006). Historical Biogeography of the Strepsirhine Primates of Madagascar. *Folia Primatologica*, 77(6), 477-487. https://doi.org/10.1159/000095393

Tattersall, I., & Schwartz, J. H. (1974). Craniodental morphology and the systematics of the Malagasy lemurs (Primates, Prosimii). *Anthropological papers of the AMNH*; 52,3.

Taylor, A.B., Groves, C.P. (2003). Patterns of mandibular variation in *Pan* and *Gorilla* and implications for African ape taxonomy. *Journal of Human Evolution*, 44(5), 529-561. https://doi.org/10.1016/s0047-2484(03)00027-7

Terhune, C.E., Mitchell, D.R., Cooke, S.B., Kirchhoff, C.A., Massey, J.S. (2022) Temporomandibular joint shape in anthropoid primates varies widely and is patterned by size and phylogeny. *Anatomical Records*. 305(9):2227-2248. doi: 10.1002/ar.24886.

Thomas, K. and John, K. (2012). Adobe Photoshop CS6. https://www.logitheque.com/windows/adobe-photoshop-cs6-21111.

Thorén, S., Lindenfors, P., Kappeler, P.M. (2006). Phylogenetic analyses of dimorphism in primates: Evidence for stronger selection on canine size than on body size. *American Journal of Physical Anthropology,* 130(1), 50-59. https://doi.org/10.1002/ajpa.20321

Venables, W.N. & Ripley, B.D. (2002). Modern Applied Statistics with S. Fourth Edition. New-York, Springer. https://doi.org/10.1007/b97626

Vieilledent, G., Grinand, C., Rakotomalala, F.A., Ranaivosoa, R., Rakotoarijaona, J-R. & Allnutt, T.F. et al. (2018). Combining global tree cover loss data with historical national forest cover maps to look at six decades of deforestation and forest fragmentation in Madagascar. *Biological Conservation,* 222, 189-197. https://doi.org/10.1016/j.biocon.2018.04.008

Viguier, B. (2004). Functional adaptations in the craniofacial morphology of malagasyMalagasy primates: shape variations associated with gummivory in the family Cheirogaleidae. *Annals of Anatomy*, 186(5-6), 495-501. https://doi.org/10.1016/S0940-9602(04)80093-1

Viguier, B., & Tort, A. (2000). Morphologie crânienne et mandibulaire des Indrinae. Apports des méthodes Procrustes et des analyses de Fourier. *Comptes Rendus de l'Académie des Sciences-Series III-Sciences de la Vie*, *323*(6), 573-582. https://doi.org/10.1016/S0764-4469(00)00148-7

Vinyard, C.J., Wall, C.E., Williams, S.H., Johnson, K.R., & Hylander, W.L. (2006). Masseter electromyography during chewing in ring-tailed lemurs (*Lemur catta)*. *American Journal of Physical Anthropology: 130*(1), 85-95. https://doi.org/10.1002/ajpa.20307

Vinyard, C.J., Ravosa, M.J., Williams, S.H., Wall, C.E., Johnson, K.R., & Hylander, W.L. (2007). Jaw-muscle function and the origin of primates. In *Primate origins: Adaptations and evolution* (pp. 179-231). Springer, Boston, MA.

Vinyard, C.J., Yamashita, N. & Tan, C. (2008). Linking laboratory and field approaches in studying the evolutionary physiology of biting in bamboo lemurs. *International Journal of Primatology*, 29, 1421-1439. https://doi.org/10.1007/s10764-007-9178-9

Winchester, J.M., Boyer, D.M., St. Clair, E.M., Gosselin-Ildari, A.D., Cooke, S.B. & Ledogar, J. A. (2014). Dental topography of platyrrhines and prosimians: Convergence and contrasts. *American Journal of Physical Anthropology*, 153(1), 29-44. https://doi.org/10.1002/ajpa.22398

Wright, B.W., & Willis M.S. (2012). Relationships between the diet and dentition of Asian leaf monkeys. *American Journal of Physical Anthropology*, 148(2), 262-275. https://doi.org/10.1002/ajpa.22081

Yamashita, N. (2003). Food procurement and tooth use in two sympatric lemur species. *American Journal of Physical Anthropology, 121*(2), 125-133. https://doi.org/10.1002/ajpa.10206

Yamashita, N., Cuozzo F.P., Sauther, M.L. (2012). Interpreting food processing through dietary mechanical properties: A *Lemur catta* case study. *American Journal of Anthropology,* 148, 205-214. https://doi.org/10.1002/ajpa.21549

Yamashita, N., Sauther, M. L., Cuozzo, F. P., & Jacky, I. A. Y. (2015). Beyond the gallery forest: contrasting habitat and diet in *Lemur catta* troops at Bezà Mahafaly Special Reserve. *Folia Primatologica*, *86*(1-2), 35-43. https://doi.org/10.1159/000368896

Yoder, A.D., Yang, Z. (2000). Estimation of Primate Speciation Dates Using Local Molecular Clocks. *Molecular Biology and Evolution*, 17(7), 1081–1090. https://doi.org/10.1093/oxfordjournals.molbev.a026389

Zelditch, M. L., Swiderski, D. L., & Sheets, H. D. (2012), *Geometric morphometrics for biologists: a primer*. academic press.

TABLE 1 Specimens of the Lemuridae family composing the sample

TABLE 2 Model selection for the mandibular shape and size variation response variables calculated on the entire sample. Significant values of R^2 are indictaed in bold. Akaike Itaeration criterion (AICc) and differences between the lower AIC value (best fitted model) and the other models are given for shape and size variation.

TABLE 3 Model selection for the mandibular shape and size variation response variables calculated on the reduced sample. Significant values of R^2 are indictaed in bold. Akaike Itaeration criterion (AICc) and differences between the lower AIC value (best fitted model) and the other models are given for shape and size variation.

FIGURE 1 Illustration of the mandibular morphology of the species composing the sample.

FIGURE 2 Data acquisition and outlining. 1 : Acquisition of mandible images in lateral view; 2: Standardisation of the images; 3: Outlining of the object of interest by the tpsDIG2 version 2.31 software; 4 : Step by step reconstruction of the mandibular outline using Momocs R package, here reconstruction by the two first hamonics.

FIGURE 3 Representative of *Prolemur simus* outlines (outlines are centered, starting point for the outline opening are indicated by arrows.)

FIGURE 4 A) Phylogenetic tree of the entire sample, obtained from Herrera & Dávalos (2016), B) Phylogenetic tree of the reduced sample

FIGURE 5 Map of the ecoregions used for the analysis (Source: Kathrin H. Dausmann, 2015. Modified in 2022)

FIGURE 6 Phylomorphospace of the entire sample computed on the genera. Larger symbols represent the group centroids. Numbers on the phylogenetic tree correspond to the nodes of the tree on figure 4. The outline of a specimen of *Eulemur rufu*s illustrates the negative values of PC1, the outline of a specimen of *Hapalemus griseus* illustrates the positive values of PC1; The outline of a specimen of *Eulemur fulvus* illustrates the negative values of PC2, the outline of a specimen of *Lemur catta* illustrates the positive values of PC2.

FIGURE 7 Phylomorphospace of the reduced sample computed on species. Species group centroids are illustrated by the small circles at the tip of the branches of the tree presented on figures 5. The outline of a specimen of *Eulemur rubriventer* illustrates the negative values of PC1, the outline of a specimen of *Lemur catta* illustrates the positive values of PC1; The outline of a specimen of *Eulemur mongoz* illustrates the negative values of PC2, the outline of a specimen of *Eulemur albifrons* illustrates the positive values of PC2.

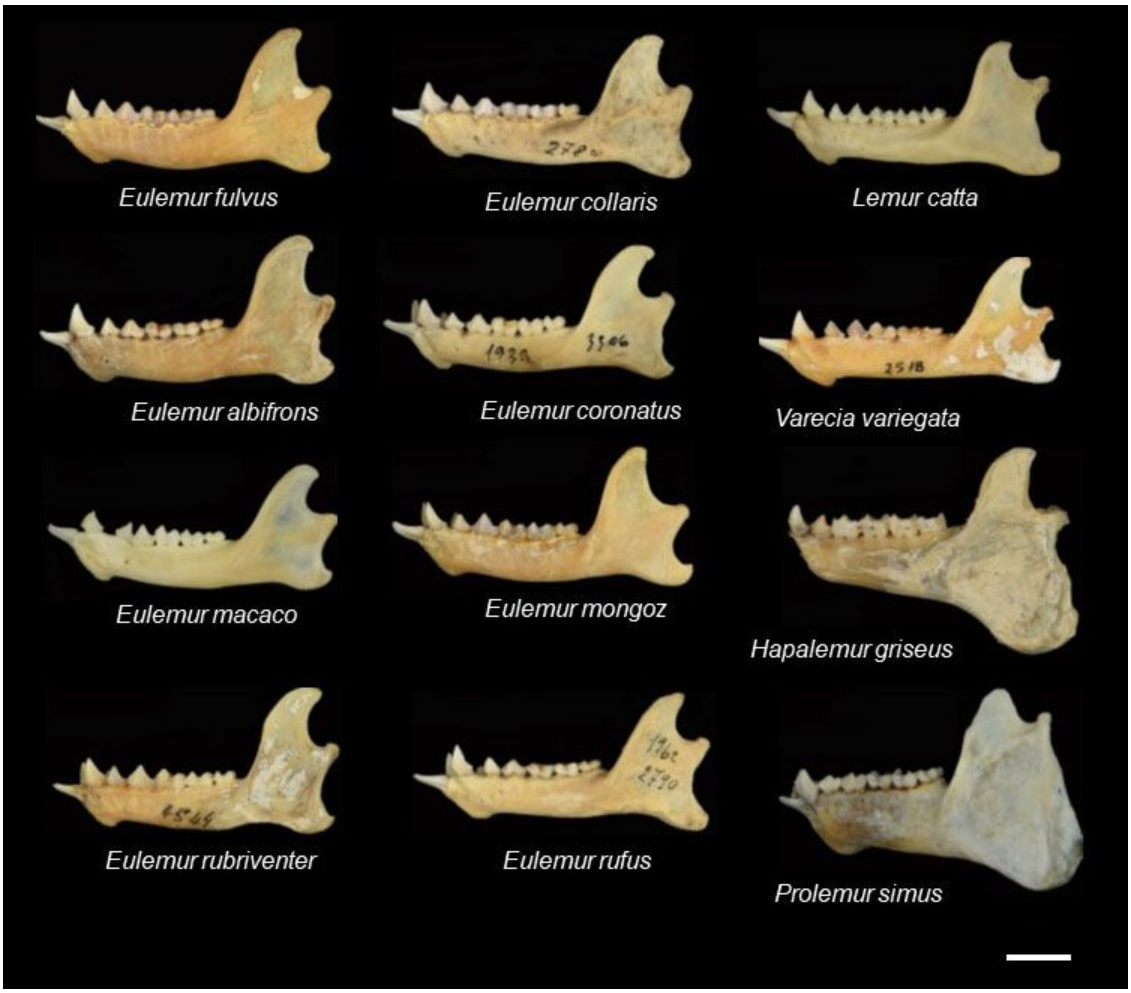
Eulemur fulvus
Eulemur collaris
Lemur catta
Eulemur albifrons
Eulemur coronatus
Varecia variegata
Eulemur macaco
Eulemur mongoz
Hapalemur griseus
Eulemur rubriventer
Eulemur rufus
Prolemur simus

FIGURE 1

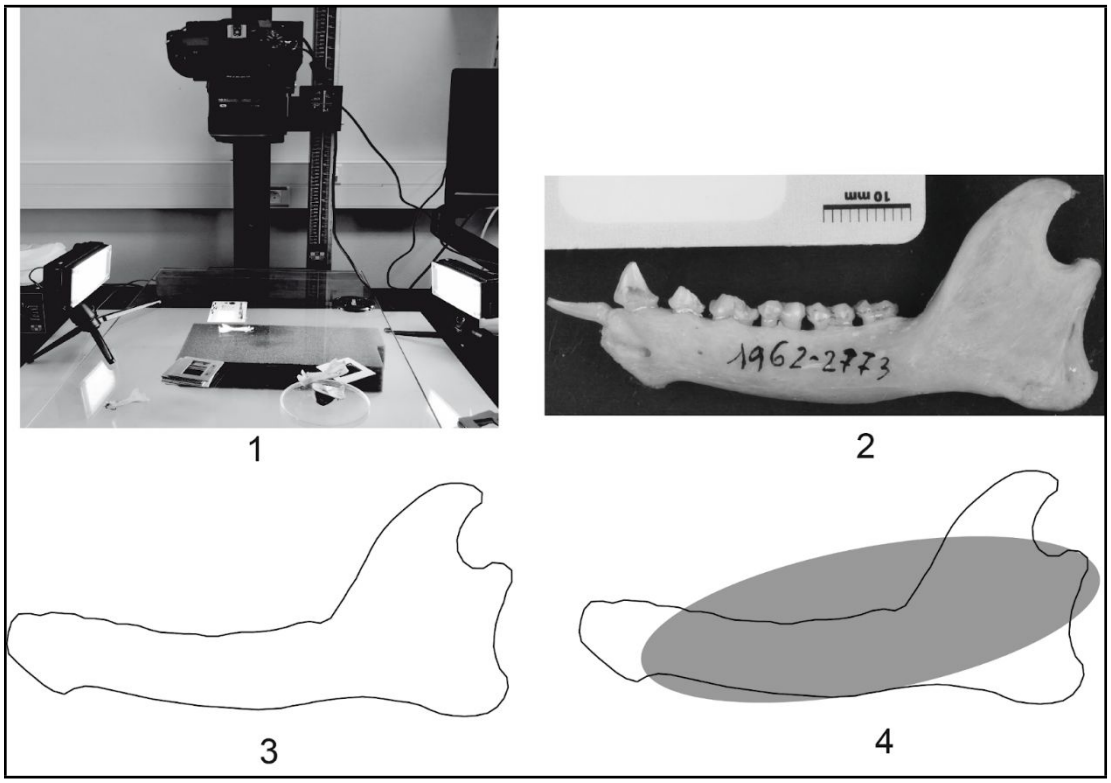

FIGURE 2

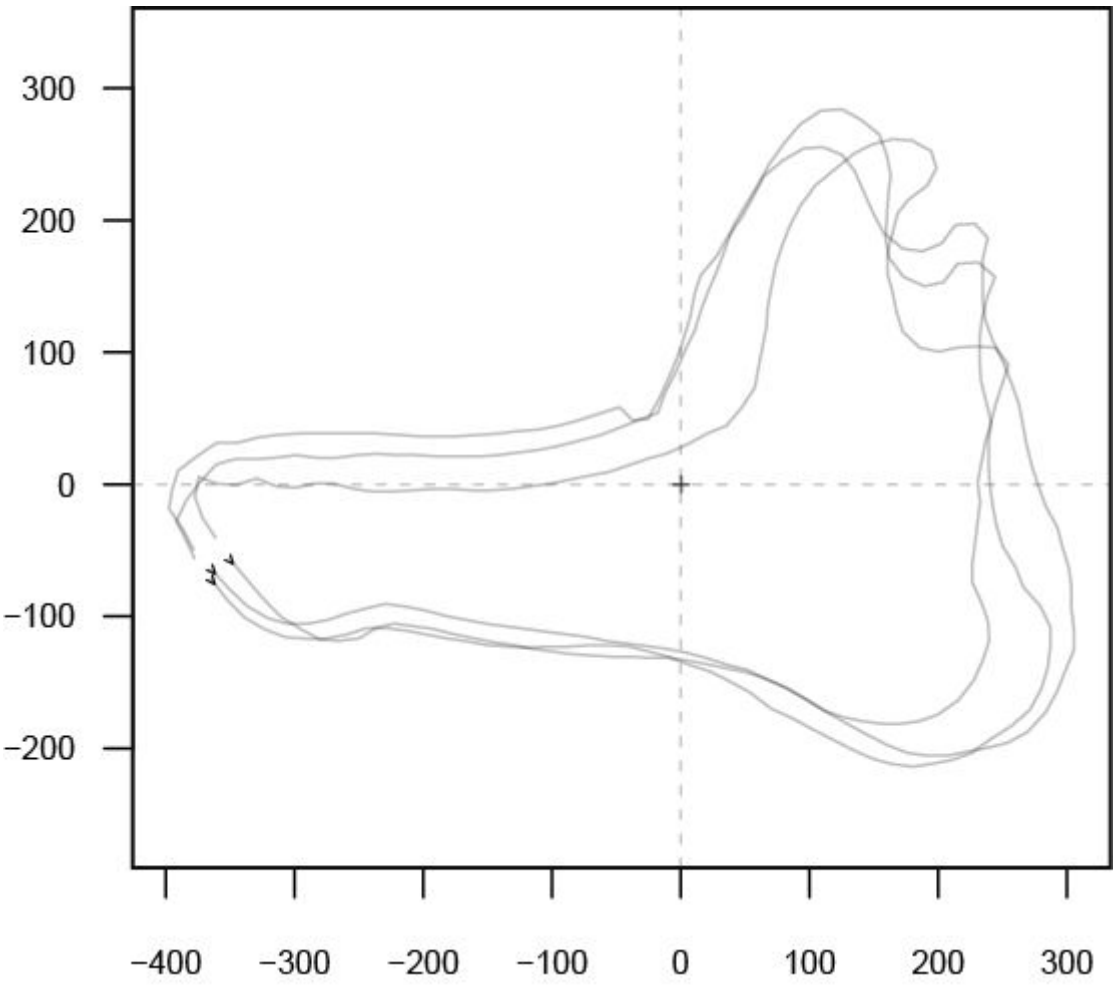

FIGURE 3

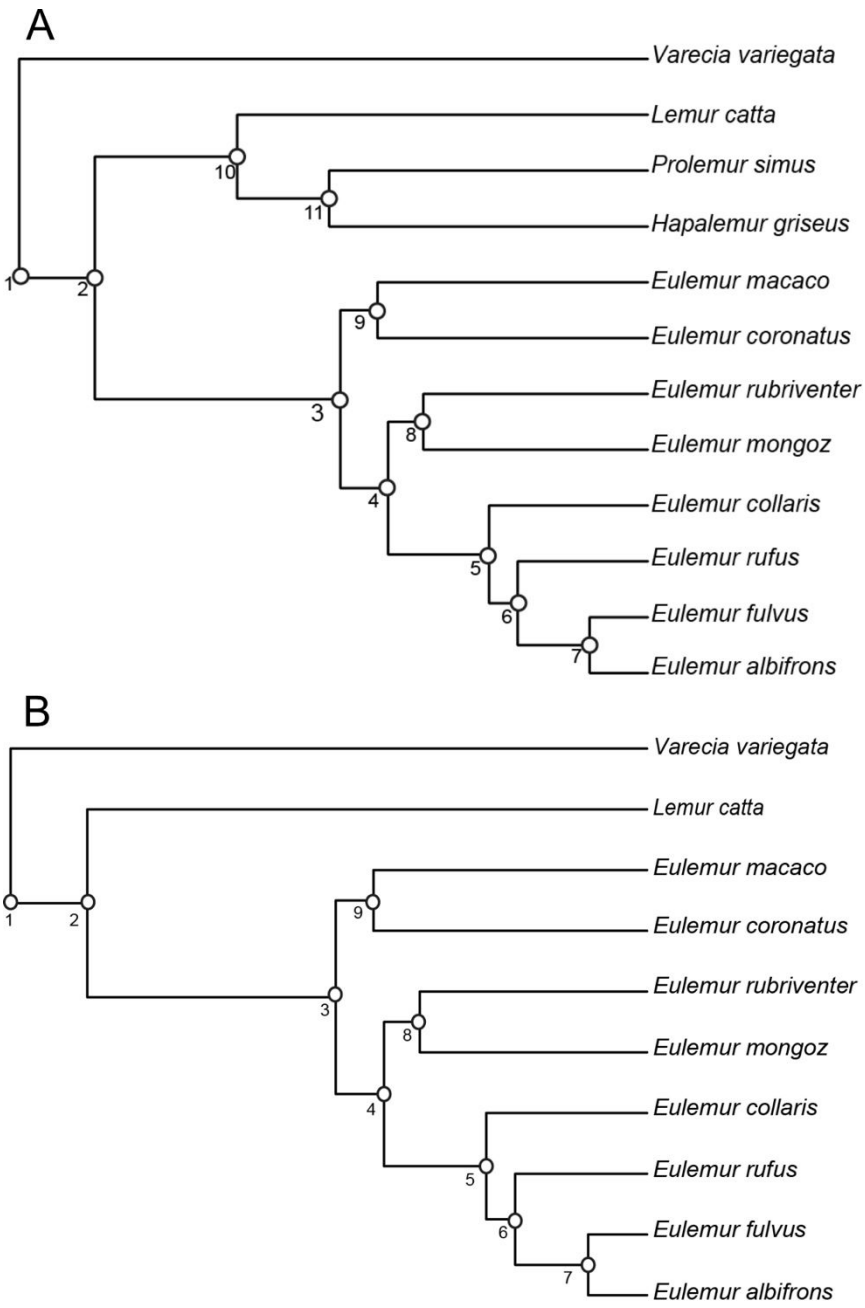
A
Varecia variegata
Lemur catta
Prolemur simus
Hapalemur griseus
Eulemur macaco
Eulemur coronatus
Eulemur rubriventer
Eulemur mongoz
Eulemur collaris
Eulemur rufus
Eulemur fulvus
Eulemur albifrons
1
2
3
4
5
6
7
8
9
10
11
B
Varecia variegata
Lemur catta
Eulemur macaco
Eulemur coronatus
Eulemur rubriventer
Eulemur mongoz
Eulemur collaris
Eulemur rufus
Eulemur fulvus
Eulemur albifrons
1
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FIGURE 4

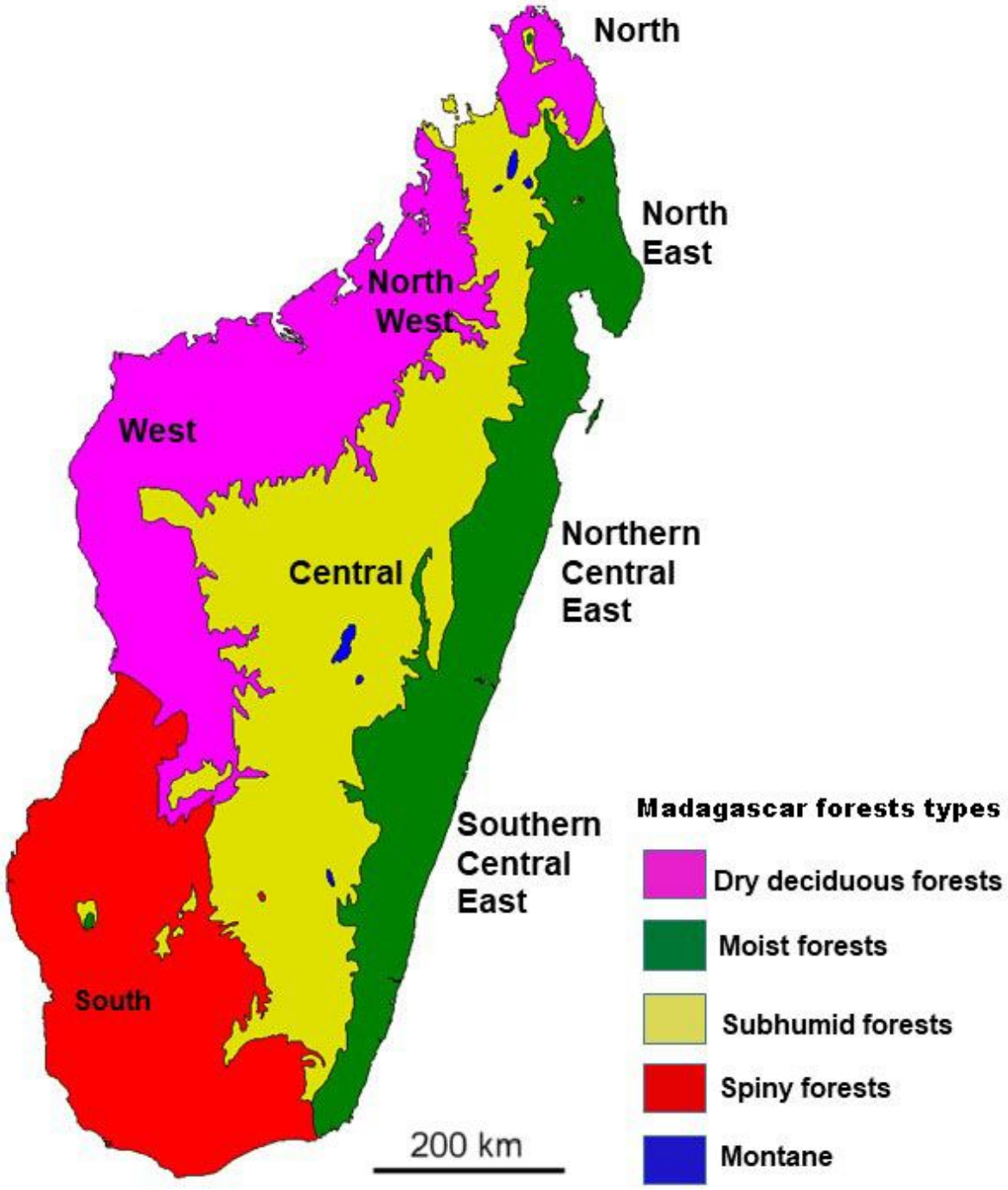
North
North East
North West
West
Central
Northern Central East
Southern Central East
South
200 km
Madagascar forests types
Dry deciduous forests
Moist forests
Subhumid forests
Spiny forests
Montane

FIGURE 5

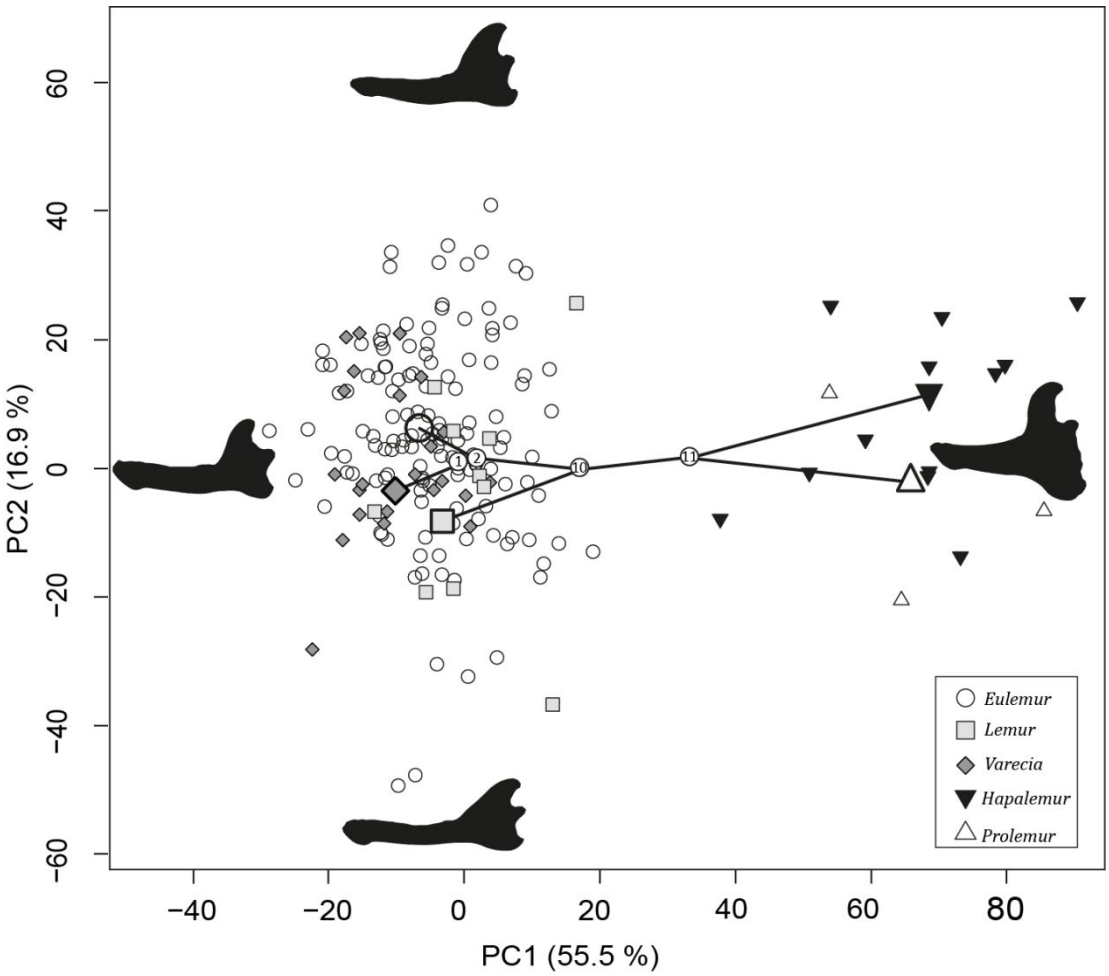
PC2 (16.9 %)
PC1 (55.5 %)
60
40
20
0
−20
−40
−60
−40
−20
0
20
40
60
80
1
2
10
11
Eulemur
Lemur
Varecia
Hapalemur
Prolemur

FIGURE 6

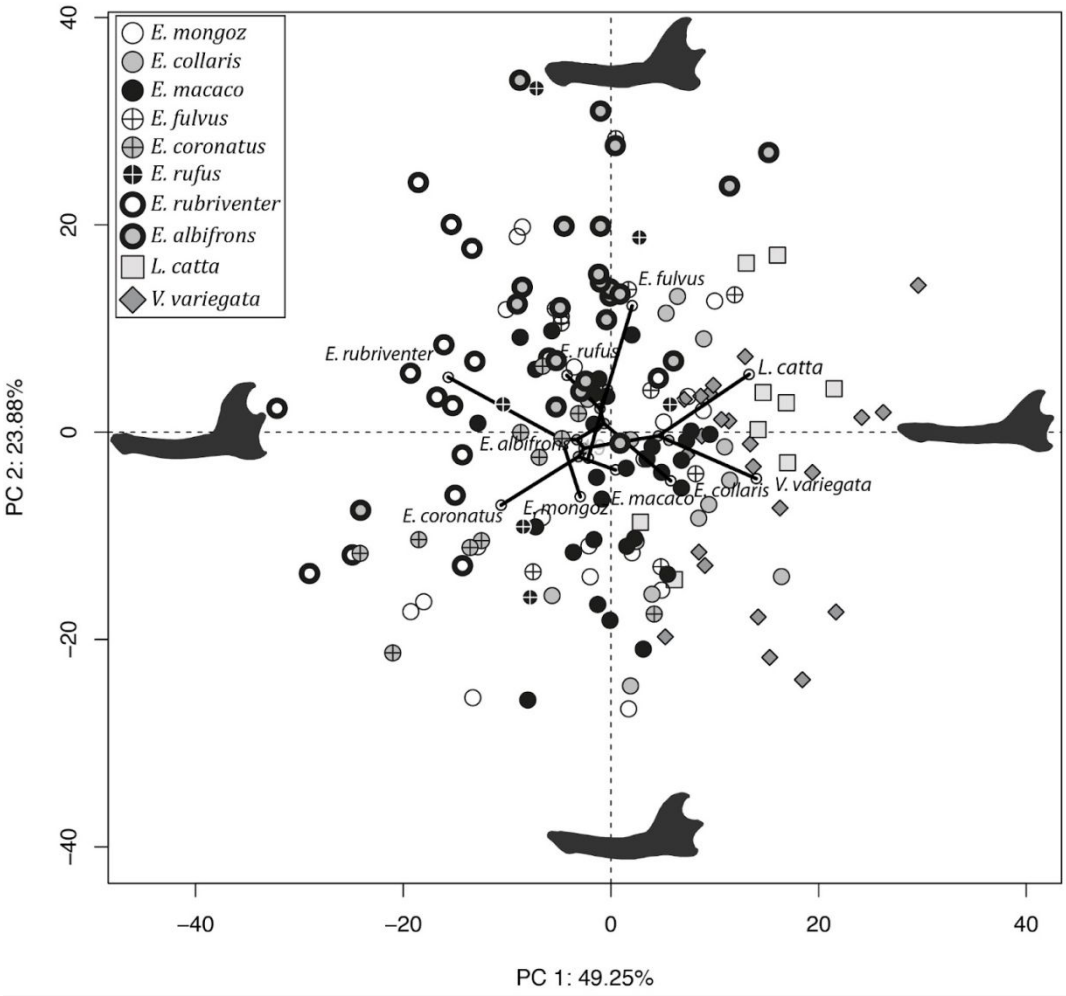
E. mongoz
E. collaris
E. macaco
E. fulvus
E. coronatus
E. rufus
E. rubriventer
E. albifrons
L. catta
V. variegata
E. fulvus
E. rubriventer
E. rufus
L. catta
E. albifrons
V. variegata
E. collaris
E. macaco
E. coronatus
E. mongoz
PC 2: 23.88%
PC 1: 49.25%
40
20
0
−20
−40
−40
−20
0
20
40

FIGURE 7

Genus	Species	Male	Female	Sex undetermined	Total
Eulemur	*E. fulvus*	8	4	0	12
	E. collaris	8	6	0	14
	E.mongoz	12	8	0	20
	E.coronatus	10	2	0	12
	E. rufus	5	1	0	6
	E. albifrons	14	9	0	23
	E. macaco	18	13	0	31
	E. rubriventer	10	7	0	17
Lemur	*L. catta*	6	4	0	10
Varecia	*V. variegata*	13	10	0	23
Hapalemur	*H. griseus*	7	4	1	11
Prolemur	*P. simus*	2	1	0	3
TOTAL					182

TABLE 1

Models (entire sample)	R^2 Shape	AICc Shape	ΔAICc Shape	R^2 Size	AICc Size	ΔAICc Size
Null model	0	4547.55	357.24	0	2494.82	1147.95
Phylogeny	**0.846**	4190.31	0.00	**0.67**	1346.86	0.00
Phylogeny + Diet	**0.846**	4190.31	0.00	**0.67**	1346.86	0.00
Phylogeny + Sex	**0.772**	4310.51	120.2	**0.609**	1398.67	51.81
Phylogeny + Habitat	**0.801**	4233.42	43.11	**0.619**	1394.57	47.71
Phylogeny + Diet + Sex	**0.817**	4226.16	35.81	**0.615**	1391.17	44.31
Phylogeny + Diet + Habitat	**0.836**	4209.49	19.18	**0.661**	1369.37	22.51
Phylogeny + Sex + Habitat	**0.826**	4220.69	30.38	**0.655**	1384.85	37.99
Phylogeny + Diet + Habitat + Sex	**0.867**	4206.58	16.27	**0.662**	1361.47	14.61

TABLE 2

Models (reduced sample)	R^2 Shape	AICc Shape	ΔAICc Shape	R^2 Size	AICc Size	ΔAICc Size
Null model	0	3848.96	90.48	0	2287.84	1049.37
Phylogeny	**0.531**	3819.19	60.71	0.152	1238.46	0.00
Phylogeny + Diet	**0.531**	3819.19	60.71	0.152	1238.46	0.00
Phylogeny + Sex	**0.557**	3793.14	34.66	0.176	1248.72	10.26
Phylogeny + Habitat	**0.693**	3782.96	24.48	0.091	1249.59	11.13
Phylogeny + Diet + Sex	**0.659**	3783.83	25.35	0.162	1249.47	11.01
Phylogeny + Diet + Habitat	**0.847**	3765.74	7.26	0.219	1249.39	10.93
Phylogeny + Sex + Habitat	**0.833**	3775.62	17.14	0.182	1250.33	11.87
Phylogeny + Diet + Habitat + Sex	**0.869**	3758.48	0.00	0.336	1245.78	7.31

TABLE 3

EIC COMMENTS

As part of your resubmission package, please provide a description addressing how you handled the points raised by the editorial board member, reviewers, and me in your revised manuscript. Please provide this description by clicking on the Resp. to Reviewers button. Additional comments, such as your response to the Editor-in-Chief letter, can be provided by clicking the Author Comments (if any) button, or in your cover letter (file) submitted to accompany the resubmitted manuscript file.

Please also revisit the "Guide for Authors" (see either the July, 2007, AJBA or the AJBA web page). In your revisions, take a look at the guide again and make all modifications that may be necessary (including those involving figure specifications, e.g., minimum resolution - 300ppi for photos and 600ppi for line graphics, inclusion of each figure as a separate file, formatted as TIF or EPS). This is one area that can delay perfectly sound manuscripts, placing them farther behind in the publication process.

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Please also note that the American Journal of Biological Anthropology and its publisher, Wiley, have recently implemented a data sharing policy. Officially, AJBA now expects, but does not absolutely require, that all data be publicly available. Authors are required to include a Data Sharing Statement in their manuscript stating whether the data underlying the manuscript are publicly available or not, and if so, where. Explicit instructions are now part of the Instructions for Authors. Any manuscript submitted or resubmitted to AJBA will now be required to follow these procedures. They will be incorporated into the Manuscript Central submission protocols as well.

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Thanks for considering the AJBA in publishing your research. I look forward to seeing your revised manuscript. Should you have any questions, do feel free to contact me. Please complete your revisions within eight weeks, no later than 21-Oct-2022. If I receive your manuscript after that date, it will be considered a new submission.

We recognise that the impact of the COVID-19 pandemic may affect your ability to return your revised manuscript to us within the requested timeframe. If this is the case, please let us know.

Sincerely,
Trudy Turner
Editor-in-Chief
American Journal of Biological Anthropology

Editorial Board Member's Comments to Author:

Associate Editor: 1
Comments to the Author:
I thank the authors for their submission, which has now been assessed by two Reviewers and the Editorial Board Member (EBM). All are positive about the study and think the topic of mandibular functional morphology in lemurs (but not the conservation framework) is relevant to readers of the AJBA. However, all reviewers raise several important concerns about the overall framing of the paper, requiring more information about the sample, morphology and function, as well as justification of the methods used (vs. 3D geometric morphometrics). I agree with this assessment and think major revisions are needed to get this manuscript to the standard suitable for publication in AJBA. Both reviewers offer detailed, helpful reviews, including specific comments in a separate PDF by R2. If the authors follow these constructive suggestions, including those from the EBM, I think the manuscript will be very much improved. I offer some additional comments below. I look forward to seeing the revised version.

We really want to thank the editorial board of AJBA for considering our manuscript and for giving us an extra month to allow us to revise our manuscript. We also want to thank the two editors that commented on the manuscript as well as both reviewers. The comments are very constructive and really helped us to rework our manuscript and improve it. We think that the manuscript is now really enhanced and hope that we will have the great pleasure to see it published in AJBA as we took each of the comments into consideration. A detailed answer is given below.

Title (and throughout the manuscript), ‘Malagasy’ should be capitalised. DONE

Abstract: the ‘Objectives’ to not actually state what morphology is to be quantified/assessed (i.e the mandible). This needs to be clarified. It is also unclear what is meant by ‘interactions of the variation with biotic and abiotic factors’ in this context.

We reworked the abstract taking this comment, as well as others into consideration.

Introduction: Currently, the Introduction begins and ends with a review of lemur species conservation. The authors make a strong link to conservation but it is not clear how this link is supported when the study is about shape variation in one side of the

mandible. Although conservation of lemurs is certainly important, AJBA is not a conservation journal and the link between conservation and mandibular morphology is tenuous.

- We really understand that AJBA is not a conservation journal and the aim of this manuscript is not focused on conservation. To us, the conservation aspect of the manuscript is a necessary opening. We have seen that AEs and reviewers stated that this conservation point was too developed. We removed the opoint about conservation in the first paragraph and the first part of the last paragraph, also focusing on conservation. We linked directly the former paragraph to the study objectives, making clearer the end of the introduction.

'World' should not be capitalised. DONE

More information about the sample is needed. The species should be listed in the text, not just Table 1. Information about sex for each taxon would be helpful.

- We added informations about the number of specimens for each species, including the number of males, females and specimens for which sex is undertermined. It appears in a new version of table 1. Moreover, we listed the species in the material part of the Material and Methods section (first paragraph of this section: "We studied 182 specimens belonging to 12 living species including *Eulemur fulvus, Eulemur rufus, Eulemur albifrons, Eulemur coronatus, Eulemur macaco, Eulemur mongoz, Eulemur rubriventer, Lemur catta, Varecia variegata, Hapalemur griseus* and *Prolemur simus* ")

Please thoroughly proofread the revised version prior to submission, as there are numerous typos and grammatical errors in this original submission.

- We really thank EB1 for these comments. We actually realized that the sent manuscript was not our final version. We really apologize about that and are grateful to the EBM and reviewers for their kindness and patience.

Editorial Board Member: 2

Comments to the Author:
Thank you for submitting the AJBA. This article was reviewed by two individuals, and both find the topic appropriate for the journal. Both reviewers highlight a number of major and minor revisions, and I encourage the authors to follow these excellent suggestions.

Both reviewers suggested changes for the introduction. I agree that lemur conservation is important, the current introduction does not provide enough background information on mandibular functional morphology to set up the study's aims. I encourage the authors to address R1's question on justifying the use of elliptical Fourier analyses rather than 3D geometric morphometrics approaches, and R2's questions on clarifying of the AIC results.

We answered every reviewers' comments as requested. Detailed answers are given below.

In order to use linear models in the analyses, the authors need to demonstrate that the data are linear, homogeneous in variance, and normally distributed.

The prerequisites for linear models must be verified, but, to be honest, it is difficult to test that the quantitative and continuous data are linear (here continuous data are harmonic coefficients) and normally distributed. For qualitative data, the covariates in our models, we should verify that the multivariate homoscedasticity (variance covariance matrix test) is respected and that the groups are independent. This is very difficult to do on such a dataset and we used a post hoc analysis of the models. Some tests exist, such as Box M test for variance/covariance matrix homogeneity. As previously said, in our case, the continuous variables are harmonic coefficients describing shape and such test is not applicable (or not really relevant). We ran the analyses and estimated the robustness of the models in a second time. We graphically verified that the mean of the residuals was close to zero, we tested the normality of the residuals, we checked the homogeneity of group variances using the residuals and looked at the leverage of points to detected potential outliers. In other words, we verified that the models really fitted the data. This cannot really be explained in the text, especially with a multivariate dataset with several covariates (and is never explained in publications). However, we understand that the idea of robustness is needed for each model. We added the R2 values for each model and added an information about the statistical significance of each linear models (p values). These statistics are given in table 2.
We added a sentence at the end of the Statistical analysis part of the Material and Methods section to indicate the fact that the model assumptions were verified ("The robustness of each model is estimated by verifying the distribution and homogeneity of variances of the residuals. We also detected the presence of potential outliers. R^2 values of each model is given in tables 2 and 3. ")

Given the high number of Eulemur samples, this sample may not be normally distributed, and non-parametric models may be needed.

We are not sure to understand EBM 2's comment. What does a sample normally distributed mean? We understand that variables can or cannot be normally distributed, but not samples. Moreover, we don't really understand the link between the high number of *Eulemur* specimens and the distribution. EBM2 is right on one point, an unbalanced number of specimens between groups (here genus) could slightly bias the models in multivariate analyses. However, as explained above, we were highly concerned by the robustness and confidence of our models and are sure that the sample heterogeneity does not affect our models.

I also encourage the authors to revise the discussion to address whether the shape differences in Hapalemur and Prolemur reflect phylogenetic differences or those associated diet and habitat given the study sample.

We understand EBM 2's comment. However, our results do not allow us to conclude on this point. *Hapalemur* and *Prolemur* are members of a same phylogenetic cluster and are the only folivorous of our sample. In this state, it is not possible to discern the origin of the differences (phylogenetic or diet). EBM2 is absolutely right on the fact that this point must be discussed, and we added a sentence about it in the first paragraph of the discussion ("As specimens of the genera *Hapalemur* and *Prolemur* are the only folivorous of our sample, it is not possible, in our case to explain the differences in mandibular shape from the other genera by a phylogenetic effect or by a difference in diet ").

Reviewers' comments to author:

Reviewer: 1

Comments to the Author
Thank you for the opportunity to review this manuscript. In this study the authors sought to establish several ecological correlates with the shape of the Lemurid mandible using elliptical Fourier analysis. The study elaborates on a 2005 Raveloson study that established a basis of the extent of variation within and among Lemurid genera. This study takes that method and approach and attempts to relate mandible variation with specific ecological correlates. I believe with revision that this manuscript could be very strong
Major comments
• While I find the results of the EF analysis interesting, the authors need to do a more thorough job of arguing why it was chosen over landmark-based 3-dimensional GM. 3D GM would allow for the authors to address issues of width of the articulated jaw and include landmarks that could be meaningful such as the attachment sites of musculature and/or ligaments.

We agree with reviewer 1 that the analysis could be done with other shape characterization methods such as 3D GM. Here, we made the choice to use EF method. To us, EF is not better or worse than other morphometric methods, it is just well adapted to characterize the shape of biological objects such as hemimandibles (essentially described by two dimensions). Moreover, our analysis is complementary to the one performed by Raveloson in 2005 and we used a similar protocol (which is a well-adapted protocol for mandibles).

EF may be considered as less trendy than 3D GM but is certainly as powerful. For instance, the analysis of flower petal shape, tree eaves or fishes otoliths are performed with standardized protocols relying on EF. For these objects it is very rare the find publications using 3D GM to characterize the shape variation. In our case, it is true that some authors (especially in recent articles) use 3D GM rather than EF. However, we don't think that the choice of one or another method could influence the signal that we analyzed.
We agree that the use of landmarks (in 2D or 3D) would be helpful in order to characterize and quantify some part of the mandible (such as muscle insertions). However, our idea was not to focus on peculiar parts of the mandible but rather to consider the entire structure.

We added a sentence at the end of the first paragraph of the “outlining” part in Material and Methods in order to justify the use of EF rather than 3D GM (“Our study is complementary to the one published by Raveloson et al. in 2005. We used a similar protocol, based on EFA (rather than 3D landmarks) in order to facilitate the comparison of results”).

- The manuscript would benefit from some reorganization. Much of the introduction is dedicated to climate change and reduction of biodiversity in Madagascar. While this is an important issue, the results of this project do not directly address this. It would be much better for the authors to use this section to establish what the outline of the mandible says about total body variation, what correlates have been established, and which correlates still need to be tested. They do this in the discussion, but some of that information needs to set up this study in the introduction.

This comment has been formulated by other reviewers and EB members. We reorganized the introducing, removing parts about conservation and adding parts about mandibular morphology and it understanding.

- Please add a hypothesis section and/or table or graphic explaining the correlates tested and criteria for each classification.

We expressed a hypothesis in the introduction. We added supplementary files containing the covariables. We detailed the different models.

- Please add a discussion of TMJ morphology, mandibular gape, and tree gouging behaviors.

We added a part about morpho-functional analysis of the mandible, TMJ, condyle morphology and chewing movements in the introduction. We did not find anything about gouging in the lemurs.

- Please add a summary table of correlates tested with significance values.

We reworked tables 2 and 3 giving more details (R2, significant values) and reworked the text.

- Please have someone proofread.

Line-by-Line comments

- Page 3 line 8 “hotspot” WC DONE
- Page 3 line 11-12 “The degree of endemicity is high in several groups of vertebrates such as fishes, birds, amphibians” Either move or remove this, it’s not relevant to your study We removed it
- Page 3 line 15 “Mammals” does not need to be capitalized unless you use the proper Mammalia DONE
- Page 3 line 15 “noticeable” WC DONE
- Page 3 line 53 “numbers” WC DONE
- Page 3 lines 53-60, reorganize into 2 or 3 sentences DONE
- Page 4 lines 51-60 this paragraph would be better at the beginning

Based on comments from reviewer 1 and other reviewers/EBM, we reorganized the introduction. This part is now at the beginning of the introduction.

- Page 4 lines 17-48 Expand this section to include some of the topics discussed above

We added a part in this paragraph to include a part about functional morphology of the mandible. Our study is not focusing on functional morphology but we agree that such part was missing in our introduction. We added a part on mandibular forces during chewing and bitting, on the presence or absence of the symphyseal fusion and about the morphology of the temporomandibular joint. This part was also related to diet, especially the consumption of leaves and hard items. We nevertheless want to highlight the fact that many of the cited studies are focusing on haplorrhines rather than strepsirrhines and that lemurids are very few represented.

- Page 5 lines 25-32 This seems beyond the scope of this paper. We don't need the abbreviations, if you want to keep this then move it to the discussion or broader impacts sections Sentence removed

- Page 6 lines 11-20 Are these the same images that were used for the 2005 Raveloson et al. paper or were new ones taken?

We worked on new images that we sampled for this work after visiting museums. We used a protocol similar to the one employed by Raveloson et al. Some specimens (very few) are present if both studies.

- Page 6 lines 33-34 "removed the parts related to the teeth" In photoshop?

Yes we did remove the teeth on our images with the help of photoshop, as precised in the following sentence.

- Page 6 line 40 Oversampling seems like the wrong word

We changed the end of the sentence. The sentence is now "Then with this same software, we converted the images in grayscale and the images were all reduced to the same size to avoid potential differences in image resolution ".

- Page 7 lines 1-38
  - What is the argument for EFA over landmark based 3D GM? It seems that you're losing data such as muscle markings and 3 dimensional depth/width and I'm not clear on what you're gaining in a structure as landmark-rich as the mandible.

We answered to this comment previously. We don't consider that we are loosing information for our study that is not focusing on muscle marking only, but rather on the overall shape of the mandible. We don't fully agree that the mandible is a "landmark rich structure". If some landmarks are easy to locate, many others are located on bone edges, along curvatures and are very challenging to locate accurately. Actually, this is a major topic in the GM community (see discussion on Morphomet: https://www.mail-archive.com/morphmet@morphometrics.org/ on this point). Semilandmarks and

random distribution of pseudolandmarks could be very helpful to cover the shape, however, to us, an accurate characterization of mandibular shape using 3D landmarks could remain problematic. We are not criticizing any authors nor any studies but just describing facts. In our case, as our work is in the framework of the one published by Raveloson et al. in 2005, we chose to work with the same protocol as long as this protocol is powerful enough to rigorously characterize mandible shape and size.

- How do you remove the teeth? Does this introduce more error?

We removed teeth because (1) this is not the aim of our study and (2) some teeth are missing. We thresholded our images on Adobe Photoshop in order to select and remove the teeth (teeth have a different colour than bone). Our error estimation took the entire data acquisition/preparation/ analysis into account. This step (teeth removal) probably introduces some error, as well as the other steps, but this error remains not significant (as given in the measurement error part of the results section).

- How did you choose which side of the mandible to do? Were there any criteria for which you may remove a specimen? Pathology? Age?

We have selected adult specimens.
We have kept the same position for the mandible (hemi-mandible left) to have the same harmonic direction of the contours
We chose, by convention, to work on the left hemimandible (following Raveloson et al. protocol). We removed specimens with broken parts, we selected adult specimens only (fully erupted and with fused cranial sutures).

- Page 7 line 51 “This hypothesis is one of the most complete analyses for strepsirrhines” wording is awkward

We changed the “phylogenetic hypothesis” into “phylogeny”.

- Page 7 lines 47-56 did the authors consider using 10k consensus tree?

No, in our case we did not use a 10K consensus tree but the majority rule consensus tree published by Herrera & Davalos in 2016. from this tree we selected the branches corresponding to the Lemuridae family. We added this precision in the text.

- Page 8 lines 18-31 You need a table for your ecological correlates. I would also like to see a graphic for your ecoregions.

We added R2 values for the models and gave an indication of the significance. We made a figure (figure 5) for the ecoregions.

- Page 9 line 45 Where do zoo specimens fall in your ecoregion breakdown?

We have 16 zoo specimens in our dataset (16/182). We checked that zoo specimens were not outliers. The we attributed the ecoregion class of wild specimens to the zoo specimens of the same species.

- Page 10 lines 32-42 Why did you not do phylogeny + sex? Or phylogeny + diet + sex?

We agree with the reviewer and added the missing models, we are now testing 9 models:

- Null model
- Phylogeny
- Phylogeny + Diet
- Phylogeny + Sex (covariables sex, sexual behaviours and mating system)
- Phylogeny + Habitat (covariables ecoregion and habitat)
- Phylogeny + Diet + sex
- Phylogeny + Diet + Habitat
- Phylogeny + sex+ habitat

  Phylogeny + Diet + Habitat + Sex

- Page 11 lines 1-7 what measurement error are you measuring here? How many individuals were included in the study?

We chose 10 specimens of the same species randomly. for these 10 specimens, we applied the complete protocol twice (positionning, picture, image preparation, outlining). Then, we tested the differences between replicate considering the difference between specimens (See Bailey and Byrnes 1990).

- Page 11 lines 12-60 Can you do a partial correlation to control for centroid size?

We are not sure to understand this question. In shape analysis, size is removed. size variation in analysed in a second time. A partial correlation would be helpful to work on the allometric pattern (which was not analysed here but is one of the perspectives of the paper).

- Page 12 lines 9-16 “The high difference between Hapalemur, Prolemur and the other genera of the family is so important that it is not easy to understand the morphological structure of mandibular shape variation in the genera Eulemur, Lemur and Varecia” Revise for wording, “high” difference between *Hapalemur*/*Prolemur* and the other genera? Or are there large differences between Hapalemur and Prolemur too? Also “important” are relative terms. DONE. High difference between *Hapalemur*/*Prolemur* and the other genera.
- Page 12 line 19 “In order to investigate this phylogenetic variation”DONE
- Page 12 lines 26-27 “developed ascending rami, with high and vertical coronoid processes, marked mandibular (sigmoid) notches”DONE

- Page 13 lines 11 weird formatting DONE
- Page 13 line 23 “accumulate” WC DONE
- Page 14 line 10-14 “Coefficients of determination for the covariates of this model are R=0.036 for Diet, R=0.09 for Ecoregion, R=0.067 for Mating system and R=0.05 for Genus.”? Make the formatting for correlation reporting consistent.

These values were wrong. we changed the text of this part. Moreover, we added the coefficient of determination for each model in tables 2 and 3.

- Page 14 lines 1-33 The capitalization of the correlates look a bit odd.

We have chosen to write in capital letters to make them visible in the manuscript but we can change if necessary.

- Page 14 line 47 “differences are likely explained by a” DONE
- Page 16 lines 1-33 Some of this information would be good to introduce in the introduction . We reorganized the introduction and used some of this part in the new version
- Page 16 line 42 “in the work published in 2005, Raveloson et al. highlighted this difference, and dissociated their analysis” Dissociated WC? We changed the sentence in “,and separated their analysis in two sub analyses”

- Page 16 line 51 “prolongation” WC DONE
- Page 16 line 51 “Raveloson et al. 2005‘s work.” Awkward wording, maybe “elaboration of Raveloson’s earlier work” DONE
- Page 16 “Here, we also report an important level of variation with the genera.” Redundant DONE
- Page 17 lines 7-15: “The direct correlation between mandibular shape variation and dietary shifts is probably enough to understand such variation. The direct link between mandibular shape variation and diet specialization is questionable.” These two sentences seem to contradict each other. Reviewer 1 is right. we changed the sentences in “The direct correlation between mandibular shape variation and dietary shifts could explain such variation. However, the direct link between mandibular shape variation and diet specialization is questionable. “ We also linked both paragraph as they develop similar ideas.

- Page 17 line 15: “Peculiar” WC DONE

- Page 17 line 19: “the expected level of variation should be low” Within folivores? Yes for the other author’s earlier work within folivores (Futuyma & Moreno, 1988) but it is not the case for our folivores sample.

- Page 17 lines 35-45: Clarify, are you saying that variation in the shape of the mandible due to musculature is independent of diet? we changed the sentences in “. Our results indicate a true covariation of mandibular morphology with diet, but also with other covariables non-dietary covariables.”

- Page 18 lines 16-18: “(I have a paper in my office to cite about this point).” I think you meant to change this, please proofread. Sorry about that. one of our comments was left in the manuscript.

- Page 18 line 21: Reduced sample – reduced from what?

At the beginning, we analyzed all of the samples (all of the genera study) and then, we removed the genera of *Hapalemur* and *Prolemur* to see the variations between the three genera *Eulemur*, *Lemur* and *Varecia*. So, we talk about the reduced samples by removing the genera *Hapalemur* and *Prolemur* analysis. Reviewer 1 is right, we gave some precisions about it in the text.

- Page 18 line 36: "Sexual behaviour does not covary with mandibular shape." DONE
- Page 19 line 16 " not a sexually dimorphic trait" DONE
- Page 19 line 23-24 "Our results indicate that mandibular shape does not vary with types of forests inhabited." DONE, actually we reworked this sentence.
- Page 19 line 34 "The second is that this covariation" What does "this" refer to?

It refers to the possible cavariation between the type of forest and mandibular shape. We reworked this sentence "The second is that the possible covariation between the type of forest and mandibular shape exists but the method to classify …"

- Page 19 lines 46-50: "Covariations of primate morphology with geographical distribution (taking ecoregions as great categories of distribution) are none for many years (Albrecht, 1990; Albrecht & Miller, 1993)." Done, we changed "none" by "known".

- Page 20 lines 2-5: "The ecogeographical difference is not only visible on size, but also on shape variation (Viguier, 2002)." Size of what? Body size? Head size? Please expand.

We agree with reviewer 1, this is not clear. We changed our sentences in "The ecogeographical difference is not only visible on size (cranial size in the case of Viguier, 2002), but also on cranial shape variation (in this case). Our results on the mandible support these findings. "

- Page 20 lines 7-10: "Madagascar's natural forest suffered a large loss of the forest cover while lemurs are distributed almost in all three of these four ecoregions (Vieilledent et al., 2017; Ganzhorn et al., 2000)." Revise for clarity DONE.

We changed the sentence in "The natural forest of Madagascar has suffered a great loss of forest cover inhabited by lemurs "

- Figure 1: Please expand and describe where in the process images 3 and 4 were taken

We added details on the figure caption in order to clarify these points.

- Figure 2: The caption is confusing, the outlines are points?

We gave precisions in the new version of the caption: "**FIGURE 2** Representative of *Prolemur simus* outlines (outlines are centered, starting points for the outline opening are indicated by arrows.)"

- Figure 5: How were the positive and negative consensus shapes produced?

We added details in the caption indicating the species for which the outlines are used for PC's variation illustrations. We did the same on figure 6.

- Table 1: how many males and females of each? We added this information in the revised version of the table.

Reviewer: 2

Comments to the Author

This paper is an interesting application of elliptical Fourier analysis to variation in mandibles in the Lemuridae. The species in this clade are widespread across the island of Madagascar, occurring in a range of habitats and varying in diet and aspects of their behavioral ecology. The authors have demonstrated a clear distinction along dietary lines in mandibular outline, with the bamboo lemurs convincingly separated from other taxa, even the closely related Lemur catta. The authors additionally present analyses excluding the bamboo lemurs in order to investigate morphological variation among the remaining species without the strong dietary effects from this unusual diet, and found some indication of other factors that influence mandibular shape.

In terms of scientific content, I would have liked more detail and discussion on previous research on mandibular morphology included in the introduction and discussion – the authors indicate that some studies have been done, but do not discuss their results in detail. Some attempt in the discussion is made to put the mandibular shape results in a wider context but this could be expanded further with more specific details on morphological trends.

We thank review 2 for this advice, we reworked our discussion taking R2 comments into account, as well as the other reviewer and EBMs ones.

The other major comment I had was that the measurement of size and use of size in some of the analyses would benefit from more explanation. While the pictures were apparently taken with a scale, it is not stated explicitly that the images were rescaled to that standard, so centroid size seems likely to reflect just proportion of area of the photograph that is occupied by the mandible, which will reflect shape and decisions made about cropping etc. I may be misunderstanding some part of the methods, but I think it should be clearer what the "size" actually means. Lemurids have a modest level of size variation compared to some of primate clades, but if someone wanted to replicate these analyses with another group this might be a more salient issue.

Size standardization is done at several steps of the protocol. First, we used a lens with a fix focal to avoid zoom effects. Second, a scale was used for each picture. Third, we set the images to the same dimension (nomber of pixel in length and width). Fourth, we used TPSDig2 to extract the outline and each picture was associated with it scale. Then, the function efourier of the package Momocs calculates the centroid size as the magnitude of the semi-major axis of the first fitting ellipse (see Bonhomme et al. 2914).

Bonhomme, V., Picq, S., Gaucherel, C., & Claude, J. (2014). Momocs: outline analysis using R. *Journal of Statistical Software*, *56*(13).

Thus, centroid size is centroid size as it seems to us that we have controlled all the potential bias.

The statement in the abstract that all of the covariables influence mandibular shape variation is a little unclear – while there were significant effects of covariables in various categories, but some of the seven covariables listed in the abstract were non-significant if I am understanding the analysis correctly. The AICc analysis could be better explained and clarified with more details on the methodology provided, and more consistent terminology when referring to covariables would be helpful..

We tried to do so in the new version.

The writing could use additional proofreading and there are places where clearer phrasing or alternate vocabulary would communicate the concepts more clearly. Some of these have been addressed in my detailed comments but more proofreading and review would be beneficial. There are also places where some of the text could be reduced in length or the relevance of that section could be made clearer. For example, the relevance of the vulnerability to extinction of various fauna in the introduction is not very clearly linked to the analysis of mandibular morphology.

Other reviewer and EBMs highlighted these points, we corrected our text and reworked the abstract, the introduction and the discussion.

Detailed comments
Abstract
Page 1: Lines 13 and 14 describe the seven covariables as sex, age, body mass, diet, ecoregion, sexual behavior and habitat. In lines 21 and 22 it is stated that in the second analysis (removing
Hapalemur and Prolemur) all of the covariables influence the morphology. In the results section on page 13, the text states that forest types, sex, and dominance pattern do not influence
morphology in the sample which seems contradictory to this statement in the abstract. Also,are ecoregions part of habitat or separate? Why is “forest region” not listed? I think it would be
helpful to clarify the description of the covariables and to increase detail on the analysis in which they are used throughout the manuscript.
We reworked the abstract and hope this new version is clearer.
For the second analysis, removing Hapalemur and Prolemur, all covariates are significant except sex.
What is called “habitat” is composed of the covariables “Ecoregion and “Forest type”. “forest region” does not exist in our text. It appears that our definition of variables is not easy to understand and work to make it clearer.

Introduction

Page 3: This part of the introduction gives a lot of specific detail on the numbers of lemur species threatened by various human activities that seems extraneous to the focus of the manuscript on factors affecting mandibular shape – I think this could be reduced a great deal or alternatively the relevance of this information to the research questions of the paper could be explained more clearly to justify the inclusion of this information. In contrast, while the authors
do list a number of papers that have shown morphological and ecological covariation in Malagasy primates, including some on page 2 that focus on the mandible, there is not much
discussion of the morphological findings which might be relevant to readers.

We removed the part about lemur species threats. We added some details about morphological findings about mandible, and especially morpho functional indications. We reworked the introduction.

Page 3: Line 44. “Factors acting on primate threat are dynamic...” “Factors threatening primates” would be a clearer phrasing. DONE
Page 3: Line 46. “... for this it is necessary” “this” here is ambiguous. For what? Also, I think this section generally should be expanded to clarify the link between extinction and studying mandibular morphology. DONE
Page 5: Line 38. Please expand with more on the way in which studies linking morphology and ecology will be useful for biodiversity conservation.

We reworked this part giving more details on this point.

Page 6: Line 14. When you describe the hemi mandibles as being in the sagittal plane, this seems contrary to the figure of the camera set up (which is a very helpful inclusion for clarifying the methodology). In anatomical position, the symphysis will be in the midline and the condyles/ ramus will be more lateral, so a median sagittal plane would intersect the symphysis and parasagittal planes would intersect the condyles and intervening portions of the
mandibular ramus, but no sagittal or parasagittal plane can intersect both the condyle and the symphysis. If the hemimandible is laid flat for photography, then the camera has to be oriented obliquely relative to the anatomical position of the mandible, because the mandibular corpus is oriented in an oblique orientation in life. I would describe your plane parallel to the long axis of the hemimandible, but not as sagittal.

Reviewer 2 is absolutely right, actually the hemimandibles were positioned in the plane parallel to there long axes, and not to the sagittal plane. We corrected our methodological part.

Page 7: Line 5. Probably you want to “retain” harmonics rather than “retrain” them. We corrected it.

Page 7: Line 26. I am confused by how meaningful centroid size will be given the earlier statement that the images were all reduced to the same size. (Page 6 line 40). See general comments.

We agree that it is not clear. After outline characterization by TPSDig2, the outline files were opened is R, size was extracted and outlines were scaled to analyze shape

variation. All these steps were done automatically by efourier function of the momocs package.

Page 7: Line 52. I think the phylogenies could be combined into one same figure- there is a lot of repeated information in the two figures if kept separate. I would also suggest adding something to the figure legend about how the node numbers on the phylogeny match the later figures.

We reorganized the figures to have both phylogeny on the same figure. We changed the text about it. We tried to have the node numbers on figure 6 but it is too difficult to make them readable.

Page 8: Line 6. here you refer to six covariables and in the abstract you said seven. It would be good to have this clarified. We have seven covariables (phylogeny, diet, sex, sexual behavior, mating system, ecoregion and forest type). We homogenized it the number of covariable is now the same in the text and in the abstract.

Page 9: Discussion of ecoregions and forest types -it would be helpful to know how this information was integrated into the analyses. Is each specimen assigned to a forest type and
ecoregion based on location data (in which case how did you handle captive specimens?) or are these assigned at the species level (in which case how did you handle instances where the same
species is present in more than one forest type or ecoregion?)

We have localities for a great majority of specimens, and used this information. When locality was not available, we used the species informations (Animaldiversity.org, Mittermeier et al. 2006, 2010, 2013). We did the same for zoo specimens and checked that they were not outliers (but we have few zoo specimens as said previously). None of the specimens of the same species were attributed to different forest type nor ecoregions. We agree with reviewer 2 that it could be a problem (that we did not have to face for this study) and that our categorizations could present some problems. We tried to categorize each specimen with rigor, avoiding biases.

Page 9: Line 35-39. The sentence that ends “where is just the rest place for lemurs” is pretty unclear- I can’t suggest alternate wording because I don’t exactly know what is intended.

We agree that this part of the sentence was not clear. We proofread the paragraph and removed this part.

Page 9: line 60. If the size was centroid size, was there any way to scale to actual size? If I am understanding the methods correctly, centroid size will largely reflect shape because the shorter deeper mandibles will have more area than the longer thinner mandibles. I don’t see the value of analyzing centroid size to any extent unless the units are standardized for all the images

We are not sure to understand R2 comment. Centroid size represents dimensions of the objects, not only length or width. The distinction between size and shape is the

basis of Geometric Morphometrics in order to avoid to analyse a mixed signal. Centroid size is not shape, it is everything but shape. However, shape can be dependent upon size (allometries). Centroid size is the best option to study overall size because it really reflects the dimensions of the objects regardless the linear measurement used.

Page 10: Please describe the methods related to the linear models in more detail, particularly related to the coding of variables and the interpretation of the models. For example, how were the categorical variables treated? When you combined different pieces of information into the model for example combining ecoregion and forest type as habitat what did that look like in the data set?

When we combined covariables in the linear models, we tested the effect of each covariable and the interactions between these covariables on the dependent variable (shape, or size). In the example given by reviewer 2 we tested the effect of ecoregion on shape, on habitat on shape and the effect of the interaction of ecoregion and habitat on shape. The model considers all these effect (not the effects independently). This is the reason why we built oll the possible models and compared them, using AIC values

Page 11: In the discussion of PCA results lines 18- 21 it would be good to state clearly in the text that which species are on the positive vs negative end of PC1 because it would be a way of
connecting the morphological shape description to the sample. This information is available in the plots but would be nice to see in the text also. It would also be nice to see examples of the
mandibles of different genera in a figure.

We added a new figure to illustrate the morphologies, we gave the name of the species used to illustrate the variation along PCs in the figure caption and in the text.

Page 11: Lines 36. I think it would be clearer to refer to the ascending ramus/ rami rather than the ascending branch here and at other points in the text, as this is the more typical terminology. Done
Page 12: did you run the MANOVA on the reduced sample? It would be interesting to know if there are still significant differences between genera without Hapalemur and Prolemur. Also, are the results of this MANOVA included in any tables?

We performed the MANOVA analysis on the reduced sample. The difference between genera is not significant (see table 3), that is to say that the phylogeny only cannot explain shape variation. Same thing for the phylogeny plus diet. However, it becomes significant when adding the other covariables and the model that fitted the best the dataset (and which is significant) if the model with all the covariables. this result indicates that the analysis of phylogeny or diet is not sufficient to understand the morphological variation of the mandible.

Page 13 : I think the AICc results here need more explanation. For a start, I don't think the acronym is spelled out anywhere in the paper. It would be nice to have a summary of what the number indicates.

AIC is the acronym for Akaike Iteration Criterion and the lower capital c indicates that it is corrected for small samples. It is the most often used for model selection. By

calculating and comparing the AIC scores of several possible models, you can choose the one that is the best fit for the data. The computation of AICc is based on the Log-likelihood values that describe how likely the model is, given the data. We added a sentence in the method section about this point.

Page 14: I still have the same questions about size in this analysis - it is difficult to know what the size variation indicates without clarification.

We show that size variation is explained by phylogeny or diet (as it is not possible to separate phylogenetic effect from diet effect) in the entire sample and it is explained by none of the covariables in the reduced sample.

Page 15: Lines 21-23. I would suggest that you phrase this as the leaf chewing _species_ vs frugivorous species rather than "the leaf chewing" and "the frugivorous" DONE

Page 15: Lines 34-35. The temporalis muscles are listed separately from the "pars temporalis" – I am not sure what the latter refers to if not part of the temporalis.
We corrected it.

Page 15: Lines 45-50. There isn't usually much resistance to depressing the mandible/ opening the mouth so I am not sure why larger muscles attaching at the symphysis would relate to chewing strength. Usually symphyseal dimensions are interpreted more as related to bending/ wishboning forces of the mandible due to the actions of the working and balancing side jaw adductors rather than to the size of the digastrics.
We found this information in (Pick & Howden, 1977).

Page 16: Line 24. I'm not sure what is meant by the "light aspect of the mandible" – does this refer to it being relatively gracile in the non-folivorous lemurids? Also I would suggest using "chewing movements" or "masticatory movements" rather than "chew movements".

We changed "light" into "gracile". we changed "chew- movements" in "chewing movements".

Page 17: Line 23 – the folivorous species rather than "the follivorous" DONE
Page 18: Lines 16-18. I think it was intended that an additional citation would be inserted here. We added the reference
Page 18: paragraphs of the discussion starting at line 21 and continuing to the next page– is there a way to use the AICc analysis to examine what aspects of the morphology are related to the covariables? I ask because there isn't really a clear mechanism by which ecoregion or mating system would affect mandibular anatomy, and understanding which morphological features are showing as associated with particular covariables might help elucidate this point.

We really understand reviewer 2's comment. We cannot answer these questions for the moment with this analysis but this is our next project.

Page 20 line 7 – "while" is an awkward phrasinng here. This sentence could use some clarification to make the intended meaning clearer. DONE

Page 20 line 16 – I think you mean to say that almost half of its forest cover has been lost since 1953 – it seems like there is something missing as the sentence is currently written. DONE

Figures and tables

As mentioned above, it would be nice to have an additional figure showing an example of each of the genera included in the analysis to give additional context to some of the PCA plots I also feel like there are more analyses described in the results than are presented in the tables.
Additional information would be helpful for understanding why some covariables were considered significant and others were not.

We added supplementary information as asked. Our covariable table is in supplementaries, we added two figures and we reworked our tables as asked.