New insights into the systematics of Malagasy mongoose-like carnivorans (Carnivora, Eupleridae, Galidiinae) based on mitochondrial and nuclear DNA sequences

Géraldine Veron[1]*, Délia Dupré[1], Andrew P. Jennings[2], Charlie J. Gardner[3], Alexandre Hassanin[1], Steven M. Goodman[4]

1: Institut de Systématique, Evolution, Biodiversité, UMR 7205 ISYEB, CNRS MNHN UPMC EPHE, Muséum National d'Histoire Naturelle, Sorbonne Universités, CP 51, 57 rue Cuvier, 75231 Paris Cedex 05, France, Phone: 33.1.40.79.48.53, Fax: 33.1.40.79.30.63, email: geraldine.veron@mnhn.fr; delia.dupre@outlook.fr; hassanin@mnhn.fr

2: SMALL CARNIVORES - Research and Conservation, 83 St. Lawrence Street, Portland, Maine, USA. Phone: 1 207 775 1192, email: smallcarnivores@yahoo.com

3: Durrell Institute of Conservation and Ecology (DICE), University of Kent, Canterbury CT2 7NR, UK. Phone: 44 1227 827056, email: cg399@kent.ac.uk

4: Field Museum of Natural History, 1400 South Lake Shore Drive, Chicago, Illinois 60605 USA / Association Vahatra, BP 3972, Antananarivo 101, Madagascar. Phone: 261 2022 27755, email: sgoodman@fieldmuseum.org

*Corresponding author: geraldine.veron@mnhn.fr

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Abstract

The Malagasy carnivorans (Eupleridae) comprise seven genera and up to ten species, depending on the authority, and, within the past decades, two new taxa have been described. The family is divided into two subfamilies, the Galidiinae, mongoose-like animals, and the Euplerinae, with diverse body forms. In order to verify the taxonomic status of Galidiinae species, including recently described taxa, as well as some recognized subspecies, we studied intrageneric genetic variation and structure, using both mitochondrial and nuclear markers. Our results suggest the recognition of four species in the Galidiinae, rendering each genus monospecific. We propose to recognize three subspecies in Galidia elegans (G. e. dambrensis, G. e. elegans, and G. e. occidentalis), two subspecies in Mungotictis decemlineata (M. d. decemlineata and M. d. lineata) and two subspecies in Galidictis fasciata (G. f. fasciata and G. f. grandidieri, the latter was recently described as a distinct species). Our results indicate also that Salanoia durrelli should be treated as a junior synonym of Salanoia concolor. Low levels of intraspecific divergence revealed some geographical structure for the Galidiinae taxa, suggesting that environmental barriers have isolated certain populations in recent geological time. All taxa, whether at the species or subspecies level, need urgent conservation attention, particularly those with limited geographical distributions, as all are threatened by forest habitat degradation.

Introduction

Madagascar's fauna is fascinating as a result of the high levels of endemism and the island's separation from continental Africa in deep geological time. These aspects make this island an excellent site to study diversification patterns in isolation. The Malagasy native carnivorans comprise seven genera and eight to ten species according to different authors (Yoder et al. 2003; Goodman 2009, 2012; Veron 2010). Recent molecular studies have brought considerable light into their evolutionary history (Yoder et al. 2003), which was not discernible based on morphological characters (Veron 2010). Three species with an assortment of body forms, Cryptoprocta ferox Bennett, 1833, Eupleres goudotii Doyère, 1835 and Fossa fossana (Müller, 1776), were previously included in the Viverridae (civets), while the mongoose-like species (belonging to the genera Galidia, Galidictis, Mungotictis, and Salanoia) were formerly included in the Herpestidae (mongooses), within the subfamily Galidiinae. The first molecular study to tackle the relationships of the Malagasy carnivorans suggested that C. ferox is closer to the Herpestidae than to the Viverridae (Veron and

Catzeflis 1993). A decade later, Yoder et al. (2003) revealed that all the native Malagasy Carnivora form a monophyletic group, which is the sister-group to the Herpestidae, and now placed in the family Eupleridae (Wozencraft 2005). This family is endemic to the island, with two recognized subfamilies: Euplerinae (Cryptoprocta, Eupleres, and Fossa) and Galidiinae (Galidia, Galidictis, Mungotictis, and Salanoia).

Recently, a new species of Galidiinae was described within the genus Salanoia, Salanoia durrelli Durbin et al., 2010, from the marshes of Lac Alaotra in the central eastern region, based on cranio-dental aspects. The congeneric species, Salanoia concolor (Geoffroy Saint-Hilaire, 1837), has a restricted distribution in the northeast and eastern regions, occurring in lowland humid forest. The description of S. durrelli was based on two specimens; the molecular data were limited (two individuals of S. durrelli and one of S. concolor) and showed little Cytochrome b divergence (0.8%) from S. concolor.

Nearly three decades ago, Wozencraft (1986) described Galidictis grandidieri from the spiny bush of the extreme southwest, which was distinguished from the only other recognized species in the genus, Galidictis fasciata (Gmelin, 1788), by its larger size, and some characteristics of the skull and coat pattern. However, its specific distinction has never been examined using molecular data. In the description of the southwestern form of Galidictis, Wozencraft (1986) proposed the name grandidiensis; subsequently, Wozencraft (1987) emended this to grandidieri, as the originally proposed name was in error, and herein, we use this spelling.

The intraspecific divergence and the genetic structure of populations of the Malagasy mongoose-like species have been little studied. Based on a fragment of the Control Region, Bennett et al. (2009) proposed a phylogeography of Galidia elegans I. Geoffroy Saint-Hilaire, 1837, the most widespread member of the Galidiinae, which is generally divided into three subspecies. Their results suggested isolation of the central western population, recognized as a separate subspecies (Galidia elegans occidentalis Albignac, 1971), but little other phylogeographical structure was found in the remaining populations, which might have been associated with the geographically limited sampling.

Based on one nuclear and two mitochondrial fragments (Beta-fibrinogen intron 7, Cytochrome b, Control Region), Jansen Van Vuuren et al. (2012) examined the genetic structure of Mungotictis decemlineata (Grandidier, 1867), a forest-restricted species. They found no strong genetic structure, but their study was only based on samples of M. d. decemlineata from a relatively limited region in the central west. Molecular data are still

lacking for the subspecies Mungotictis decemlineata lineata Pocock, 1915, which is known only from a limited area in the southwest (Hawkins et al. 2000; Goodman et al. 2005).

The aim of this study was to examine intraspecific diversity and genetic structure within all species of Galidiinae, in order to: 1) verify the taxonomic status of the recently described S. durrelli, and 2) assess the level of differentiation and phylogeographical patterns within other genera with respect to current specific and subspecific designations. For these purposes, we analyzed two mitochondrial and one nuclear fragments: Cytochrome b, Hypervariable region 1 of the Control Region, and Beta-fibrinogen intron 7. These data also provide insight into the role of environmental factors in shaping the geographical structure between and within species of Malagasy euplerids. Owing to the rarity and difficulty in capturing many of these taxa, we have relied heavily on museum specimens, many decades old, which in turn has imposed some limitations on sample sizes.

Materials and Methods

Sampling, extraction, PCR, and sequencing

We analyzed fresh (hair or tissue) and museum samples (skin or tissue taken from skulls) from 33 individuals of all species of Eupleridae (Table 1, Figure 1). Total genomic DNA was isolated following a cetyl trimethyl ammonium bromide (CTAB)-based protocol (Winnepenninckx et al. 1993). For museum samples, we added dithiothreitol (DTT 1M, ca 8-15 µL per extract) during tissue lysis to break up disulfide bonds, and we increased the lysis time (up to 72 hours).

We sequenced two mitochondrial fragments: Cytochrome b gene (Cytb) and the Control Region (CR; HVR1), using previously described primers (Cytb: Veron and Heard, 2000; Veron et al. 2004; 2014; Wilting and Fickel 2012; CR: Palomares et al. 2002). To provide an evolutionary assessment independent from mitochondrial markers, we amplified the nuclear marker Beta-fibrinogen intron 7 (FGB) using the primers of Yu and Zhang (2005). Primers' sequences are provided in Supporting information Table S1.

Polymerase chain reactions (PCRs) were performed as in Patou et al. (2010), with annealing temperatures of 50°C for Cytb, 61°C for CR, and 59°C for FGB. PCR products were sent to Eurofins Genomics (Ebersberg, Germany) for purification and sequencing (on Applied Biosystem® 3730XL). Sequences were edited and then aligned manually using Bioedit (version 7; Hall 1999).

Phylogenetic and haplotypic network analyses

Phylogenetic analyses were performed using neighbour joining (NJ), maximum likelihood (ML), and maximum parsimony (MP), as implemented in MEGA6 (Tamura et al. 2013), and Bayesian inference (BI) using MrBayes 3.2 (Ronquist et al. 2012). We rooted the phylogenetic analyses of the Galidiinae with three Euplerinae, C. ferox, E. goudotii, and F. fossana, and one Herpestidae, Herpestes ichneumon (Linnaeus, 1758).

For ML, the best-fitting model was estimated prior to the analyses using MEGA6, following the Akaike information criterion (AIC). The selected model was then implemented in the ML analyses, in which node robustness was assessed through 1,000 bootstrap replicates. For BI, we used Reversible Jump Markov Chain, to sample across the 201 substitution models, and gamma distribution (Lset nst = mixed rates = gamma option) to sample the posterior distribution of trees and to take into account the substitution model uncertainty. We used default priors for branch lengths and ran the chains for 10,000,000 Metropolis-coupled MCMC generations, with trees sampled every 1000 generations, and a burn-in of 25%. Two independent Bayesian runs were performed for each dataset, and the posterior probabilities were checked to ascertain that the chains had reached stationarity.

Trees were visualized and edited using FigTree 1.4.0 (Rambaut 2012). We compared resulting topologies and their node support; nodes were considered as supported when posterior probabilities were ≥ 0.99 and bootstrap values were $\geq 70\%$.

We used DNAsp5.10 (Librado and Rosas 2009) for defining haplotypes. NETWORK (v 4.6, www.fluxus-engineering.com) was used to construct haplotype median-joining networks (Bandelt et al. 1999) for each of the three genes. We computed genetic distances (within and between groups) and genetic diversity (haplotype and nucleotide diversity) using MEGA6 and DNAsp5.10.

Results

All new sequences were deposited in GenBank (Accession numbers: KX592614 to KX592671; Table 1). A total of 99 individuals were used in this study, including data obtained from GenBank (Table 1). Given the elusive nature of certain species of Galidiinae and their apparent rarity, we relied extensively on museum specimens (e.g. seven out of eight Salanoia samples were from museum specimens, some being many decades old), in addition to field collections of tissue or hairs, from which DNA was extracted. Owing to the degraded nature of DNA retrieved from certain specimens, only partial sequences could be obtained,

and nuclear DNA could not be amplified by PCR from museum specimens. New sequence data were obtained for Cytb (n=28), CR (n=11), and FGB (n=18) (see Table 1).

Our Cytb phylogeny of the Galidiinae is shown in Figure 2 and contains all species and one individual per haplotype (length of the alignment, number of variable positions, number of parsimony-informative sites, number of samples: l: 1140 bp, v: 223, pi: 203, n=38 without outgroups; model GTR+G+I). The results confirmed the monophyly of the Galidiinae, the position of Galidia as the sister group to all other Galidiinae, a sister-group relationship between Mungotictis and Salanoia, and Galidictis as sister to the latter two genera. The intergeneric Cytb distances within the Galidiinae ranged from 4% between Mungotictis and Salanoia to 13.5% between Galidia and Mungotictis.

The FGB fragment (l: 665 bp, v: 6, pi: 3, n= 19, without outgroups; model: TN93) showed no intraspecific variation for Galidia, contained only one polymorphic site in Galidictis, which was found to be heterozygous in both species, and showed low variation in Mungotictis (see Supporting Information Figure S1).

Within Salanoia, the Cytb tree including all samples (l: 1140 bp, v: 10, pi: 9, n=10; model: GTR+G+I; Figure 3) provided two well-supported groups of three individuals each, while the position of the four other specimens was poorly supported. We obtained three Cytb haplotypes (due to missing data, only 248 sites were included; see Table 2 for DNA polymorphism and Figure 3 for haplotype network): H1, with individuals from the Sianaka Forest (also known as the Sihanaka Forest); H2, with individuals from the Sianaka Forest and from an unknown location; and H3, corresponding to individuals from Lac Alaotra (i.e., S. durrelli). H1 and H2 are separated by two mutations, while H3 is separated by only one mutation from H1 and by three mutations from H2. It was not possible to amplify CR and FGB from museum samples of Salanoia and only one fresh sample was available.

Within Galidia, the Cytb phylogeny with all individuals (l: 1140 bp, v: 41, pi: 36, n=12; model: GTR+G+I; Figure 4) provided: A) a well-supported group composed of all sequenced individuals from a limited area in the north, including the dry forests of Ankarana and the humid forests of Montagne d'Ambre; B) a poorly-supported group with individuals from the humid forests of Ranomafana, Andringitra, and Andohahela, covering a latitudinal swath of about 375 km; C) unresolved position of the remaining individuals from Ranomafana, likely due to missing data (only 252 bp were retrieved from these poorly preserved hair samples). The CR phylogeny (l: 564 bp, v: 68, pi: 34, n=13; model: HKY+G+I, Supporting Information Figure S2) also provided a well-supported clade with individuals from Ankarana (north) and Andringitra (central southeast), while the clade containing the

Montagne d'Ambre individuals was more distant. Another well-supported clade grouped individuals from Tsinjoarivo (central east) and Ranomafana (southeast), sites separated by about 180 km straight-line distance. The position of the other individuals was poorly supported. We obtained five Cytb haplotypes (Table 2, Figure 4): one haplogroup from northern Madagascar (H2, Ankarana; H3, Montagne d'Ambre), separated by one mutation, and one haplogroup from the east (H1, H4, H5), separated by one to two mutations. These two haplogroups are separated by four mutations. We obtained 11 CR haplotypes (Table 2, Figure 4), and the individuals from Ankarana (H1, H11) and those from Montagne d'Ambre (H9) were not closely related. All other haplotypes are separated by at least seven mutations. The haplotype from western Madagascar (Bemaraha, H4) is the most distant (37 mutations to H1). A haplogroup (H5, H6, and H8) from Tsinjoarivo and Ranomafana is also quite divergent (23 mutations to H9).

Within Galidictis, the Cytb phylogeny (l: 1140 bp, v: 27, pi: 24, n=8, model: GTR+G; Figure 5) provided two sister clades, one corresponding to G. grandidieri and the other to G. fasciata. We obtained five Cytb haplotypes (see Table 2), which fall into two haplogroups (Figure 5), one corresponding to G. fasciata and the other to G. grandidieri, separated by 21 to 24 mutations. Galidictis fasciata haplotypes are separated by three to six mutations, and G. grandidieri haplotypes are separated by one mutation. The CR phylogeny (l: 637 bp, vi: 52, pi: 11, n=6, model: GTR+I, Supporting Information Figure S3) revealed a similar geographical structure. The CR fragment used to compute haplotype networks (l: 385 bp, v: 1, pi: 0, n=4) provided only two haplotypes separated by one mutation, one representing G. fasciata and the other G. grandidieri.

Within Mungotictis, the Cytb phylogeny (l: 1140 bp, v: 36, pi: 10, n=56; Figure 2), revealed no geographical structure amongst a large sample set obtained in the Menabe Region, which formed the sister group to one individual (MdTC731) from the Manombo River Valley of the Mikea Region (extreme southern limit of this species' range). The CR phylogeny (l: 563 bp, v: 41, pi: 31, n=51; model: HKY+G+I, Supporting Information Figure S4) also showed no geographical structure; all clades included specimens from the different sampled localities (CR was not retrieved for MdTC731). The FGB dataset (l: 591 bp, v: 2, pi: 0, n=46; Supporting Information Figure S1) lacked phylogenetic information, and MdTC731 is, as with Cytb, divergent from the other individuals. We obtained six Cytb haplotypes for Mungotictis, separated by one or two mutations, apart for H5 (MdTC731), which is separated by 23 mutations from all the others (see Table 2 for DNA polymorphism). The network has a star-like structure (Figure 6), with the main haplotype H2 (including individuals from five

localities), separated by one mutation from H1 and H3, and by 23 mutations from H5. H1 (including individuals from five localities) is separated by one mutation from H4, and H3 is separated by one mutation from H6. We obtained 19 CR haplotypes, separated by one to 13 mutations (Figure 6), structured into four groups: one haplogroup (including individuals from four localities, one of which is only found in this group) with H3 at the centre, separated from H4 and H17 by one mutation, and from H16 and H18 by two mutations; one haplogroup (including individuals from five localities, one of which is only found in this group), with H5 at the centre, with seven haplotypes separated from it by one to three mutations; and a secondary group separated from H5 by three mutations (H2, H13), and another one separated by two to three mutations (H9, H12); a separate haplotype, H1 (two localities) is separated from H5 by 14 mutations; and another one H11 (one locality) by nine mutations (see Table 3 for details on geographical distribution of haplotypes and Supporting Information Table S2 for the list of CR haplotypes). With FGB, we obtained four haplotypes, separated by one mutation. H1-H3 grouped 10 individuals from four different localities, H2 grouped 35 individuals from six different localities, and H4 corresponds to only one individual (MdTC731) from the southern limit of this species' range (which was also divergent in Cytb).

The haplotype and nucleotide diversity was the highest for Galidia, followed (in descending sequence) by Galidictis, Salanoia, and Mungotictis (see Table 2); Cytb distances observed within each genus were the highest for Galidia and smallest for Mungotictis (see Table 4). The Cytb distances between individuals assigned to S. durrelli and S. concolor ranged from 0.3±0.1% (to S. concolor H1) to 1±0.2% (to S. concolor H2), while the divergence between the two haplotypes of S. concolor was 0.7%. As a point of comparison, between the five Cytb haplotypes of Galidia, distances ranged from 0.4% to 2.9%; the smallest distance was between H1 (southeast) and H5 (central east), and the largest between H1 and H3 (Montagne d'Ambre, in the far north). Both H2 (Ankarana) and H3 showed considerable divergence with other sampled populations (respectively 1-2.8% and 1-2.9% from the other haplotypes; and 1% between H2 and H3), while H1, H4 (central west), and H5 have lower distances separating them (0.4-0.8%). In Galidictis, the Cytb divergence between G. fasciata and G. grandidieri ranged from 1.1 to 1.2%, while intraspecific divergence was <0.3% between the six individuals of G. fasciata and null between the two individuals of G. grandidieri. In Mungotictis, the individual from the far southwest (MdTC731) was found to be highly divergent (Cytb distances ranging from 1.8 to 2.0%), while other individuals showed a low polymorphism (0-0.6% of Cytb pairwise distances).

Discussion

Although examining the relationships within the Eupleridae was not the initial intent of this project, our phylogenetic analyses, with greater taxonomic sampling than the previously published studies by Yoder et al. (2003) and Poux et al. (2005), confirms the monophyly of the Galidiinae. Galidia is the first to branch off, then Galidictis, and our results show that Salanoia is sister to Mungotictis. Morphologically, Salanoia and Mungotictis share the presence of a first upper premolar, which is absent in other Galidiinae.

Within the different genera of the Galidiinae, we were able to assess polymorphism for several genes, examine geographical structure, and test the validity of proposed species and subspecies. The three genera Galidictis, Mungotictis, and Salanoia show low levels of polymorphism in the Cytb gene (< 2%), while more divergent haplotypes (up to 2.9%) were detected in Galidia, the only genus considered monotypic without debate. The higher divergence between populations of Galidia can be explained by its broader distributional range. Within the three other genera, the level of Cytb divergence is only up to 2% (Mungotictis), and less for the two other genera (Salanoia and Galidictis). Considering the criteria for mammal species recognition, specifically the level of Cytb divergence (>5%, Baker and Bradley 2006; >1.5-2.5%, Tobe et al. 2010), on the basis of current data, it is best to consider these genera as monotypic. Moreover, while FGB has been proven to vary between species of mammals (e.g. Bezerra et al. 2016), and especially in Carnivora (e.g. Veron et al. 2015a,b), it showed no or very little variation among Galidiinae genera.

Within Salanoia, the results showed that the population from the marshlands around Lac Alaotra, which was described as a separate species, S. durrelli (molecular data from the type specimen was included in our dataset), is less divergent from one of the Cytb haplotypes of S. concolor, than are the two S. concolor haplotypes from each other. In any case, the amount of Cytb divergence within Salanoia (0-1.2%) falls within the range of intraspecific variation as estimated by Baker and Bradley (2006) for mammals and below that of other Carnivora species (e.g. Veron et al. 2015a,b). Furthermore, in comparison, intraspecific Cytb diversity was higher in the other studied Galidiinae, in particular G. elegans (0-2.9%). Our samples of known origin for the genus Salanoia came from a limited geographical area, mostly from the lowland Sianaka Forest, which is in close proximity to the marshlands of Lac Alaotra.

Among the morphological characters outlined by Durbin et al. (2010) for separating S. durrelli and S. concolor, which we compared to specimens held in the MNHN (list of specimens available at https://science.mnhn.fr/institution/mnhn/collection/zm/item/search,

and see below), the foot structure of S. durrelli (in particular, the larger pads on the fore and hind feet and the elongated thenar and hypothenar pads on the hind feet) is similar to what we have observed in specimens of S. concolor from different localities (e.g. MNHN-ZM-MO 1866-233, 1880-2554, 1880-2553, 1962-325). Furthermore, the foot of a specimen of S. concolor (BMNH 1925.4.10.10) illustrated by Durbin et al. (2010), and compared to that of S. durrelli, seems not typical of S. concolor, based on the MNHN material. We found that the coat coloration in S. concolor varies from dark brown to rufous or light brown, with speckling in some individuals (e.g. MNHN-ZM MO-1866-233, 1962-325, 1880-2553), and thus, the colour differences highlighted for S. durrelli by Durbin et al. (2010) seem to fall within the range of variation of S. concolor.

Aspects of skull shape in S. concolor vary in the MNHN specimens (most likely associated with intraspecific variation, perhaps related to age and sex) and, hence, the differences highlighted by Durbin et al. (2012) for S. durrelli may not readily separate the two named forms. The presence of an extra cusp on P4 highlighted by Durbin et al. (2010) in the holotype of S. durrelli, was not found in any of the 13 skulls of S. concolor in the MNHN collections. However, with only one specimen of S. durrelli currently available, it is not possible to confirm if this tooth cusp character can be considered as diagnostic for S. durrelli or part of intraspecific variation within S. concolor sensu lato.

Our molecular data suggested that the Lac Alaotra population of Salanoia should not be considered a separate species. Moreover, the Lac Alaotra individuals were genetically closer to some S. concolor individuals from the adjacent Sianaka Forest, than individuals obtained from the Sianaka Forest were to each other. Hence, considering the Lac Alaotra population as a separate species or a subspecies would render the Sianaka Forest population polyphyletic. However, the Lac Alaotra samples in our study formed a monophyletic group, and this population may be physically isolated from some other S. concolor populations. The Lac Alaotra marshland habitat, as well as the humid forest habitat, are in need of conservation attention, especially in the view of the restricted range of S. concolor (Goodman 2013).

Within Galidia, our results showed that the populations are well structured, with up to 3% Cytb divergence between the most divergent individuals, which is presumably related to its larger distributional range. Two northern Galidia populations, from Ankarana and Montagne d'Ambre, were notably divergent from other sampled populations. The western population of Galidia, which was not sequenced in this study and is represented only by a CR haplotype (Bennett et al. 2009), was also notably divergent. The eastern populations form a separate haplogroup with Cytb, but, with CR, the structure is more complex, with populations

from the central east being closer to those from the north than to southeastern populations. The CR results for Galidia need to be considered with caution, given the differences to those from Cytb. As CR consists frequently of repeated fragments (which seems the case in Malagasy taxa, see Hassanin and Veron 2016), homology of sequenced fragments can be problematic, particularly in the absence of longer amplifications for double-checking the sequences, which was not possible to do for poorly preserved samples.

On the basis of the data (in particular Cytb), as well as taking into account the results of Bennett et al. (2009), we suggest that the northern populations (Montagne d'Ambre and Ankarana regions) be recognized as G. e. dambrensis, the western population as G. e. occidentalis, and the eastern populations as G. e. elegans, although the latter might prove to have a more complex structure. These subspecies were described based on coat colour variation, but there is some variation even within G. e. elegans (Albignac 1973). The separation of these subspecies and populations is presumably associated with geographical distances and their potential low dispersal capacity, as well as the different forest types and historical habitat connections (such as the former continuous corridor of humid forest in the east and the isolated deciduous forest in the central west). Remnant Galidia populations will continue to become further isolated due to human-induced habitat destruction, underlining the clear need for heightened conservation attention. As a case in point, Muldoon et al. (2009) found subfossils of Galidia in Ankilitelo Cave, in the southwest and outside the modern range of this genus, that, based on C14 analysis, were dated to about 500 years ago; its range reduction could be best explained by human degradation of the environment.

Within Galidictis, we obtained two separate clades corresponding to the two described species, G. fasciata and G. grandidieri, which showed relatively low levels of genetic divergence (1.1 to 1.2% for Cytb), and no divergence in the nuclear marker. Differences between these two species have been shown for their habitat preferences, life history traits, behaviour, size, and pelage coloration (Goodman 2003), although more work has been conducted on G. grandidieri (Andriatsimietry et al. 2009; Marquard et al. 2011), and further information is needed to provide greater insight into these presumed differences. The absence of nuclear variation and the low mitochondrial divergence between these two species suggest they are best separated at the subspecific level (G. f. fasciata and G. f. grandidieri).

The morphological differences of the two Galidictis species, as described by Wozencraft (1986), concern mainly size and coat pattern (with wider spaces between the longitudinal stripes). The G. f. fasciata skins available in the MNHN (MNHN-ZM-MO 1880-1962, 1882-1613, 1882-1615, 1932-3539, 1955-601) demonstrate variation in stripe colours

(brown or black) and in the width and number of stripes (six, but the two median stripes can split into two on the second half of the back).

Galidictis f. grandidieri was originally described from two specimens, and was compared to 15 specimens of G. f. fasciata, and none from the southern part of its range, based on the map presented in Wozencraft (1986). Since then, additional specimens have been obtained for both subspecies. In particular, Marquard et al. (2011) captured 43 individuals of G. f. grandidieri (30 being adults), for which males and females showed differences in body mass. This highlights the need to take sexual dimorphism into account when assessing the morphological differences within Galidictis, which was not done by Wozencraft (1986). Marquard et al. (2011) gave the range of total length in G. f. grandidieri as 685 to 752 mm for 19 males, and 707 to 758 mm for eight females, which fits the measurements for this taxon included in our study (FMNH specimens, 703 mm for one male and 706 mm for one female). For G. f. fasciata, the total length of the FMNH museum specimens included in our molecular study ranged from 581 to 632 mm for two males, and from 558 to 610 mm for four females; specimens of this subspecies in the MNHN showed, however, important size variation (although measurements taken from fresh specimens were not available, so exact data cannot be provided). More external measurement and body mass data are needed for G. f. fasciata across its range, which should then be compared to those of G. f. grandidieri to better evaluate aspects of sexual dimorphism and size differences between these two forms.

The morphological differences between these two Galidictis lineages might be related to the isolation of G. f. grandidieri in the southwestern spiny bush, while G. f. fasciata is found in the eastern humid forests. Moreover, G. f. fasciata is sympatric with other Galidiinae species across its range, while G. f. grandidieri does not co-occur with any other Galidiinae, and some character release may have taken place in absence of competition (as is known in Asian mongooses, Simberloff et al. 2000; Veron et al. 2007), which might explain the size difference observed.

Recently, Muldoon et al. (2009) identified some cave deposit specimens of Late Holocene age in the southwest of Madagascar as G. f. grandidieri, 50 km north of its present known range. This area has been recently surveyed for mammals (S. Goodman, unpublished data) and the absence of this species nowadays indicates how rapid distributional changes can occur in small carnivorans.

Within Mungotictis, the main divergence was found between populations currently assigned to two subspecies, M. d. decemlineata (central Menabe Region) and M. d. lineata (extreme southwest), with 1.8 to 2% Cytb divergence between the two forms. Within M. d.

decemlineata, which was sampled across a limited area, the populations are not structured, and we found many shared haplotypes at different localities, which is not surprising for a species with such a restricted range. The morphological characteristics proposed to separate the two forms of Mungotictis (Pocock 1915; Albignac 1973) include coat colour and the number and conspicuousness of the dorsal stripes. However, MNHN specimens referable to M. d. decemlineata (MNHN-ZM-MO 1881-288, 1961-975, 1961-976, 1964-236) exhibit variation in coat coloration and patterns. Until further data are available, we propose to maintain these two forms as subspecies. We underline that, due to their restricted ranges in deciduous and spiny forest habitats, which are rapidly declining (Grinand et al. 2013), they require conservation attention, in particular M. d. lineata.

In conclusion, our molecular results suggest the recognition of four species in the Galidiinae, rendering each genus monospecific. The level of genetic divergence between populations within genera is limited and most species have a low genetic polymorphism, but some did show geographical structure. We propose to recognize three subspecies of Galidia elegans (G. e. dambrensis, G. e. elegans, and G. e. occidentalis), two subspecies of Mungotictis decemlineata (M. d. decemlineata and M. d. lineata), and two subspecies of Galidictis fasciata (G. f. fasciata and G. f. grandidieri). Concerning Salanoia, we place S. durrelli as a junior synonym of S. concolor. It is critical to point out that the Lac Alaotra population of S. concolor and the Mikea Region population of M. d. lineata, and in a general sense all taxa of Galidiinae, need increased attention associated with field studies to understand aspects of their natural history and apply this information to concrete conservation actions.

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Figure Legends

Figure 1: Generalized distribution of the four recognized genera of Galidiinae based on IUCN (2016) and localities of samples (dots) used in this study.

Figure 2: ML tree of the Galidiinae based on complete Cytb sequences (1140 bp), with bootstrap proportions, and BI posterior probabilities ≥ 0.99 indicated by stars. The maps next to each clade indicate the distribution of each genus, with the recent species or debated subspecies in red (Salanoia durrelli, Mungotictis decemlineata lineata, Galidictis grandidieri).

Figure 3:
a: ML tree of Salanoia indicating the locality of samples based on complete Cytb sequences (1140 bp), with bootstrap proportions, and BI posterior probabilities ≥ 0.99 indicated by red stars below the branches;
b: Median joining network of Cytb haplotypes for Salanoia concolor and Salanoia durrelli. The size of each circle is proportional to the haplotype frequency; the shortest link corresponds to one mutation. Black: S. concolor, Sianaka Forest; red: S. durrelli (Lac Aloatra); grey: S. concolor of unknown location;
c: Distribution map of S. concolor (black) and S. durrelli (red, region of Lac Alaotra).

Figure 4:

a. ML tree of Galidia indicating the locality of samples based on complete Cytb sequences (1140 bp), with bootstrap proportions, and BI posterior probabilities ≥ 0.99 indicated by red stars below the branches;

b. Median joining network for Galidia elegans of Cytb haplotypes (top) and CR haplotypes (bottom). The size of each circle is proportional to the haplotype frequency; the shortest link corresponds to one mutation. Black: north (Ankarana & Montagne d'Ambre); dark grey: east/northeast (Namarafana, Zahamena); light grey: east/southeast (Andringitra); red: west (Bemaraha);

c. Distribution map of Galidia elegans (with the same colour code as the networks).

Figure 5:

a: ML tree of Galidictis indicating the locality of samples based on complete Cytb sequences (1140 bp), with bootstrap proportions, and BI posterior probabilities ≥ 0.99 indicated by red stars below the branches;

b: Median joining network of Cytb haplotypes for Galidictis. The size of each circle is proportional to the haplotype frequency; the shortest link corresponds to one mutation. Black: G. f. grandidieri; dark grey: G. f. fasciata from Andohahela; light grey: G. f. fasciata from Midongy-Sud; white: G. f. fasciata from Ivohibe;

c: Distribution map of Galidictis (black: G. f. grandidieri, grey: G. f. fasciata).

Figure 6:

a. Median joining network of Cytb haplotypes (top) and CR haplotypes (bottom) for Mungotictis. The size of each circle is proportional to the haplotype frequency; the shortest link corresponds to one mutation. In the Cytb network, H5 is MdTC731 (from the Manombo River Valley in the Mikea Region); MdTC731 did not yield a CR sequence;

b. Distribution of Mungotictis (green outline) and localities of samples (dots, with the same colour code as the networks).

643 **List of Supporting Information**

644

645 Supporting information Table S1: Primers used in this study.

646

647 Supporting information Table S2: List of CR haplotypes in Mungotictis.

648

649

650 Supporting information Figure S1: ML tree for all Galidiinae genera, represented by one
651 individual per locality for each species, for the FGB fragment (665 bp), with bootstrap
652 proportions, and BI posterior probabilities ≥ 0.99 indicated by red stars below the branches.

653

654 Supporting information Figure S2: ML tree of Galidia elegans for the CR fragment (564 bp),
655 with bootstrap proportions. Sample localities are indicated.

656

657 Supporting information Figure S3: ML tree of Galidictis fasciata for the CR fragment (637
658 bp), with bootstrap proportions. Sample localities are indicated.

659

660 Supporting information Figure S4: ML tree of Mungotictis decemlineata, for the CR fragment
661 (563 bp), with bootstrap proportions. Sample localities are indicated. MdTC731 did not yield
662 a CR sequence.

663

664 **Tables**

665

666 Table 1: List of the samples included in this study. For each sample, we report the
667 identification number, the specimen/sample number (AMNH: American Museum of Natural
668 History, New York; FMNH: Field Museum of Natural History, Chicago; ISEM: Institut des
669 Sciences de l'Evolution, Montpellier; MCZ: Harvard Museum of Comparative Zoology,
670 Harvard University, Cambridge; MNHN: Muséum National d'Histoire Naturelle, Paris;
671 NHM: The Natural History Museum, London), the GenBank (Gbk) number, and locality (ND:
672 no data; NP: National Park; Res: Reserve, SR: Special Reserve). GenBank numbers in bold
673 represent new sequences produced in this study; others from: Yoder et al. (2003), Gaubert et
674 al. (2004), Bennett et al. (2009), Patou et al. (2009), Durbin et al. (2010), Jansen Van Vuuren
675 et al. (2012), Hassanin and Veron (2016).

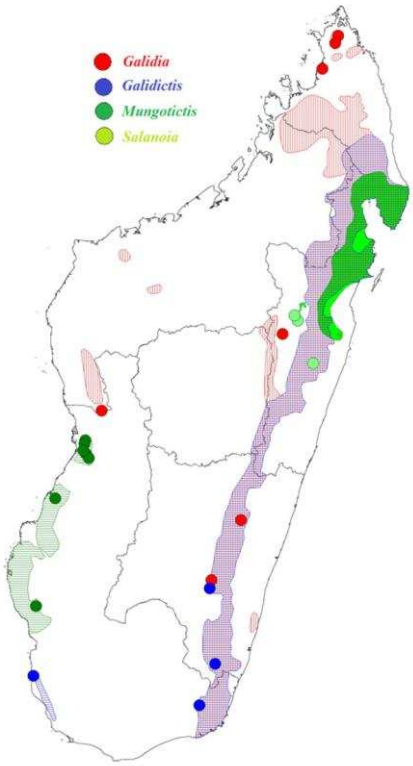
676

Table 2: Genetic diversity estimates within the four genera of Galidiinae. N: number of samples; n: number of sites used; h: number of haplotypes; Hd: haplotype diversity, Pi: nucleotide diversity; S: number of polymorphic sites; k: average number of nucleotide differences.

Table 3: Number and identification number of CR haplotypes for Mungotictis for each locality. N: number of individuals in the analysis, n: number of haplotypes.

Table 4: Summary of pairwise Cytb distances within the four studied genera.

688 Fig. 1



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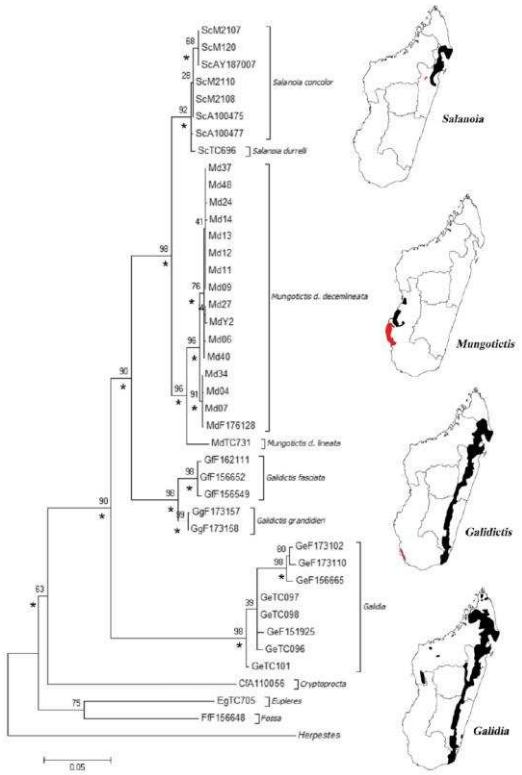
691 Fig 2



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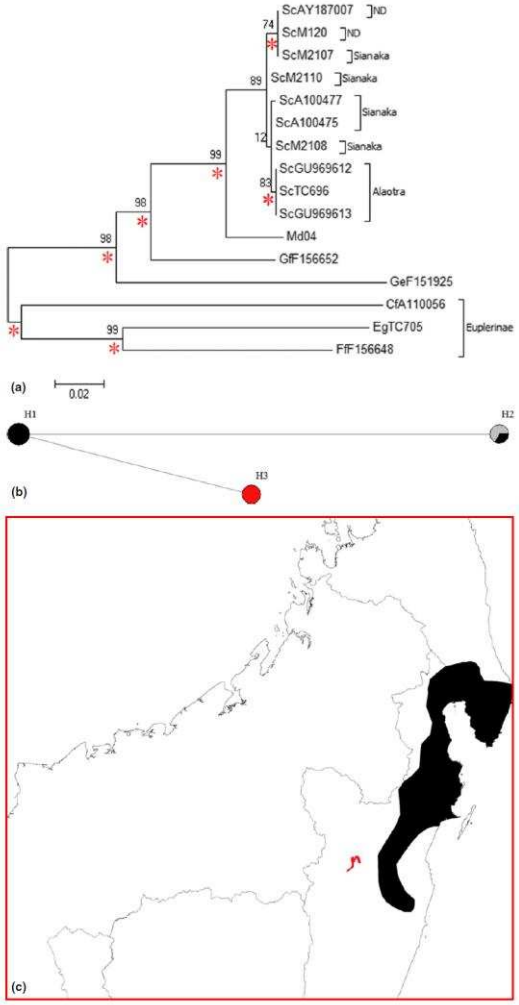
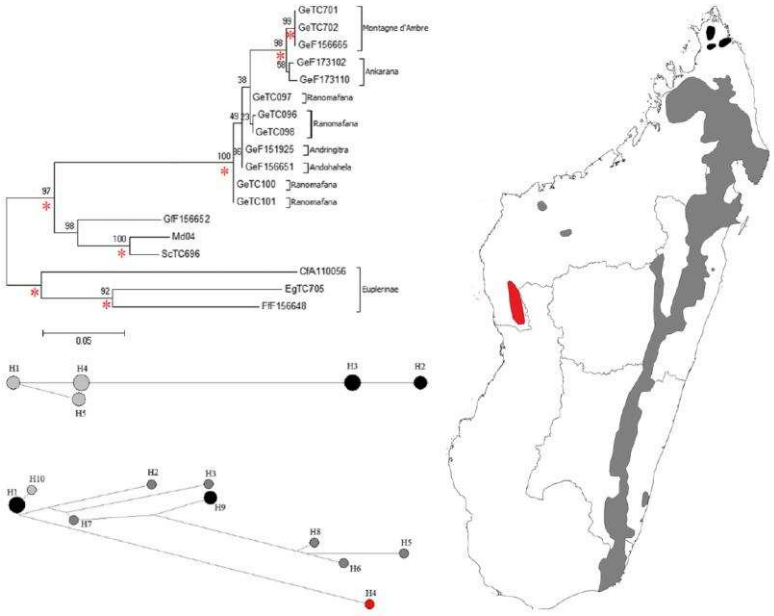
694 Fig 3



695

696

697 Fig 4



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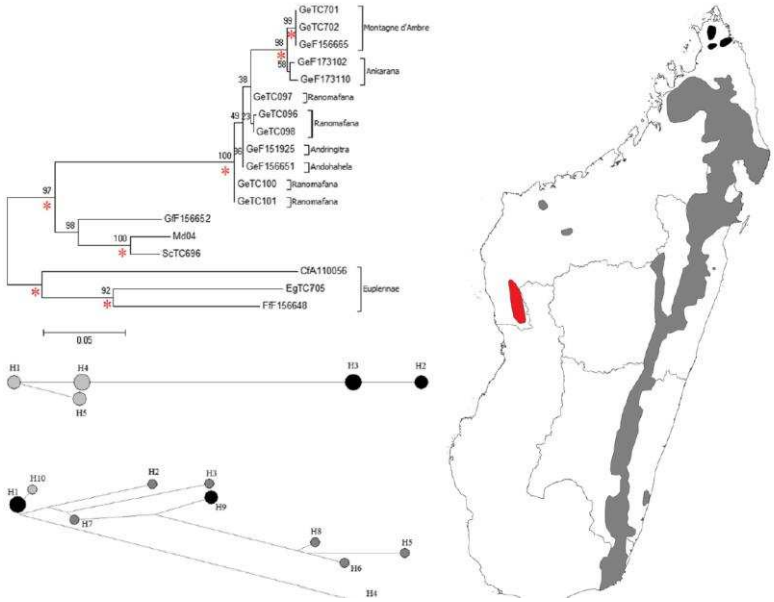
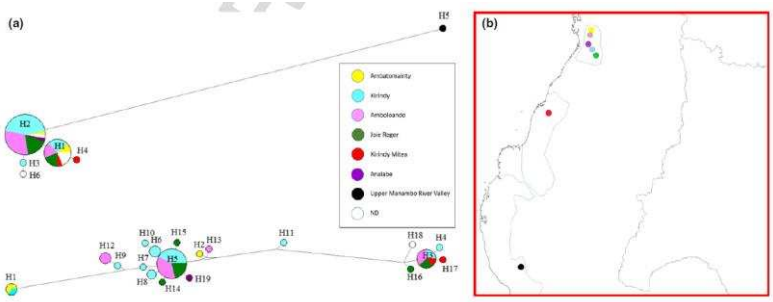
700 Fig 5


GeTC701
GeTC702
GeF156665
Montagne d'Ambre
GeF173102
GeF173110
Ankarana
GeTC097
Ranomafana
GeTC096
GeTC098
Ranomafana
GeF151925
GeF156651
Andohahela
GeTC100
GeTC101
Ranomafana
GlF156652
Md04
ScTC696
CfA110056
EgTC705
FfF156648
Euplerinae
0.05
H1
H4
H3
H2
H5
H10
H2
H3
H1
H9
H7
H8
H5
H6
H4

701

Fig 6


(a)
(b)
H1
H2
H3
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H5
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H8
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H14
H15
H16
H17
H18
H19
Amalaoe
Upper Manambolo River Valley
ND

705 Fig 6

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707