



# Imperilled phylogenetic endemism of Malagasy lemuriformes

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## ABSTRACT

**Aim** To highlight where in Madagascar the phylogenetically and spatially rare lemur species at risk of extinction are concentrated.

**Location** Madagascar.

**Methods** Phylogenetic endemism (PE) is a combined measure for apportioning a phylogenetic tree across a landscape used to identify those geographical locations that contain spatially rare phylogenetic diversity (Rosauer *et al.*, 2009). We present a simple extension (imperilled phylogenetic endemism) that scales this phylogenetic diversity by the probability of its loss to extinction. We apply these measures to a composite phylogeny of all confirmed Malagasy lemuriform species using International Union for Conservation of Nature (IUCN) extent of occurrence and threat status data.

**Results** We find that, because nonimperilled species are scattered about the lemuriform tree, interior branches of the tree are still quite secure: this also means that areas of high phylogenetic endemism for Madagascar lemuriformes are often the same areas as those of high imperilled PE (IPE), as both are heavily weighted by branches nearer the tips. However, although the North of Madagascar holds the largest amount of spatially rare evolutionary history using both PE and IPE, there are additional pockets of imperilled history in the south and west.

**Main Conclusions** Correlations of endemism and threat status with phylogenetic isolation are modest across lemurs and so are not substitutable conservation values. They might best be integrated on the landscape using IPE. As illustrated here, IPE successfully highlights areas containing species which are at once threatened with extinction and that are phylogenetically and spatially rare.

## Keywords

Conservation, endemism, lemurs, Madagascar, phylogeny, risk.

## INTRODUCTION

Endemic species, species unique to a specific geographical location, or sets of such species, have often been used to assign conservation priority to one geographical locale over another (Myers *et al.*, 2000). Phylogenetic diversity (PD) identifies the amount of genetic diversity represented by sets of species found at different locales (Faith, 1992), and this has been presented as a complementary method for focusing conservation efforts on the landscape: this genetic diversity is often considered a proxy for combined feature diversity (Faith, 1992; Forest *et al.*, 2007) or ecological breadth (Cadotte *et al.*, 2008). Recently, Rosauer *et al.* (2009) combined endemism and PD into a metric called phylogenetic

endemism (PE), which identifies geographical areas that contain the most spatially restricted phylogenetic diversity.

Practically, PE is calculated by dividing all the branches of a phylogenetic tree and apportioning them onto a landscape grid such that each branch length ( $L_c$ ) is divided equally among the grid cells in which that branch is represented ( $R_c$ ). The sum of these partial branch lengths in a particular cell is its total PE (Mooers & Redding, 2009; Rosauer *et al.*, 2009). Rosauer *et al.* (2009) presented the formula of PE as follows:

$$\text{Phylogenetic endemism} = \sum_{(c \in C)} \frac{L_c}{R_c} \quad (1)$$

A grid cell containing a highly endemic species not represented in other grid cells will be allocated the entire branch on the tree leading to that species, which will be longer to the extent that the species has no close relatives, whereas a more common species will share its unique branch among all the grid cells in which it occurs. Internal branches on the tree (those leading to clades rather than to species) are divided up among all the grid cells in which their clade members are found. Thus, PE measures areas that contain the species that are both phylogenetically distinct and spatially rare.

A third and common way in which species and areas are prioritized for conservation is risk of extinction, such that more imperilled species are given higher priority (see, e.g., Purvis *et al.*, 2000; Rodrigues *et al.*, 2006). This is especially important for landscapes with endemic species, as they are irreplaceable (Brooks *et al.*, 2006). Here, we introduce an extension of PE – imperilled phylogenetic endemism (IPE) – that combines PE with measures of species threat status. We test the application of this metric using a new composite phylogeny of all confirmed species of Malagasy lemuriform primates ( $n = 67$ ).

The Malagasy lemuriformes provide a valuable system for testing and applying PE and IPE: they are restricted to a relatively small area (the island of Madagascar), their geographical ranges are fairly well known, and a variety of gene sequence data are available for most species ( $n = 57$  of 67 confirmed species). Furthermore, many lemur species are threatened with extinction: 51 confirmed lemur species have been assigned a threat status by the IUCN, and 23 are either endangered or critically endangered. In addition, conservation planning is urgently needed for the lemurs of Madagascar more generally: many species are threatened by subsistence hunting (Lehman and Wright, 2000), logging, and habitat degradation due to agricultural activities (Johnson & Overdorff, 1999) throughout their ranges. Only 10–20% of the original forest of Madagascar used by many lemur species remains and that in small and fragmented areas (Whitmore, 2000; Ganzhorn, 2001; Lehman *et al.*, 2006; Harper *et al.*, 2007).

Below we report the first complete composite phylogenetic tree of Malagasy lemuriform primates based on sequence data and use it to calculate and compare the quantities of phylogenetic endemism and IPE on the landscape. Because species-level range maps for lemurs need refining (Harcourt & Thornback, 1990), we do not aim to present a prioritization for Madagascar reserves. Rather, we quantify the effects of novel and existing conservation methods on identifying areas of conservation worth, in the hopes that better maps can be generated soon and that managers will subsequently consider these methods.

## METHODS

### Lemuriform taxonomy and data set

The IUCN Red List (2008; <http://www.iucnredlist.org/>, accessed 2 February 2011) comprises 93 lemur species; how-

ever, 26 of these are delineated solely through consideration of genetic distance or geographical separation of ranges, and the IUCN calls for confirmation of their species status (IUCN, 2008; see also Tattersall, 2007). Thus, there are 67 confirmed lemur species. Genetic data for 57 of these 67 species were available on GenBank. We reconstructed phylogenetic relationships among Malagasy Lemuriformes from coding and noncoding regions of 12 mitochondrial genes (Cytochrome B, Cytochrome Oxidase Subunit II, Cytochrome Oxidase Subunit III, tRNA-Gly, ND3, tRNA-Arg, ND4, tRNA-His, tRNA-Ser, tRNA-Leu, D-loop and 12S) and six nuclear genes and introns (Short-wave Sensitive type 1 Opsin, Fibrinogen Alpha, Adenosine Receptor A3, ENO1, Interphotoreceptor Retinoid-Binding Protein and von Willebrand Factor), all obtained from Genbank. We aligned each gene separately, employing the local alignment tool MAFFT (Kathoh *et al.*, 2009). We then inspected the alignment of each gene by eye using Se-AL (Rambaut, 1996). Aligned sequences for each gene for each species were then concatenated into a supermatrix using the sequence-handling functions found in the R package APE (Paradis, 2006), with the remainder of the matrix filled with missing data.

### Phylogenetic analyses

We employed both maximum likelihood (ML) and Bayesian methods of phylogenetic tree reconstruction. For ML and Bayesian analyses, we employed codon position (CP) models (Shapiro *et al.*, 2006) for protein coding sequences and estimated the best-fit model of molecular evolution for intron sequences and nonprotein coding DNA using MrModeltest 3.04 (Nylander, 2004). ML analyses were conducted using RAxML version 7.04 (Stamatakis, 2006) employing the combined ML search and bootstrapping function implemented on the SFU IRMACS computing cluster. We conducted Bayesian analyses using MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003). Two runs employing four chains (three heated and one cold) were run for  $10 \times 10^6$  generations, and trees were sampled every 1000 generations. We assessed convergence using the standard deviation of the split frequencies between runs, and graphically using the program Tracer (Rambaut & Drummond, 2007) and AWTY (Nylander *et al.*, 2008). Bayesian posterior probabilities and ML bootstrap values were utilized to assess support for recovered nodes. We then rate-smoothed the consensus tree from the posterior distribution using penalized likelihood and a smoothing parameter ( $\lambda$ ) value of one as implemented in APE.

### Completing the tree

We added ten species that are recognized by the IUCN but for which we lacked sequence data to our ultrametric penalized likelihood tree based upon their taxonomy (see also Day *et al.*, 2008; Lanfear & Bromham, 2011). We first used the relevant modern taxonomic treatments to place each species

on the tree next to its sister species: *Phaner electromontis* with *P. furcifer* (Groves & Tattersall, 1991); *P. parienti* with *P. furcifer* (Groves & Tattersall, 1991); *Avahi betsileo*, *A. meridionalis*, *A. peyeriasi*, and *A. ramanantsoavani* with *A. laniger* (Andriantompohavana *et al.*, 2007); *Cheirogaleus adipicaudatus* with *C. medius* (Groves, 2000); *C. minusculus* and *C. ravis* with *C. major* (Groves, 2000); and *Eulemur rufifrons* with *E. rufus* (Mittermeieri *et al.*, 2008). Given that all genera (complete and incomplete) were monophyletic on our tree, assuming monophyly for lemuriiform genera with missing species seems justifiable. We then assigned branch lengths to these new species by first assuming a simple pure-birth model of evolution for the new subclade and then estimating the species age given the ML estimate of the local diversification rate  $\lambda$  for a subtree with  $n$  tips and stem age  $t$ , which is  $\ln(n)/t$  (see, e.g. Magallon & Sanderson, 2001). The estimated species age is  $1/(2*\lambda)$  (Steel and Mooers, 2010) (see results below). Thus, our final composite tree contains all 67 species recognized by the IUCN. An alternative approach, which ultrametricizes the posterior distribution of trees and then adds in the 10 species using the techniques of Kuhn *et al.*, (2011) yields indistinguishable trees. This full distribution of trees (which may be of use for a wider range of analyses, has been deposited on TreeBase (<http://purl.org/phylo/treebase/phylo/study/TB2:S12843>).

### Geo-spatial analysis

The spatial data comprising the geographical range of each lemur species were taken from the IUCN database for spatial data of mammals (<http://www.iucnredlist.org> data, accessed 27 August 2010). We obtained range data for 66 of the 67 confirmed lemur species (no spatial data on *Mirza zaza* are available, and so it was dropped from the tree). Maps for every branch in the tree (both external and internal) were generated using the mapping functions of R (R Development Core Team, 2011) (packages: maps, mapdata, maptools, sp, rgdal, spatial, spdep, shapefiles and mapproj) using an equi-rectangular projection and a grid cell size of 30 arc minutes (1/4 degree) of latitude and longitude, producing a sample size of 206 grid cells in which at least one lemur species was found. Grid cells differ slightly in size due to this projection, thus the average size of grid cells is 2920 km<sup>2</sup> with a standard deviation of 68 km<sup>2</sup>. The lemur IUCN maps have a coarse resolution and are advised to be only used for very large global or continental scales (Hoffmann *et al.*, 2008). Hurlbert & Jetz (2007) suggest that the grid cell size chosen for coarse range size maps should be at least 2° to avoid overestimation of species richness. However, Madagascar can be divided into 26 grid cells when the size is 2°. As our study is primarily heuristic, we chose the grid cell size of a quarter-degree, resulting in 206 grid cells that contain lemurs. Our results are reported for this scale only, and top-ranking grid cells were then identified on a map of natural and human used environment in Madagascar developed by Bidgrain (2010).

### Phylogenetic endemism

We calculated phylogenetic endemism for the Malagasy lemurs by first obtaining the length of each branch in the phylogenetic tree using the tree-handling functions in the APE. Second, the number of grid cells in which the branch or clade occurs was calculated using a program written in C# (Map Reader, available on request), and each branch of the tree was divided equally among that number. Using Excel, these branch fractions were then apportioned to each grid cell with Map Reader, using the maps of each species or the union of such maps for deeper branches). The resulting distribution of quantities of lemuriiform evolutionary history on the landscape was then visualized using heat maps (Wilkinson & Friendly, 2009) in R projected on to the map of Madagascar using the original 30 arc minutes grid. We also constructed heatmaps for species richness per grid cell (SR), and Weighted Endemism per grid cell, calculated by taking the inverse of the number of grid cells in which a species  $\times$  occurs ( $Q_x$ ) and summing the total for all  $S$  species in a grid cell, following Kerr (1997); Crisp *et al.*, 2001):

$$WE = \sum_{x=1}^S Q_x^{-1}. \quad (2)$$

### Risk-weighted phylogenetic endemism

We can extend the idea of apportioning the tree to the landscape to explicitly include the current probability of loss of portions of that tree. Suppose we have a tree with  $E$  total branches, each branch (or edge)  $e$  assigned a length  $l(e)$ , and we assign a probability of extinction ( $p(ext)_x$ ) to each tip  $x$  in the tree. Witting & Loeschke (1995) present a simple equation for the expected total loss of phylogenetic diversity from that tree:

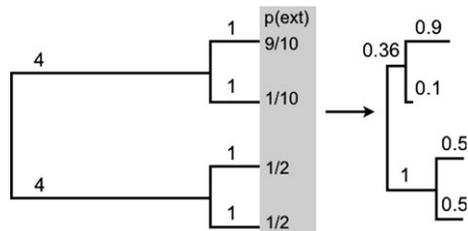
$$E(Loss) = \sum_{e \in E} (l(e) \prod_{x \in C(e)} p(ext)_x) \quad (3)$$

where  $E(Loss)$  is the expected loss, and  $C(e)$  denotes the set of tips descendent from branch  $e$ . For external branches (leading to species), the expected loss is simply the length of the branch (the first term in the summation) times its probability of extinction. The expected loss for an internal branch is its length multiplied by the probability that all the species it subtends go extinct (hence the product term). The expected total loss is then the sum of the expected loss terms for each branch. Magnuson-Ford *et al.* (2010) make explicit that this quantity is closely related to the expression for PD that is expected to remain in the future ( $E(PD)$ ; Faller *et al.*, 2008) such that  $E(PD) = PD - E(Loss)$ . The concept of expected loss is also behind the 'edge of existence' conservation programme, which highlights threatened species representing unique evolutionary history (see Redding and Mooers, 2006; Isaac *et al.*, 2007; Collen *et al.*, 2011).

Assuming one can assign  $p(ext)$  to the tips of the tree, equation (2) can be used to produce a tree whose branch lengths are in units of expected loss (see Fig. 1). If we apply the PE algorithm to this transformed tree, we quantify where on the landscape at-risk evolutionary history is concentrated. We call this mapped quantity ‘IPE’ or IPE. Following Table 1 from Mooers *et al.* (2008) and drawing on Redding and Mooers (2006) and Isaac *et al.* (2007), we applied two transformations to IUCN categories to produce rough  $p(ext)$  values (see our Table 1). Both use a nominal 100-year window and sets  $p(ext)$  for vulnerable species = 0.1, with the IUCN transformation having a much steeper slope between shifts in IUCN categories and  $p(ext)$ . Rather than assign a single  $p(ext)$  value to the 15 ‘data deficient’ species, we followed Magnuson-Ford *et al.* (2010) and used information on their ecology to estimate their threat status (Table S1 contains all taxonomic and threat status information). We report results from both transformations, with a focus on the IUCN transformation.

### Correlations among measures

To examine how different methods of reserve rankings might differ, we compared PD, PE, IPE, species richness and endemism with Pearson’s correlations as well as with linear and polynomial regressions across all 206 grid cells containing lemur species using R (R Development Core Team, 2011). As PD, PE and IPE are (weighted) sums of branch lengths found in a grid cell, we expect them to share variation between them and with raw species richness and weighted



**Figure 1** The production of a tree whose branch lengths are in units of expected loss. Because the probability of loss of internal branches is the product of the probabilities of loss of all tips above it, their worth (length) decreases relative to external branches, and their relative lengths can also change substantially.

**Table 1** Species extinction probabilities based on IUCN and Isaac transformations

IUCN category	IUCN	Isaac
Least concern	0.0001	0.025
Near threatened	0.01	0.05
Vulnerable	0.1	0.1
Endangered	0.667	0.2
Critically endangered	0.999	0.4

endemism (Mooers & Redding, 2009). We also considered the overlap between the measures for the top 20 cells – that is for those cells with greatest conservation worth.

## RESULTS

### Sequence data set

Our sequence data set consisted of 302 sequences for 57 lemur taxa comprising a total of 13,127 positions (Table S2). The matrix is 39.4% complete. Of the 13,127 positions 4062 characters were variable, and 3062 characters were parsimony informative. Taxon and character sampling was heterogeneous among higher-level lemurs. We included the pygmy slow loris (*Nycticebus pygmaeus*) and thick-tailed galago (*Otolemur crassicaudatus*) as outgroup taxa. Genbank accession numbers for all genes included in our analyses are listed in Table S2.

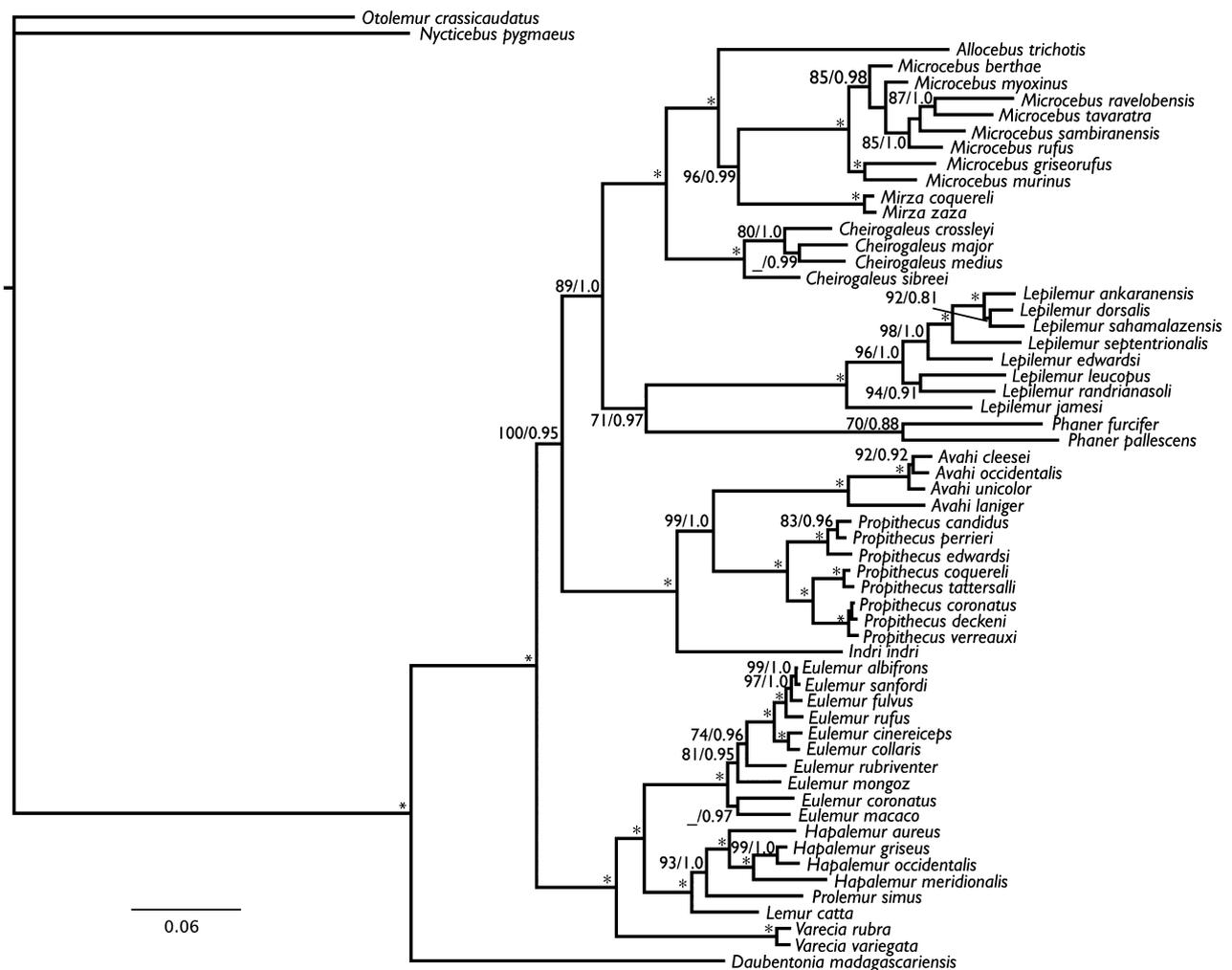
### Phylogeny of Malagasy lemuriformes

We summarized trees sampled during the last 2 million generations of mcmc sampling. Both ML (RAxML) and Bayesian (MrBayes 3.1.2) methods converged on nearly identical topologies. Similarly, with few exceptions, support for familial and generic relationships as judged by both ML (RAxML) bootstrapping and Bayesian posterior clade probabilities were robust throughout the topology (Fig. 2). No well-supported nodes differed among analyses. The composite ultrametric phylogeny of Malagasy lemuriform primates that includes species lacking sequence data that were added to the tree is presented in Fig. 3. Importantly, the average branch lengths of internal and external branches in the complete portions of the tree are very similar, supporting our use of a Yule model for placing the missing species on the tree. Based on our results, the Malagasy lemuriformes are composed of five major clades broadly corresponding to families. Among families, lemur genera were each reciprocally monophyletic, receiving Bayesian posterior clade probabilities of 1.0 (RAxML bootstrap values of 100), with the exception of the placement of the genus *Phaner* (supported with 0.97 posterior probability, RAxML bootstrap value of 73). There were few instances of poorly supported relationships within lemur genera, notably within *Eulemur*, *Propithecus* and among the *Microcebus* species (Fig. 2).

Figure 4 depicts the corresponding risk-weighted tree using the IUCN transformation for probabilities of extinction; Fig. S1, presents the tree under both the Isaac and IUCN transformations for comparison.

### Phylogenetic endemism

The heat map for PD is given in Fig. 5d, and PE is depicted in Fig. 5e. The 20 grid cells with the highest PE values are primarily found in the north and the east of the Island of Madagascar. Interestingly, although PE does show significant clustering, as might be expected (Join Count test on the top



**Figure 2** Bayesian consensus phylogeny of 57 Malagasy lemuriform species based on 12 nuclear and mitochondrial genes. Support values are as follows: \* are placed at nodes receiving both 100 ML bootstrap support and 1.0 Bayesian posterior probabilities, otherwise ML bootstrap/Bayesian posterior probability values are reported; ‘\_’ represent values for nodes receiving ML or Bayesian support values <70.

20 cells,  $Z = 31$ ,  $P < 0.001$ ), some of the highest-ranked grid cells are dispersed rather than clustered (e.g. the grid cell with the highest and third highest PE can be found in the east of Madagascar, whereas the second highest PE level is found in the north). The 20 highest ranking grid cells all contain differing amounts of fragmented areas of forest (Bidgrain, 2010). Half of the top 20 PE grid cells contain national and special reserves, and six of the 20 contain national parks. Forest in Madagascar is concentrated in the north, east and along the west coast (Fig. 5a); although relatively species-rich (Fig. 5b), the west does not seem to harbour exceptional amounts of lemur PE.

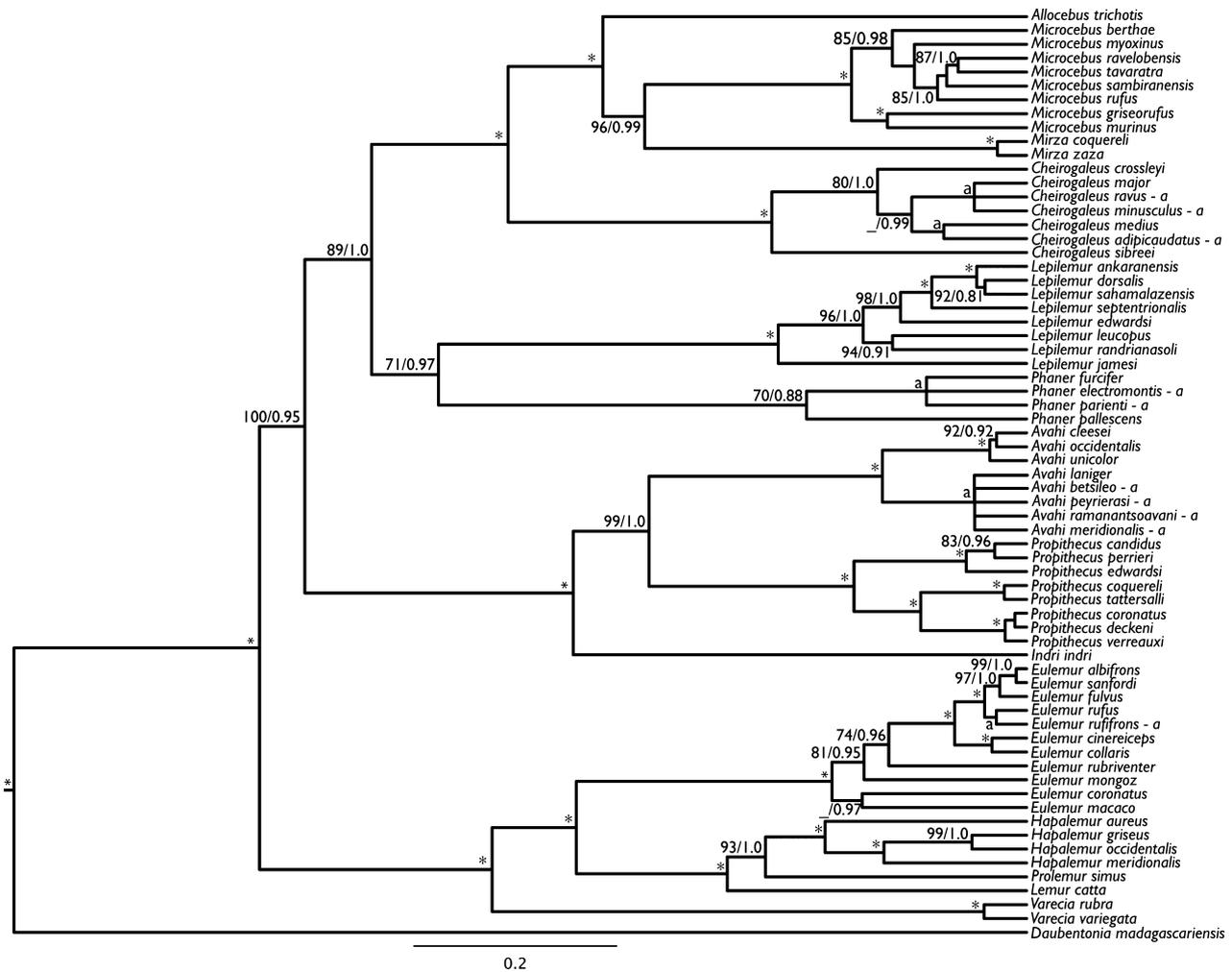
### Imperilled phylogenetic endemism

Figure 5f presents the heat maps of the risk-weighted lemur trees mapped onto the landscape using the IPE algorithm and the IUCN transformation, and the corresponding map for the IPE algorithm and the ‘Isaac’ transformation can be found in Fig. S2. Eight of the top 20 IPE<sub>IUCN</sub> grid cells are

found in the north of Madagascar, with the remainder scattered in the east ( $n = 11$ ) and west ( $n = 1$ ). The top 20 IPE<sub>IUCN</sub> grid cells all contain forested habitat, two of these 20 grid cells also contain conservation reserves, and national parks occur in nine grid cells (Bidgrain, 2010).

### Correlations among measures

Heatmaps of species richness (SR), weighted endemism (WE), phylogenetic diversity (PD), phylogenetic endemism (PE) and Imperilled PE (IPE<sub>IUCN</sub>) are presented in Fig. 5 (and IPE<sub>Isaac</sub> is presented in Fig. S2). Pairwise rank correlation coefficients across all the measures are presented in Table 2, and pairwise scatterplots are presented in Figs S3 and S4. Rank correlations were generally high to very high, especially among the various phylogenetic endemism measures. The weakest relationship was between PD and Weighted endemism ( $r = 0.5$ ). Nonlinear models on transformed data improved the pairwise fit of relationship between variables somewhat (Table S3); for instance,  $R^2$  for the relationship between phylogenetic



**Figure 3** Rate-smoothed composite phylogeny of Malagasy lemuriform primates based on 12 nuclear and mitochondrial genes. Nodal support values are as follows: \* are placed at nodes receiving both 100 ML bootstrap support and 1.0 Bayesian posterior probabilities, otherwise ML bootstrap/Bayesian posterior probability values are reported, ‘\_’ represent values for nodes receiving ML or Bayesian support values < 70. Lineages lacking sequence data that were added by hand are denoted with ‘- a’ after the species name and an ‘a’ at the nodes.

endemism and species richness improves from 0.67 in a simple linear model to 0.70 in a quadratic framework. Even with nonlinear fits, there is appreciable nonoverlapping variation (on the order of 30%) between phylogenetic and nonphylogenetic measures of conservation worth.

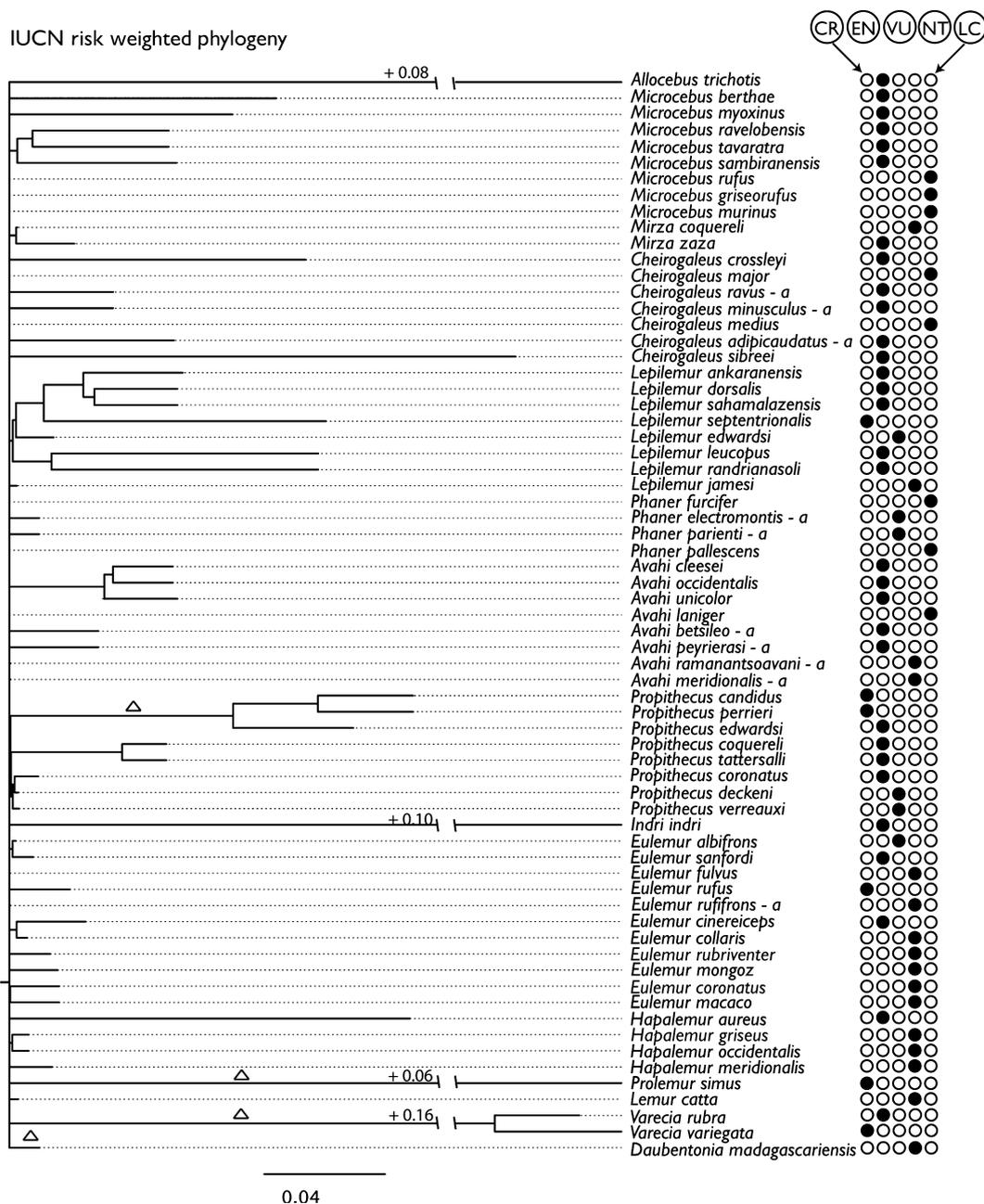
Moreover, if we confine our analyses to the top 20 cells, the overlap among metrics is only moderate (Table 3). Overall, maps of species richness and weighted endemism are not very congruent, having only 12 cells in common. This is due to the fact that the relevant species show high variance in range size. PE and IPE show mild overlap with each other (see discrepancies in particular the Northwest); this is also expected because IPE incorporates an additional independent variable. PE also shows only mild overlap with SR and WE. Seventeen of the top 20 cells are common to the three phylogenetic methods (PE, IPE<sub>IUCN</sub> and IPE<sub>Isaac</sub>), and 10 grid cells are common in the top 20 across SR, WE and the three range-weighted phylogenetic methods. The overlap of

with PD with the other metrics is low, and only six grid cells are common across all six metrics.

## DISCUSSION

### Phylogeny of Malagasy lemuriformes

We present the most complete, best-supported phylogeny of the Malagasy lemuriformes to date. Our tree is broadly in agreement with previous phylogenies: the five lemur families are shown to be monophyletic and *Daubentonia madagascariensis* diverges early as the sister to all other lemurs. Our topology is completely congruent with that of Horvath *et al.* (2008), with stronger support (posterior of 1.0 vs. 0.77) for the clade including *E. rufus*, *E. rubriventer*, *Hapalemur griseus* and *Lemur catta*. While our topology and that of the consensus tree of Chatterjee *et al.* (2009) are not fully congruent (having an NNI or Nearest Neighbour Interchange distance of 9;



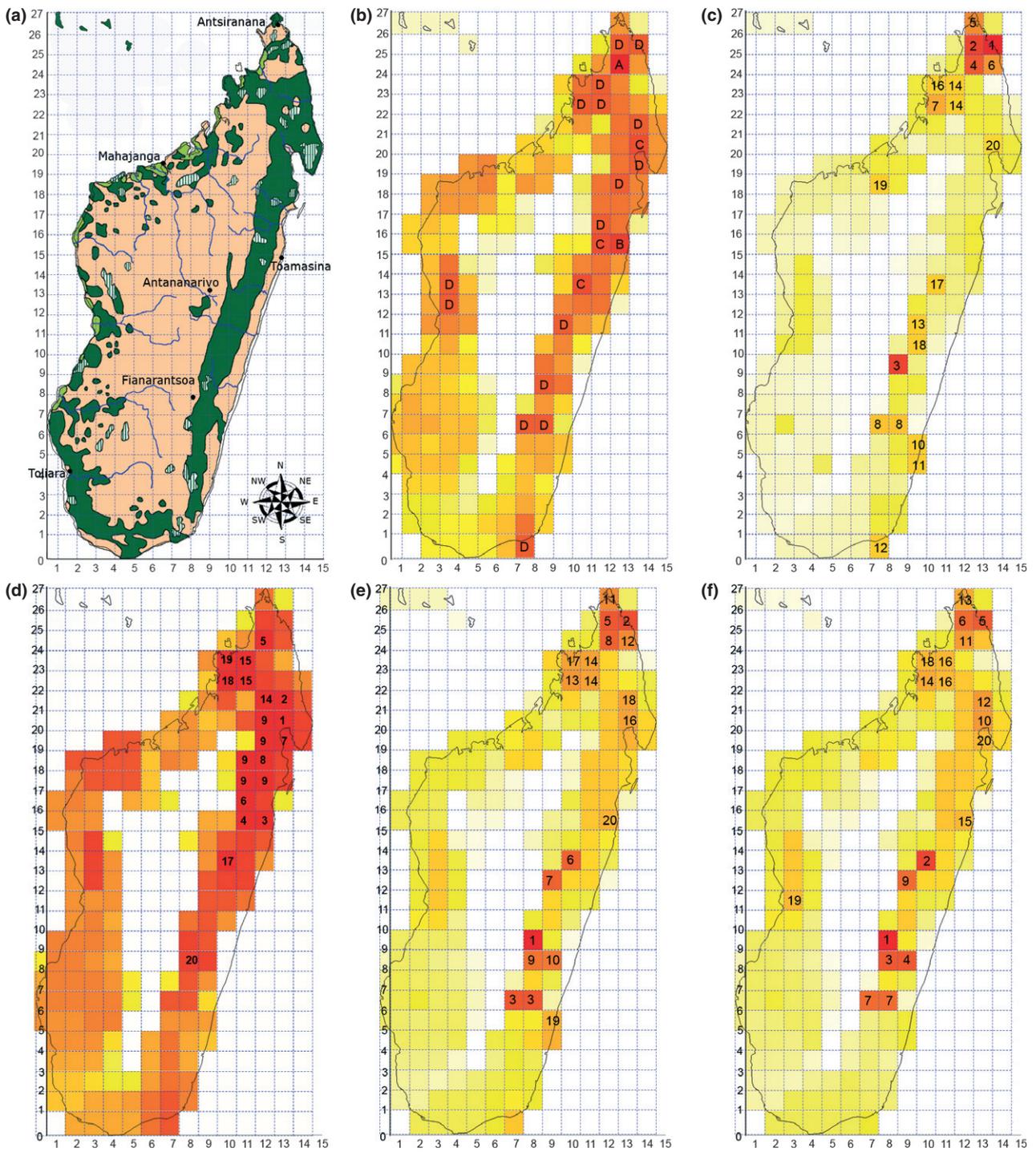
**Figure 4** The tree from figure 2 with branch lengths in units of expected loss, using the International Union for Conservation of Nature (IUCN) weighting scheme: longer branches signify more 'at-risk' evolutionary history (in relative time units) than do shorter branches. Open triangles above branches indicate branches whose lengths are very different when comparing IUCN versus Isaac probabilities of extinction (see Fig. S1).

see Felsenstein, 2004), the differences are almost exclusively in areas of lower support on one or both trees. For instance, differences within the *Lepilemur* genus have no support on the Chatterjee *et al.* (2009) tree (with bootstraps <50%), while the arrangement near *Microcebus myoxinus* has little support from our data. Importantly, a Shimodaira-Hasegawa test (1999) as implemented in PAUP\* 4.0b10 (Swofford, 2002) shows that our consensus topology and that of Chatterjee *et al.* (2009) are not significantly different on our (more extensive) data set ( $\Delta \ln L = 19.02$ ,  $P = 0.34$ ). While all trees are conditioned on

available data, we hope the present one will serve as a basis for more evolutionary ecology and conservation work in this important and charismatic group.

### Phylogenetic endemism and risk-weighted phylogenetic endemism

Similarly to PE, IPE can assess rare biodiversity consistently and independently of politically and biologically defined regions (Rosauer *et al.*, 2009). IPE adds to PE the capacity to further



**Figure 5** (Heat) maps of conservation measures with an equirectangular projection. The numbers represent the rank within the top 20, with 1 being the highest rank. Red grid cells contain the highest levels, white grid cells contain the lowest levels. Grid cells are quarter-degree squares (30 arc minutes) with the (1,1) coordinate being (43.2°W, -25°S). (a) Geopolitical map of Madagascar: dark green: forest; light green: mangroves; reserves: hatched; managed environments: tan. (b) Species Richness – here the cells marked ‘A’ = rank 1 (16 species); cells marked ‘B’ = rank 2 (14 species); cells marked ‘C’ = tied rank 3 (13 species); marked ‘D’ = tied rank 4 (12 species); (c) Weighted Endemism; (d) Phylogenetic Diversity; (e) Phylogenetic Endemism; (f) Imperilled Phylogenetic Endemism [International Union for Conservation of Nature (IUCN) transformation].

identify locations where concentrations of phylogenetically distinct and spatially and numerically rare endangered species are concentrated, which allows for more in-depth scrutiny.

For instance, the grid cell in the mid-east of Madagascar (coordinates 8, 9) ranked first for PE and IPE<sub>IUCN</sub> and third for both IPE<sub>Isaac</sub> and weighted endemism contains

**Table 2** Standard correlation coefficients among conservation metrics for all grid cells containing lemurs ( $N = 206$ )

	PE	IPE <sub>IUCN</sub>	IPE <sub>Isaac</sub>	SR	WE	PD*
Phylogenetic endemism (PE)	0.96	0.93	0.89	0.82	0.72	
Imperilled PE (IPE <sub>IUCN</sub> ) <sup>†</sup>		0.95	0.81	0.82	0.72	
Imperilled PE (IPE <sub>Isaac</sub> ) <sup>†</sup>			0.88	0.80	0.82	
Species Richness (SR)				0.64	0.95	
Weighted Endemism (WE)					0.50	

\*PD, Phylogenetic Diversity of a grid cell (Faith, 1992).

<sup>†</sup>See Table 1 for transformations of IUCN categories to nominal extinction probabilities.

**Table 3** Top-20 grid cell overlap among conservation metrics\*

	PE	IPE <sub>IUCN</sub>	IPE <sub>Isaac</sub>	SR	WE	PD <sup>†</sup>
Phylogenetic endemism (PE)	90	85	65	75	50	
Imperilled PE (IPE <sub>IUCN</sub> ) <sup>‡</sup>		90	70	65	55	
Imperilled PE (IPE <sub>Isaac</sub> ) <sup>‡</sup>			80	65	55	
Species Richness (SR)				60	65	
Weighted Endemism (WE)					35	

\*Entries are percentage of the top twenty grid cells that are common to a pair of metrics.

<sup>†</sup>PD: Phylogenetic Diversity of a grid cell (Faith, 1992).

<sup>‡</sup>See Table 1 for transformations of IUCN categories to nominal extinction probabilities.

*Cheirogaleus sibreei*, a relatively isolated species on the tree that is endangered and restricted to only two disjunct grid cells. This species and its habitat might be worthy of new attention in conservation planning exercises.

### Correlations among measures

Closely related species of lemurs tend to be broadly allopatric, and species ages are generally similar on the tree (with the exception of the broadly distributed *D. madagascariensis*, and *Indri* and *Phaner* species). This drives high correlations between phylogenetic and nonphylogenetic metrics generally. For instance, weighted endemism is concentrated in the north of Madagascar. The highest-ranked grid cell for WE harbours 12 species, eight of which have range sizes of eight grid cells or fewer (the average number of grid cells is 21, while the median is 8). This high level of weighted endemism also leads to a high rank for PE (2nd), and IPE<sub>IUCN</sub> (5th)

and IPE<sub>Isaac</sub> (1st). The exception here is the phylogenetic metric PD, which shows a weak correlation with WE ( $r = 0.50$ ). Given the strong relationship between PD and SR, this shows the strong influence of range size on PE.

Focussing on the newer phylogenetic measures, external branches contribute most to both PE and IPE. Thus, areas of high phylogenetic endemism should be congruent with those of high imperilled PE. This congruence is increased for Madagascar Lemuriformes because species with low current extinction probability are distributed throughout our phylogeny (see also Magnuson-Ford *et al.*, 2010), safeguarding internal branches and so down-weighting them in IPE calculations (indeed, most internal branches are of negligible length under the IPE transformation (Figs 3 & S1) attesting to their small chance of being lost. Application of IPE to taxa with a more uneven distribution of extinction probability (specifically clustering of high extinction risk within small clades) is likely to lead to internal branches that are less secure and possibly even more mismatch between PE and IPE.

What mismatches there are between PE and IPE are driven by the interaction among range size, branch length and threat status. So, grid cells containing phylogenetically distinct and spatially rare species contribute to high PE scores. If these species are relatively secure, however, then they will contribute little to IPE. For example, the 19th highest rank grid cell for PE (coordinates 9,5) is ranked 58th under IPE<sub>IUCN</sub>. The distinct and small-range *Lepilemur jamesi*, which contributes to the high rank for PE there, has low relative extinction probability, and so this grid cell is not flagged as a high-priority area.

PE correlates more strongly with IPE<sub>IUCN</sub> than with IPE<sub>Isaac</sub>. This is expected, because the IUCN weighting scheme ranges over four orders of magnitude vs. only two under the Isaac *et al.* scheme (note the different scales for the two trees in Fig. S1). However, and somewhat counter-intuitively, the more extreme IUCN weighting scheme increases the correspondence between IPE and species richness: grid cells with high species richness have a higher probability of containing at least one endangered or critically endangered species.

While the risk-weighted trees using the two transformations are very congruent, there are a few clear outliers: the *Varecia* clade, the *Propithecus* clade and *Prolemur simus* are all endangered or critically endangered, resulting in higher relative weights under the IUCN than under the Isaac transformation. On the other hand, *D. madagascariensis* has a very long external branch, but is listed only as Near Threatened, and so has a lower relative extinction probability for IPE<sub>IUCN</sub> than for IPE<sub>Isaac</sub>.

Given the strong impact of external branch length and range size on phylogenetic endemism scores, it is instructive to consider the relationships among external branch length, range size (measured as number of grid cells occupied) and  $p(ext)$  across species (Fig. S5). The correlation across all 206 grid cells is significant for  $\ln(range\ size)$  and  $\ln(p(ext))$ :  $r = -0.62$  and  $-0.63$  (IUCN and Isaac transformations,

respectively). However, more than 35% of the variation in  $p$  ( $ext$ ) is independent of range size. For the other two measures, there is no correlation at all:  $\ln(\text{external branch length})$  and  $\log(\text{range size})$ :  $r = 0.12$ ;  $\ln(\text{external branch length})$  vs.  $\ln(p(ext))$ :  $r = -0.17$ . It is this independence that suggests that IPE may be a useful method to highlight the areas that contain the rarest species overall.

## CONCLUSION

Most conservation metrics, including those used here (weighted endemism, species richness, phylogenetic diversity and phylogenetic endemism) are contingent on a clear view of what constitutes a lineage worth preserving. As highlighted with the Madagascar Lemuriformes (with 67 well-supported species, but over 90 putative species), this is a very vexing issue. Although we found strong correspondence in what areas are ranked high using two different sets of extinction probabilities, such probabilities are neither static nor fully quantifiable and more work is needed (see also Mooers *et al.*, 2008). Regardless, phylogenetic endemism metrics speak to a growing realization that all lineages are not equal and that geographical and evolutionary redundancy should be considered explicitly in conservation alongside threat, especially in triage situations (Welnitz & LeRoyPoff, 2001; Isaac *et al.*, 2007; Marris, 2007). Although we included PD of a grid cell as a further measure of conservation worth for comparison, and phylogenetic endemism has Faith's (1992) phylogenetic diversity (PD) concept at its core, the two approaches are distinct. Faith's PD measure was designed with marginal gains in mind (see, e.g. Forest *et al.*, 2007); Rosauer *et al.* (2009) were clear that PE was not designed with this goal. For instance, protecting the top-ranked areas in Fig. 4 would not necessarily protect the maximum amount of lemur PD overall (see also Faith, 2008). Like species richness and weighted endemism, (I)PE must be treated as an additional point measure of biodiversity value on the landscape. Given that it does include endemism and edge lengths explicitly, it would be interesting to test its ability to capture cumulative PD. Regardless, phylogenetic endemism may be a useful tool for helping identify potential areas for conservation reserves, especially when good spatial and phylogenetic data are available. PE and IPE would serve as useful layers when prioritizing areas for conservation action, especially in a planning exercise that mapped and combined metrics of interest on the landscape first and added planning boundaries later (Rosauer *et al.*, 2009).

## ACKNOWLEDGEMENTS

We are especially grateful to Tom Sanders for assistance with computer programming and designing the C# program that divided branches among grid cells based on occurrence maps. We are grateful to Simon Reader for facilitating this collaboration, to Walter Jetz and the fab-lab at SFU for input, and to Walter Jetz, Dan Rosauer, Karen Magnuson-Ford, Simon Reader, Tom Sanders, Dan Faith and two anonymous reviewers

for commenting on previous versions of this manuscript. The IRMACS centre provided world class facilities and computing resources. This work was supported by an NSERC Discovery grant to AOM.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Table S1** Taxonomic and threat status data for Malagasy Lemuriformes.

**Table S2** Genbank Accession numbers for Malagasy Lemuriforme sequence data.

**Table S3** Comparisons between variables using linear and nonlinear (quadratic) models. Abbreviations as in the main text.

**Figure S1** IUCN risk-weighted tree of lemurs (left), Isaac risk-weighted tree (right) and IUCN designation in the centre (note scale difference between trees).

**Figure S2** The heatmaps of  $IPE_{IUCN}$  (A) and  $IPE_{Isaac}$  (B). The top 20 with the highest ranking are numbered.

**Figure S3** Scatterplots across the three phylogenetic conservation measures and Phylogenetic Diversity across 206 grid cells for Malagasy lemuriformes. A.  $IPE_{IUCN}$  vs. PE; B.  $IPE_{Isaac}$  vs. PE; C.  $IPE_{Isaac}$  vs.  $IPE_{IUCN}$ ; D. PE vs. PD; E.  $IPE_{Isaac}$  vs. PD; F.  $IPE_{IUCN}$  vs. PD. See main text for abbreviations.

**Figure S4** Scatterplots of phylogenetic vs. nonphylogenetic conservation measures across 206 grid cells for Malagasy lemuriformes. Endemism = Weighted Endemism; IPE, Imperilled Phylogenetic Endemism. See Table 1 in main text for further details.

**Figure S5** Scatterplots of across-species measures of 'rarity' for Malagasy lemuriformes. 'Pendant Edge' is the relative length of the external branch leading from the species to the rest of the phylogenetic tree; Range size is the number of grid cells occupied by a species. P(ext) is the transformation of IUCN threat status from Table 1 of the main text.

## BIOSKETCHES

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Editor: Jeremy Austin